WITH OBESITY BECOMING THE NEW NORMAL, WHAT SHOULD WE DO?

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WITH OBESITY BECOMING THE NEW NORMAL, WHAT SHOULD WE DO?

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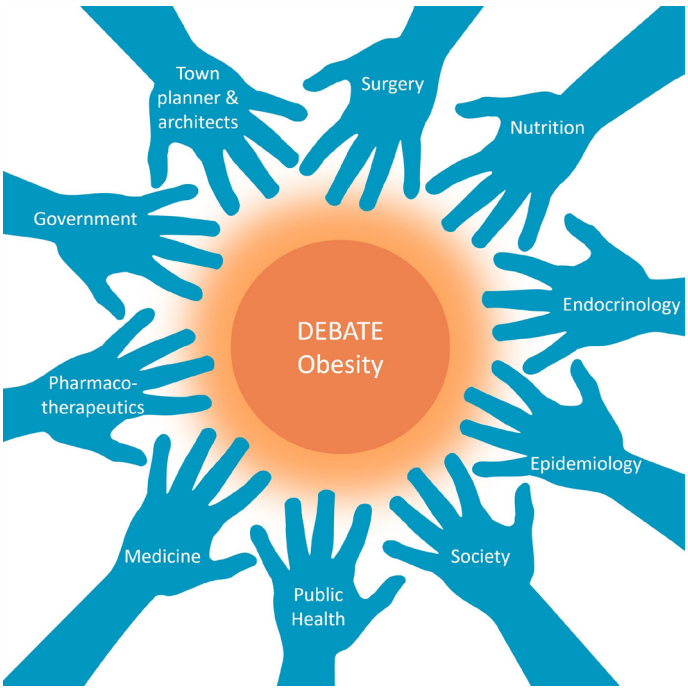

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Obesity is a global epidemic and an urgent health crisis impacting human health and health services, with the economic consequences of loss of human capital. It is a crisis for health professionals, health economists and government officials managing finite resources and the economy with premature loss of life and economic productivity. In this Frontiers Research Topic, researchers from a breadth of disciplines internationally contributed reviews, meta-analyses and novel data on the challenges obesity presents in attempts to stimulate debate on strategies and solutions for this crisis.

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EDITORIAL
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Editorial: With Obesity Becoming the New Normal, What Should We Do?

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The Editorial on the Research Topic

With Obesity Becoming the New Normal, What Should We Do?

In just a blink oft Earth's eye (approximately three decades), obesity has become a global epidemic and an urgent health crisis due to its impact on health services and the loss of human capital. It is not just a crisis for health professionals, health economists, and government officials managing finite resources and considering the economics of premature loss of life and economic productivity: it is a major societal concern that challenges the way we think about and manage resources. These resources can be considered in terms of health, human, budgetary, financial, and primary products. In this timely Frontiers Research Topic, researchers from a breadth of disciplines internationally contributed reviews, meta-analyses, and novel data on the challenges obesity presents in attempts to stimulate debate on ways forward.

The impact of modernity on body composition homeostasis is reviewed by Tremblay who challenges us to reconsider the paradigm that imbalances between food intake and physical activity are the only determinants of the obesity epidemic, given that complexities within the energy balance equation prevent a reductionist approach. Factors influencing or regulating the endocrine responses to energy-in and energy-out are discussed, including the impacts of contemporary lifestyle: alterations in the sleep-wake cycle, shortened sleep duration, environmental pollutants, high mental cognitive work, and stress. The challenge of weight loss maintenance in the face of an endocrine system that has evolved to defend body fat stores and promote weight regain is examined. The solution? In the face of the obesity pandemic, promotion of the healthiest lifestyle possible. We should not be limited by focusing solely on energy balance, but holistically intervene on sleep hygiene, reducing stress responses, and limiting environmental exposure to pollutants and endocrine disruptor chemicals, all with the aim of down-regulating the central-, adrenal-, and adipokine-regulating hormonal responses that mitigate an individual's hard won weight loss. Whilst government intervention is required to address some of these factors (such as creating safe environments), clinicians can play an important role in guiding patients to battle against the regulatory pathways and modernity factors that promote weight regain. With this in mind, clinicians must realize that the work is not done when weight loss is achieved: we keep our obese patients for our working life, as they will require ongoing guidance, supervision, and intervention.

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Where governments have shown leadership in developing and implementing policies to address obesity, the presence of bias or influence is a significant concern. In an environment where everyone is an "expert" on diet and obesity [informed from diverse sources including media, talk-show hosts, reality and lifestyle programs, advertorials, internet blogs and (occasionally) real science], do obesity policy makers and service deliverers bring their own prejudices to the execution of professional duty? Pengilley and Kelly demonstrate that uninformed opinion does influence policy implementation. They recommend that development and design of obesity policy requires a strict, robust, and transparent governance framework to prevent these prejudices from derailing the efficacy, reach, and delivery of obesity policy. Their findings and comments are prophetic, published just months before The Lancet published evidence of undue influence by a multinational sweetened beverage corporation on obesity science and policy in China (1) and, separately, Milbank Quarterly published evidence of efforts by a multinational beverage corporation to influence the US Centers for Disease Control and Prevention (2). A strongly worded editorial in Lancet Oncology very recently stated that "Governments must not allow their public health strategies to be unduly influenced by powerful multinationals who might be more concerned with protecting their own interests than helping to solve this ongoing health crisis " (3).

Where government has implemented population-based obesity prevention interventions, proof of cost-effectiveness is vital, given precious health resources. The paper from Döring et al. evaluated the cost-effectiveness of PRIMROSE, a program aimed at Swedish pre-school children that addressed healthy eating and physical activity, with the primary outcome of BMI at age 4. Cost-effectiveness was not established, due to the cost of educating nurses to implement the program and parental income lost to attend. These results raise a number of questions for intervention implementation (e.g., utilizing trained but less expensive staff). As obesity impacts on sick leave and reduced productivity at work, employers are understandably focused on their employees' health. Feldman et al. examined the effectiveness of a workplace wellness program in obese attendees and found that whilst the program was associated with only very modest weight loss, the weight loss was clinically meaningful in attendees who were ready for change. These findings assist in determining weight loss resource allocation for greatest impact. Thus, publicly and privately funded initiatives for obesity reduction are likely to make very little or only a modest dint in the obesity epidemic. The serious fundamental question in the modern epidemic of obesity is: does the solution lie in influencing the choices individuals make in an obesogenic environment (marketing works commercially, especially for foods), or is it altering or limiting the choices available to individuals, using legislation if the food industry insufficiently responds to the obesity crisis? Can we learn anything from the efficacy of government legislations that, for example, restricted the sale of alcohol (Sweden), highly taxed cigarettes (Ireland), or introduced plain packaging and graphic warnings on tobacco products (Australia)? (4). Each represented a government legislative response to harmful products and have reduced consumption and the adverse health outcomes associated with each product. Sugar taxes are hotly debated internationally. A recently published review showed that excise taxation of tobacco, alcohol, and sweetened beverage works in reducing consumption (5). Provocative food for thought.

Following on from the theme of childhood obesity prevention are three papers that report factors associated with childhood obesity and the surprising lack of cardiometabolic risk reduction with weight loss intervention. Corica et al. present sobering data in obese children aged 2–17 years, demonstrating high rates of obesity and cardiometabolic disease within the family; extreme youth did not protect against a deteriorated metabolic profile. Dalla Valle et al. similarly reported an almost universal presence of at least one cardiometabolic risk factor in obese children aged 2–17 years; obesity intervention did not improve these without a very substantive weight loss. When we consider obesity-accelerated diseases such as diabetes, heart disease, and cancer and the known consequent reduced life expectancy, these data indicate the urgent need for effective public health preventative measures, in addition to family-based interventions addressing intergenerational factors and treating not only the afflicted children, but also their parents.

Relevant to childhood obesity is consideration of the impact of obesity on reproductive health and the intrauterine environment, since excess maternal weight and weight gain in pregnancy adversely affects birth weight and subsequent childhood obesity risk. Obesity impacts the risk for polycystic ovary syndrome and reduces fertility and success of assisted reproduction. Additionally, maternal obesity impacts the risk of pre-eclampsia and gestational diabetes, unplanned Cesarean section and fetal and neonatal outcomes: not limited to premature delivery, neonatal hypoglycaemia, macrosomia, and childhood-onset of obesity. The impact of obesity on the efficacy and outcomes of assisted reproduction, reviewed by Tziomalos and Dinas examines these issues in polycystic ovary syndrome, including the evidence for weight reduction prior to assisted reproduction, recommending deferment of conception until a healthier weight is achieved.

When the impact of intrauterine over-nutrition and macrosomia on obesity risk in childhood are considered, a rationale for public health measures for healthy maternal weight throughout the reproductive years is justified. Farpour-Lambert et al. examined the evidence for lifestyle interventions during pregnancy and postpartum impacting maternal and neonatal outcomes, reporting that they reduce gestational weight gain, pregnancy-induced hypertension, the need for Cesarean section and neonatal respiratory distress syndrome, without any risk of harm to the mother or neonate. These benefits were observed across all BMI categories. Dietary interventions were associated with the greatest reduction of gestational diabetes, pregnancy-associated hypertension and preterm birth. The synthesis of the evidence reviewed substantiates that a low glycaemic load and 30–60 min of physical activity most days should become health policy directives for women during pregnancy and postpartum. In response to the Farour-Lambert paper, Skouteris et al. call attention to the lack of any international consensus guidelines on weight management pre-conception. The impact of weight

status in the pre-conception phase on fertility, pregnancy and subsequent maternal and infant outcomes is reviewed. The importance of efforts to improve the lifestyle and weight status of mothers-to-be is highlighted, with calls for international efforts. Thus, healthy lifestyle and weight in the pre-conception period and during pregnancy are critical factors in assisting newborns get off to the best start as they enter our brave new obesogenic world.

Moving away from policy, politics, and lifestyle interventions, this Research Topic includes papers enlarging our scientific understanding of energy homeostasis and its links to other organ systems. A challenge to clinicians treating and researchers investigating people with obesity is predicting energy expenditure. In a large population of morbidly obese participants, Cancello et al. examined existing models for energy expenditure calculation against indirect calorimetry. They found most models inaccurate and recommended indirect calorimetry.

The fascinating interface of gut hormone regulation in response to nutrient intake and how these hormones regulate appetite and metabolism are reviewed by Hope et al. particularly in how these hormones respond to fasting, bariatric surgery and with pharmaceutical analogs. Future therapies are discussed; particular consideration is given to the co-targeting of multiple regulatory pathways in order to tackle the many different ways that human physiology has evolved to defend energy stores in the face of (potentially life-threatening) energy deficiency. Thus far, science is yet to detect a hormonal or chemical signal that effectively says: "stop eating, our adipose stores are full." We are challenged to determine how to enhance the effects of the homeostatic effects of important gut hormones.

The central regulation of food intake is a key factor in obesity development: what makes some people say "enough" and other to say "more please" when faced with the same meal or more food? Over three decades, the number of players regulating the physiology of appetite and satiety has expanded rapidly resulting in a greater understanding of the role of numerous newly discovered gut peptides and the contribution of circadian and stress hormones. Lasschuijt et al. present novel data examining the impact of oro-sensory and central pathways involved in the endocrine response to food ingestion, specifically, the effects of the duration of chewing food and sweet taste intensity on satiety and endocrine responses. In a series of elegant experiments, healthy weight participants consumed isocaloric foods of differing textures and sweetness but with similar macronutrient composition followed by measurement of hormonal responses and subsequent food intake. Modest increases were found in pancreatic polypeptide following hard-sweet food ingestion. Interestingly, eating and spitting out the food was associated with higher acute insulin responses. These data suggest that hormonal responses are affected by food texture, not just sweetness or caloric content. The act of chewing impacts hormonal responses to food, even without ingestion. It is possible lingual sweet taste receptors may contribute, as we start to understand the unique endocrinology of the tongue. More research in this fascinating area is awaited.

Physicians and metabolic researchers are increasingly aware of the important role the liver plays, not only as a synthetic factory for essential proteins and glucose and a passive reservoir for excess lipid, but as a key regulator of many aspects of metabolism. It is not only an innocent bystander in the physical onslaught of obesity but also the victim of the inflammatory effects of chronic excessive nutrient intake. Obesity is now the commonest cause of chronic liver disease in countries spared the epidemic of hepatitis B. The widespread metabolic associates of the spectrum of non-alcoholic fatty liver disease (NAFLD) are currently being delineated. Tarantino et al. demonstrate associations between the presence of obesity-associated NAFLD and carotid artery intima media thickness, an index of atherosclerosis, apparently mediated by the degree of visceral adiposity, age, and haematocrit. These data add to our understanding in the clinical evaluation of obese patients.

Depression is closely linked to obesity. Ouakinin et al. review the evidence for neuroendocrine links between adipose tissue, the hypothalamus-pituitary-adrenal axis, the sympathetic nervous system, and circulating and tissue-based inflammation. The review provides a framework to deepen our understanding of how energy homeostasis, stress, and neuroendocrine responses are connected, to the degree that both obesity and depression share biological mechanisms and pathogenesis.

The search for blood proteins in the early detection of cancers is a promising area that may improve health outcomes. As obesity is a major contributor to the risk of many cancers, the discovery of novel or sentinel markers for early cancer detection is important. Xu et al. showed that serum zinc-α2-glycoprotein levels, a newly recognized adipokine, were higher in Chinese patients with colorectal cancer. Validation of this interesting finding in larger populations and evaluation of its efficacy as an early cancer biomarker is awaited.

Epigenetics is an expanding area of interest particularly in understanding how environmental factors up- or down-regulate genetic susceptibility to obesity. Zhu et al. review transcription factors implicated in obesity pathogenesis and mechanisms of epigenetic modification. This area presents a number of possible mechanisms by which future interventions may mitigate against the impact of environmental factors on obesity susceptibility. It represents an exciting area for future research.

The potential metabolic benefits for traditional Chinese medicine contribute to the pharmacotherapeutic armamentarium to battle obesity as reviewed by Huang et al. examining the evidence for some medicinal components to improve metabolism. Challenges in translating plant and animal-based extracts into traditional Western pharmaceuticals are discussed. Many of our patients purchase supplements with claims of benefit in an unregulated, frequently not evidence-based, but highly profitable, supplements environment. Clinicians who are well-informed on the evidence for supplements of traditional Chinese medicine are best placed to advise their patients on where there may or may not benefit.

As editors of this Research Topic, we invited authors to examine the challenges we face in the broad areas that obesity impacts. We were rewarded by high quality contributions across the breadth of medicine, clinical, and fundamental research and public health. We anticipate the reader is informed by this collection of articles that embrace the many challenges obesity

holds for all of us in basic and clinical science, as clinicians, public health planners, policy makers and consumers. To answer the question posed by this Research Topic: what should those of us in the health sciences do? Our mandate is clear, as obesity will erode all the progress we have made in human health over the last 100 years. First, we require a huge skilled health industry workforce and physical resources to tackle the health issues that people with obesity experience today and into the immediate future: we must train scientists, doctors, and allied health professionals to effectively treat, alleviate or palliate those suffering from obesity today and tomorrow. Second, our governments and industries need to be provided with evidence, so that they understand they will require deep pockets to support the immense loss of human and health capital that is occurring now and will do so for the immediate future, impacting economies internationally. Third, we must lobby for leadership from forward-looking and enterprising food industries and corporations to create products for harm minimization: food of lower energy content, smaller portion size, foods that promote satiety not hunger, an ethos of not promoting over-consumption. We must encourage food industry "disruptors": enterprising food producers and manufacturers ready to exploit the opportunities presented in a market of consumers demanding better foods, when "old school" food industry doesn't respond with real change (only cosmetic or cynical change). Finally, we suggest that our policy makers and politicians should respond with taxation, levies, and laws that protect one of the most valued resources our society has: human health. Many governments have done so with other toxic substances, such as cigarettes and alcohol, occupational and environmental hazards. Such legislations are present already in some countries and cities, with sugar taxation and portion size limits: these could be extended elsewhere. Imagine a world however, where these measures were not required. Imagine a world where food is nourishing and beneficial, not harmful.

AUTHOR CONTRIBUTIONS

KS drafted this editorial. HT, MG, JlC and JMPH each contributed by revising and editing.

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PERSPECTIVE
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Obesity Management: What Should We Do If Fat Gain Is Necessary to Maintain Body Homeostasis in a Modern World?

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The prevalence of overweight has substantially increased over the last decades despite the intent of health professionals and the general population to prevent this trend. Traditionally, this phenomenon has been attributed to unhealthy dietary macronutrient composition and/or to the decrease in physical activity participation. Beyond the influence of these factors, it is more than likely that other factors have influenced energy balance in a context of modernity. These include inadequate sleep, demanding cognitive effort, chemical pollution, and probably others which also have the potential to promote a positive energy balance but which are also part of the reality of success and productivity in a globalized world. As discussed in this paper, many individuals may become conflicted with themselves if they wish to prevent weight gain while influencing factors which are determinants of their socioeconomic success. In this regard, this paper reminds us of the contribution of adipose tissue gain in body homeostasis which is essential to permit energy balance, especially under lifestyle conditions promoting overfeeding. From a clinical standpoint, this imposes the consideration of a weight loss program as a search for compromise between what can be changed to promote a negative energy balance and what can be tolerated by the body in terms of fat loss. Furthermore, if we also consider the impact of pollution on energy balance for which we currently do not hold solutions of reversibility, we probably must accept that the mankind of today will have to be more corpulent than its ancestors. In this pessimistic environment, there are still possibilities to do better; however, this will probably require the revisiting of lifestyle practices according to what the human body and planet can tolerate as deviation from optimal functioning.

Keywords: environment, adipose tissue, body weight, pollution, sleep

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INTRODUCTION

Obesity has become a major preoccupation over the last decades because of its important increase in its prevalence all around the world. This reality has obviously mobilized researchers and health professionals who have significantly improved their knowledge and skills about obesity management. However, population statistics clearly show that this progress has not been sufficient since the prevalence of obesity continues to increase in most countries, including those where famine and malnutrition have traditionally been the main food-related problems. Furthermore, currently available estimates suggest that obesity prevalence may continue to increase in the foreseeable future (1).

Thus, it is not unreasonable to emphasize in the title of the special issue of this journal that obesity becomes the new normal human condition. In fact, this title is particularly relevant for the physiologist who perceives adipose tissue as an important organ for the maintenance of body homeostasis. As described in the present paper, adipose tissue contributes to the control of appetite and thermogenesis, plays an active role in the secretion of hormones involved in metabolic regulation, and acts as a storage site of lipid soluble pollutants. Thus, a physiological perception of obesity considers that excess body fat is the problem of individuals who rely too much on body fat gain to maintain adequate body functioning. According to the theme of this journal issue, the question that emerges is "What should we do when body fat gain is needed to maintain body homeostasis in today's life?" To document this issue, four proposals of responses are presented about "what we should do" together with some relevant implications.

PROPOSAL 1: TO BETTER UNDERSTAND THE DETERMINANTS OF OBESITY AND TO INTERVENE ACCORDING TO RELEVANT CAUSES

It is a truism to indicate that body fat gain happens in accordance to the first law of thermodynamics (i.e., it results from an excess energy intake over energy expenditure). This observation has prompted the simplistic deduction that body fat gain results from gluttony and laziness or, in a broader sense, from suboptimal macronutrient intakes and insufficient exercise. From a clinical standpoint, this has dictated the emergence of diet prescriptions such as low and very low calorie diets which completely disregard the tolerability of the body regarding these dietary practices. As further discussed, this ignorance of body homeostatic factors has generally resulted in weight regain to a value frequently exceeding pre-intervention body weight. In addition, recent research has highlighted a significant weight gaining effect promoted by non-traditionally considered lifestyle factors. These factors are not necessarily inducing a direct change in caloric intake or expenditure, but rather represent a source of stimuli that more discretely affect energy balance *via* an impact on some regulatory systems.

According to results of the Quebec Family Study (2), short sleep duration appears on top of the list of non-traditionally considered determinants of excess body weight. While population studies have clearly shown a secular decrease in sleep duration in many countries (3), laboratory-based studies have documented a clear link between insufficient sleep and the proneness to a positive energy balance, as described in **Table 1**. In addition, our research experience has showed that overweight is particularly pronounced in the short sleeper when it is accompanied by a high disinhibition eating behavior profile being related to reduced appetite control and food overconsumption (4).

Beyond the impact of inadequate sleep habits on energy balance and body composition, many other non-traditional determinants of overweight seem to be influential in a modern lifestyle. These include persistent organic pollutants (13–15), inadequate feeding behaviors (16–18), demanding cognitive effort (19, 20), and suboptimal micronutrient intake (21), especially low calcium intake (22–24). As emphasized above, these factors are not vectors of high caloric input or output but like for sleeping, they exert a significant impact on regulatory mechanisms affecting energy balance.

TABLE 1 | Summary of the effects of short sleep duration on energy balance and related variables.

Effect	Reference
• Reduces the plasma concentration of the anorectic hormone leptin	Spiegel et al. (5); Chaput et al. (6)
• Increases the plasma concentration of the orexigenic hormones ghrelin and cortisol	Spiegel et al. (7); Spiegel et al. (5)
• Promotes mild hypoglycemia	Chaput et al. (8)
• Increases spontaneous energy intake	Brondel et al. (9)
• Reduces spontaneous physical activity	Schmid et al. (10)
• Interferes with the outcome of a diet restriction on body fat	Nedeltcheva et al. (11); Chaput and Tremblay (12)

From a clinical standpoint, "what should we do?" must focus on actions directly targeting factors promoting excess energy intake. For instance, if short sleep duration or low sleep quality appears to have a causal link with the body weight status of a patient, the primary focus of clinical actions should target optimal sleep habits. On the other hand, it might well be counter-productive to rely on traditional approaches such as diet restriction and physical activity to intervene in this case. After all, exercise does not seem the optimal remedy for people who are vulnerable because of fatigue and the lack of adequate body recovery.

The multifactorial nature of the environmental determinism of excess energy intake also emphasizes the necessity to broaden multidisciplinary collaboration in the management of obesity. This *a priori* requires a more detailed evaluation and characterization of obese individuals before implementing an obesity management program. If successful, this approach should permit a more individualized management, possibly *via* the use of new technologies, that should contribute to the well-being of individuals as well as their success in body weight loss and maintenance.

PROPOSAL 2: TO BE PREOCCUPIED BY AN ADEQUATE BALANCE BETWEEN PHYSICAL AND COGNITIVE EFFORT

A discussion pertaining to obesity rarely challenges the adequacy of the socioeconomic context to which most people are confronted with today. As previously described (25, 26), we now have to deal with a globalized environment for which key words like performance and productivity are the main targets for many workers. This reality has also been accompanied by changes in the modalities of daily labor which now mostly relies on cognitive effort instead of physical work. From a biological standpoint, there is a major difference in the metabolic flexibility that is allowed to physical and mental work. Physical work relies on muscle cells that have the flexibility to use carbohydrate or lipid as fuel for ATP production. Furthermore, they also have the possibility to accentuate the use of a substrate versus the other one depending on carbohydrate availability. This is in contrast with mental work

that is based on the contribution of neurons which depend on carbohydrate availability for their activity. In this case, metabolic flexibility is reduced and the resulting work may depend more acutely on glucose availability. This observation guided us toward the hypothesis according to which prolonged demanding cognitive effort would increase energy intake to sustain carbohydrate availability. This hypothesis was initially investigated by our team in female students at Laval University who were tested under conditions of demanding mental work which were compared to a control relaxed condition. The main results obtained in these studies are presented in **Table 2**. Globally, the observed effects support the idea that demanding cognitive effort has the potential to induce a substantial acute positive energy balance. This is in agreement with the results reported by McCann et al. (27) who found that episodes of high cognitive effort in researchers preparing grant applications promoted stress and hyperphagia. This is also concordant with a recent study demonstrating that long periods of stressful homework are associated with an increase in body fat in male children (20).

Our research experience reveals that physical activity could represent an appropriate solution to prevent the hyperphagia induced by demanding cognitive effort. We have reported that a timely inclusion of a brief period of exercise between cognitive effort and mealtime prevents the hyperphagia induced by mental work (31). Concordant results have been recently reported by other investigators (32). This is also consistent with the success obtained with sport/study programs that contribute to improve fitness and prevent weight gain without altering academic success even if school time is reduced (33).

Another option to prevent the positive energy balance resulting from demanding cognitive effort is provided by pharmacology. This is the case for methylphenidate which is the active agent of drugs like Ritalin and Concerta. It has been shown that Ritalin reduces by about 50% the increase in carbohydrate utilization induced by mental work (34). This is in agreement with the observation that methylphenidate decreases spontaneous *ad libitum* energy intake (35). In individuals with obesity known to be resistant to weight loss, the supplementation of Concerta was found to result in substantial weight loss when compared to individuals receiving the same nutritional supervision with a placebo (36). Taken together, these observations suggest that methylphenidate helps to deal with demanding cognitive effort while preventing its hyperphagic effect. Spontaneously, this fits with the preoccupation of the health professionals treating obesity although it remains unclear if such a practice should be part of "what we should do in obesity management."

TABLE 2 | Acute effects of demanding cognitive effort on components of energy balance and related variables.

Effect	Reference
• Increase in energy intake without changes in appetite sensations	Chaput and Tremblay (19)
• Very weak enhancing effect on energy expenditure	Chaput and Tremblay (19); Chaput et al. (28)
• Increase in energy intake associated with increased cortisolemia and glycemia instability	Chaput et al. (29)
• Decrease in parasympathetic nervous system activity	Pérusse-Lachance et al. (30)

PROPOSAL 3: TO RECOGNIZE THE IMPORTANCE OF THE REGULATORY ROLE OF ADIPOSE TISSUE ON ENERGY BALANCE AND TO PLAN REALISTIC INTERVENTIONS

Lipid storage is the main function that has been traditionally attributed to adipose tissue. Even if we have been aware of the existence of ectopic fat deposition, adipocytes have been the target of obesity management because of their large capacity to store lipids and to release fatty acids in the context of dietary restriction. Several decades ago, the perception of adipose tissue had to be revisited with the discovery of leptin (37) which was the first hormonal messenger secreted by fat cells that was documented for its properties to affect energy balance. Immediately after its discovery, leptinemia was found to fluctuate according to energy balance (38). Accordingly, an increase in body fat was reported to be associated with an increase in leptinemia that suggested a state of leptin resistance in obese individuals (39).

The changes of plasma leptin induced by body weight/fat loss are of particular interest for the discussion of the present paper. In this regard, our experience and that of others showed that weight loss is associated with an increase in hunger (40, 41) as well as a greater than predicted decrease in energy expenditure both in the resting (42) and active (43, 44) states. Furthermore, the observation that leptin administration to weight-reduced individuals with obesity reverses these effects (41) should be viewed as a clear proof of concept pertaining to the regulatory role of fat cell secretion on energy balance. From a clinical standpoint, these observations also suggest that messengers of adipose tissue like leptin may contribute to body weight restabilization in the context of overfeeding and to the occurrence of resistance to further lose body fat in response to a prescribed negative energy balance. This also emphasizes the relevance to use some molecules such as leptin in drug formulation to attenuate the physiological vulnerability of weight-reduced obese individuals.

What does it mean in terms of "what we should do" today in the management of obesity? From a clinical standpoint, the first implication of a concept focusing on the protective role of adipose tissue is the necessity to improve lifestyle practices to prevent fat regain in the weight-reduced obese individuals. As explained above, variations in fat mass affect both energy intake and expenditure. Thus, the weight-reduced obese individual should improve his/her food, physical activity, and sleep habits with the hope that the resulting gains in functionality will be sufficient to compensate for the impact of fat loss on energy balance. According to the experience of some members of the US National Weight Loss Registry, lifestyle changes can permit the maintenance of large body weight loss over years provided that they adhere to a new lifestyle in a weight-reduced obese state. For instance, McGuire et al. (45) found that members of the registry reporting a low-fat diet (25% dietary energy as fat) and a regular

physical activity participation were able to maintain a 30 kg weight loss for 5.7 years. However, this does not mean that these new life habits will be sufficient to bring back body weight to pre-obese values. Our experience reveals that healthy eating and vigorous physical activity participation can induce a substantial weight loss up to resistance to further lose fat which was, however, not sufficient to reach baseline pre-obese body weight values (46, 47). As further discussed in the next section, we cannot exclude that humans in a modern world should accept to be more corpulent than their ancestors.

PROPOSAL 4: TO CONSIDER THAT SOME ENVIRONMENTAL POLLUTANTS CAN INTERFERE WITH A HEALTHY REGULATION OF ENERGY BALANCE AND TO TRY TO COUNTERACT THIS EFFECT

The comfort and productivity that have been provided by modernity have at least partly been achieved *via* chemistry innovation that allowed the development of high performance chemicals. In agriculture, these compounds have been used as insecticides to improve the outcome of crops. In the industrial sector, they have been successfully used in the formulation of paints or for their insulating properties in the development of electrical transformers. In fact, their great usefulness at low price has promoted their use throughout the planet. However, we now realize that the enthusiasm that favored the dissemination of these compounds exaggeratedly dominated the knowledge of their side effects that had the potential to prevent their use. Several decades after the beginning of their commercialization, the evidence demonstrating their link with the development of hormone-dependent cancers was sufficiently strong to justify their banishment in many countries. Despite their withdrawal, their long half-life and the liposolubility of some of them confer a clear interest in the study of obesity and its complications.

According to Lee et al. (48), the old pollutants of the family of organochlorines are particularly detrimental regarding the proneness to metabolic dysfunctionality. In addition, they are of great interest for the obesity management since body fat represents the dilution space of these compounds in the body. This issue becomes a problematic matter in weight-reducing programs since body fat loss results in the increase of their concentrations in blood and tissues (13–15, 49).

About 20 years ago, we initiated a research program oriented toward the study of the fat loss-induced increase in circulating organochlorine pollutants on the energy metabolism in individuals with obesity subjected to weight loss. In brief, the strategy used consisted of measuring organochlorine concentrations in blood and adipose tissue before and after a weight-reducing program. The observed changes in concentrations were correlated with those of different biomarkers. This was the best available approach to examine the possibility of a causal link between pollutant changes and those in energy metabolism in humans. Thus, following a weight loss of about 10 kg in individuals with obesity, we observed the following adaptations that were correlated with the increase in the blood concentration of some pollutants: (1) a decrease in resting metabolic rate being significantly greater than the changes predicted by weight loss (50); (2) a decrease in weight-adjusted sleeping metabolic rate being explained more by changes in blood pollutants than by those in leptinemia (51); (3) a decrease in circulating levels of thyroid hormones (50); and (4) a decrease in skeletal muscle oxidative enzymes (52). Taken together, these observations suggest that the body thermogenic capacity had become more vulnerable with weight loss possibly because of the detrimental associations with changes in blood pollutants.

The issue of "what we should do" to promote sustainable solutions is particularly challenging for the pollutant-obesity connection. It is irrelevant to hope that the natural clearance of these compounds will re-establish a rapid return of homeostasis since the half-life of some of them lasts several decades. On the other hand, since it is not realistic to omit losing weight to prevent pollutant body hyperconcentration, the stimulation of body clearance remains the only practical option of treatment. Up to now, the lipid analog Olestra has been the main documented compound for its potential detoxifying properties regarding lipid soluble pollutants. In severely contaminated individuals with TCDD (2,3 7,8-tetrachlorodibenzo-p-dioxin), this non-digestible dietary fat substitute accelerated the intestinal excretion of TCDD by 8–10 times the expected clearance which was sufficient to decrease from 7 to 1.2 years the elimination half-life of this compound (53). This is in agreement with the results reported by Redgrave et al. (54) who also demonstrated a strong detoxifying effect of Olestra in an individual subjected to a large amount of weight loss. In individuals with obesity, our experience with the use of Olestra was more limited. Indeed, following a body fat loss of about 3 kg, Olestra accentuated the clearance of β-hexachlorocyclohexane which significantly differed from its increase in blood concentrations following a comparable fat loss without Olestra. However, we did not find significant differences for changes in 18 other pollutants in response to Olestra supplementation (55).

There is also no clear evidence showing that specific dietary modifications can exert a substantial body detoxifying effect. For instance, when comparing blood organochlorine concentrations between omnivores and vegans, only small differences favoring vegans were observed between the two groups (55). Interestingly, a relationship between the concentrations of body pollutants and some gut bacteria was also recently reported (56). However, this does not permit yet realistic inferences that a stimulation of the gut microbiota could favor body detoxification. Globally, these observations suggest that as far as the pollutant-obesity issue is considered, there is no clear solution that would permit some actions improving body homeostasis in a palpable manner.

CONCLUSION

The evidence summarized in this paper suggests that it is maybe realistic to consider that overweight has become the new normal scenario of mankind. Indeed, the changes in our lifestyle are so deep in terms of the way of living and were so dictated by the search of comfort and well-being that there is no perspective for a spontaneous return toward a less obesogenic traditional lifestyle.

On the other hand, as explained in this paper, body fat gain appears as a homeostatic adaptation that compensates for some effects of this lifestyle on some components of the body's biology such as the regulation of energy balance. In this context where obesity seems to be a condition which clearly places many individuals in conflict with themselves, the most realistic "what we should do" is to promote the healthiest lifestyle as possible with the hope that adipose tissue compensations will be minimally solicited to permit body homeostasis.

AUTHOR CONTRIBUTIONS

AT is the unique author of this manuscript. Therefore, he took in charge all the sections of this paper.

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Building the Machine: The Importance of Governance in Obesity Policy

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The Australian Capital Territory (ACT) is a small Australian jurisdiction with a single tier of government and a population of approximately 400,000 people. Despite enjoying comparatively high levels of income, education, physical amenity, and access to nutritious food, overweight and obesity is the most prevalent risk factor for chronic disease in the ACT. From 2011, the ACT Government Health Directorate (ACT Health) led the development of a whole of Government plan (the Action Plan) to address obesity. A political imperative to take such action and recent administrative reform assisted the development of a plan with specific actions to be undertaken by different government agencies. Obesity is a "wicked problem" with a diversity of opinion about its causes and potential solutions. These opinions remained influential even when an official course of action had been decided upon. Strong decision making and accountability processes were therefore necessary to support the development of the Action Plan. A lack of understanding beyond the health sector in relation to the evidence for effective, population level interventions to address obesity and a tendency to try and address population health risks by scaling up client-centered models of Government services also proved problematic. This experience highlights the critical importance of designing obesity policy within a robust governance framework in order to ensure progress is made in a highly contested environment. Whilst the observations included here are strongly influenced by local contextual factors, there are important lessons which can be applied elsewhere.

Keywords: obesity, government, overweight, governance, policy

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INTRODUCTION

Overweight and obesity is the most prevalent chronic disease risk factor in the Australian Capital Territory (ACT), affecting two-thirds of adults and one quarter of children (1). These rates have increased dramatically over the past 25 years, despite the ACT having extensive green space, a relatively well-educated and affluent population and access to high quality food (1). While there is a socio-demographic gradient in the prevalence of overweight and obesity in the ACT, the fact that the majority of the population is affected suggests that the obesity epidemic is not caused by socio-economic determinants alone.

Reducing rates of overweight and obesity is a government priority due to the associated increased risk of chronic illness and the significant health services burden (2).

It is widely recognized that effective action requires collaboration beyond the health sector in areas such as transport, planning and education (3). The theoretical frame for cross-sectoral approaches to wicked problems in health has been documented by de Leeuw (4). She argues that, since the conceptualization of "healthy public policy" more than 30 years ago, most analyses have remained abstract, focused on arguing the case for the approach rather than showing how it can be done and grounding these examinations in political theory. This paper presents a practical, real world example of where such a cross-sectoral approach to the "wicked problem" of obesity was achieved at the local level.

METHOD

A reflective practice method (5) was employed to document and analyze the key components of the lived experience of the authors, who were participant-observers tasked with leading the development of the *Toward Zero Growth: Healthy Weight Action Plan (the Action Plan)* (6). From the initiation of this work in February 2011 to the public launch of the Action Plan in October 2013, the authors and other members of the steering group within ACT Health met at least weekly to discuss progress and to plan the next steps. At each meeting, notes were made and key learnings as well as progress were documented. In preparation for the paper, these notes were reviewed and major themes were extracted, compared, refined and then triangulated with the authors' individual reflective notes, as well as those of other members of the steering group. External researchers involved with qualitative studies of the related ACT Healthy Weight Initiative also reviewed the paper and provided valuable insights.

THE AUSTRALIAN CAPITAL TERRITORY: LOCAL CONTEXT

The ACT is a small jurisdiction in south-eastern Australia in which Canberra, a low density urban area (160 people/km^2) with a population of approximately 412,000 people is located (7). Uniquely in Australia, the ACT has one tier of government, with both local municipal and provincial level responsibilities.

WHOLE OF GOVERNMENT ACTION: THE AUTHORIZING ENVIRONMENT

Under the ACT *Public Health Act 1997*, the Chief Health Officer is required to report on trends and indicators in health status for the ACT population (8). The *2010 Chief Health Officer's Report* indicated a high prevalence of overweight and obesity across the life-course and shortly after the publication of this report (9), the then Health Minister requested that the Chief Health Officer develop a "whole of government response" to this issue.

The initial approach to this was to write to the administrative heads of all ACT Government agencies and request information about what actions their respective agencies were taking to address obesity. The responses reinforced the impression that other agencies considered obesity to be a "health agency problem".

Whole of government action to address obesity may well have stopped there, but for two important developments in the authorizing environment to support coordinated action in early 2011. Firstly, the Health Minister became the head of government (Chief Minister). Secondly, a comprehensive review of the ACT Public Service (ACTPS) was undertaken, examining its capacity to support strategic advice, and to coordinate cross-government service delivery (10). The review recommended a range of reforms to centralize strategic direction including the establishment of a strategic board to be comprised of all agency heads and chaired by the head of the Chief Minister's directorate (10).

Having a new Chief Minister (who was also the Health Minister) who was passionate about addressing obesity, together with this new bureaucratic structure for whole-of-government collaboration presented a unique window of opportunity. Centralized authority provided an opportunity to resolve competing government agendas, and reduced the complexity of managing multi-agency action.

However, this level of political support imposed an almost immediate expectation to provide tangible outcomes to justify the Government's focus on obesity. This significantly constrained the length and scope of consultation which could be undertaken before proposing specific actions. Additionally, it did not allow time for incremental change in the culture and understanding of colleagues from non-health backgrounds. The Chief Minister was clear, however, that this work must not merely result in a "forgettable summit," nor produce a policy document to sit on a shelf. Rather, the aim was to design a suite of new initiatives, coordinated across government, to meaningfully contribute to halting the rise in the rate of overweight and obesity.

ADDRESSING OBESITY: WHICH MODEL TO CHOOSE?

The development of the Action Plan was informed by two existing models already operating in other Australian states; whole of government strategic plans and Health in All Policies.

Whole of Government Strategic Plan

In 2011, two Australian states had well-developed whole of government strategic plans auspiced by their respective Premier's Department, namely *Toward Q2: Tomorrow's Queensland* and *South Australia's Strategic Plan* (11, 12). These plans specified mutually supportive key performance indicators and targets for health and other agencies within their government structures.

The strength of this approach was to embed a clear element of co-production for co-benefit across portfolios. The non-health agencies had relevant performance targets set in their own areas of responsibility which in turn provided strong anchor points for collaboration on health issues. For example, an environmental agency tasked with reducing carbon emissions could collaborate with a transport agency tasked with increasing use of public transport, which in turn provided

an opportunity to leverage active transport to address obesity. Discussions with departmental officers in these states indicated that over several years, cross-agency advocacy and collaboration became entrenched in the process of bidding for human and financial resources, thereby further enhancing cross-agency cooperation.

This approach was proposed for the Action Plan but was not adopted by the Strategic Board. This was primarily due to the absence, at that time, of an overarching plan which gave sufficient policy clarity in areas other than obesity prevention.

Health in All Policies

The World Health Organization endorsed Health in All Policies (HiAP) approach has been extensively evaluated in a range of settings (4). The South Australian Government established a HiAP unit in 2007 (13) and ran in tandem with the implementation of *South Australia's Strategic Plan* by applying a "health lens" to policies across government. In theory, through the process of looking at policy in terms of what it could contribute to health outcomes, or what barriers it may present to achieving desired health outcomes, the policy environment could be reoriented to be health promoting. This is consistent with the Ottawa Charter which places "building healthy public policy" as a core aim of health promotion (14). HIAP advocates have emphasized the need for strong governance and centralized authority to effectively drive policy reform—something that *South Australia's Strategic Plan* provided.

The establishment of a HIAP unit in the ACT context was considered, but the likely utility of such a unit was deemed to be limited. Indeed, the aforementioned review of the ACTPS noted.

"There was a clear and consistent view in consultation that the ACT Government has too many plans, leading to a propensity for the ACTPS to 'tie itself in knots' with snowballing layers of plans, strategies, action plans, implementation plans, statements of intent, frameworks and performance agreements" (10).

Our experience suggested that official policy statements do not necessarily reflect the power relationships or depths of division which determine what gets done in government. The problem is not necessarily unhealthy policy *per se*, but the effectiveness of the policy process to ensure that competing priorities and resource limitations are addressed. It was therefore considered important to embed the policy intent of the Action Plan in a governance structure based on the established, hierarchical lines of authority and accountability in the bureaucracy. This connected the Minister directly to service delivery across government wherever that delivery could most effectively and efficiently take place to achieve the desired outcome. Thus, rather than setting up a mechanism for ensuring healthy policy, the Action Plan became a policy designed to achieve a single health objective through multiple complementary actions.

THE ACT MODEL: WHAT WE DID AND HOW WE DID IT

The Action Plan developed using a phased approach in which the scope of the plan was decided, then a working group agreed actions and finally teams were convened to implement those actions. Between each of these stages the strategic board was asked to approve the progress of the work and the commencement of the next phase (**Figure 1**).

There were two main reasons for adopting this process. Firstly, the range of options to address obesity only became clear through the development of the Action Plan and regular executive oversight provided several points at which the feasibility and acceptability of proposals could be assessed. Without tight executive control, it would be difficult to drive progress beyond a discussion of the issue to the successful implementation of actions. Secondly, enforcing decision points between the

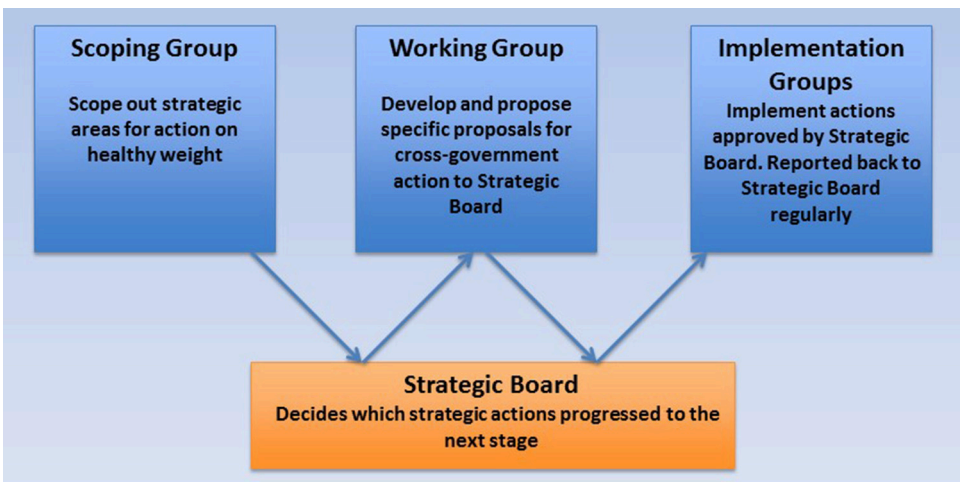

FIGURE 1 | Healthy Weight Action Plan governance model.

development of proposals and their implementation provided a means of avoiding "scope creep" or prolonged discussion of proposals prior to their implementation. The implementation teams were therefore tasked to focus on how to achieve actions they had been given rather than revisiting whether these actions should be undertaken.

There is considerable public interest in obesity and politicians and public servants bring their own views regarding its causes and potential solutions to the policy process. From the outset, the prevailing view was that obesity was an issue primarily for the health agency to deal with. Consequently, causes and potential solutions remained contested throughout the process of the Action Plan development. Non-health staff tended to perceive morbid obesity, that is the upper extreme of the problem, as the primary target for action and tended to under-appreciate the difficulty of scaling intensive interventions to the population level.

Thus, a discussion was necessary across Government about the scale of the problem, the comparative role of nutrition versus exercise as proximal determinants, the importance of the environment in shaping decisions of individuals and the goals which an Action Plan might reasonably achieve. The staged approach ensured that the Executive endorsed a consensus view on the definition, extent, causes and consequences of overweight and obesity prior to discussing the question of how to address the issue. This approach was intended to ensure that the discussion of interventions was less clouded by diverging understandings of the issue to be addressed.

Having all the agency heads collectively oversight the development of the Action Plan also allowed staff time to be allocated across government using existing lines of authority and with direct accountability to the Chief Minister. It was envisaged that this high level and strongly supportive authorizing environment would streamline the process by removing the need to create a new bureaucratic structure and ensure a relatively simple process incorporating existing structures within a simple project management framework.

DEVELOPING THE ACTION PLAN: MAKING IT WORK

Several issues were encountered during the Action Plan development process, delaying the Action Plan development by almost 18 months. One significant issue was the low level of public health literacy (15) among staff from non-health agencies. This limited the ability of agencies to identify which areas of work might best be incorporated in the Action Plan. To address this, a study was commissioned to identify projects and programs currently underway across government which impacted on levels of physical activity or nutrition. The study identified important gaps. Almost all existing programs targeted physical activity, with the exception being ACT Health-led school-based programs. Existing programs also demonstrated an emphasis on individual service delivery rather than population-wide interventions such as regulation.

Several existing strategy documents included targets relevant to reducing obesity rates, but implementation processes and reporting mechanisms associated with these documents were unclear. This suggested that there was a "baseline" level of cross-government action already taking place, but that significant value could be added by filling the gaps identified by the study. The lack of interventions that focused on improving nutrition and had population-wide reach were particularly noted. It was thought that the Action Plan might also provide a "nudge" to existing programs where high level executive support could be used to enhance reach and scale. For example, the process for including active living principles in the design of new residential developments was not well aligned with the specifications for infrastructure required by other government agencies. By targeting this relatively minor point of bureaucracy, significant improvements in urban planning could result, which in turn could contribute to addressing obesity.

The relative paucity of population-level interventions in the nutrition sphere was not entirely unexpected. Policies aimed at reducing the consumption of energy dense, nutrient poor food, and drink are likely to involve placing restrictions on the production, distribution and/or promotion of these products and therefore could be viewed as a limitation of personal liberty. Conversely, physical activity is promoted by a range of businesses such as gyms, sporting organizations, and equipment shops and thus promotion of active living could be viewed as choice-enhancing and as a business-promoting stimulus for the local economy. The survey of existing programs suggested that it had been easier for program managers to work collaboratively with the promoters of physical activity than to actively oppose the interests of food businesses. This suggested that one of the potential benefits of a whole of government plan would be to offer high level, inter-sectoral support for measures to address the food environment. Regulation, financial intervention or changes in business practice were the most difficult for individual areas to undertake, but also comprised the actions which could most influence the high proportion of the population who were overweight or obese. Therefore, the Action Plan developers concentrated in these areas.

The next step was to ensure that the working group was provided with contemporary evidence on obesity prevention initiatives which were likely to be effective in the ACT context. Thus, an independent review of the literature was commissioned (16). Whilst this provided useful guidance, determining how to apply the information gleaned from this review required extensive cross-government socialization of the findings, including individual meetings between senior Health officials and each agency head to explain the desired co-benefit methodology.

There was, in general, a degree of resistance to regulatory approaches or interventions in the commercial sector, particularly in the food environment. In most cases, non-health agencies proposed actions which were extensions of existing services, representing an expansion of effort rather than a change of approach. This reflected a level of comfort with prevailing models of Government action such as education about lifestyle choices, time-limited programs, or the provision of funding for individuals to purchase services or products. These were not interventions which had necessarily been proven to be effective or easily scalable to a population level.

TABLE 1 | List of actions included in the Healthy Weight Action Plan (4).

Theme/lead agency	Actions
Theme: Workplaces Lead: Central agency	1. Implement a Chief Minister's award scheme that rewards healthy workplaces and food outlets. 2. Improve the availability of healthy food and drink choices and reduce unhealthy choices at ACT Government workplaces, facilities and government-funded events. 3. Implement a program of health risk assessments for ACT Government staff and explore options for extending this to the private sector. 4. Create new incentives for ACT workers and/or workplaces to participate in physical activity or active travel. 5. Update requirements for new commercial buildings to contain facilities which encourage physical activity and improve access to these facilities for existing buildings.
Theme: Urban planning Lead: Agency responsible for planning and environment	6. Promote and prioritize active travel through the implementation of the *Transport for Canberra* plan and master planning processes. 7. Incorporate active living principles into the Territory Plan Codes and the Territory and Municipal Services Standards for public realm design and development works. 8. Create car parking and other incentives which encourage active travel (walk/cycle/bus) and discourage private transport for entire journeys into town centers.
Theme: Schools Lead: Education agency	9. Develop an ACT Government school food and drink policy with supporting guidelines that will mandate the implementation of the National Healthy School Canteen Guidelines in ACT Schools. 10. Improve the measurement, capacity to deliver and curriculum support for physical education in all ACT schools.
Theme: Food environment Lead: Health agency	11. Restrict the advertising of unhealthy foods within the government's regulatory control. 12. Regulate the sale of sugar-sweetened drinks. 13. Enact a mandatory code for supermarkets to require at least one checkout aisle be identified as free of energy dense, nutrient poor (EDNP) foods. 14. Improve the availability of free drinking water in public places and food outlets.
Theme: Social inclusion Lead: Social and community services agency	15. Create new incentives for targeted populations to increase the uptake of healthy food and/or active travel options. 16. Improve awareness, skills and capability across the ACT in buying and preparing healthy food.
Theme: Evaluation Lead: Health agency	17. Develop and maintain a web-based information resource for workplaces, primary care providers and the community about opportunities to improve physical activity and nutrition levels. 18. Collect and evaluate usage and demand data about walking and cycling infrastructure to guide actions that increase use. 19. Improve the collection and assessment of biometric data in General Practice.

Throughout the process, health officials were conscious of the need to avoid the perception of "health imperialism" that may stem from appearing to instruct other government agencies to change their business to specifically address a health issue, in this case, obesity. The preferred approach was to find actions which would have co-benefits and achieve positive health and non-health outcomes simultaneously. Improved nutrition and or physical activity levels among school children could, for example, improve educational outcomes (17, 18). It was felt that without this approach, the actions included would be resisted and not be given priority across government.

To help gain cross-government legitimacy, it was decided to allow all agencies to propose actions and then for the final plan to be based on a vote by working group members and key non-Government organizations. Nineteen actions with the strongest support and which were also supported by evidence of likely population level effect were proposed and then endorsed by the strategic board and the Chief Minister. These were then grouped into six thematic streams and included in the Action Plan (**Table 1**).

This process highlighted the importance of engaging with an ongoing discussion about potential measures with the working group to balance recommendations based primarily on evidence of effectiveness on the one hand, with feasibility and political acceptability in the local context on the other.

WHAT WE LEARNT

The Action Plan represents an attempt to effect whole of government action to halt the rising rates of overweight and obesity in the ACT. Lessons can be learnt from the process of developing the Action Plan, including the importance of political commitment and a clear governance framework in achieving meaningful population level action. Whilst much of what is

presented here was influenced by particular temporal, political and structural factors, the lessons learnt have wider practical implications.

As there is no single cause for the rising rate of obesity, there is a need for broad action to address the issue which has in turn prompted calls for whole of Government plans (2). However, this same contested narrative about the determinants and the solutions required to address them meant that particularly strong governance structures were required to gain policy coherence to support implementation of meaningful actions (19, 20).

A specific decision was made early in the process not to formally adopt a HiAP approach, but rather to use existing formal lines of government authority, thereby increasing the chance of executives and operational areas having a common understanding of what to implement. This is possible only when there is a strong political mandate to align bureaucratic structures to focus on an issue such as obesity. This was present at the highest level of government and represented a significant "window of opportunity" to gain cross-sectoral support for the Plan (21).

Ultimately the Action Plan did conform to many of the principles of HiAP – a plan conceived by public health experts but eventually co-owned by a wider group, with an agreed way of working and a commitment to co-production for mutual benefit (4, 22–24). This highly structured approach to decision making, a limited level of health public health literacy and unfamiliarity with working in a whole of government process meant it took a significant amount of time and energy to "build the machine" prior to effective measures to address obesity being implemented. In our experience, this was at odds with the demand for action to commence rapidly once the political decision to develop the Action Plan was made, and ultimately required a degree of "bludgeoning persistence" despite a strong wish to work collaboratively. Political support, while necessary to implement a large scale plan, can sometimes be at odds with time and knowledge constraints within government. This was also observed during the formulation of the plan. Embedding a plan within the machinery of government sufficiently strongly to allow complex and sustained actions to address complex issues such as obesity may require more time than the political cycle can accommodate.

AUTHOR CONTRIBUTIONS

Both authors were actively involved in the work described in this paper. The paper itself was conceived by PK, the first draft was written by AP and subsequently amended by PK. Both authors read and agreed to the final version as submitted and agree to be accountable for the content.

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Economic Evaluation of PRIMROSE—A Trial-Based Analysis of an Early Childhood Intervention to Prevent Obesity

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Background: Childhood obesity is a major clinical and economic health concern. Alongside the clinical understanding of obesity, there is a growing interest in designing and implementing interventions that are worth their money given the scarce resources in the health care sector. This study is one of the first efforts to provide evidence by assessing the effects and costs of a population-based primary prevention intervention targeting pre-school children attending child health centers in Sweden.

Methods: The economic evaluation is based on the PRIMROSE cluster-randomized controlled trial aiming to establish healthy eating and physical activity among pre-school children (9–48 months of age) through motivational interviewing applied by trained nurses at child health centers. The cost-effectiveness is assessed over the trial period from a societal perspective. The primary outcome was BMI at age 4. Cost data was prospectively collected alongside the trial. Scenario analyses were carried out to identify uncertainty.

Results: The estimated additional mean total costs of the PRIMROSE intervention were 342 Euro (95% CI: 334; 348) per child. During pre-school years direct costs mainly consist of training costs and costs for the additional time used by nurses to implement the intervention compared to usual care. Early indirect costs mainly consist of parents' absence from work due to their participation in the intervention. The incremental cost-effectiveness ratio in the base case analysis was 3,109 Euro per 1 BMI unit prevented.

Conclusion: We cannot provide evidence that the PRIMROSE intervention is cost-effective, given the uncertainty in the effect measure. Until further evidence is provided, we recommend resources to be spent elsewhere within the field of obesity prevention. Furthermore, to achieve valid and reliable cost-effectiveness results, the economic evaluation of obesity prevention programs in early childhood should incorporate the life time impact to capture all relevant costs and benefits.

Keywords: economic evaluation, childhood, obesity, prevention, RCT

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INTRODUCTION

Despite signs of stabilization (1, 2), the burden of childhood overweight is still considerable in many westernized countries. Severe health concerns for the individual (3), but also significant societal and economic consequences (4, 5) have raised awareness among policy makers and researchers likewise to address childhood obesity through primary prevention strategies already at early ages. Still, the evidence base around the prevention of childhood obesity is far from conclusive (6–10). Nevertheless, primary health care providers and (pre-) school settings may be encouraged to address and implement behavioral counseling and other interventions as long as they do not cause harm. Yet, given the scarce resources of most health care systems, decision makers need to prioritize and shed light on opportunity costs. So far, little is known about the costs of universal population-based primary childhood obesity prevention interventions, especially in the European settings (11). Health economic evaluations are often neglected when designing and conducting intervention studies. The lack of relevant, individual, and prospectively collected data hamper the meaningful conduction of cost-effectiveness analyses. In a recent systematic review, only six studies addressing the cost-effectiveness of obesity prevention programs in early childhood were identified, and only three of them were based on a randomized trial (11) This paper aims to critically assess the costs and evaluate the economic benefits of a population-based primary prevention intervention embedded in regular child health services targeting first time parents and their children.

MATERIALS AND METHODS

Study Design and Setting

This paper describes an economic evaluation of the PRIMROSE cluster-randomized primary prevention trial, where the costs and outcomes of the intervention were compared with those of usual care from a societal perspective. A societal perspective implies that costs also outside the health care system were included in addition to direct health care costs, i.e., productivity losses, due to participation in the trial. The present economic evaluation is an analysis of the costs and health effects of the PRIMROSE trial during the intervention period only, i.e., up to age 4.

The PRIMROSE Cluster-Randomized Controlled Trial

The PRIMROSE trial evaluated the effectiveness of an early childhood obesity intervention delivered in the first 4 years of life, embedded in regular child health services in Sweden. Details of the study design and the intervention components have been reported previously (12, 13). In brief, the study included 1,355 families with 1,369 infants. The intervention took place at child health care centers (CHCs), which were randomized into interventions ($n = 31$) and control units ($n = 28$). The intervention consisted of nine sessions in a time frame of approximately 39 months delivered by specially trained nurses. The intervention aimed to assist first time parents in promoting healthy food and physical activity habits in their children and in changing their own health behaviors if needed through the application of motivational interviewing (MI). The intervention was targeting eating pattern (i.e., regular meals together with the family, no force feeding/eating), food choices (i.e., consumption of fruit and vegetables, reduced consumption of soft drinks and snacks), and physical activity (i.e., incorporating physical activity in the everyday routine, reducing sedentary time). The intervention components were mainly targeted at the parents to become role models for their children and to increase parental self-efficacy for behavioral change. Prior to intervention, nurses attended a 5-day workshop, including an introduction to healthy nutrition, physical activity, learning theory, and social cognitive theory (SCT), as well as training in MI. During the course of the intervention, nurses received extensive and tailored feedback on their MI performance (14). Ethical approval (2006/525-31/2) was obtained from the Regionala Etikprövningsnämnden Stockholm (The Ethical Review Board Stockholm). All parents of the participating children gave informed written consent.

Comparator

Families in the control CHCs were only offered the regular age-related health check-ups of Swedish child health services, which focused on physical development and immunizations and less attention is paid to children's health behavior (15). Swedish CHCs are free or charge and attended by nearly all families in Sweden.

Measurement of Clinical Outcomes

Children's weight, height, and waist circumferences were objectively measured by study nurses at each visit to the CHC. The primary outcome was BMI at age four, applying the IOTF references for defining cut-offs (16). Secondary outcomes were mother's objectively measured anthropometrics as well as children's and mother's physical activity and food habits (13). For the current analysis, only the primary outcome, i.e., BMI at age 4, was considered.

Measurement of Costs

Costs of the intervention program included costs of a 5-day workshop offered to intervention nurses, costs of MI training, and supervision of nurses and costs of implementation. The costs of the workshop were obtained from collected invoices and salary contracts. We collected prospectively data on costs to deliver the intervention, including staff's time to deliver the intervention and parents' time to take part. This information was then supplemented with parents' average net salaries to estimate productivity losses due to participation in the intervention, based on the human capital approach. In line with current guidelines (17), we excluded the costs for research and development and any costs associated with evaluation or administration of the trial. Costs are indexed to the year 2015 and displayed in Euro using the average exchange rate from 2015 (1 Euro = 9.3 SEK).

Statistical Analysis and Uncertainty Analysis

We compared the total costs of the PRIMROSE intervention to the costs of usual care. Costs and effects were derived from participant-level data. The incremental cost-effectiveness ratio (ICER) was expressed as cost per 1 BMI unit prevented. The method of non-parametric bootstrapping was applied using EXCEL, where 1,000 costs and outcome pairs were generated (with replacement) (18). The results were illustrated by using cost-effectiveness acceptability curves, in which the probability that the PRIMROSE intervention is cost-effective was illustrated for different theoretical willingness-to-pay (WTP) levels for prevention of 1 BMI unit.

Scenario Analysis

We conducted two types of scenario analysis for calculating the intervention costs. First, intervention costs were calculated based on a per-protocol basis. Instead of individual uptake and duration of meetings, we assumed full uptake (seven face-to-face meetings and two telephone meetings) and the duration of meetings according to the manual specification as previously reported (13). Missing information on parents' attendance was imputed based on the observed distribution of parents' attendances during respective meetings. In a second scenario analysis, we halved the observed duration of meetings. This is to partly account for potential overlap with usual health care during the intervention meetings, but also to allow for a shorter duration of intervention meetings if implemented in the current CHC practices. The effect measure was kept constant.

Decision-Making Beyond Cost-Effectiveness

In addition to the quantitative assessment of the cost-effectiveness, we applied the criteria developed by the ACE-Obesity Working Group, which are intended to incorporate other, broader aspects of decision-making. The criteria included were "strength of

TABLE 1 | Summary of unit cost information, data sources, and assumptions for education and training of nurses.

Type of cost	Description	Unit costs	Source	Assumptions
Education				
Personnel time				
Nurse	10 nurses per education, 40 h	17.9 Euro/h	Statistics Sweden, salary statistics Primrose database	Average wage rate
Nutritionist	Food habits and physical activity training, 3 h	21.5 Euro/h	Statistics Sweden, salary statistics Primrose database	Average wage rate
Psychologist	SCT, learning theory, and some CBT training, 3 h	23.9 Euro/h	Statistics Sweden, salary statistics Primrose database	Average wage rate
MI trainer (psychologist)	28 h	23.9 Euro/h	Statistics Sweden, salary statistics Primrose database	Average wage rate
Instructor	2 instructors, 16 h	23.9 Euro/h	Statistics Sweden, salary statistics Primrose database	Average wage rate
Supervisor (MINT)	5 supervisors, 8 h	19.9 Euro/h	Statistics Sweden, salary statistics Primrose database	Average wage rate
Project coordinator	40 h	16.6 Euro/h	Primrose database	
Other costs				
Catering and materials	Includes coffee/tea, lunches, and snacks	3,023 Euro	Primrose database	Calculation based on invoices made for one education session
Travel	Two-way train ride	55 Euro	Estimate	No information on mode of transportation; Average costs for medium distance train ride
	Per nurse Per child			1,441.4 Euro 110.8 Euro
Training				
Personnel time				
Nurse	9 occasions, 30 min feedback on the telephone	17.9 Euro/h	Statistics Sweden, salary statistics Primrose database	Average wage rate, full maintenance
Supervisor (MINT)	9 occasions, 1 h preparation, 30 min on the telephone	19.9 Euro/h	Statistics Sweden, salary statistics	Average wage rate
Other costs				
Coding of MI conversations	6 codings	55 Euro	Invoice	
Recording device	One recording device per nurse	22 Euro	Invoice	
	Per nurse Per child			867.9 Euro 66.6 Euro

SCT, social cognitive theory; CBT, cognitive behavioral therapy; MI, motivational interviewing; MINT, motivational interviewing network of trainers.

evidence," "equity," "feasibility of implementation," "acceptability of stakeholders," "sustainability," and "side-effects" (19).

RESULTS

At follow-up, there were 1,148 children with data on weight and height at age 4. Intervention and control children at follow-up were very similar with regards to demographic characteristics and baseline characteristics (12).

Main Intervention Effect

The main results of the trial have been published elsewhere (12). In brief, there was no statistical significant indication for improvement in the primary outcome measure of children's BMI at age 4. While the intervention effect pointed in the "right" direction, the estimate was too small to reach statistical significance with respect to group differences in the children's BMI at age 4 [$\beta = -0.11$, 95% confidence interval (CI): −0.31 to 0.08] (12).

Total Costs

The estimated mean total costs per participant in the intervention group were 453 Euro (Min = 177, Max = 740) in comparison to 111 Euro (Min = 0, Max = 246) in the control group for the usual care. The mean additional costs for carrying out this interventions were 342 Euro (95% CI: 334; 348) per participant. The main costs components of the education program were costs of the workshop, costs of MI training and supervision, (**Table 1**) and costs of implementation of the intervention program (**Table 2**). The largest component of PRIMROSE costs arose from delivery of the intervention within the CHC settings. The large intervention costs variation is mainly driven by meeting uptake.

Cost of Education

In total, 67 nurses received the PRIMROSE education, which involved a 5-day workshop, including an introduction to nutrition, physical activity, learning theory, and SCT, as well as training in MI. The MI training part of the workshop consisted of 3.5 days, with 8 h of training per day. Seven workshops were conducted, each with an average of 10 participating nurses. The workshops were led by a senior clinical psychologist with extensive experience in leading MI workshops, and membership of the Motivational Interviewing Network of Trainers. On average, two more licensed clinical psychologists assisted as workshop instructors. On the last day, participants' supervisor's joined the workshop. In total, there were 10 supervisors, which were each responsible for on average five nurses. On average five supervisors participated per workshop (20).

Costs of Further MI Training and Supervision

After the workshop intervention, nurses were offered feedback on their MI performance at nine occasions (four training sessions before the PRIMROSE intervention and five sessions with children

TABLE 2 | Summary of unit cost information, data sources, and assumptions for intervention delivery.

Type of cost	Description	Unit costs	Source	Assumptions
Group meeting				
Number of group meetings	On average five parental units per group meeting with both parents being present	1	Primrose manual	
Length of meeting[a]		1 h and 23 min	Primrose database	
Nurse		17.9 Euro/h	Statistics Sweden, salary statistics Primrose database	Average wage rate
Parents[b]				
Father (7.7%) Mother (53.2%)		18.5 Euro/h 14.3 Euro/h	Statistics Sweden, salary statistics, estimate	Average wage rate
Other costs				
Travel	Two-way ride	5 Euro	Estimate	Average collective communication cost for short distance
Individual meeting				
Number of individual meetings		6	Primrose manual	
Length of meeting[a]		53 min	Primrose database	
Nurse		17.9 Euro/h	Statistics Sweden, salary statistics Primrose database	Average wage rate
Parents[b]				
Father (7.8%) Mother (54.1%)		18.5 Euro/h 14.3 Euro/h	Statistics Sweden, salary statistics, estimate	Average wage rate
Telephone meeting				
Number of telephone meetings		2	Primrose manual	
Length of meeting[a]		22 min	Primrose database	
Nurse		17.9 Euro/h	Statistics Sweden, salary statistics Primrose database	Average wage rate
Parents[b]				
Father (8.9%) Mother (77%)		18.5 Euro/h 14.3 Euro/h	Statistics Sweden, salary statistics, estimate	Average wage rate

[a]*Mean length reported in the trial.*

[b]*Percentage refers to parental presence during the meetings, otherwise both parents were present.*

in the PRIMROSE intervention group). 35 of 51 nurses (69%) completed all nine supervision sessions that were planned to last for 30 min and to be based on at least 20 min of audio-recorded session time with parents of young children. In total, six sessions (one training session and five intervention sessions) were coded for quality of MI performance by the motivational interviewing treatment integrity-code (14).

Costs of Implementation

The PRIMROSE intervention consisted of one group meeting, six individual meetings and two telephone meetings. These meetings were conducted as add-on of the usual care provided at the CHC. In **Table 2**, the mean duration of visits is reported. On average, 54% of all CHC visits were done by mothers only. If both parents were present, the meetings were on average 15 min longer. To calculate the costs of implementation, we used individual trial data concerning uptake of meetings, parental presence, and duration of meetings.

In the RCT, the point estimate of the ICER was 3,109 Euro per 1 BMI unit prevented. The bootstrapped estimates of incremental costs and incremental benefits (represented by BMI units prevented) of the PRIMROSE interventions are presented in the cost-effectiveness plane (**Figure 1**). About 11% of the bootstrapped pairs were dominated, meaning the PRIMROSE intervention costs more for less effect. Yet, the vast majority of the bootstrapped ICER estimates indicate increased benefits and greater costs.

Regarding the scenario analyses, the per-protocol analysis resulted only in marginal differences in intervention costs with a corresponding ICER of 3,553 Euro per BMI unit. When we assumed the meeting time to be halved, the ICER was reduced to 2,128 Euro per BMI unit. The cost-effectiveness acceptability curve presents the probability of cost-effectiveness of the PRIMROSE intervention given different levels of WTP per avoidance of 1 BMI unit for all three scenarios (**Figure 2**). It shows that by halving the meeting times the probability of cost-effectiveness can be increased by approximately 20%. With increasing WTP, one can

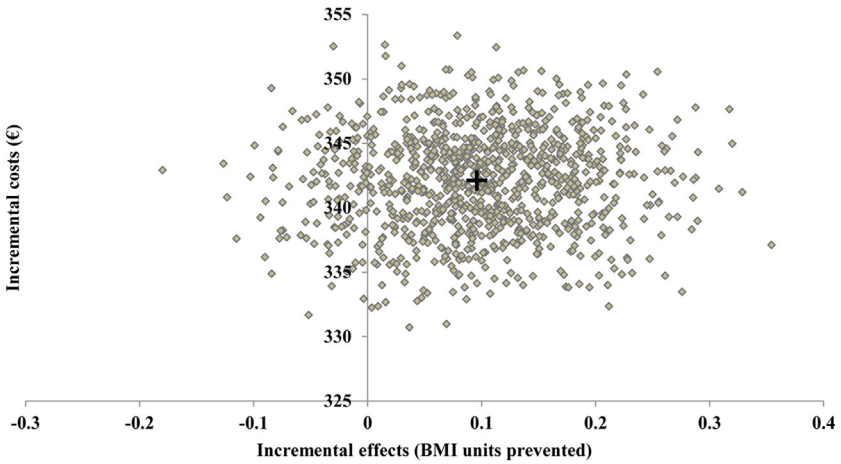

FIGURE 1 | Incremental costs and incremental effects of the PRIMROSE intervention on the cost-effectiveness plane. Results of 1,000 Monte Carlo simulations. + = point estimate.

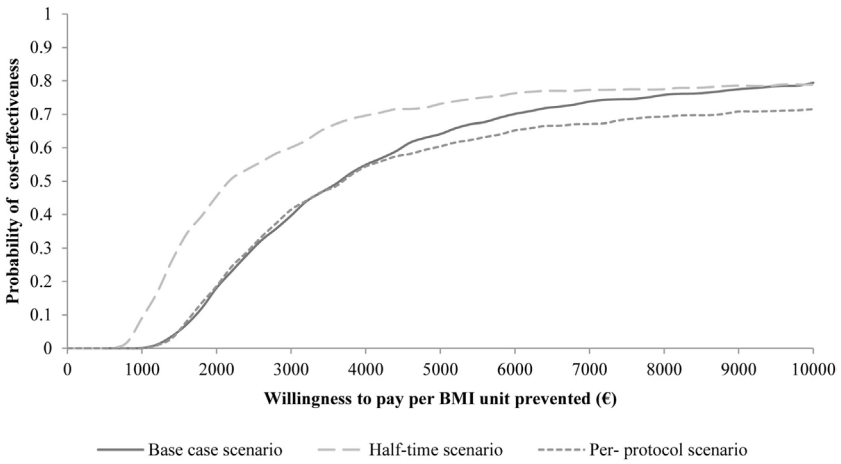

FIGURE 2 | Cost-effectiveness acceptability curves of PRIMROSE for three scenarios "Base case scenario," "Half-time Scenario," and "Per-protocol Scenario."

TABLE 3 | Criteria for decision-making.

Strength of Evidence	Equity	Acceptability	Feasibility	Sustainability	Side-effects
– Large cluster-RCT – Results not statistically significant	– PRIMROSE population has higher SES compared to the general population – Targeted to Swedish speaking families only	– Positively received by nurses in the trial – No stigmatization (primary prevention) – On-top of regular child care	– Embedded in regular child health services – Potential problems concerning additional time required	– On-going training of nurses during the intervention, high quality manual – Possible issues: updating of manual, ensuring an adequate workforce of trained nurses, motivational interviewing competence	– Positive spill-over: potential impact on weight and health behavior on other family members – Possible unintended negative: potential feeling of lack of self-efficacy of parents, however, unlikely
Major concerns	Minor concerns	No concerns	Some concerns	Some concerns	No concerns

observe an approximation of probabilities of cost-effectiveness for all three scenarios.

Decision-Making Beyond Cost-Effectiveness

When looking beyond the cost-effectiveness, key concerns relevant for decision-making were around the strength of evidence, feasibility, and sustainability (**Table 3**). While the intervention effect pointed in the right direction, the group difference was not statistically significant. The evidence was limited and additional, possible larger, RCTs might be needed to confirm the results. Furthermore, more research is needed to reflect on the clinical relevance of small effects on BMI during early childhood. The additional time needed by health professionals cannot be disregarded and need to be carefully evaluated. In addition, there are some issues that require resolutions to ensure sustainability, including, among others, the need of ongoing training and supervision of nurses. When implemented in practice, it can be assumed that equity issues can be neglected given that nearly 100% of the Sweden living population attends the regular CHC meetings, irrespective of ethnicity or SES (21).

DISCUSSION

This is the first European trial-based economic evaluation of an early childhood obesity prevention intervention. While the intervention effect pointed in the favorable direction, there was no statistical significant BMI difference at age 4 between intervention and control groups. From a societal perspective, the incremental costs of the intervention were estimated to 342 Euro per participating family over 4 years. The corresponding ICER was 3,109 Euro per BMI unit prevented. As discussed elsewhere (12), the reasons for the non-significant effect size can be manifold and do not necessarily reflect an ineffective intervention. However, given the uncertainty combined with considerable opportunity costs, the current trial-based economic evaluation of PRIMROSE suggest that resources might be better used elsewhere within the field of obesity prevention.

The PRIMROSE intervention study and its economic evaluation has a number of strengths, including the large number of participants in the RCT and the prospective planning of the economic evaluation, which allowed the inclusion of detailed individual cost data. Combined with detailed measurements of individual participation time and national statistics on age adjusted mean salary information, we were able to also include individual productivity losses. However, we need to acknowledge that we had no accurate information on the individual employment situation (i.e., unemployment or parental leave), and, therefore, cannot exclude the possibility that parental productivity losses might be under- or overestimated. Given that societal costs often outweigh the direct health care costs, we recommend for future economic evaluation of RCTs a prospective and detailed collection of all relevant economic information. Furthermore, we did not have access to individual health care utilization data during the trial period, in addition to the healthcare provided by the CHCs. One may, however, assume that the vast amount of obesity related health care costs (and savings by prevention) occurs later in life, which was confirmed in the study of Hayes et al. showing only marginal differences in health care costs up to age 2 (22). However, over the subsequent 3 years, total health care costs of children with obesity were 1.62 (95% CI 1.12–2.36) times higher than among children with normal weight, which was driven by the higher risk of hospitalization (23). When comparing only children who were hospitalized, the differences were non-significant between the BMI groups. Therefore, more research on health care utilization during the early childhood is needed to also capture the possible short-term benefits of obesity prevention.

We are aware of only two other studies that conducted an economic evaluation that was restricted to the costs and effects during trial period for that age group (22, 24). The Australian trial-based economic evaluation of "Healthy Beginnings" reported an ICER of AUD 4,230 (≈2,950 Euro) per BMI unit prevented (22). Their intervention was conducted only over a period of 2 years, yet with a similar intensity of 8 home visits, in comparison to 7 meetings and 2 telephone meetings in the PRIMROSE intervention. The economic evaluation of the live, eat, and play (LEAP) intervention showed intervention costs close to AUD 5,000 borne by both the health care sector and the families (24). However, they also included costs borne by the family by changing the diet or physical activity habits. When excluding those, the costs over the 15 months trial period AUD 973, still more costly than

PRIMROSE. The LEAP economic evaluation did not report any ICER, given their insignificant observed effect size. This is a similar case as in the PRIMROSE intervention, yet we followed the approach suggested by Drummond who argues for the importance of conducting economic evaluations even if the effect size does not reach statistical significance (17). This approach is also followed by Moodie et al. in their economic evaluation of the "Be active eat well intervention," who calculated an ICER of AUD 547 (≈381 Euro) per BMI unit prevented during the trial period (25). Yet, the costs and effects were aggregated to the school level in a somewhat older population, which may hinder the direct comparison to our results.

Despite similar results to other studies, the judgment of whether such an intervention is cost-effective depends on the WTP of decision makers. Currently, there is no national or international threshold on WTP for the prevention of a BMI gain in childhood. Given the challenges of calculating QALYs for this age group (11, 26), we hope that our calculated ICER, similar to the one calculated by Hayes et al. (22) can serve as comparator for future economic evaluations, especially in the European setting. Next to the choice of outcome measure for economic evaluations during early childhood, the preferred choice of time horizon is also debatable. There are arguments to restrict economic evaluation of early childhood obesity prevention to the observation period. These include the lack of evidence on effect maintenance, the lack of evidence on the independent association of childhood obesity and adult onset of diseases, and the methodological challenges of linking childhood obesity to utility values. Furthermore, decision makers may also be interested in the immediate costs (or savings) of an intervention. At the same time, these calculations are likely to be very conservative and important costs and health parameters are missing that allow meaningful decision on resource allocation. To truly capture the cost-effectiveness of an intervention all consequences need to be considered. For preventive obesity intervention studies during early childhood this includes health consequences and societal costs over the entire life time. A model-based simulation study is a way of synthesizing the best available data on health effect and costs in the long-run also including consequences beyond the clinical trial period. In future economic evaluations of strategies for preventing obesity in early childhood, we recommend to combine clinical trial data with data from outside the trial using a modeling framework, taking into account the consequences on costs and benefits in the long-run.

CONCLUSION

The economic evaluation of the PRIMROSE intervention demonstrated that even small intervention effects would be value for money under current modeling assumptions. However, given the uncertainty around the effect measure, resources might be better used elsewhere within the field of obesity prevention, until further evidence on effectiveness is provided. In addition, more research in the phases of design, implementation, evaluation, and maintenance of early childhood interventions for obesity is needed to provide policymakers and decision makers with the information they seek to allocate scarce resources in a more efficient and sustainable way. Furthermore, to achieve valid and reliable cost-effectiveness results, the economic evaluation of obesity prevention programs in early childhood should incorporate the life time impact to capture all relevant costs and benefits.

ETHICS STATEMENT

The trial was approved by the Ethical Review Board in Stockholm, Sweden and was recorded in the ISRCTN registry (ISRCTN16991919). All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

ND substantially contributed to the design of the study, carried out the analysis, and drafted the initial manuscript. DS substantially contributed to the design of the study and the analysis, and provided substantial and critical feedback on the manuscript. JM and PT provided substantial feedback on the manuscript. NZ supported the data analysis and provided substantial feedback on the final manuscript. FR is the PI of the PRIMROSE intervention and contributed to the design of this study and provided substantial feedback on the final manuscript.

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Predictors of Weight Change: Findings From an Employee Wellness Program

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Introduction: Employers are instituting employee wellness programs that include educational, lifestyle coaching, and weight and other condition management components to address obesity-related issues in the workplace. However, the findings of such wellness initiatives have been mixed. The purpose of this exploratory study is to determine whether the readiness for change measures are important predictors of weight loss in an employee wellness program.

Methods: Retrospective data analysis of an employee wellness program conducted in the United States was conducted using data collected between 2014 and 2015 for people with BMI $\geq$ 30. These participants were assigned to one of two subprograms: weight management or condition management. We assessed the weight change within each program. Further, the relationship between weight change and readiness for change variables for weight, diet, and physical activity were examined by applying multiple linear regression and logistic regression models. The multivariable model included subprogram; gender; age; systolic and diastolic blood pressure; risk factor count; readiness for change for weight, activity, and diet; and stress level as covariates.

Results: There were 209 participants in the weight management program and 243 participants in the condition management program who met the criteria for obesity, resulting in a final sample of 452 participants. On average, the weight change for these participants was −0.28 pounds (SD = 15.55) and there was no statistical difference between the weight change in the two programs. When compared to the reference group (maintenance), participants at the action stage of physical activity, on average, lost weight (b = −4.59, p = 0.02). Likewise, participants at the pre-contemplation stage of physical activity lost weight when compared to the maintenance group (b = −26.24, p = 0.000). Participants at the pre-contemplation stage of physical activity had higher odds of achieving at least 5% weight loss than participants at the maintenance stage (OR = 5.80, p = 0.053).

Conclusion: Readiness for change for activity may be a predictor of weight change, and may predict the likelihood of achieving clinically significant weight loss. These findings can assist in targeting subjects for participation in such programs. The findings regarding the relationship between readiness for change and weight loss are counterintuitive, and further research is warranted in this area.

Keywords: obesity, employee wellness, weight loss, readiness for change, health behavior

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INTRODUCTION

Obesity (BMI $\geq$ 30 kg/m^2) prevalence has increased globally within the last four decades (1) and the United States has one of the highest rates of obesity (2). Furthermore, the economic burden of obesity is "considerable and rising" (3). The global economic impact of obesity is an estimated $2 trillion (US dollars), or 2.8% of gross domestic product (GDP) (4). Indirect societal costs of obesity are a result of increased absence from work and reduced productivity (4–6), as well as workplace injuries and disability payments (3, 4).

Employee wellness programs have become a popular mechanism to address health behaviors and reduce chronic conditions, including obesity, that impact workplace performance, and healthcare costs, especially for self-insured organizations (7). The workplace provides an ideal setting for employee wellness programs because nearly 60% of American workers receive their health insurance through their employer (8) and approximately 50% of waking hours are spent at work (9). The workplace also provides the necessary communication channels and social support for these programs to develop (10). For workers with obesity, in particular, employers pay more due to expenses related to medical claims, disability, and absenteeism (9). In the United States, over 37% of employees are considered overweight (they have a BMI in the range of 25–29.9 kg/m^2), and 29% are classified as obese (6, 11, 12). Taken together, these factors provide an impetus for employers to take the lead in implementing programs to address obesity.

There is a general consensus on the need to reduce obesity rates, but developing and implementing effective strategies and policies has proven to be a difficult task (1). A RAND Health Quarterly study (5) examined various aspects of employee wellness programs, including the prevalence of these programs, how they are designed, their impact on health outcomes, the role of incentives, and factors that facilitate these programs. Employee wellness programs are common: approximately 50% of U.S. companies offer employee wellness programs (5, 13). These programs vary in their complexity, but most incorporate wellness screenings and interventions to educate participants about making healthy lifestyle choices (5). Many employee wellness programs offer incentives in order to increase participation (7, 10). These incentives come in various forms, including cash payments and discounts (7, 13). In addition to enhancing program participation, incentives can promote desirable outcomes, such as healthy eating and physical activity (13–15).

There is much debate surrounding the effectiveness of employee wellness programs (16). However, research indicates that the transtheoretical model (TTM), or the stages of change model, can be a useful framework for wellness programs that involve behavioral change (17–19). The transtheoretical model for change suggests that, "health behavior change involves progress through six stages of change: precontemplation, contemplation, preparation, action, maintenance, and termination" (20). Further, a recent randomized controlled trial found that there was no weight change in a behavioral weight loss program coupled with motivational interviewing compared to only the behavioral weight loss program (21). The authors indicated the need to tailor motivational interviewing according to the participant's baseline motivation to observe improvement in weight loss (21). Hence, in this exploratory study we will assess the average weight change observed in an employee wellness program and whether the baseline measure of readiness for change is a predictor of weight loss. In addition, we will assess whether other demographic and clinical factors are associated with weight loss.

The stages of change model is used to "explain and predict how and when individuals change behaviors" (17): it gauges individuals' readiness for change. Readiness for change emerges from the stages of change model (22), which is commonly used in the health promotion literature. Originally, the model was applied to smoking cessation, but it has been used to facilitate change in other behaviors, including diet and physical activity (23). According to the model, individuals are typically at different stages with regard to adopting and adhering to health behaviors. The model outlines five stages: (1) precontemplation (not thinking seriously about modifying current behavior); (2) contemplation (thinking about modifying current behavior); (3) preparation (finding the determination to modify current behavior; (4) action (changing habits and/or their environment); and (5) maintenance (successfully maintaining new habits and behaviors) (22). Based on this model, it is expected that individuals at earlier stages (e.g., precontemplation or contemplation) will be less likely to adopt healthier default behaviors than those in later stages (e.g., preparation and action) (24).

Studies have shown that the stages of change model can predict behavior modification in smoking cessation programs and, "to a lesser degree," weight loss and maintenance programs (25). However, the model has received criticism. Sutton (26) acknowledged that finding significant differences in reported outcomes based on the participants' stage of change would support the model, but this approach ignores the possibility that different factors could have a more substantial effect at different stages (26, 27). A review of the literature on this model revealed that higher levels of self-reported readiness did not predict better treatment adherence, nor did it predict greater weight loss (28). Although the findings have been mixed, there are studies that support the use of interventions based on the stages of change model. For example, wellness interventions that accounted for participants' stage of change at baseline have been associated with increased physical activity (29, 30).

Physical inactivity is common among Americans, but interventions based on the stages of change model have been found to enhance physical activity in adults (30–32). However, Prochaska and DiClemente (33) have suggested that the model is cyclical rather than linear. Because individuals often fail to "establish and maintain lifestyle changes" (34, 35), it is common for them to regress back to an earlier stage of change (34). Research suggests that interventions based on the stages of change model are effective in initiating change in physical activity (34, 36). However, the effectiveness of these programs with regard to maintaining an active lifestyle is less clear: self-reported measures that gauge physical activity generally focus

on short-term maintenance, but they fail to account for physical activity over longer periods of time (36). Other studies indicate that interventions developed from the stages of change model affect behavioral change rather than maintenance (34, 37).

Diet change is key to improving various health outcomes. Cummins et al. (38) connect the increase in obesity prevalence in the United States to "changes in the food system" (283); they suggest that interventions designed to reduce caloric intake and improve the quality of food that is consumed should be part of a larger effort to reduce obesity prevalence. However, many individuals are resistant to adopting healthy eating habits (39). Other factors, including personal routines and social norms, can complicate diet change (40, 41). As such, simply increasing access to more nutritional food options fails to produce the desired results with regard to health outcomes (38).

The literature references several barriers to healthy eating, including limited access to resources; lack of nutrition literacy; and other factors such as price, taste, and tradition that are often attached to food (42). Among a sample of adults with type 2 diabetes, individuals who were actively improving their diet perceived fewer barriers to making these changes than people who were at the preparation stage; often, the latter group becomes discouraged and reverts back to poor eating habits (39). A recent study evaluated the effectiveness of an intervention on various outcomes, including readiness to change dietary habits. Participants in the intervention group showed statistically significant progress in readiness for change between baseline and follow-up. The results further indicated that the improvement in dietary habits was significantly different between the intervention and control groups (43).

Determining individuals' readiness to lose weight is necessary, and designing interventions that are tailored to participants' level of readiness could result in successful weight loss and maintenance (44). Readiness for change can vary from one individual to another, even if these individuals share similar risk profiles. Ghannadiasl et al. (44) found that obese women in Iran were at different stages of readiness for change with regard to weight loss. Alakaam et al. (45) examined the relationship between individuals' perception of their weight and stage of change for weight loss; the findings revealed that participants who were classified as overweight or obese and who perceived themselves as such were more likely to be at the action stage with regard to weight loss. Similarly, in the Ghannadiasl et al. (44) study, obese women at the precontemplation stage of weight loss had a lower waist-to-hip ratio than women at other stages of change (44). For this exploratory study, we sought to determine whether the readiness for change measures for weight, physical activity, and diet are important predictors of weight loss in an employee wellness program.

MATERIALS AND METHODS

The program was designed to guide, support, and educate individuals in making necessary lifestyle changes for better health and condition management. One of the primary goals was for the participants to lose excess weight, which is associated with comorbidities. This section describes the employee wellness program and then discusses how the program participants were recruited and assigned to each of the four subprograms. Coaching techniques are briefly described. Finally, methods of statistical analysis are detailed.

Employee Wellness Program Description

The employee wellness program that was used in this study is comprised of four subprograms: (1) low-risk, (2) lifestyle behavior, (3) weight management, and (4) condition management. In this paper we analyze data from the weight management and condition management programs only because these programs shared comparable factors: both were aimed at employees with obesity, both had similar age and gender distribution, and both had similar cardiometabolic risk profiles.

The primary area of focus for the weight management program is maintaining a healthy weight, which addresses issues related to obesity risk, portion control, water intake, caloric intake, and choosing healthy snacks. Similarly, the program encourages healthy eating: it promotes the Mediterranean diet, encourages participants to read food labels and use food trackers, and, when necessary, to consult with a dietitian. Additionally, the weight management program aims to assist participants with increasing physical activity (e.g., by developing a fitness routine and using fitness trackers), developing healthy sleep patterns, stress management, and smoking cessation.

The condition management program shares many components with the weight management program: these components include maintaining a healthy weight, healthy eating, increased physical activity, improved sleep patterns, stress management, and smoking cessation. In addition to these components, the condition management program promotes knowledge of various chronic conditions (diabetes, hypertension, and heart disease) and self-management of these conditions through practices such as blood glucose and blood pressure monitoring, knowledge and awareness of symptoms associated with these conditions, and developing an action plan for treatment. The condition management program also addresses issues related to medication adherence and encourages the use of self-monitoring devices, such as glucometers and blood pressure cuffs.

Study Participants

Program participation was open to employees and their spouses who were beneficiaries of the employer-sponsored health insurance plan. Participants were recruited into the program through a variety of mechanisms: these included, but were not limited to emails, huddles, and flyers. The recruitment period for the program opened on September 16, 2013, and closed on December 13, 2013. Employees who were interested in the program accessed a website to register for participation. Program participants were paired with a coach, and they received phone calls during the 6 month engagement period (January 2014 to July 2014). Baseline measures were collected between April 1, 2014, and August 31, 2014. Post-intervention measures were collected between April 1, 2015, and August 31, 2015. All baseline and post-implementation measures (blood pressure, height, weight,

waist circumference, HbA1c, and LDL) were collected by a third party vendor that was sub-contracted to perform measurements during employee wellness screenings. Because measurements were conducted by a third party vendor, the authors do not have access to the actual instruments that were used; however the vendor assured us that the same instruments for measure were used at both times and that instruments are calibrated in accordance with industry guidelines. It should be noted that risk of measurement error may occur between measurements.

An initial call gauged each participant's readiness for change (RFC) and set SMART goals (e.g., decrease in weight and waist circumference). The RFC instrument was included in the initial health assessment as completed by the employee and was derived from transtheoretical model (22) and included five of the six stages: precontemplation, contemplation, preparation, action, and maintenance. SMART goals are desired in behavior change based programs because they are Specific, Measureable, Achievable, Relevant, and Time-limited. In addition, each subprogram provided relevant educational resources for participants (e.g., various print, web, and electronic tools).

Based on a proprietary algorithm that considered BMI, HbA1c, LDL, systolic and diastolic pressures, and the American Heart Association My Life Check score, participants were stratified into low-, moderate-, and high-risk groups. Participants who were stratified into the low-risk group were assigned to the lifestyle management program. Participants stratified into the moderate- and high-risk groups were assigned to either the weight management program or the condition management program. These programs differed in that the condition management program primarily focused on teaching participants to manage diabetes, hypertension, and heart disease. The coaches employed motivational interviewing techniques to guide participants to adopt healthier default behaviors. For the purposes of the statistical analyses, we only examined participants who were classified as obese at baseline. All of these participants were either assigned to the weight management or condition management group.

Program participants consented to program participation as part of the program onboarding process. This paper used deidentified secondary data and was approved by the Institutional Review Board of University of Alabama at Birmingham IRB # 170421002.

Statistical Analyses

The study conducted exploratory statistical analyses that included descriptive analyses, two-group comparisons, and multivariable regression models. While the statistical significance was at 0.05, we report the exact *p*-values, thus allowing the readers to interpret the findings based on their choice of multiple testing.

Descriptive statistics were generated for the outcome variables of interest for the weight management and condition management subprograms. These variables include weight, BMI, A1C levels, systolic and diastolic blood pressure, and waist circumference. In addition, gender and age composition for each of the subprograms were computed. A paired *t*-test was conducted to compare average weight for participants at baseline and at follow-up. Subsequently, we conducted separate paired *t*-tests within the weight management and condition management subprograms.

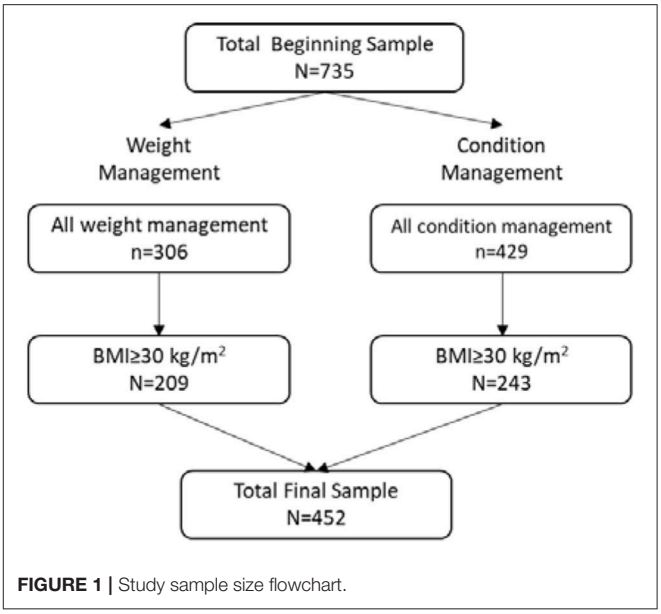

FIGURE 1 | Study sample size flowchart.

Next, we generated a variable for weight change: the difference between final weight and baseline weight was calculated. Welch *t*-tests were conducted in order to determine whether there was a significant difference in average weight change within the weight management and condition management subprograms. Then, linear regression models were run to determine predictors of weight change.

Finally, a binary variable was created to indicate whether participants met the criteria of clinically significant weight loss (46). "Clinically significant weight loss" refers to a loss of five percent or more of body weight and is expected to improve a number of health outcomes in individuals with obesity (47). Participants were assigned a value of 1 if they lost at least 5% of their baseline body weight. Otherwise, they were assigned a value of 0. A series of logistic regression models were run in order to determine factors that increase the likelihood that participants will attain clinically significant weight loss. Odds ratios for predictors of clinically significant weight loss are reported and interpreted in the Results section.

RESULTS

A flowchart that depicts how the final sample size for this study was obtained is provided in **Figure 1**. Our total beginning sample size consisted of 735 participants across the weight management and condition management subprograms (**Table 1**), out of which 306 were enrolled at the start of the weight management program. Of those participants, 275 (89.87%) were female, and 31 (10.13%) were male. The average age of weight management program participants at baseline was 46.09 years ($SD = 11.23$); participant age ranged from 23 to 66 years. Analysis of baseline biometric data of all participants by program revealed

TABLE 1 | Baseline characteristics of participants.

	Weight Mgmt. ($n = 306$)	Cond. Mgmt. ($n = 429$)	Total ($n = 735$)
Age (years)	46.1 (11.2)	52.5 (9.9)	49.9 (10.9)
Gender	Male: 31 (10.13%)	Male: 63 (14.69%)	Male: 94 (12.79%)
	Female: 275 (89.87%)	Female: 366 (85.31%)	Female: 641 (87.21%)
A1C	5.5 (0.4)	5.9 (1.0)	5.7 (0.9)
Systolic BP	119.5 (12.8)	125.5 (15.8)	123.1 (15.0)
Diastolic BP	79.9 (9.4)	80.7 (9.9)	80.4 (9.7)
Weight (lbs.)	206.2 (39.7)	193.6 (44.8)	198.9 (43.2)
Waist circum. (in.)	40.0 (5.4)	38.8 (6.2)	39.3 (5.9)
BMI	34.2 (6.5)	32.3 (7.2)	33.1 (6.9)
BMI $\geq$ 30 kg/m^2	$n = 209$	$n = 243$	$n = 452$

TABLE 2 | Predictors of weight change.

Predictor	Coefficient (95% confidence interval)	t-stat	*P*-value
Subprogram	−0.134 (−3.414, 3.145)	−0.08	0.936
Gender	−0.636 (−5.654, 4.382)	−0.25	0.803
Age	−0.088 (−0.237, 0.061)	−1.16	0.246
Systolic BP	−0.071 (−0.197, 0.054)	−1.12	0.262
Diastolic BP	−0.112 (−0.291, 0.068)	−1.22	0.223
Risk factor count	0.473 (−0.493, 1.438)	0.96	0.337
Number of observations = 417			

A linear regression model was run to determine if any descriptive measures (gender and age), subprogram participation, and/or cardiometabolic factors are significant predictors of weight change. BP, blood pressure.

no statistically significant difference in terms of BMI. However, there were significant differences in A1C, systolic, and diastolic blood pressure. Participants in the condition management group, on average, had higher A1C, systolic, and diastolic BP at baseline than participants in the weight management group. This finding is consistent with the proprietary algorithm used for participant program selection, as A1C and systolic and diastolic blood pressure were determinants for condition management vs. weight management.

Next, the condition management program composition is examined. Four hundred twenty-nine participants were enrolled at the start of the condition management program. Three hundred sixty-six (85.31%) of the participants were female, and 63 (14.69%) were male. The condition management cohort was older than the weight management cohort: average age at the start of the program was 52.53 years ($SD = 9.88$). Participant age in the condition management group ranged from 23 to 76 years.

In terms of biometric measures, baseline measures for A1C, systolic and diastolic blood pressure, weight, waist circumference, and BMI were obtained at the initial screening. The means and standard deviations for these biometric measures (A1C, systolic and diastolic blood pressure, weight, waist circumference, and BMI), as well as age and gender composition, are reported below (see **Table 1**). These measures are reported for the weight management and condition management subprograms independently and in total.

For the purposes of the statistical analyses, we only examined participants who were classified as obese at baseline (BMI $\geq$ 30 kg/m^2). This resulted in 209 participants in the weight management group and 243 participants in the condition management group. The total final sample was 452 participants. The RFC Diet level 4 had only one participant and therefore was excluded from the regression analyses.

Weight Change Across Subprograms

The initial paired *t*-test compared baseline and final weight for participants in both subprograms. There were 451 paired observations. The average baseline weight was 219.65 pounds ($SD = 37.14$). Average weight after the intervention was 219.37 pounds ($SD = 39.76$). On average, participants who were classified as obese lost 0.28 pounds ($SD = 15.55$) over the course of the intervention. The weight loss was not statistically significant.

Weight Management Program Outcomes

There were 209 paired observations in the weight management subprogram. The average baseline weight in this cohort was 219.02 pounds ($SD = 38.75$). Average weight after the intervention was 219.70 pounds ($SD = 41.47$). On average, participants in the weight management subprogram who were classified as obese gained 0.69 pounds over the course of the intervention; the weight gain was not statistically significant.

Condition Management Program Outcomes

There were 242 paired observations in the condition management subprogram. The average baseline weight in this cohort was 220.19 pounds ($SD = 35.76$). The average final weight was 219.08 pounds ($SD = 38.30$). On average, participants in the condition management subprogram who were classified as obese lost 1.11 pounds over the course of the intervention; the weight loss was not statistically significant.

Weight Change by Subprogram: Welch *t*-Test Results

The average weight change in the weight management subprogram was +0.69 pounds ($SD = 15.66$). The average weight change in the condition management subprogram was −1.11 pounds ($SD = 15.44$). We also compared whether the changes in weight differed across the two subprograms. The results of the Welch *t*-test, as well as the regression model (after adjusting for risk factors), indicate that there is no significant difference in weight change between the two subprograms (see **Table 2** for the regression model).

Predictors of Weight Change: Linear Regressions

Next, a series of linear regressions were conducted, in which weight change was the outcome (dependent variable). The initial model included subprogram (weight management or condition management); gender; age; various clinical measures;

TABLE 3 | Weight change predictors (regression model results).

Predictor	Coefficients (95% confidence interval)	t-stat	*P*-value
Subprogram	−0.379 (−3.669, 2.911)	−0.23	0.821
Gender	−1.855 (−6.951, 3.240)	−0.72	0.475
Age	−0.120 (−0.270, 0.029)	−1.58	0.114
Systolic BP	−0.076 (−0.202, 0.050)	−1.19	0.236
Diastolic BP	−0.099 (−0.276, 0.078)	−1.10	0.272
Risk factor count	0.711 (−0.326, 1.748)	1.35	0.178
RFC Weight (1: action stage)	0.517 (−7.970, 9.005)	0.12	0.905
RFC Weight (2: preparation stage)	−5.309 (−15.006, 4.387)	−1.08	0.282
RFC Weight (3: contemplation stage)	−3.629 (−17.902, 10.644)	−0.50	0.617
RFC Weight (4: precontemplation stage)	4.552 (−12.441, 21.546)	0.53	0.599
RFC Activity (1: action stage)	−4.592 (−8.453, −0.731)	−2.34	0.020
RFC Activity (2: preparation stage)	−2.856 (−7.832, 2.120)	−1.13	0.260
RFC Activity (3: contemplation stage)	−5.657 (−14.392, 3.078)	−1.27	0.204
RFC Activity (4: precontemplation stage)	−26.242 (−37.925, −14.561)	−4.42	0.000
RFC Diet (1: action stage)	3.578 (−1.323, 8.479)	1.44	0.152
RFC Diet (2: preparation stage)	3.884 (−2.709, 10.475)	1.16	0.247
RFC Diet (3: contemplation stage)	8.659 (−2.006, 19.325)	1.60	0.111
Number of observations = 403			

*This linear regression model is an extension of the analysis conducted in **Table 2**. In addition, we examined the RFC measures for weight, physical activity, and diet as predictors of weight change. BP, blood pressure; RFC, readiness for change. For this model, we had to exclude the RFC Diet (level 4, which is the precontemplation stage because of very small sample size and to allow the models to converge).*

and readiness for change (RFC) variables for weight, physical activity, and diet as covariates. For our study, we considered five stages of change: maintenance (0); action (1); preparation (2); contemplation (3); and precontemplation (4). The reference category for the RFC variables was maintenance.

The results of the regression model are provided in **Table 3**. RFC for physical activity was a significant predictor of weight change. Participants at the action stage of physical activity lost weight when compared to the reference group (b = −4.59, $p = 0.02$), as did participants at the precontemplation stage of physical activity (b = −26.24, $p = 0.000$). Similar patterns emerged when we accounted for stress level in the regression model. Participants at both the action stage (b = −4.37, $p = 0.029$) and the precontemplation stage (b = −25.89, $p = 0.000$) of physical activity lost weight over the course of the intervention.

It is possible that baseline weight could be associated with subsequent weight loss. Taking this point into consideration, an additional linear regression model was run that included baseline weight as a predictor of weight change. Counter to intuition, baseline weight did not emerge as a significant predictor of weight change (b = −0.001, $p = 0.951$). To further compare the linear regression models with and without baseline weight included as a covariate, we generated the Akaike information criterion (AIC) to examine model fit. The AIC for the model without baseline weight was 3337.6; the AIC for the model with baseline weight included was 3339.6. The results of the sensitivity analysis indicate that including baseline weight does not enhance the model substantially and that our original findings are robust.

Logistic Regression

Finally, a logistic regression model was run where the dependent variable was a binary variable that measured whether participants achieved clinically significant weight loss or not. Of the participants who were classified as obese, only 73 (16.19%) achieved clinically significant weight loss. The logistic regression model included gender, age, subprogram, clinical measures, and the readiness for change variables for weight, physical activity, and diet as covariates. Participants at the precontemplation stage of weight change (four observations) and diet change (one observation) were excluded from the analysis due to small sample size. The odds of clinically significant weight loss in participants at the precontemplation stage of physical activity were nearly six times higher than the participants at the maintenance stage; this finding approaches statistical significance ($OR = 5.80$, $p = 0.053$). These results are presented in **Table 4**.

Similar to the linear regression models, we ran an additional logistic regression model to test the idea that baseline weight could have an association with the likelihood that an individual would achieve clinically significant weight loss. Again, baseline weight did not emerge as a significant predictor of clinically significant weight loss (OR = 0.9997, z = −0.07, $p = 0.947$). Again, we generated AIC values for the logistic regression models with and without baseline weight included as a covariate to examine model fit. The AIC for the logistic regression model without baseline weight was 371.2; the AIC for the model with baseline weight included was 373.2. Again, the results of the sensitivity analysis indicate that the model was not enhanced by the inclusion of baseline weight as a predictor, and our original findings are robust.

DISCUSSION

This study estimated the average weight changes in an employee wellness intervention who were classified as obese, and compared these weight changes between two programs: weight management and condition management. The average weight loss for participants in both the weight management and condition management subprograms was 0.28 pounds. With regard to weight loss, the results of this employee wellness program are consistent with other published findings (21, 43). Specifically, Mache et al. (43) found that during a 12 month workplace intervention, the average weight loss was 0.5 kilograms, or approximately 1.10 pounds. Additionally, only 7% of the intervention group achieved clinically significant weight loss; an additional 3% lost at least 10% of their original body weight (43).

TABLE 4 | Predictors of clinically significant weight loss.

Predictor	Odds ratio (95% confidence interval)	Z-statistic	*P*-value
Subprogram	0.904 (0.489, 1.672)	−0.32	0.748
Gender	0.877 (0.328, 2.350)	−0.26	0.795
Age	0.993 (0.965, 1.021)	−0.49	0.628
Systolic BP	1.017 (0.994, 1.042)	1.43	0.153
Diastolic BP	1.010 (0.976, 1.044)	0.56	0.576
Risk factor count	0.968 (0.798, 1.175)	−0.33	0.745
RFC Weight (1: action stage)	0.854 (0.161, 4.545)	−0.18	0.854
RFC Weight (2: preparation stage)	2.378 (0.378, 14.960)	0.92	0.356
RFC Weight (3: contemplation stage)	2.152 (0.165, 27.989)	0.59	0.558
RFC Activity (1: action stage)	2.080 (0.924, 4.681)	1.77	0.077
RFC Activity (2: preparation stage)	1.181 (0.421, 3.309)	0.32	0.752
RFC Activity (3: contemplation stage)	2.631 (0.567, 12.201)	1.24	0.216
RFC Activity (4: precontemplation stage)	5.802 (0.976, 34.487)	1.93	0.053
RFC Diet (1: action stage)	0.511 (0.210, 1.248)	−1.47	0.141
RFC Diet (2: preparation stage)	0.452 (0.138, 1.476)	−1.32	0.188
RFC Diet (3: contemplation stage)	0.328 (0.045, 2.419)	−1.09	0.274
Number of observations = 399			

The logistic regression model was run to determine whether there are any significant predictors of clinically significant weight loss (5% or more of original weight). BP, blood pressure; RFC, readiness for change.

Further, the study explored whether RFC measures were predictors of weight change. The RFC measures for physical activity may help to predict weight change. The findings related to the physical activity of readiness for change will be discussed in the following paragraphs.

Participants at the action and precontemplation stages of change lost weight when compared to those at the maintenance stage. Based on the literature, the former is to be expected. Macchi et al. (48) propose the following: "Applied to weight management, weight loss occurs during the action stage and weight-loss maintenance occurs during the maintenance stage" (48).

With regard to participants at the precontemplation stage, the findings are counterintuitive. Individuals at more advanced stages of change are more likely to enroll in and complete workplace physical activity challenges (49, 50). Programs that do not increase physical activity gradually could deter participation from individuals at lower stages of change (e.g., precontemplation and contemplation). Tsai et al. (28) offer a possible explanation for this finding: "Individuals may overestimate their readiness because they do not clearly understand what behaviors are needed to make them successful, or because they greatly desire the outcome of weight loss" (28). From this perspective, it is plausible that participants who report higher levels of readiness for change might fail to achieve the desired results, and those at earlier stages might actually report better outcomes at the end of the intervention. However, additional research is needed to understand the weight change within the precontemplation group.

Every attempt was made to address or mitigate limitations. However, this study is limited in terms of participant recruitment, as the sample was heavily skewed toward females and did not include a control group. Because the study utilizes observational data, it is an exploratory study; we are unable to make any generalizations about causal relationships between participants' stage of change and weight loss. Moreover, testing of multiple hypotheses as part of an exploratory study can lend to false discoveries and it may be that RFC on physical activity may be a false positive. We provide exact *p*-values so that the readers can use their preferred approach and interpretation with respect to multiple testing. Another limitation of the study is that it only accounts for baseline and final (post-intervention) measures. A more useful approach would be to collect data (e.g., weight, blood pressure, waist circumference) from study participants at multiple time points, similar to the Moss et al. (21) study. For this study, we collected self-reported data for the RFC measures. The use of self-reported data introduces the possibility of various biases, the most likely of which is social desirability bias. When social desirability bias is present, participants report inaccurately in an effort to present themselves in a positive light. The presence of bias limits the validity of our results and could partially explain the counterintuitive findings of the study.

In addition to potential validity issues, the exploratory nature of the study and the use of self-reported data could restrict the generalizability of our findings (51). Even with the limitations, this study provides valuable information for group wellness programs as employers begin to think about offering wellness programs to employees.

It is worth noting that this study did not compare the costs of the program to savings generated from reduced absenteeism.

Future research would benefit from following participants over a longer period of time, including a more rigorous design that includes a control group, ideally randomized controlled studies, and more objective and measured measures to address the issues of bias, measurement error, and generalizability. Pragmatic designs, including Sequential Multiple Assignment Randomized Trial (SMART) designs that can be used to develop adaptive interventions, need to be leveraged in developing effective wellness programs (52, 53). Finally, analyses such as cost-effectiveness, cost-benefit, and miscrosimulations can be used to evaluate the short-term and long-term economic impact of wellness programs.

CONCLUSION

This study highlights the importance of considering readiness for change within physical activity as a potential predictor of weight change. There is at least a moderate level of evidence that suggests use of the stages of change model as a framework for

employee wellness interventions and more research in this area is warranted to test this hypothesis using a more rigorous design.

AUTHOR CONTRIBUTIONS

SF conceived the design of the study, conducted data collection, contributed to data interpretation, and provided overall direction and planning. RC contributed to the data analysis. TM provided oversight for data analysis and interpretation activities. SF, RC, and TM contributed to the manuscript writing, editing, and revising.

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Does Family History of Obesity, Cardiovascular, and Metabolic Diseases Influence Onset and Severity of Childhood Obesity?

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Objectives: The objectives were to evaluate (1) the metabolic profile and cardiometabolic risk in overweight/obese children at first assessment, stratifying patients according to severity of overweight and age; and (2) to investigate the relationship between family history (FH) for obesity and cardiometabolic diseases and severity of childhood obesity.

Methods: In this cross-sectional, retrospective, observational study, 260 children (139 female), aged between 2.4 and 17.2 years, with overweight and obesity were recruited. Data regarding FH for obesity and cardiometabolic diseases were collected. Each patient underwent clinical and auxological examination and fasting blood sampling for metabolic profile. Homeostasis model assessment of insulin resistance (HOMA-IR), triglyceride-to-high-density lipoprotein cholesterol ratio, and atherogenic index of plasma were calculated. To evaluate the severity of obesity, children were divided into two groups for BMI standard deviation (SD) ≤2.5 and BMI SD >2.5. Moreover, study population was analyzed, dividing it into three groups based on the chronological age of patient (<8, 8–11, >11 years).

Results: BMI SD was negatively correlated with chronological age ($p < 0.005$) and significantly higher in the group of children <8 years. BMI SD was positively associated with FH for obesity. Patients with more severe obesity (BMI SD >2.5) were younger ($p < 0.005$), mostly prepubertal, presented a significantly higher HOMA-IR ($p = 0.04$), and had a significantly higher prevalence of FH for arterial hypertension, type 2 diabetes mellitus, and coronary heart disease than the other group.

Conclusion: (1) Family history of obesity and cardiometabolic diseases are important risk factors for precocious obesity onset in childhood and are related to the severity of obesity. (2) Metabolic profile, especially HOMA-IR, is altered even among the youngest obese children at first evaluation. (3) Stratification of obesity severity, using BMI SD, is effective to estimate the cardiometabolic risk of patients.

Keywords: childhood obesity, parental obesity, insulin resistance, cardiometabolic risk, body mass index

INTRODUCTION

Childhood obesity (ChO) is one of the major public health issues worldwide and is known to be associated with an increased risk of severe long-term complications in adulthood (1–3). Obese children have a greater risk of developing arterial hypertension (AH), dyslipidemia, impaired glucose tolerance, insulin resistance (IR) or type 2 diabetes mellitus (T2DM), and precocious atherosclerosis (4–6). Long-term prediction of cardiometabolic risk is strictly conditioned by the severity of obesity (7).

BMI has been correlated with total body fat and cardiovascular risk factors (8); therefore, it would appear useful to evaluate the degree of obesity in the clinical setting. Furthermore, several metabolic and inflammatory factors are involved in the pathogenesis of cardiovascular and metabolic risk in obese children. IR is an important link between obesity and associated cardiovascular and metabolic risk (9). Triglyceride-to-high-density lipoprotein (HDL) cholesterol ratio (TG/HDL ratio) (10) and the atherogenic index of plasma (AIP) (11) may be associated to cardiovascular risk in ChO; however, only few papers have evaluated these parameters in children.

Aims of our cross-sectional, retrospective, observational study were (1) to evaluate, at first assessment, metabolic profile and cardiometabolic risk in overweight/obese children, stratifying them according to the severity of overweight and age; (2) to investigate the relationship between family history (FH) for obesity and cardiovascular and metabolic diseases and severity of ChO.

MATERIALS AND METHODS

Subjects

Two hundred and sixty overweight and obese children and adolescents (139 female), aged between 2.4 and 17.2 years, referred by their family pediatrician, were admitted to the Pediatric Endocrinology Outpatient Clinic at the University of Messina, from January 2010 to December 2013. Analysis of data was carried out retrospectively and completely anonymously. Retrospective analysis of data was notified to the Ethics Committee.

The inclusion criteria for the present study were chronological age between 2 and 18 years, BMI SD >2 from 2 to 5 years (12), and BMI SD >1 over 5 years of age corrected for sex (13), born as healthy full-term infant adequate for gestational age, blood pressure within normal range corrected for age. Exclusion criteria were genetic and endocrinological causes of obesity, born as preterm or postterm, diagnosis of chronic diseases, chronic therapies, and smoking.

Methods

Detailed history from the parents and from clinical records and family pediatrician data was obtained. Data on FH for obesity, AH, T2DM, dyslipidemia, coronary heart disease (CHD) in parents, siblings, and grandparents were also collected. The following measurements were obtained with standard methods: height, weight, and BMI. Body weight was determined to the nearest 0.1 kg on accurate and properly calibrated standard beam scales, in minimal underclothes and no shoes. Height was measured to the nearest 0.1 cm on standardized, wall-mounted height boards, according to standardized procedures. The children stood with the head aligned in the Frankfort plane, barefoot, with feet placed together and flat on the ground, heels, buttocks, and scapulae against the vertical backboard, arms loose and relaxed with the palms facing medially. BMI was calculated using the equation: body weight (kg)/height $(m)^2$. We considered BMI >2.5 SD (>99th percentile corrected for age and sex) to define severe obesity (14). The subjects underwent a detailed physical examination and pubertal evaluation, assessed by five Tanner stages of breast development in girls and testicular volume in boys (15), performed by pediatric endocrinologists. A fasting blood sampling for plasma triglycerides, HDL, low-density lipoproteins (LDL), total cholesterol, glucose, and insulin was performed at least 8 h after the last meal. These parameters were analyzed with standard techniques: triglycerides were measured enzymatically, the HDL-cholesterol fraction was obtained after precipitation using a phosphotungistic reagent, glucose was measured using a glucose oxidase method; serum insulin was determined by a chemiluminescence immunoassay. We considered abnormal levels of triglycerides, total cholesterol, LDL, HDL as defined by the National Cholesterol Education Panel (14): total cholesterol >170 mg/dl, LDL >130 mg/dl, HDL <40 mg/dl, triglycerides >110 mg/dl. We considered abnormal fasting glucose >100 mg/dl and fasting insulin >15 μUI/ml. IR was measured through homeostasis model assessment of insulin resistance (HOMA-IR). This index was calculated using the equation: fasting insulin (μU/ml) × fasting glucose (mg/dl)/405 (16). AIP was calculated using the equation: [LOG (triglycerides/HDL cholesterol)] (17). We considered the following cutoffs to define the three indices of cardiometabolic risk as abnormal: HOMA-IR >2.5 (16), AIP >0.11 (17), and TG/HDL ratio >1.25 (18). This study was carried out in accordance with the World Medical Association's Declaration of Helsinki.

Statistical Analysis

Numerical data are expressed as mean and standard deviation (SD) and categorical variables as numbers and percentages. Most of the examined variables were normally distributed as verified by Kolmogorov–Smirnov test; consequently, the parametric approach was used. In order to compare the two groups (BMI SD ≤2.5 vs BMI SD >2.5) with reference to categorical variables, the chi-squared test was applied; with reference to numerical parameters, Student's *t*-test was estimated. Some univariate linear regression models were estimated to assess the possible dependence of BMI SD on some potential explicative variables, such as chronological age, sex, FH for obesity, T2DM, AH, dyslipidemia, CHD, total cholesterol, LDL, HDL, HOMA-IR, AIP, or TG/HDL ratio. A stepwise multivariate regression model was estimated to identify the most significant predictors of BMI SD. To evaluate the distribution of BMI SD, according to familial risk categories, Student's *t*-test was estimated. Some univariate logistic regression models were estimated to verify the possible dependence of dichotomous variables, such as HOMA-IR (<2.5 and ≥2.5), AIP (<0.11 and ≥0.11), and TG/HDL ratio (<1.25 and ≥1.25), on some potential explicative variables such as age, sex, FH for obesity, T2DM, AH, dyslipidemia, CHD, total cholesterol, LDL, HDL, and HOMA-IR, AIP, or TG/HDL ratio, respectively. For

each parameter, a stepwise multivariate logistic regression model was estimated to identify the most significant predictors.

Furthermore, the whole population was stratified into three groups based on age of patients, and numerically balanced: group 1 (<8 years), group 2 (8–11 years), and group 3 (>11 years). Differences among these strata were evaluated by ANOVA (for numerical parameters) and chi-squared test (for categorical variables). For only variables that significantly differ in comparison among three groups, the pairwise comparisons were performed, using Student's *t*-test (for numerical parameters) and chi-squared test (for categorical variables); in this context, we applied the Bonferroni correction to control the multiplicity effect.

Statistical analyses were performed using SPSS 17.0 for Window package. $P < 0.050$ two-sided was considered to be statistically significant.

RESULTS

The mean age of study participants was 9.2 ± 2.9 years. One hundred and thirty-nine subjects (53.4%) were female. One hundred and sixty-one subjects (61.9%) were prepubertal. FH for obesity, T2DM, AH, dyslipidemia, CHD in parents, siblings, and grandparents was positive in 196 subjects (75.4%). The mean values of BMI and BMI SD were 29.7 ± 4.6 and 2.6 ± 0.5 SD, respectively.

Laboratory Parameters

Total cholesterol >170 mg/dl was seen in 105 patients (40.4%), and in 29 subjects (11.2%), it was >200 mg/dl. LDL was >130 mg/dl in 30 subjects (11.5%); triglycerides were >110 mg/dl in 37 children (14.2%). HDL was <40 mg/dl in 40 children (15.3%). The mean value of TG/HDL ratio was 1.7 ± 0.84. TG/HDL ratio was >1.25 in 166 subjects (63.8%). The mean value of AIP was −0.18 ± 0.2; AIP was >0.11 only in 14 patients (5.4%). Twenty-nine patients had fasting blood glucose >100 mg/dl; those subjects underwent an oral glucose tolerance test that excluded diabetes and confirmed a condition of impaired glucose tolerance. Fasting insulin was >15 μUI/ml in 88 children (33.8%). None of the patients received a diagnosis of metabolic syndrome according to Weiss et al. criteria (19). The mean value of HOMA-IR was 3 ± 2.1. Considering children divided for HOMA-IR <2.5 or ≥2.5, HOMA-IR ≥2.5 was positively correlated with age of patients ($p < 0.05$), triglycerides ($p < 0.05$), AIP ($p < 0.05$), and TG/HDL ratio ($p = 0.02$).

Evaluation According to BMI SD

BMI SD was negatively correlated with age of patients ($p < 0.005$). A negative association between BMI SD and age of patient was confirmed by univariate linear regression analysis ($p < 0.005$) and by stepwise multivariate regression analysis ($p < 0.005$). Moreover, a positive association between BMI SD and FH for obesity was demonstrated by univariate linear regression analysis ($p = 0.002$) and by stepwise multivariate regression analysis ($p = 0.007$).

Furthermore, the evaluation of BMI SD distribution according to familial risk categories confirmed a significantly higher BMI SD among children with a positive FH for obesity and for dyslipidemia (**Table 1**).

To evaluate FH, clinical characteristics, and metabolic profile according to the severity of obesity, children were divided into two groups with BMI SD ranging from >2 to ≤2.5 (group A, median 2.3) and from >2.5 to 5.2 (group B, median 2.7), respectively. Detailed results concerning the two groups are presented in **Table 2**. Patients of group B were significantly younger than of group A, and most of them were prepubertal. Furthermore, children of group B had a significantly higher positive FH for T2DM, AH, and CHD and a significantly higher mean value of fasting insulin and HOMA-IR compared to those of group A. Other parameters did not present significant differences between groups (**Table 2**). Univariate logistic regression analysis showed a negative association between BMI SD >2.5 and age of patients ($p < 0.005$), and a positive association among BMI SD >2.5 and FH for AH ($p = 0.02$), FH for T2DM ($p = 0.003$), and HOMA-IR ($p = 0.046$). These associations were confirmed by stepwise multivariate logistic regression analysis (**Table 3**).

TABLE 1 | BMI SD distribution (mean ± SD) in children according to familial risk factors.

	Positive FH	Negative FH	*p*-value
FH for obesity	2.7 ± 0.5	2.5 ± 0.4	0.005
FH for T2DM	2.6 ± 0.3	2.5 ± 0.5	n.s.
FH for AH	2.6 ± 0.4	2.5 ± 0.5	n.s.
FH for CHD	2.6 ± 0.4	2.6 ± 0.5	n.s.
FH for dyslipidemia	2.7 ± 0.6	2.5 ± 0.4	0.013

n.s., not statistically significant; FH, familiar history; AH, arterial hypertension; CHD, coronary heart disease; T2DM, type 2 diabetes mellitus.

TABLE 2 | Family history, clinical, and biochemical data according to BMI SD ≤2.5 or >2.5.

	Group A BMI SD ≤2.5 (*n* = 126) (mean ± SD)	Group B BMI SD >2.5 (*n* = 134) (mean ± SD)	*p*-Value
Age (years)	10.5 ± 2.3	7.9 ± 2.8	<0.0005
Gender (M/F)	63/63	58/76	n.s.
Prepubertal/pubertal	63/63	98/36	<0.0005
FH for obesity (yes/no)	37/89	53/81	n.s.
FH for AH (yes/no)	46/80	68/66	0.02
FH for T2DM (yes/no)	47/79	75/59	0.003
FH for dyslipidemia (yes/no)	26/97	43/91	n.s.
FH for CHD (yes/no)	14/112	27/107	0.04
Fasting insulin (μUI/ml)	12.8 ± 8.7	15 ± 9.3	0.04
Fasting glucose (mg/dl)	87.4 ± 9.2	87.7 ± 13.4	n.s.
Total cholesterol (mg/dl)	166.7 ± 34.1	162.5 ± 30.7	n.s.
HDL (mg/dl)	50.9 ± 9.7	49 ± 9.3	n.s.
LDL (mg/dl)	99.7 ± 30.7	97.6 ± 26.2	n.s.
Triglycerides (mg/dl)	79.1 ± 41.8	79.7 ± 28.5	n.s.
HOMA-IR	2.8 ± 1.9	3.3 ± 2.3	0.04
AIP	−0.2 ± 0.2	−0.2 ± 0.2	n.s.
TG/HDL ratio	1.6 ± 0.9	1.7 ± 0.7	n.s.

Differences among groups were evaluated by Student's t-test (for numerical parameters) and chi-squared test (for categorical variables: gender, pubertal stages, and FH).
"Yes/No" refer to the number of patients.
Male (M), Female (F), not statistically significant (n.s.), familiar history (FH), arterial hypertension (AH), coronary heart disease (CHD), type 2 diabetes mellitus (T2DM), homeostasis model assessment of insulin resistance (HOMA-IR), triglyceride-to-HDL cholesterol ratio (TG/HDL ratio), atherogenic index of plasma (AIP), low-density lipoprotein (LDL), high-density lipoprotein (HDL).

TABLE 3 | Stepwise multivariate logistic regression analysis for BMI SD, HOMA-IR, TG/HDL ratio, and AIP.

Predictors	*B*	Odds ratio (CI 95%)	*p*-Value
BMI *SD ≤2.5 or >2.5*			
Age	−0.487	0.615 (0.536–0.705)	<0.005
FH for T2DM	0.710	2.034 (1.123–3.684)	0.019
FH for AH	0.738	2.091 (1.142–3.829)	0.017
HOMA-IR	0.284	1.328 (1.114–1.583)	0.002
HOMA-IR *<2.5 or ≥2.5*			
Age	0.25	1.292 (1.13–1.47)	<0.005
Sex (F)	−0.922	0.39 (0.23–0.68)	0.001
BMI SD	0.87	2.39 (1.15–4.97)	0.01
TG/HDL ratio *<1.25 or ≥1.25*			
Age	0.289	1.335 (1.153–1.154)	<0.005
Sex (F)	−0.992	0.371 (0.207–0.664)	0.001
BMI SD	1.171	3.224 (1.394–7.456)	0.006
LDL	0.018	1.019 (81.008–1.029)	0.001
AIP *<0.11 or ≥0.11*			
Total cholesterol	0.05	1.053 (1.006–1.101)	0.02

Confidence interval (CI), Arterial hypertension (AH), coronary heart disease (CHD), type 2 diabetes mellitus (T2DM), Homeostasis model assessment of insulin resistance (HOMA-IR), triglyceride-to-HDL cholesterol ratio (TG/HDL ratio), atherogenic index of plasma (AIP), low-density lipoprotein (LDL), Female (F).

Evaluation According to Age Subgroups

To verify the precocious incidence of obesity and metabolic complications related to obesity, we divided our population into three groups, numerically balanced, according to age of patients: *group 1* (<8 years; 86 subjects), *group 2* (8–11 years; 102 subjects), *group 3* (>11 years; 72 subjects). In **Table 4**, the results obtained from group comparisons for both categorical variables and numerical parameters are reported. The pairwise comparisons demonstrated significant differences in BMI SD and BMI SD >2.5, and in fasting insulin, HOMA-IR, and HOMA-IR ≥2.5, reported in **Table 5**.

DISCUSSION

In our observational, cross-sectional, retrospective study, we analyzed a large cohort of children with simple overweight and obesity, included in a wide range of ages, brought to our outpatient clinic for first evaluation.

The main findings of this study are the more severe obesity in younger children and the relationship between FH for obesity, cardiovascular, and metabolic diseases and the severity of obesity. Our findings suggest that the problem of ChO is not only related to an increase in the number of diagnoses among children but also concerns an earlier insurgence of obesity.

Furthermore, we suggest that FH for obesity, AH, T2DM, dyslipidemia, and CHD should be considered risk factors for early onset and a major severity of obesity in children. The findings that FH for obesity significantly increases the risk of ChO have been consistently reported in other studies (20–24). This association is likely the result of a combination of genetic and epigenetic mechanisms and environmental factors, such as shared family lifestyle characteristics.

Our results support the role of FH for obesity, suggesting also the involvement of FH for cardiovascular and metabolic diseases in determining ChO.

TABLE 4 | Comparison among three groups according to age of patients.

	Group 1 <8 years (*n* = 86)	Group 2 8–11 years (*n* = 102)	Group 3 >11 years (*n* = 72)	*p*-Value
Age (years)	6.07 ± 1.3	9.4 ± 0.86	12.7 ± 1.6	<0.05
Gender (F/M)	57/29	52/50	31/41	<0.05
Prepubertal/pubertal	81/5	59/43	21/51	<0.05
FH for obesity (yes/no)	35/51	35/67	20/52	n.s.
FH for AH (yes/no)	38/48	44/58	32/40	n.s.
FH for T2DM (yes/no)	43/43	51/51	28/44	n.s.
FH for dyslipidemia (yes/no)	27/59	28/74	17/55	n.s.
FH for CHD (yes/no)	11/75	15/87	15/57	n.s.
BMI SD	2.9 ± 0.5	2.4 ± 0.2	2.3 ± 0.3	<0.05
HOMA-IR	2.8 ± 2.1	2.7 ± 1.5	3.6 ± 2.5	<0.05
Fasting insulin (μUI/ml)	13.1 ± 2.1	12.9 ± 6.8	16.4 ± 11.3	<0.05
Fasting glucose (mg/dl)	86.7 ± 9.8	87.3 ± 9.9	88.9 ± 14.9	n.s
Total cholesterol (mg/dl)	164.7 ± 29.5	167.3 ± 36.5	160.6 ± 29.1	n.s.
HDL (mg/dl)	50.3 ± 9.7	49.9 ± 9.4	49.5 ± 9.5	n.s.
LDL (mg/dl)	98.9 ± 26.9	101.2 ± 31.8	94.5 ± 25	n.s.
Triglycerides (mg/dl)	75.5 ± 27.6	80.3 ± 36.7	82.7 ± 42	n.s.
AIP	−0.19 ± 0.2	−0.17 ± 0.19	−0.16 ± 0.2	n.s.
TG/HDL ratio	1.5 ± 0.7	1.7 ± 0.8	1.7 ± 0.9	n.s.
BMI SD >2.5 (yes/no)	73/13	38/64	23/49	<0.05
HOMA-IR >2.5 (yes/no)	41/45	46/56	47/25	<0.05
AIP >0.11 (yes/no)	6/80	5/97	3/69	n.s.
TG/HDL ratio >1.25 (yes/no)	50/36	65/37	51/21	n.s.

Differences among groups were evaluated by ANOVA (for numerical parameters) and chi-squared test (for categorical variables: gender, FH, BMI SD >2.5, HOMA-IR >2.5, AIP >0.11, TG/HDL ratio >1.25). "Yes/No" refer to the number of patients. Male (M), Female (F), not statistically significant (n.s.), arterial hypertension (AH), type 2 diabetes mellitus (T2DM), coronary heart disease (CHD), homeostasis model assessment of insulin resistance (HOMA-IR), triglyceride-to-HDL cholesterol ratio (TG/HDL ratio), atherogenic index of plasma (AIP), low-density lipoprotein (LDL), high-density lipoprotein (HDL).

TABLE 5 | Pairwise comparisons among three groups according to age of patients.

	p-Value Group 1 vs 2	*p*-Value Group 1 vs 3	*p*-Value Group 2 vs 3
BMI SD	<0.001	<0.001	n.s.
HOMA-IR	n.s.	0.006	0.006
Fasting insulin (μUI/ml)	n.s.	0.007	0.006
BMI SD >2.5	<0.001	<0.001	n.s.
HOMA-IR >2.5	n.s.	n.s.	0.009

*For only variables that significantly differ in comparison among three groups (**Table 4**), the pairwise comparisons were performed, using Student's t-test (for numerical parameters) and chi-squared test (for categorical variables); in this context, we applied the Bonferroni correction to control the multiplicity effect (adjusted α is 0.017).*

In this context, family pediatricians, aware of familiar risk factors for early onset of ChO, play a primary role in identifying children's overweight in a precocious phase, to start prevention programs. Moreover, although prevention remains the first target in ChO management, pediatricians should focus on the early identification of obesity-associated complications, which may already occur precociously in children.

Early onset of severe obesity increases the risk of long-term obesity, cardiovascular, and metabolic complications (1, 2, 7, 25).

Overweight and obesity from childhood to adulthood have been related to an increased risk of T2DM, AH, dyslipidemia, and carotid-artery atherosclerosis. However, it has been reported that in subjects who were overweight or obese in childhood, but had normal weight in adulthood, cardiovascular risk in adulthood is comparable to the general population (4). Therefore, an early, multidisciplinary approach (pediatric, endocrinological, nutritional) in overweight and obese children is necessary to reduce the development of cardiovascular and metabolic complications. IR, previously considered a problem in adulthood, becomes a serious issue also in children. In a cohort of preschool children, Aristizabal et al. reported IR as the most frequent metabolic alteration, supporting the use of BMI to identify children with IR (26). Also in our population, BMI was an efficacious screening method, easy for routine use, to identify children at risk of early obesity complications. IR represents an important link between obesity and associated cardiovascular risk (9) and has been indicated as one of the first mechanisms involved in the development of endothelial dysfunction in obese youths (5). Giannini et al. showed that early changes in glucose metabolism, detected in obese prepubertal children, could be one of the factors leading to the increase of intima media thickness (5). Moreover, IR has been related to the metabolic syndrome incidence in childhood. Weiss et al. demonstrated the association between the severity of obesity and the diagnosis of metabolic syndrome, and the negative effect of obesity worsening on each component of the metabolic syndrome (19). In our cohort, HOMA-IR was abnormal in 51.9% of participants, significantly higher among those with the most severe obesity, despite them being younger and mainly prepubertal.

Furthermore, it is suggested that IR influences the link between obesity and dyslipidemia. Vukovic et al. demonstrated that HOMA-IR was strictly related to lipid profile in their pediatric population (27). Giannini et al. reported a correlation between IR and TG/HDL ratio (5). In our cohort, we demonstrated a correlation between HOMA-IR and lipid profile, in particular with triglycerides, AIP, and TG/HDL ratio. Recently, the importance of TG/HDL ratio as a marker of cardiovascular risk and inflammatory status in children has been demonstrated (10). TG/HDL ratio was frequently abnormal among our patients, even if a significant difference according to the severity of obesity has not been demonstrated. TG/HDL ratio is an easily calculated marker, useful in monitoring obese children at risk of metabolic complications. AIP, considered a new marker of atherogenicity, has been shown to significantly correlate with cardiovascular disease and metabolic syndrome in adulthood (28); however, it has been little studied in children. Vrablik et al. demonstrated the correlation of AIP with BMI and HOMA-IR in children (11). Conversely, we did not confirm these data in our patients, among whom only in few cases was AIP abnormal.

Some limitations of this study need to be acknowledged. In particular, socioeconomic factors have not been considered. Waist circumference was not systematically evaluated during the assessment; therefore, we were not able to estimate the incidence of metabolic syndrome in comparison with other pediatric diagnostic criteria (29), other than Weiss et al. criteria (19). In addition, our cohort of patients comes from southern Italy, which may limit the generalization of results; on the other hand, our population represents a sample with homogeneous features.

To summarize, we suggest the importance of adopting prevention programs to contrast ChO development and, when ChO is already diagnosed, start an early multidisciplinary medical approach in these children, who, even if so young, may already show the first signs of metabolic disorders, possible preluding cardiovascular and metabolic disease development in young adulthood.

We conclude that (1) an FH for obesity, AH, T2DM, or CHD is an important risk factor for precocious obesity onset in childhood and influences the severity of obesity; (2) metabolic profile, especially HOMA-IR, is altered even among the youngest obese children at first evaluation; (3) stratification of the severity of obesity, using BMI SD, is effective to estimate the cardiometabolic risk of patients and to program a specific and multidisciplinary follow-up for each patient.

ETHICS STATEMENT

This study was carried out in accordance with the World Medical Association's Declaration of Helsinki. Retrospective and anonymous analysis of data was notified to the Ethics Committee.

AUTHOR CONTRIBUTIONS

MW and FD conceived the study. TA, MV, and MM contributed to data collection. AA carried out data analysis. DC, MW, TA, and FD carried out data interpretation. DC and MW were involved in literature search and writing of the manuscript. All authors approved the submitted version of the manuscript.

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Girls and Boys Have a Different Cardiometabolic Response to Obesity Treatment

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Background: Childhood obesity exposes individuals to cardiometabolic disturbances. We analyzed how family-based multidisciplinary obesity treatment influenced children's cardiometabolic health.

Materials and methods: In this retrospective, two-year, follow-up study of 654 2- to 18-year-old children treated for obesity in three Finnish pediatric clinics in 2005–2012, blood pressure (BP), metabolic parameters, and the influence of sex, puberty and a change in body mass index standard deviation score (BMI SDS) were analyzed.

Results: At baseline, at least one cardiovascular risk factor was present in 474 (80%) cases. Boys presented with more significant changes in cardiometabolic parameters than girls during the treatment. Boys' total cholesterol (TC) improved by 12 months ($P = 0.009$), and their low-density lipoprotein C (LDL-C) and glycosylated hemoglobin ameliorated by 12 months ($P = 0.030$ and 0.022, respectively) and 24 months ($P = 0.043$ and 0.025, respectively). Boys' triglycerides, insulin, homeostasis model assessment for insulin resistance (HOMA-IR) and systolic BP deteriorated at 24 months ($P < 0.001$, 0.004, 0.002, and 0.037, respectively). In all children, the number of acceptable TC, LDL-C, insulin, and HOMA-IR values increased if BMI SDS reduced 0.25 or more by 12 months.

Conclusion: Minor cardiometabolic improvements were found during the obesity treatment. These findings indicate the need to assess treatment methods and focus on prevention.

Keywords: childhood obesity, specialist care, cardiometabolic, treatment outcomes, blood pressure, fatty liver, metabolism, BMI SDS

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INTRODUCTION

Childhood obesity increases morbidity and premature mortality (1, 2). A wide spectrum of physical symptoms, psychosocial disturbances, and cardiovascular (CV) risk factors, even diseases, are related to childhood obesity (3–6). Moreover, childhood obesity tracks easily into adulthood (7). Due to the strong correlation between childhood and adulthood obesity, it is difficult to determine the independent effects of childhood obesity (8).

The processes of atherosclerosis and obesity-related complications already begin in childhood. Left ventricle hypertrophy and dysfunction have been documented in children with severe obesity (4, 9, 10). The components of the metabolic syndrome, such as dyslipidemia, hyperinsulinism, and hypertension, are common in adolescents with obesity (11). Moreover, fatty liver is a frequent consequence of childhood obesity (9, 12). As the disease processes begin in childhood, prevention and treatment for obesity should start early. It has been established that the risks for CV diseases and type 2 diabetes significantly decrease if an obese child does not become an obese adult (6, 11).

In randomized controlled clinical trials, the cardiometabolic short-term outcomes of childhood obesity treatment are moderately efficient, but the data on the long-term outcomes of these trials and on the outcomes of studies conducted in everyday clinical practice are insufficient (13–17). There is an urgent need to analyze efficacy of obesity treatments and to find better intervention methods, which could be implicated in clinical practice.

The purpose of this study was to analyze the cardiometabolic outcomes of a family-based multidisciplinary behavioral treatment of up to 2 years in length for childhood obesity in three Finnish pediatric units, to examine the influence of sex and puberty on the outcomes, and to explore the influence of body mass index standard deviation score (BMI SDS) change during treatment on the metabolic profile.

MATERIALS AND METHODS

Sample and Study Design

This is a retrospective, register-based longitudinal study of 654 children aged 2 to 18 years treated for obesity in the period 2005–2012 in three pediatric units of Eastern Finland (Kuopio University Hospital, Mikkeli Central Hospital, or North Karelia Central Hospital). These hospitals are responsible for pediatric secondary and tertiary care in their hospital districts. The participants in this study were included in our first report on children ($n = 900$) evaluated for obesity and cardiometabolic profile at the time of the baseline visit in specialist care (18) and in our second report ($n = 654$) on the BMI SDS outcomes of the obesity treatment (19). The children in the present study had to have one or more follow-up visits with a pediatrician during the study period. The data were analyzed at three different time points: at baseline (the first visit), 12 months (6–17.9 months, $n = 521$) from baseline, and 24 months (18–30 months, $n = 345$) from baseline. The included and excluded children did not differ in terms of sex {Mann-Whitney U [M-W U], $P = 0.631$}, age (M-W U, $P = 0.087$), puberty {Pearson chi-square [χ^2], $P = 0.375$}, or BMI SDS (M-W U, $P = 0.844$) at the time of their baseline visit.

The treatment, described in detail in our previous report, was carried out by a multidisciplinary team, which consisted of a pediatrician, specialist nurse, dietician, physiotherapist, psychologist, and family therapist (19). The treatment was planned individually with each family and child according to the regional obesity treatment programs based on the Finnish National Current Care Guidelines on Childhood Obesity (20). Parental involvement, motivation, and long-term adherence to the protocol were important components of the treatment. Ambulatory treatment lasted approximately a year, but children with severe obesity and children who already had significant metabolic disturbances remained in specialist care for a longer time.

The Research Ethics Committee of the Hospital District of Northern Savo (Kuopio, Finland) has approved the study protocol. Permission to use the patient registers was obtained from the National Institute for Health and Welfare and from the participating hospitals.

Assessments

The children's height and weight were measured and recorded on visits by an experienced nurse. Height was measured by using a wall-mounted Harpenden stadiometer (Holtain Ltd, Crymyck, UK) with an accuracy of 0.1 cm. The mean of the closest two out of three measurements of barefoot height was used. Weight was measured in light underwear using a calibrated electronic scale with an accuracy of 0.1 kg as the mean of two measurements. BMI was calculated as weight divided by height squared. Height and BMI values were converted into body height SDS and BMI SDS according to Finnish gender- and age-specific population standards (21).

The children were classified at baseline into the following age groups: 2–6.9 years (13%), 7–9.9 years (20%), 10–14.9 years (53%), and 15–18 years (14%). Children who were 10 years old or older were considered adolescents (22). Pubertal status was recorded by physicians using the Tanner staging method (23, 24). For the purpose of this study, children were classified as prepubertal (45%) and pubertal (55%) at baseline and as prepubertal (25%) and pubertal (75%) over the 2-year follow-up. Girls with palpable breast tissue and boys with testicular volume >3 ml were designated as pubertal (25). BMI SDS was used to classify children into four obesity categories: overweight, obesity, severe obesity, and morbid obesity. According to Finnish growth standards the BMI SDS cut-offs for each category for girls were 1.16, 2.11, 2.76, and 3.24, respectively, and for boys 0.78, 1.70, 2.36, and 2.85, respectively. These values corresponded to BMIs of 25, 30, 35, and 40 kg/m^2, respectively, at the age of 18 years (21). At baseline, 9% of the children were overweight, 47% had obesity, 31% had severe obesity, and 13% had morbid obesity. Using the cut-offs of the International Obesity Task Force, 6% of the children were overweight and 94% obese (26). The BMI SDS changes from baseline were defined at 12 and 24 months. Study subjects were categorized at 12 months in three groups according to the BMI SDS reduction from baseline: BMI SDS reduction $\geq$ 0.25 units (good), 0–0.24 units (borderline), and no reduction (poor). BMI SDS reduction $\geq$ 0.25 has been reported as necessary to improve CV risk factors in overweight children (27).

Blood Pressure

Blood pressure (BP) was measured two or three times using the Criticon Dinamap Vital Signs monitor 1846 SX with a suitable Duracuff (8–13 or 38–50 cm) from the right arm in the supine position after a recommended 15 min of rest while seated, and the lowest recording was registered. Systolic BP (SBP) and diastolic

BP (DBP) were classified at baseline as normal, high normal, and hypertensive (stages 1 and 2). The height percentile-, age-, and gender-specific percentile cut-offs were used (<90th, 90th but less than 95th and ≥ 95th), as recommended by the fourth report from the National High Blood Pressure Education Program (NHBPEP) Working Group on Children and Adolescents (28). For clinical purposes, the hypertensive category was divided into two subgroups: stage 1 hypertension (BP between the 95th percentile and the 99th percentile plus 5 mmHg) and stage 2 hypertension (BP above the 99th percentile plus 5 mmHg).

Laboratory Analyses

The laboratory analyses done in the 6-month periods before and after the first visit, 12-month visit, and 24-month visit were included. All samples were taken after a recommended 12-h overnight fast. Each hospital carried out the analyses in their own laboratories in 2005–2007. From 2008, all laboratory analyses were performed in one regional laboratory, the Eastern Finland Laboratory Center. We calculated possible differences between analyses carried out before and after the move to central laboratory analysis using separate general linear models for boys and girls, controlling for age, pubertal status and obesity status.

Total plasma cholesterol (P-TC) and plasma triglyceride (P-TG) were analyzed with a colorimetric enzymatic assay, and plasma low-density lipoprotein cholesterol (P-LDL-C) and plasma high-density lipoprotein cholesterol (P-HDL-C) were analyzed with a homogeneous colorimetric enzymatic assay (both Roche Diagnostics GmbH, Mannheim, Germany). The IFCC kinetic method was used to quantify plasma alanine aminotransferase (P-ALT) (Roche Diagnostics GmbH, Mannheim, Germany). Plasma glucose (P-Gluc) was analyzed by the hexokinase method, and blood glycosylated hemoglobin (B-HbA1c) was analyzed with a turbidimetric inhibition immunoassay (both Roche Diagnostics GmbH, Mannheim, Germany). Serum insulin (S-INS) was analyzed using an electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany).

Fasting (f) P-Gluc concentrations were classified according to the International Society for Pediatric and Adolescent Diabetes (ISPAD) Clinical Practice Consensus Guidelines 2014 Compendium (29) as normal, impaired fP-Gluc (IFG), or diabetic: <5.6 mmol/l, 5.6–6.9 mmol/l, and ≥ 7 mmol/l, respectively. A dichotomous classification of fP-Gluc of normal (< 5.6 mmol/l) and abnormal (≥ 5.6 mmol/l) was used in McNemar's test. P-Gluc values following a 2-h oral glucose tolerance test (OGTT; a load of 1.75 g/kg anhydrous glucose up to a maximum of 75 g, dissolved in water) were classified as normal (<7.8 mmol/l), impaired glucose tolerance (IGT) (7.8–11.0 mmol/l), or diabetic (≥ 11.1 mmol/l). B-HbA1c% was classified as normal (< 5.8%), prediabetic (5.8–6.4%), or diabetic (≥ 6.5%). Prediabetes was recognized in cases where there was IFG or IGT, or HbA1c in the prediabetic range (29). Fasting S-INS concentrations were categorized as normal or hyperinsulinemic (HI) using the following pubertal stage-specific cut-offs for hyperinsulinemia: prepubertal, >15 mU/l; pubertal, >30 mU/l; and postpubertal, >20 mU/l (30). The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated by the formula fS-INS (mU/l) × fP-Gluc (mmol/l)/22.5. The cut-offs for normal and abnormal HOMA-IR values were 2.67 for prepubertal boys and 5.22 for pubertal boys, and 2.22 and 3.82 for prepubertal and pubertal girls, respectively (30).

Lipid levels were classified as acceptable, borderline, or high (or low for HDL-C), in accordance with the Summary Report (2011) of the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (28), as follows: fP-TC: < 4.40, 4.40–5.17, and > 5.18 mmol/l, respectively; fP-LDL-C: < 2.84, 2.84–3.35, and ≥3.36 mmol/l, respectively; fP-TG for children under 10 years old, < 0.84, 0.84–1.12, and ≥1.13 mmol/l, respectively; for adolescents, < 1.02, 1.02–1.46, and ≥1.47 mmol/l, respectively. The cut-offs of fP-HDL-C sub-groups were acceptable, borderline and low, defined as > 1.17, 1.04–1.17, and <1.04 mmol/l, respectively. Fasting P-ALT data were classified as elevated at ≥ 40 IU/l (31).

CV risk factors comprised hypertensive SBP or DBP, high P-TC, high P-LDL-C, high P-TG, low P-HDL-C, and diabetes or prediabetes.

Statistical Analyses

Descriptive data were analyzed according to sex, and the data were either presented as the means and 95% confidence intervals (CIs) or as medians and interquartile ranges (IQR), according to the distribution of the data variables. The distributions for normality were tested with the Shapiro-Wilk test and visualized with the histograms. Metabolic parameters and BP were presented also in staged distributions. Continuous variables were compared using the independent samples *t*-test for normally distributed variables and the M-W U test for non-normally distributed variables. The Pearson χ^2 test was used to compare distributions at one time point.

Because of the large variety in timing of the visits and repeated cardiometabolic measurements, cardiometabolic outcomes were analyzed using linear mixed model analysis. To obtain a better normality in the distributions of metabolic parameters, the log-transformed values were used and to avoid negative metabolic values, a constant value (one) was added to all metabolic values before the logarithms were calculated. To analyze whether the study subjects benefitted from treatment, cardiometabolic measurements at baseline were compared with those over the entire treatment period using linear mixed model analyses. Consequently, three time points were categorized dichotomously (baseline group and 12 and 24 months together as another group). Continuous cardiometabolic parameters were introduced one by one in the model as a dependent variable, time points as fixed effects, and study subjects as random effects. Because age and BMI SDS could influence the levels of dependent variables, all analyses were adjusted for age and BMI SDS at baseline including these covariates as fixed effects to the models. Linear mixed model analyses were conducted for all study subjects and separately for girls and boys. Moreover, all the analyses were conducted for all prepubertal and pubertal children and separately for prepubertal

and pubertal girls and boys. The results were represented as the mean differences and 95% CIs. Residuals' normality was used to evaluate the validity of the assumptions of the used mixed models.

Furthermore, to analyze cardiometabolic changes at time points, the comparisons between 12 months and baseline and between 24 months and baseline were performed using linear mixed model analysis as described above with an exception of time points. In these comparisons, all three time points (baseline, 12 and 24 months) were included in the models as fixed effects. The analyses were conducted for all study subjects and separately for girls and boys. The mean differences between time points were represented using the traffic light method where the green color indicated improvement and the red color deterioration of the cardiometabolic parameter.

The influence of the change in BMI SDS on the changes of metabolic parameters was studied using categorized variables and the McNemar's test. The categorized metabolic parameters (acceptable, borderline, and high/low or normal and abnormal) at baseline were compared to their pairs at 12 months in three subgroups of BMI SDS change at 12 months from baseline (good, borderline, and poor). These comparisons of metabolic distributions were done using both authentic paired data and data created by the intention-to-treat approach to avoid the problem of missing measurements (data not shown). The influence of BMI SDS change was analyzed only at 12 months. At 24 months, the number of cases in some subgroups was too small for reliable comparisons. The results were represented in bar graphs.

Statistical analyses were performed using SPSS 21.0, (IBM Corporation, New York, USA). A p-value of <0.05 was considered statistically significant.

RESULTS

Table 1 describes the background clinical characteristics of study subjects. Of the 654 children (53% boys) at baseline, 68% were adolescents and 55% were pubertal. Over the entire follow-up, 463 (75%) study subjects were pubertal. The median (IQR) age was 11.9 (9.2, 14.1) years, and the mean (95% CI) BMI SDS was 2.52 (2.48, 2.56). There were significantly more boys than girls among subjects with severe and morbid obesity at baseline and at both 12 and 24 months (**Tables 1**, **2**). There was no sex difference in BMI SDS reduction and BMI SDS decreased by at least 0.25 in 24% of children both from baseline to 12 months and to 24 months as reported in our previous study (19).

To describe the cardiometabolic status of study subjects over the treatment, BP measurements and metabolic measurements and their distributions into acceptable, borderline, and high or low categories are presented in **Table 3**. Boys had more elevated P-ALT than girls at baseline, at 12 and 24 months (χ^2, 0.003, 0.019, and 0.003, respectively) and more IFG at baseline and at 24 months (χ^2, 0.048, and 0.029, respectively). Elevated P-ALT at baseline was associated with acanthosis nigricans (χ^2, $P = 0.003$) and moreover, it was more frequent in children whose HOMA-IR indicated insulin resistance than in children whose HOMA-IR was normal (32 vs. 20%; χ^2, $P = 0.035$). At baseline, HOMA-IR was non-acceptable in 108 (58%) boys and in 138 (83%) girls (χ^2, $P < 0.001$), and it was significantly related to acanthosis nigricans (χ^2, $P < 0.001$). There were no significant sex differences in the distributions of lipids or in the

TABLE 1 | Background characteristics of the study subjects.

	Girls *n* (%)	Boys *n* (%)	*P*
All *n* (%)	302 (46.2)	352 (53.8)	
Age[a], years	11.6 (8.9, 14.1)	12.0 (9.3, 13.9)	0.580[d]
AGE, YEARS			
2–6.9	43 (14.2)	38 (10.8)	0.204[e]
7–9.9	63 (20.9)	66 (18.8)	
10–14.9	149 (49.3)	202 (57.3)	
15–18	47 (15.6)	46 (13.1)	
Adolescence[b]			
Children	106 (35.1)	104 (29.6)	0.129[e]
Adolescents	196 (64.9)	248 (70.4)	
Pubertal stage[g] recorded	299	347	
Prepubertal	99 (33.1)	189 (54.5)	<0.001[e]
Pubertal	200 (66.9)	158 (45.5)	
BMI SDS[c,h]	2.59 (2.53, 2.64)	2.45 (2.40, 2.51)	
Obesity stage[d] *n*	302	352	
Overweight	43 (14.2)	15 (4.3)	<0.001[e]
Obesity	164 (54.3)	143 (40.6)	
Severe obesity	69 (22.8)	134 (38.1)	
Morbid obesity	26 (6.7)	60 (17.0)	
Obesity (IOTF)[e] *n*	302	352	
Overweight	17 (5.6)	20 (5.7)	0.977[e]
Obesity	285 (94.4)	332 (94.3)	
Height SDS[c,h]	0.5 (0.4, 0.7)	0.5 (0.3, 0.6)	
SBP and DBP *n*	242	281	
SBP[c,i], mmHg	121 (119, 123)	123 (121, 125)	
Normal	88 (36.4)	110 (39.1)	0.689[e]
High normal	27 (11.1)	26 (9.3)	
Stage 1	91 (37.6)	97 (34.5)	
Stage 2	36 (14.9)	48 (17.1)	
DBP[c,i], mmHg	70 (69, 72)	70 (69, 71)	
Normal	158 (65.2)	207 (73.6)	0.206[e]
High normal	43 (17.8)	35 (12.5)	
Stage 1	37 (15.3)	35 (12.5)	
Stage 2	4 (1.7)	4 (1.4)	
Acanthosis nigricans	225	249	
Yes	139 (61.8)	157 (63.1)	0.775[e]
No	86 (38.2)	92 (36.9)	

Results for girls and boys are represented as n (%) for distributions, and as [a]median and interquartile ranges (IQR25,75) for not normally distributed age and as [c]means and 95% confidence intervals (CI) for normally distributed continuous variables. [d]Mann-Whitney test; [e]Pearson's chi-square test. A P-value <0.05 is statistically significant. Categorized on the basis of [b]the definition by WHO (22), [g]the Tanner method (23, 24), [h]the Finnish growth references (21), and n the [i]NHBPEP Working Group on Children and Adolescents; Stages 1 and 2 are hypertensive (28). BMI SDS, body mass index standard deviation score; IOTF, International Obesity Task Force; SBP, systolic blood pressure; DBP, diastolic blood pressure; NHBPEP, The National High Blood Pressure Education Program.

TABLE 2 | BMI SDS, changes in BMI SDS from baseline, and obesity stage distributions at 12 and 24 months from baseline in girls and boys.

	12 months ($n = 521$)			24 months ($n = 345$)		
	Girls	**Boys**	***P***	**Girls**	**Boys**	***P***
n (%)	243 (46.6)	278 (53.4)		157 (45.6)	188 (54.4)	
BMI SDS[a,b]	2.48 (2.42, 2.55)	2.38 (2.32, 2.43)		2.50 (2.44, 2.56)	2.38 (2.32, 2.44)	
Change in BMI SDS from baseline[c]	−0.10 (−0.14, −0.06)	−0.09 (−0.12, −0.05)		−0.09 (−0.13, −0.04)	−0.08 (−0.04, 0.05)	
REDUCTION IN BMI SDS						
≥0.25 (good)	63 (25.9)	60 (21.6)	0.144[d]	42 (26.8)	41 (21.8)	0.297[d]
0.24 to 0 (borderline)	83 (34.2)	118 (42.4)		43 (27.4)	65 (34.6)	
<0 (poor)	97 (39.9)	100 (36.0)		72 (45.8)	82 (43.6)	
OBESITY STAGE[b]						
Normal weight	2 (0.8)	1 (0.4)	<0.001[d]	2 (1.3)	0	0.007[d]
Overweight	46 (18.9)	15 (5.4)		23 (14.6)	16 (8.5)	
Obesity	125 (51.5)	117 (42.1)		79 (50.3)	74 (39.4)	
Severe obesity	58 (23.9)	105 (37.7)		40 (25.5)	70 (37.2)	
Morbid obesity	12 (4.9)	40 (14.4)		13 (8.3)	28 (14.9)	

[a]BMI SDS is normally distributed and represented as means and 95% CIs. [b]Based on the Finnish growth references (21). Distributions are represented as n (%). [c]Revealed in linear mixed model analysis adjusted for age at baseline, expressed as mean difference (95% CI) from baseline, a negative value signifies a reduction, P for all comparisons was <0.001 (19) [d]Pearson's chi-square test for differences between boys and girls. A P value > 0.05 is statistically significant. BMI SDS, body mass index standard deviation score; CI, confidence interval.

P-LDL-C to P-HDL-C and the P-TG to P-HDL-C ratios at any time point.

The changes in metabolic parameters and BP from baseline throughout the entire two-year follow-up revealed in linear mixed model analyses adjusted for age and BMI-SDS at baseline are presented in **Table 4**. In all children, P-TC and P-LDL-C levels and B-HbA1c% values decreased significantly. In boys, P-TC, P-LDL-C, and P-HDL-C levels decreased ($P = 0.008$, 0.008, and 0.029, respectively), and P-TG levels increased ($P = 0.008$). Moreover, in boys, B-HbA1c% decreased, but S-INS levels and HOMA-IR values increased ($P = 0.007$, 0.024, and 0.011, respectively). In P-Gluc or P-ALT levels, no significant changes were detected throughout follow-up. Neither SBP nor DBP changed significantly. Boys presented with more significant changes in cardiometabolic parameters than girls during the treatment. **Table 4** also presents the effect of puberty on cardiometabolic changes. The increase in S-INS levels and HOMA-IR was detected in both sexes only in prepuberty. Girls' P-TC and P-LDL levels decreased in prepuberty but in boys, they decreased in puberty. P-TG levels decreased in pubertal girls but increased in pubertal boys. Puberty did not influence BP levels over the entire treatment.

The changes in metabolic parameters and in BP at 12 and 24 months from baseline revealed in linear mixed model analyses are represented in **Figure 1** using the traffic light method. In girls, there were no significant changes in any metabolic parameter or in BP at 12 or 24 months. In boys, a significant improvement (dark green) was detected at 12 months in P-TC levels ($P = 0.009$) and both at 12 and 24 months in P-LDL-C levels ($P = 0.030$ and 0.043, respectively) and in HbA1c% values ($P = 0.013$ and 0.025, respectively). Moreover, in boys, P-TG and S-INS levels and HOMA-IR values deteriorated (dark red) at 24 months from baseline ($P < 0.001$, 0.004, and 0.002, respectively). Boys' SBP levels increased significantly at 24 months ($P = 0.037$), but DBP levels remained unchanged both at 12 and 24 months.

The influence of BMI SDS changes on the categorized metabolic parameters at 12 months compared with those at baseline is represented in **Figure 2**. When the reduction in BMI SDS was poor, no significant favorable change in any metabolic profile was detected. Whereas when the BMI SDS reduction was good, 0.25 or more, the proportion of acceptable P-TC, P-LDL-C, S-INS, and HOMA-IR values were more prevalent at 12 months than at baseline (McNemar's test, $P = 0.016$, 0.007, 0.021, and 0.004, respectively). When the BMI SDS reduction was borderline (0–0.24), the acceptable proportions of P-Gluc, S-INS, HOMA-IR, and P-TG were augmented ($P = 0.087$, 0.052, 0.065, 0.063, respectively) but only the profile of TC was ameliorated significantly ($P = 0.048$). The comparison of the influence of BMI SDS change on the distributions of metabolic parameters at 12 months and at baseline was also done using the intention-to-treat method, and the results of this approach were very similar to the results presented in **Figure 2**.

DISCUSSION

In this study, the cardiometabolic outcome of the multidisciplinary family-based lifestyle treatment for childhood obesity presented some interesting findings. Boys presented with more significant changes in cardiometabolic parameters than girls during the treatment. In boys, P-TC, P-LDL-C, and HbA1c improved, but P-HDL-C, P-TG, S-INS, HOMA-IR deteriorated. The boys' significant deteriorations of TG, INS, HOMA-IR, and moreover, of SBP, were detected only at 24 months. P-Gluc, P-ALT, and DBP levels remained stable over the course of treatment both in girls and boys. At 12 months, for all children, the acceptable proportions of P-TC, P-LDL-C,

TABLE 3 | Metabolic and blood pressure measurements in girls and boys at baseline, at 12 and 24 months.

	Girls *n* (%)[a]			Boys *n* (%)[a]		
	Baseline	**12 months**	**24 months**	**Baseline**	**12 months**	**24 months**
fP-ALT[b], *n*	177	88	60	227	94	82
IU/l	24 (18, 35)	23 (17, 35)	21 (15, 35)	29 (29, 47)	33 (22, 61)	34 (25, 55)
<40	144 (81.4)	70 (79.5)	48 (80.0)	155 (68.3)	60 (63.8)	46 (56.1)
>40	33 (18.6)	18 (20.5)	12 (20.0)	72 (31.7)	34 (36.2)	36(43.9)
fP-TC[c], *n*	222	113	79	247	102	92
mmol/l	4.45 (3.90, 5.10)	4.50 (3.80, 5.10)	4.60 (4.10, 5.10)	4.50 (3.70, 5.10)	4.50 (4.00, 5.20)	4.45 (3.80, 5.00)
Acceptable	100 (45.0)	51 (45.1)	28 (35.4)	114 (46.2)	42 (41.2)	43 (46.7)
Borderline	70 (31.5)	37 (32.6)	33 (41.8)	75 (30.4)	33 (32.4)	32 (34.8)
High	52 (23.5)	25 (22.3)	18 (22.8)	58 (23.4)	27 (26.4)	17 (18.5)
fP-LDL-C[c], *n*	212	112	79	233	99	92
mmol/l	2.80 (2.30, 3.38)	2.75 (2.20, 3.36)	2.90 (82.23, 3.38)	2.80 (2.28, 3.30)	2.85 (2.40, 3.40)	2.70 (2.23, 3.38)
Acceptable	121 (57.1)	63 (56.2)	37 (46.8)	126 (54.1)	49 (49.5)	52 (56.5)
Borderline	38 (17.9)	21 (18.8)	21 (26.6)	55 (23.6)	24 (24.2)	17 (18.5)
High	53 (25.0)	28 (25.0)	21 (26.6)	52 (22.3)	26 (26.3)	23 (25.0)
fP-HDL-C[c], *n*	216	112	79	244	100	93
mmol/l	1.13 (0.97, 1.33)	1.12 (0.99, 1.33)	1.11 (0.92, 1.28)	1.12 (0.95, 1.35)	1.06 (0.89, 1.33)	1.05 (0.88, 1.32)
Acceptable	97 (44.9)	45 (40.2)	30 (38.0)	100 (41.0)	39 (39.0)	37 (39.7)
Borderline	51 (23.6)	31 (27.7)	20 (25.3)	53 (21.7)	19 (19.0)	18 (19.4)
Low	68 (31.5)	36 (32.1)	29 (36.7)	91 (37.3)	42 (42.0)	38 (40.9)
fP-TG[c], *n*	219	110	78	245	97	88
mmol/l	1.21 (0.90, 1.68)	1.19 (0.81, 1.68)	1.19 (0.91, 1.76)	1.09 (0.77, 1.57)	1.25 (0.83, 1.65)	1.27 (0.82, 1.82)
Acceptable	65 (29.7)	37 (33.6)	25 (32.1)	101 (41.2)	32 (33.0)	30 (34.1)
Borderline	67 (30.6)	31 (28.2)	24 (30.8)	59 (24.1)	25 (25.8)	21 (23.9)
High	87 (39.7)	42 (38.2)	29 (37.1)	85 (34.7)	40 (41.2)	37 (42.0)
fP-Gluc[d], *n*	222	124	84	249	111	93
mmol/l	5.30 (5.10, 5.70)	5.40 (5.10, 5.68)	5.30 (5.10, 5.60)	5.50 (5.20, 5.80)	5.50 (5.20, 5.80)	5.50 (5.20, 5.90)
< 5.6	152 (68.5)	82 (66.1)	57 (67.9)	145 (58.2)	65 (58.6)	47 (50.5)
5.6-6.9 (IFG)	67 (30.2)	39 (31.5)	26 (31.0)	102 (41.0)	46 (41.4)	46 (49.5)
> 7.0	3 (1.3)	3 (2.4)	1 (1.1)	2 (0.8)	0 (0.0)	0 (0.0)
2-h-OGTT-Gluc[d], *n*	69	26	24	80	34	35
mmol/l	6.4 (5.50, 7.30)	5.50 (4.95, 6.10)	5.75 (5.30, 6.98)	6.35 (5.50, 7.30)	6.75 (5.78, 7.73)	6.70 (5.50, 7.60)
<7.8,	57 (82.6)	21 (80.8)	21 (87.5)	66 (82.5)	26 (76.5)	27 (77.1)
7.8–11.0 (IGT)	10 (14.5)	4 (15.4)	3 (12.5)	13 (16.3)	8 (23.5)	8 (22.9)
>11.1	2 (2.9)	1 (3.8)	0	1 (1.2)	0	0
B-HbA1c[d], *n*	80	57	38	81	66	42
mmol/l	35 (33, 38)	36 (33, 37)	36 (33, 37)	36 (34, 39)	36 (34, 38)	36 (33, 38)
<5.7	73 (91.2)	51 (89.5)	35 (92.1)	66 (81.6)	57 (86.4)	36 (85.7)
5.8–6.4	4 (5.0)	4 (7.0)	3 (7.9)	12 (14.8)	8 (12.1)	6 (14.3)
>6.4	3 (3.8)	2 (3.5)	0	3 (3.6)	1 (1.5)	0
fS-INS[e], *n*	168	105	69	191	88	61
mU/l	24 (16, 32)	24 (16, 34)	25 (18,36)	17 (11, 27)	23 (15, 34)	22 (16, 36)
Normal,	85 (50.6)	54 (51.4)	29 (42.0)	110 (57.6)	50 (56.8)	30 (49.2)
High	83 (49.4)	51 (48.6)	40 (58.0)	81 (42.4)	38 (43.2)	31 (39.8)
HOMA-IR[f,g]	6.0 (4.0, 8.1)	5.6 (3.6, 8.2)	6.6 (4.4, 8.6)	4.1 (2.7, 7.0)	5.5 (3.5, 8.4)	5.3 (4.0, 8.7)
Acceptable	27 (16.4)	21 (21.4)	8 (12.1)	76 (41.3)	31 (36.9)	21 (35.6)
High	138 (83.6)	77 (78.6)	58 (87.9)	108 (58.7)	53 (63.1)	38 (64.4)
BP, *n*	243	171	134	279	188	161
SBP[h], mmHg	121 (119, 123)	120 (119, 122)	122 (120, 125)	123 (121, 125)	124 (122, 126)	124 (122, 126)
DBP[h], mmHg	70 (69, 72)	70 (69, 72)	70 (68, 72)	70 (69, 71)	70 (69, 72)	70 (69, 72)

[a]All continuous metabolic parameters are non-normally distributed and represented as medians and interquartile ranges (IQR25,75). [h]Normally distributed SBP and DBP are represented as means and 95% confidence intervals (CIs) for girls and boys at baseline, at 12 and 24 months from baseline. Categorized parameters and their distributions are represented as n (%). [b]Classified as elevated > 40 IU/l (31). [c]Categorized on the basis of NCEP (28). [d]fP-Gluc, IFG, 2-h-OGTT, IGT, and B-HbA1c classified on the basis of ISPAD (29). [e]High: > 15 mU/l in prepuberty, > 30 mU/l in puberty, and > 20 mU/l in postpuberty (30). [f]Calculated using the formula: fS-INS (mU/l) x fP-Gluc (mmol/l)/22.5 (30). [g]High: > 2.67 in prepubertal boys and > 5.22 in pubertal boys, and in girls > 2.22 and > 3.82, respectively (30). fP-ALT, fasting plasma alanine transferase; fP-TC, fasting plasma total cholesterol; fP-LDL-C, fasting plasma low-density lipoprotein cholesterol; fP-HDL-C, fasting plasma high-density lipoprotein cholesterol; TG, triglyceride; fP-Gluc, fasting plasma glucose; 2-h-OGTT-Gluc, 2-h oral glucose tolerance test; B-HbA1c, blood glycosylated hemoglobin; fS-INS, fasting serum insulin; HOMA-IR, homeostasis model assessment for insulin resistance; SBP, systolic blood pressure; DPP, diastolic blood pressure; NCEP, National Cholesterol Education Program Expert Panel; ISPAD, International Society for Pediatric and Adolescent Diabetes.

TABLE 4 | Comparisons between cardiometabolic measurements during two-year follow-up and at baseline revealed in linear mixed model analyses adjusted for age and BMI SDS at baseline in all study subjects, girls, boys, and in prepubertal and pubertal children.

	All[a]			Girls[a]			Boys[a]		
	Mean difference	**95% CI for mean difference**	***P***	**Mean difference**	**95% CI for mean difference**	***P***	**Mean difference**	**95% CI for mean difference**	***P***
fP-ALT, IU/L	−0.007	−0.033, 0.018	0.570	−0.038	0.077, 0.001	0.055	0.019	−0.015, 0.052	0.272
Prepubertal	−0.015	−0.057, 0.027	0.478	−0.330	−0.109, 0.042	0.369	−0.005	−0.057, 0.047	0.859
pubertal	−0.005	−0.037, 0.027	0.756	−0.037	−0.082, 0.009	0.110	0.028	−0.015, 0.071	0.205
fP-TC, mmol/l	−0.010	−0.016, −0.004	**0.002**	−0.008	−0.017, 0.000	0.061	−0.011	−0.019, −0.003	**0.008**
Prepubertal	−0.015	−0.026, −0.004	**0.011**	−0.031	−0.050, −0.011	**0.003**	−0.006	−0.020, 0.008	0.404
Pubertal	−0.007	−0.014, 0.000	0.052	−0.003	−0.013, 0.007	0.573	−0.013	−0.023, −0.002	**0.019**
fP-LDL-C, mmol/l	−0.011	−0.019, −0.003	**0.005**	−0.008	−0.019, 0.003	0.171	−0.015	−0.026, −0.004	**0.008**
Prepubertal	−0.016	−0.031, −0.001	**0.032**	−0.039	−0.065, −0.013	**0.005**	−0.004	−0.021, 0.014	0.670
Pubertal	−0.009	−0.018, 0.000	0.063	−0.001	−0.013, 0.012	0.927	−0.019	−0.034, 0.005	**0.009**
fP-HDL-C, mmol/l	−0.004	−0.009, 0.001	0.096	−0.001	−0.008, 0.006	0.823	−0.007	−0.014, −0.001	**0.029**
Prepubertal	−0.006	−0.016, 0.004	0.226	−0.010	−0.030, 0.011	0.341	−0.003	−0.014, 0.007	0.522
Pubertal	−0.003	−0.008, 0.003	0.324	0.002	−0.006, 0.009	0.635	−0.008	−0.017, 0.000	0.060
fP-TG, mmol/l	0.006	−0.006, 0.019	0.342	−0.012	−0.030, 0.006	0.193	0.024	0.006, 0.041	**0.008**
Prepubertal	0.018	−0.003, 0.040	0.097	0.031	−0.004, 0.066	0.080	0.011	−0.018, 0.040	0.444
Pubertal	0.001	−0.014, 0.016	0.902	−0.021	−0.042, −0.001	**0.043**	0.027	0.005, 0.050	**0.017**
fP-Gluc, mmol/l	−0.001	−0.005, 0.002	0.491	−0.002	−0.006, 0.003	0.523	0.000	−0.005, 0.004	0.887
Prepubertal	0.002	−0.006, 0.010	0.555	0.014	0.004, 0.023	**0.010**	−0.004	−0.014, 0.006	0.458
Pubertal	−0.002	−0.006, 0.002	0.319	−0.004	−0.009, 0.001	0.123	0.001	−0.004, 0.007	0.610
fS-INS, mU/l	0.023	−0.004, 0.050	0.089	−0.001	−0.037, 0.036	0.975	0.046	0.006, 0.085	**0.024**
Prepubertal	0.123	0.067, 0.179	**<0.001**	0.158	0.068, 0.249	**0.002**	0.105	0.033, 0.176	**0.005**
Pubertal	−0.003	−0.33, 0.027	0.864	−0.020	−0.058, 0.018	0.298	0.022	−0.027, 0.070	0.385
HOMA-IRb	0.023	−0.003, 0.049	0.078	−0.002	−0.037, 0.033	0.912	0.051	0.012, 0.090	**0.011**
Prepubertal	0.113	0.064, 0.162	**<0.001**	0.152	0.073, 0.232	**0.001**	0.096	0.035, 0.158	**0.003**
Pubertal	−0.001	−0.031, 0.028	0.921	−0.021	−0.057, 0.015	0.250	0.028	0.020, 0.077	0.247
B-HbA1c, mmol/l	−0.010	−0.019, −0.002	**0.020**	−0.001	−0.008, 0.005	0.719	−0.010	−0.017, −0.003	**0.007**
Prepubertal	−0.024	−0.056, 0.007	0.131	NAc	NAc	NAc	−0.034	−0.075, 0.006	0.097
Pubertal	−0.007	−0.015, 0.002	0.111	−0.001	−0.013, 0.011	0.852	−0.012	−0.022, −0.002	**0.018**
SBP, mmHg	0.569	−0.637, 1.776	0.354	−0.082	−1.828, 1.663	0.926	1.391	−0.267, 3.049	0.100
Prepubertal	0.805	−1.366, 2.977	0.465	0.058	−3.108, 3.223	0.971	1.210	−1.729, 4.148	0.416
Pubertal	0.423	−1.043, 1.888	0.571	−0.026	−2.051, 1.999	0.980	1.347	−0.748, 3.441	0.207
DBP, mmHg	−0.217	−1.072, 0.639	0.619	−0.399	−1.708, 0.909	0.547	−0.038	−1.149, 1.091	0.959
Prepubertal	0.687	−0.999, 2.373	0.422	−0.113	−2.996, 2.770	0.938	1.432	−0.678, 3.542	0.181
Pubertal	−0.584	−1.599, 0.431	0.259	−0.456	−1.936, 1.024	0.545	−0.739	−2.123, 0.644	0.294

Metabolic parameters were not normally distributed and to reach normal distributions they were log. transformed before analyses. [a]The results are represented as mean difference from baseline and its 95% CI in all study subjects, girls, boys, and in prepubertal and pubertal children (23, 24). A negative mean difference expresses a reduction from baseline. A P value < 0.05 is statistically significant (in bold font). [b]HOMA-IR was calculated using the formula: fS-INS (mU/l) × fP-Gluc (mmol/l)/22.5. [c]The number of events was too small for reliable analyses. BMI SDS, body mass index standard deviation score; CI, confidence interval; fP-ALT, fasting plasma alanine transferase; fP-TC, fasting plasma total cholesterol; fP-LDL-C, fasting plasma low-density lipoprotein cholesterol; fP-HDL-C, fasting plasma high-density lipoprotein cholesterol; fP-TG, fasting plasma triglyceride; fP-Gluc, fasting plasma glucose; fS-INS, fasting serum insulin, HOMA-IR, homeostasis model assessment for insulin resistance; B-HbA1c, blood glycosylated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure.

S-INS, and HOMA-IR augmented significantly only if BMI SDS decreased at least 0.25 from baseline. On the other hand, even if there was no BMI SDS reduction at 12 months, no deterioration occurred in the distributions of lipids, Gluc, INS, HOMA-IR, or ALT.

Many cardiometabolic parameters change by age and puberty. Insulin resistance rises progressively from age 7 years, a few years before the first pubertal signs, and increases further at puberty (32). At puberty there is also a switch: prepubertal girls have higher insulin resistance but boys exhibit a higher metabolic risk by the end of puberty (33). These findings may partly explain the complexity of our results: S-INS and HOMA-IR increased in prepubertal girls and boys, despite of treatment. Surprisingly no change in these parameters was detected in pubertal children. This may be due to a low proportion of subjects with impaired glucose tolerance (14.5% in

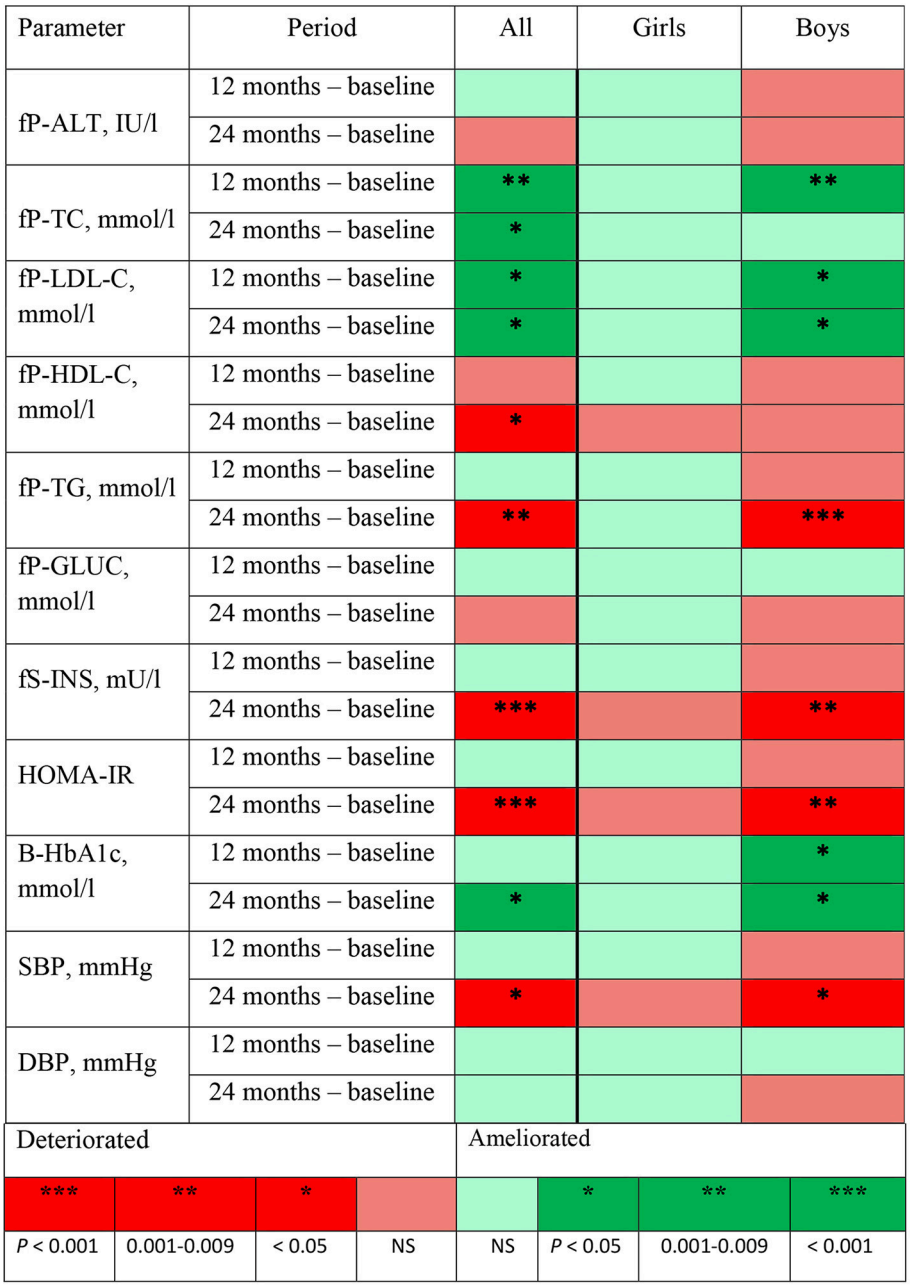

FIGURE 1 | Changes in metabolic and BP measurements, their directions, and the significance of the change at 12 and 24 months from baseline revealed in mixed model analyses adjusted for age and body mass index standard deviation score at baseline presented using the traffic light method. fP-ALT, plasma alanine transferase; fP-TC, fasting plasma cholesterol; fP-LDL-C, fasting plasma low-density lipoprotein; fP-HDL-C, fasting plasma high-density lipoprotein; fP-TG, fasting plasma triglyceride; fP-Gluc, fasting plasma glucose; fS-INS, fasting serum insulin; HOMA-IR, homeostasis model assessment for insulin resistance calculated by using the formula fS-INS (mU/l) × fP-Gluc (mmol/l)/22.5; B-HbA1c, blood glycosylated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure.

girls; 16.3% in boys) at baseline. Plasma lipids show significant physiological changes by age (34). P-TC and P-HDL-C reach their maximum levels by age 9 years and thereafter decline together with P-LDL-C. Unlike cholesterol levels, P-TG increases clearly in boys during puberty. In our study, P-TC and P-LDL-C decreased in prepubertal girls and pubertal boys. Whereas this prepubertal decrease in girls may well be due to lifestyle changes, the decrease in pubertal boys is not necessarily due to the treatment but may simply reflect a physiological change. The difference between the change of P-TG levels in pubertal boys and girls may also be due to a natural course of P-TG during puberty. The complexity of our findings highlights the importance of a proper control group, especially around puberty.

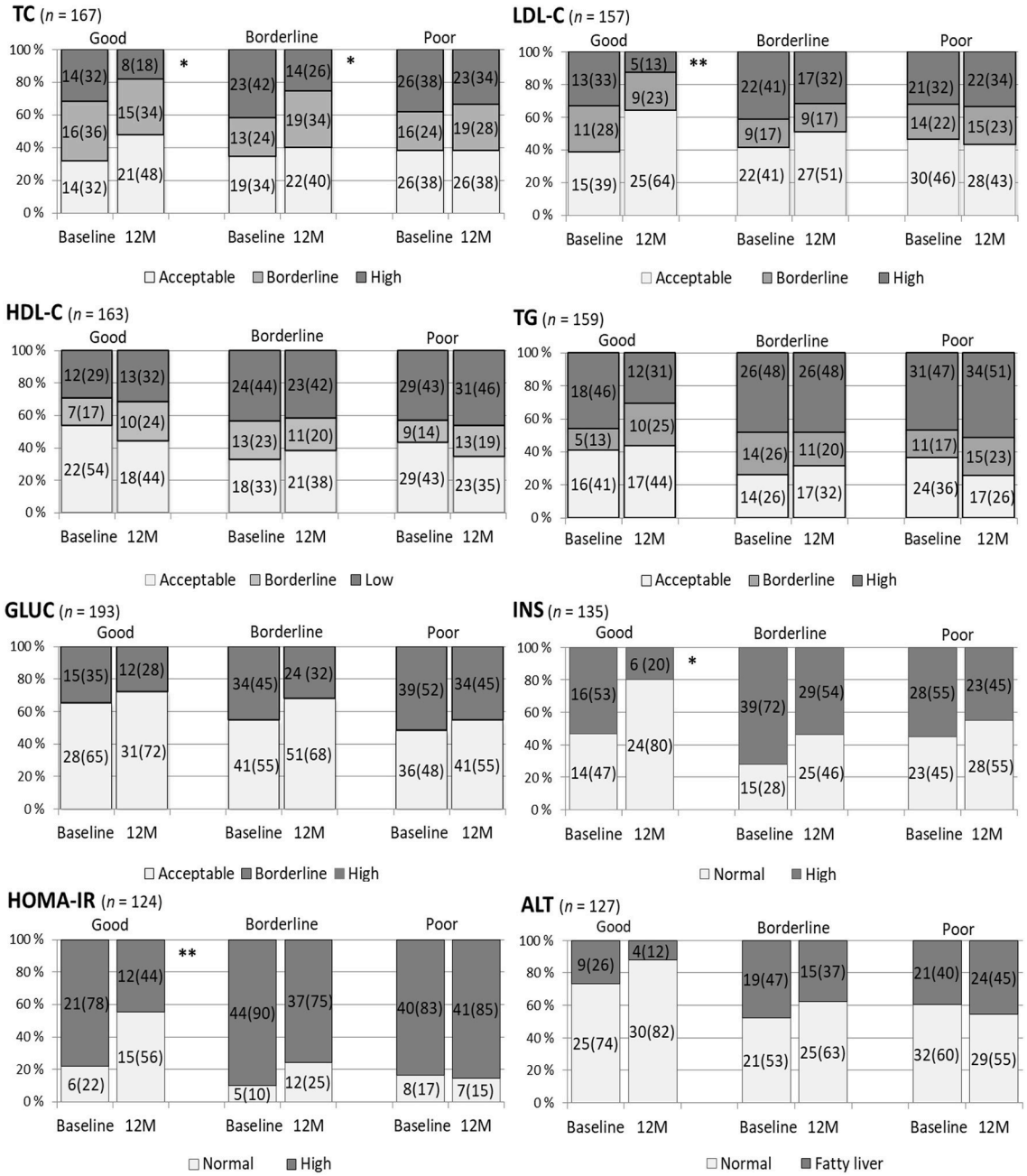

FIGURE 2 | The influence of the staged change in body mass index standard deviation score (BMI SDS) on categorized metabolic variables at 12 months (M) compared to the situation at baseline. A BMI SDS reduction of 0.25 units or more from baseline was defined as good change, 0–0.24 borderline, and no reduction as poor. Plasma total cholesterol (TC) in mmol/l was categorized: acceptable < 4.40, high ≥ 5.18; plasma low-density lipoprotein cholesterol (LDL-C) in mmol/l, acceptable < 2.84, high ≥ 3.36; plasma high-density lipoprotein cholesterol (HDL-C) in mmol/l, acceptable > 1.17, low < 1.04; plasma triglyceride (TG) in mmol/l for < 10-year-olds, acceptable < 0.84, high ≥ 1.12 and for ≥ 10-year-olds, acceptable < 1.02 and high ≥ 1.47 (28). Plasma glucose (GLUC) cut-off for normal and elevated was 5.6 mmol/l (29). Serum insulin (INS) was classified as normal or high with cut-offs: in pre-puberty 15 mU/l, puberty 30 mU/l, and in postpuberty 20 mU/l (30). Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated by the formula: fS-INS (mU/l) × fP-Gluc (mmol/l)/22.5 and its cut-offs for normal and high were 2.67 in prepubertal boys, 5.22 in pubertal boys and in girls 2.22 and 3.82, respectively (30). Plasma alanine transferase (ALT) values ≥ 40 IU/l expressed fatty liver (31). McNemar's test, $^{**}P$ 0.001–0.010, $^{*}P < 0.05$.

In our study, the prevalences of adverse levels of lipids were higher than those reported from Central Europe (35). In the meta-analysis published in 2013, lifestyle interventions led to significant improvements in P-LDL-C and P-TG up to one year from baseline, as well as improvements in P-HDL-C if the treatment included both dietary and exercise interventions (36).

However, the results presented in different meta-analyses were not unequivocal. In a recent meta-analysis on childhood obesity interventions, no significant changes in lipids were reported (15). In recent prospective studies, a BMI SDS reduction of 0.25 or more was needed to improve P-TG and P-HDL-C significantly in a one-year lifestyle intervention (27, 37). In our study, TC and LDL-C were ameliorated, but HDL-C and TG deteriorated during the treatment even when the BMI SDS reduction was good. This finding is worrying, as the lipid levels in childhood are strongly correlated with the levels in adulthood, and this correlation, which is stronger in boys, was independent of the age at the time of measurements (38). Our results were also concerning because worsened P-HDL-C and P-TG are considered a part of the metabolic syndrome in all of its definitions.

Plasma glucose levels did not improve significantly in this study, although the high prevalence of IFG at baseline demanded its improvement. However, IFG was less frequent at 12 months than at baseline irrespective of the change in BMI SDS. Glucose levels did not improve in many other similar studies or meta-analyses (15, 36). Insulin resistance, defined by HOMA-IR, decreased at 12 months according to our results and this decrease was related to the degree of BMI SDS reduction (**Figure 2**). This finding is in line with those of many studies of insulin resistance in childhood (30, 39). Unfortunately, in our study, the levels of insulin and HOMA-IR in both sexes increased at 24 months, with a significant increase in boys. Better short than long term results in glucose metabolism could be due to more intensive lifestyle treatment during the first year of intervention. The boys' increased insulin resistance, together with the deteriorations of SBP and obesity lipids HDL-C and TG, evoke a concern of an early metabolic syndrome especially in boys (40).

Elevated P-ALT is considered a predictor of chronic nonalcoholic fatty liver disease (NAFLD). NAFLD is strongly related to visceral obesity, IR, dyslipidemia, and hypertension and is more frequent in boys. Moreover, in a recent meta-analysis, NAFLD was identified as a risk factor for subclinical abnormalities in the myocardium and in left ventricular function already in childhood (9). Many prospective cohort studies focusing on lifestyle treatment for NAFLD have reported a significant reduction in P-ALT related to a reduction in weight and IR (13, 41). However, poor compliance with the treatment worsened the outcomes (42). In accordance with other studies, we found that elevated P-ALT was associated with high HOMA-IR and acanthosis nigricans, but P-ALT did not ameliorate even if the reduction in BMI SDS was good and the lifestyle treatment included all the substantial elements. We can only speculate whether this finding was due to other concurrent metabolic disturbances, such as the high prevalence of IFG and elevated HOMA-IR, or perhaps the treatment time of 1 year was too short for liver tissue recovery.

Although there was a possibility of overestimation in the high prevalence of hypertensive SBP values (52% at baseline) because of an oscillometric measuring method and fear of measurements in children, the levels of BP remained high over the follow-up in comparison with other reports (3). Furthermore, SBP levels even significantly deteriorated in boys over the entire follow-up. In clinical practice, this finding indicates that there were many hypertensive children who should have been examined thoroughly to define the cause and the importance of high BP for assessing the need for antihypertensive medication. A 24-h ambulatory blood pressure monitoring should be performed in subject with elevated BP at rest, as it is the gold standard measure in children and adolescents. An important question is whether adequate attention is paid to hypertensive BP values in clinical practice. In other studies, variable BP outcomes in obesity treatment were described, and in most studies, SBP significantly decreased (36, 43).

Our study had some obvious limitations. It was a retrospective analysis of obesity treatment in clinical practice, and therefore, there was no standardized intervention protocol with a control group. A control group would have separated changes in cardiometabolic parameters caused by age and puberty from those caused by treatment. Because of individually programmed treatment, it was not possible to obtain data on all study subjects over the entire follow-up time. We could not know how well the participating families managed to carry out the advised lifestyle changes. Moreover, because there was no standardized protocol for laboratory analyses, these analyses were conducted only when necessary. This procedure might have caused a decrease in the normal range values rather than in the values out of reference range. The change in laboratory usage at the beginning of 2008 had a small effect on mean levels of P-HDL-C and P-Gluc in girls and on P-LDL-C in boys. It was, however, unlikely that these effects would have influenced the results. Finally, our study was conducted among obese children treated in specialist care, and therefore, the results may not be generalized to all overweight and obese children.

The concise strengths of this study were a relatively large number of study subjects, the cardiometabolic follow-up data up to 24 months, and the cardiometabolic outcome of childhood obesity treatment in real-life conditions. The data included all children who were treated in specialist care for obesity in the study region during the study period, and therefore, the results were representative of this group of patients. Furthermore, the study subjects were a homogenous group originating from Eastern Finland, and thus, children's cardiometabolic outcomes were not influenced by the difference in the ethnic background. Although this study was retrospective, the data up to the two-year follow-up were very reliably constructed because of the common treatment strategies in the participating clinics (20) and well-documented data and measurements. The growth data, for instance, were available in 100% of all clinical visits. In our study, the percentage of dropouts, namely, the number of those who left care without a recorded reason, was 3.6% of the original data. This is a very small percentage compared with other similar studies on childhood obesity. Finally, this is the first large study in Finland to describe the cardiometabolic outcomes of pediatric obesity treatment.

Against our expectations, there were only a few improvements in cardiometabolic status in this study. However, difficulty in achieving good cardiometabolic responses to treatment corresponded with findings from the recent meta-analyses done on the randomized controlled studies, which often had a very

limited number of study subjects, varying treatment protocols, a notable number of dropouts, and usually no follow-up time at all (14–16). Our study supported previous statements of the need for at least a 0.25 reduction in BMI SDS (27, 37).

An interesting finding in our study was the different cardiometabolic response in boys and girls to the obesity treatment even after adjusting for age and although there was no significant sex difference in BMI SDS reduction over treatment time, and moreover, no significant sex difference in the adherence to the protocol (19). In girls, most metabolic parameters, except S-INS and HOMA-IR, which were already worse in girls than in boys at baseline, improved during the treatment, although not significantly. Most of the deteriorations in metabolism occurred in boys. Surely there is no simple explanation for the sex difference in metabolic outcomes. We can only speculate whether the girls already had more healthy diets and dietary habits before coming into care, and thus, the lifestyle changes were less effective for them, or if body composition was different in boys and girls and enabled the treatment responses. Were the boys perhaps less obedient to the lifestyle advice, or are the results pure reality and anticipation for males' increased risk for the early CV events and higher male prevalence of the adult metabolic syndrome? This kind of clear difference in the response to pediatric obesity treatment between sexes has not been described thoroughly in previous reports and warrants further research.

Our results emphasize early treatment and prevention of childhood obesity, a need for new treatment strategies, especially for boys and for those who already have several clustered CV risk factors related to childhood obesity.

AUTHOR CONTRIBUTIONS

MDV, TL, and JJ: designed the study; MDV, HP, PN, and JJ: performed collection of the data; MDV: handled and analyzed the data, wrote the first and the final draft; MDV, TL, and JJ revised the manuscript. All the authors discussed the data and accepted the final draft of the manuscript.

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Obesity and Outcome of Assisted Reproduction in Patients With Polycystic Ovary Syndrome

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Assisted reproduction, including *in vitro* fertilization and intracytoplasmic sperm injection, is increasingly being used for the management of infertility in patients with polycystic ovary syndrome (PCOS). However, there are limited data regarding the association between obesity and the outcome of assisted reproduction in this specific population as well as on the effects of weight loss. The aim of the present review is to summarize the existing evidence on the association between obesity and the outcome of assisted reproduction in patients with PCOS. Accumulating data suggest that obesity is associated with lower pregnancy and live birth rates in patients with PCOS who are undergoing assisted reproduction therapy. However, it remains unclear whether weight loss improves the outcome of this therapy. Notably, recent guidelines state that the health benefits of postponing pregnancy to achieve weight loss must be balanced against the risk of declining fertility with advancing age. Therefore, if weight loss is not achieved within a reasonable time period, assisted reproduction therapy should be offered in adequately selected patients with PCOS, regardless of the presence of obesity.

Keywords: obesity, polycystic ovary syndrome, weight loss, assisted reproduction, *in vitro* fertilization

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INTRODUCTION

In recent decades, the prevalence of obesity has increased globally and reached pandemic proportions (1). In high-income countries, approximately one-third of adults are obese and one-third are overweight (1). This rise in the prevalence of obesity has important health-care implications, since obesity is an important risk factor for cardiovascular disease and all-cause mortality (2, 3).

In addition to its cardiometabolic sequelae, obesity is implicated in the pathogenesis of polycystic ovary syndrome (PCOS) (4, 5). Approximately 40–70% of patients with this syndrome are either overweight or obese (4–6). The prevalence of PCOS is also almost four times higher in overweight and obese patients than in lean subjects (7). However, other studies reported that obesity only minimally increases the risk of PCOS (4, 8). Moreover, it has been reported that PCOS might also increase the risk of obesity, potentially by a reduction in basal metabolic rate and an impairment in appetite regulation (9–11).

Polycystic ovary syndrome is the commonest endocrine disorder in women of reproductive age (8, 12, 13) and the leading cause of anovulatory infertility (14). Several studies showed that obese patients with PCOS have more impaired ovulation and lower pregnancy rates than normal-weight patients with this syndrome (15–17).

Assisted reproduction, including *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI), is increasingly being used for the management of infertility in patients with PCOS (14). However, there are limited data regarding the association between obesity and the outcome

of assisted reproduction in this specific population as well as on the effects of weight loss. The aim of the present review is to summarize the existing evidence on the association between obesity and the outcome of assisted reproduction in patients with PCOS.

EFFECTS OF OBESITY ON THE OUTCOME OF ASSISTED REPRODUCTION IN PATIENTS WITH PCOS

A number of studies evaluated the association between obesity and the outcome of assisted reproduction in patients with PCOS (**Table 1**). However, most studies were small and retrospective. Moreover, most studies included patients with various causes of fertility and did not perform separate analyses of patients with PCOS.

In a small retrospective study ($n = 16$), lean patients with PCOS had less retrieved oocytes than obese patients with PCOS, even though they required fewer gonadotropin ampoules (18). In contrast, implantation, clinical pregnancy and live birth rates did not differ between the two groups, possibly due to the small number of patients (18). Indeed, in a larger retrospective study ($n = 72$), patients with PCOS and body mass index (BMI) $\geq$ 40 kg/m^2 had significantly lower clinical pregnancy rates after IVF than patients with PCOS and lower BMI (32 vs. 72%, respectively) and a trend for lower live birth rates (32 vs. 60%, respectively) despite the absence of difference in number of embryos transferred and implantation rates between the two groups (19). Moreover, the former required higher doses of gonadotropin and had fewer oocytes retrieved than the latter (19). In another study that included 55 patients with PCOS, implantation and pregnancy rates declined with increasing BMI (20). More specifically, implantation rates in patients with BMI $<$ 18.5, 18.5–24.9, 25.0–29.9, and $\geq$30 kg/m^2 were 75.0, 50.7, 57.1, and 27.3%, respectively ($p = 0.016$) whereas ongoing pregnancy rates were 100.0, 68.8, 66.7, and 41.7%, respectively ($p = 0.039$) (20). Notably, the total gonadotropin dose and the number of oocytes retrieved did not differ between the different BMI categories (20).

In a retrospective study in 79 patients with PCOS undergoing IVF, obese patients with PCOS had 69% lower odds of clinical pregnancy per cycle start than patients with PCOS and normal BMI ($p = 0.02$) and 77% lower odds of clinical pregnancy per embryo transfer ($p = 0.008$) (21). In addition, the odds of live birth were 71% lower in obese patients per cycle start ($p = 0.02$) and 77% lower per embryo transfer ($p = 0.01$) (21). On the other hand, a trend for lower incidence of ovarian hyperstimulation syndrome (OHSS) was observed (19.6, 10.5, and 3.2% in normal weight, overweight, and obese patients, respectively) (21).

In the largest study performed specifically in patients with PCOS ($n = 100$), patients with PCOS and BMI $>$ 25 kg/m^2 undergoing IVF had lower fertilization rates than patients with PCOS and BMI $\leq$ 25 kg/m^2 undergoing IVF (44 $\pm$ 22 vs. 62 $\pm$ 18, respectively) and also had lower clinical pregnancy rates (11.8 vs. 44.4%, respectively) (22). Interestingly, these differences were present in both patients undergoing ovarian stimulation with gonadotrophin-releasing hormone (GnRH) agonist and in those undergoing stimulation with GnRH antagonist (22). Of note, GnRH agonists prevent premature luteinizing hormone (LH) surge, thereby increasing the number of retrieved oocytes and pregnancy rates and decreasing the number of cycle cancelations (23). However, they might increase the risk for OHSS (23). On the other hand, GnRH antagonists can competitively block GnRH receptors and cause rapid suppression of gonadotropin release, resulting in fewer complications but appear to be less effective than GnRH agonists (23). Notably, follicle-stimulating hormone (FSH) preparations might offer a more physiologic approach in patients with PCOS, since the LH/FSH ratio is frequently elevated in this population (24).

There are very limited data on the association between obesity and the outcomes of ICSI in patients with PCOS. In a small study in 56 patients with PCOS undergoing IVF or ICSI, obesity was independently related to a lower oocyte count and increased FSH requirement (25). However, pregnancy and live birth rates were not reported and outcomes were not reported separately in patients receiving IVF or ICSI (25).

TABLE 1 | Studies evaluating the association between obesity and the outcome of assisted reproduction in patients with polycystic ovary syndrome.

Reference	*n*	Outcome
(18)	16	Retrieved oocytes (lean and obese patients): 22.2 ± 9.2 vs. 14.3 ± 4.9 (p = 0.04) Implantation rates: 0.55 ± 0.39 vs. 0.20 ± 0.37 (p = NS) Live birth rates: 83.3 vs. 45.5% (p = NS)
(19)	72	Clinical pregnancy rates (patients with BMI ≥ 40 kg/m^2 and <40 kg/m^2): 32 vs. 72% (RR 0.44, 95% CI 0.22–0.87) Live birth rates: 32 vs. 60% (RR 0.52, 95% CI 0.26–1.05)
(20)	55	Implantation rates (patients with BMI < 18.5, 18.5–24.9, 25.0–29.9 and ≥30 kg/m^2): 75.0, 50.7, 57.1 and 27.3% (p = 0.016) Ongoing pregnancy rates: 100.0, 68.8, 66.7, and 41.7% (p = 0.039)
(21)	79	Clinical pregnancy per cycle start (patients with BMI 18.7–24.9 and ≥30 kg/m^2): 56.9 vs. 35.5% (OR 0.31, 95% CI 0.11–0.86) Live birth per cycle start: 49.0 vs. 32.3% (OR 0.29, 95% CI 0.10–0.84)
(22)	100	Fertilization rates (patients with BMI ≤ 25 and >25 kg/m^2): 62 ± 18 vs. 44 ± 22 (p = 0.02) Clinical pregnancy rates: 44.4 vs. 11.8% (p = 0.02)

BMI, body mass index; RR, relative risk; CI, confidence interval; OR: odds ratio.

EFFECTS OF WEIGHT LOSS ON THE OUTCOME OF ASSISTED REPRODUCTION IN PATIENTS WITH PCOS

Several small ($n = 18$–67) and uncontrolled studies showed that lifestyle-induced weight loss restores ovulation in patients with PCOS (26–29). Weight loss also increased spontaneous pregnancy rates in these studies (27, 28). Case series also suggested that patients with PCOS undergoing bariatric surgery were able to conceive postoperatively (30, 31).

There are very few randomized controlled study (RCTs) that evaluated the effects of weight loss on the outcome of assisted reproduction in patients with PCOS. An early RCT in 38 patients with various causes of infertility showed no benefit of diet and exercise-induced weight loss on pregnancy and live birth rates (32). However, weight loss was modest (3.8 kg) and the change in waist circumference was similar in patients who implemented lifestyle changes and in controls (32). In contrast, a more recent and larger RCT ($n = 49$) reported higher pregnancy and live birth rates in patients with various causes of infertility who lost approximately 6.6 kg with diet, exercise, and behavioral modification (33). Notably, fewer ART cycles were required to achieve these higher rates in patients who lost weight (33). Nevertheless, neither of these studies reported the effects of weight loss in the subgroup of patients with PCOS (32, 33).

In a secondary analysis of the pregnancy in PPCOS II trial ($n = 187$) and the treatment of hyperandrogenism versus insulin resistance in infertile PCOS (OWL PCOS) trial ($n = 142$), lifestyle modification (caloric restriction, antiobesity medication, behavioral modification, and exercise) followed by treatment with clomiphene resulted in higher rates of ovulation and live birth compared with immediate treatment with clomiphene (risk ratio 1.4 and 2.5, respectively) (34). Of note, clomiphene is the treatment of first choice for induction of ovulation in women with PCOS (35, 36). Clomiphene induces ovulation through its anti-estrogen action, which results in a change in GnRH pulse frequency, release of FSH from the anterior pituitary and consequent follicular development (24). Treatment with clomiphene results in ovulation in 75–80% of patients and increases the likelihood of live birth approximately six times more than placebo and three times more than metformin (35–38).

The promising findings of the latter studies were not confirmed in the largest RCT that evaluated the role of weight loss in infertile patients receiving assisted reproduction treatment. In this study, 577 infertile women (201 with PCOS) were randomized to receive a 6-month lifestyle-intervention program followed by 18 months of infertility treatment or prompt infertility treatment for 24 months (39). The primary outcome (vaginal birth of a healthy singleton at term within 24 months after randomization) occurred in a smaller percentage of the women who followed a lifestyle program than in those who received prompt infertility treatment (27.1 vs. 35.2%, respectively; rate ratio 0.77, 95% confidence interval 0.60–0.99, $p = 0.04$) (39). However, weight loss was rather small (4.4 kg) and only 37.7% of patients randomized to lifestyle changes lost >5% of their body weight (39). Moreover, a considerable proportion of patients in the lifestyle arm discontinued treatment (21.8%) (39). Importantly, rates of pregnancy resulting from natural conception were higher in women assigned lifestyle changes (26.1 vs. 16.2% in patients assigned prompt infertility treatment) (39). Accordingly, the use of ovulation induction or other infertility treatment was less frequent and the number of infertility treatment cycles was lower in the lifestyle arm (39). Subgroup analyses among the 201 PCOS patients that were included in this study were not reported (39). However, among women with anovulatory infertility ($n = 269$), the rates of the primary outcome, live birth, and ongoing pregnancy did not differ between the intervention and control group (39).

CONCLUSION

Accumulating data suggest that obesity is associated with lower pregnancy and live birth rates in patients with PCOS who are undergoing assisted reproduction therapy. However, it remains unclear whether weight loss improves the outcome of this therapy. Notably, the American Society for Reproductive Medicine recently concluded that the health benefits of postponing pregnancy to achieve weight loss must be balanced against the risk of declining fertility with advancing age (40). Therefore, if weight loss is not achieved within a reasonable time period, assisted reproduction therapy should be offered in adequately selected patients with PCOS, regardless of the presence of obesity.

AUTHOR CONTRIBUTIONS

KT drafted the mini review. KD critically revised the draft.

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Obesity and Weight Gain in Pregnancy and Postpartum: an Evidence Review of Lifestyle Interventions to Inform Maternal and Child Health Policies

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Background: Maternal obesity, excessive gestational weight gain (GWG) and post-partum weight retention (PPWR) constitute new public health challenges, due to the association with negative short- and long-term maternal and neonatal outcomes. The aim of this evidence review was to identify effective lifestyle interventions to manage weight and improve maternal and infant outcomes during pregnancy and postpartum.

Methods: A review of systematic reviews and meta-analyses investigating the effects of lifestyle interventions on GWG or PPWR was conducted (Jan 2009–2018) via electronic searches in the databases Medline, Pubmed, Web of Science and Cochrane Library using all keywords related to obesity/weight gain/loss, pregnancy or postpartum and lifestyle interventions;15 relevant reviews were selected.

Results: In healthy women from all BMI classes, diet and physical activity interventions can decrease: GWG (mean difference −1.8 to −0.7 kg, high to moderate-quality evidence); the risks of GWG above the IOM guidelines (risk ratio [RR] 0.72 to 0.80, high to low-quality evidence); pregnancy-induced hypertension (RR 0.30 to 0.66, low to very low-quality evidence); cesarean section (RR 0.91 to 0.95; high to moderate-quality evidence) and neonatal respiratory distress syndrome (RR 0.56, high-quality evidence); without any maternal/fetal/neonatal adverse effects. In women with overweight/obesity, multi-component interventions can decrease: GWG (−0.91 to −0.63 kg, moderate to very low-quality evidence); pregnancy-induced hypertension (RR 0.30 to 0.66, low-quality evidence); macrosomia (RR 0.85, 0.73 to 1.0, moderate-quality evidence) and neonatal respiratory distress syndrome (RR 0.47, 0.26 to 0.85, moderate-quality evidence). Diet is associated with greater reduction of the risks of GDM, pregnancy-induced hypertension and preterm birth, compared with any other intervention. After delivery, combined diet and physical activity interventions

reduce PPWR in women of any BMI (−2.57 to −2.3 kg, very low quality evidence) or with overweight/obesity (−3.6 to −1.22, moderate to very low-quality-evidence), but no other effects were reported.

Conclusions: Multi-component approaches including a balanced diet with low glycaemic load and light to moderate intensity physical activity, 30–60 min per day 3–5 days per week, should be recommended from the first trimester of pregnancy and maintained during the postpartum period. This evidence review should help inform recommendations for health care professionals and women of child-bearing age.

Keywords: obesity, weight gain, pregnancy, postpartum, physical activity, nutrition, intervention, systematic review

INTRODUCTION

Overweight and obesity are increasing steadily in all age groups worldwide, especially in low- and middle income countries (1). Pre-pregnancy obesity (body mass index, BMI $\geq$ 30 kg/m^2), excessive gestational weight gain (GWG) and post-partum weight retention (PPWR) are seen as new public health challenges, given the association with negative short- and long-term maternal and child outcomes (2). These outcomes include obstetrical or neonatal complications, obesity, type 2 diabetes (T2D) and cardiovascular diseases (CVD) later in life (3–10) (see **Table 1**).

Large for gestational age neonates have a 50% risk of developing obesity and a metabolic syndrome between 6 and 11 years of age (33), and a 35% risk of dying prematurely of CVD (22). To reduce the detrimental intergenerational cycle of obesity and associated non-communicable diseases (NCDs), weight management during pregnancy and postpartum should be prioritized across countries, with an increased commitment for concerted, coordinated and specific actions. The aim of this overview is to draw together systematic review evidence examining the effectiveness of different intervention approaches for the management of maternal weight and the improvement of maternal and child health outcomes.

Maternal Obesity as a Global Health Issue

In 2016, a world report from the Non-Communicable Diseases Risk Factor Collaboration indicated that age-standardized prevalence of obesity increased from 3.2% in 1975 to 10.8% in 2014 in men, and from 6.4 to 14.9% in women (1). In the United States of America (U.S.A), pre-pregnancy obesity prevalence increased by an average of 0.5% point per year from 17.6% in 2003 to 20.5% in 2009 (34). Currently, 31.9% of reproductive age women in the U.S.A. have obesity and 55% have obesity or are overweight, with a higher prevalence in non-Hispanic black and Mexican American women (35). In the European region, the current prevalence of maternal obesity ranges from 7 to 25% (36), and it is expected to increase to 37% by 2020 (22). First trimester maternal obesity is significantly increasing over time in the United Kingdom too, having more than doubled from 7.6 to 15.6% over 19 years (1989 and 2007) (22).

Abbreviations: BMI, Body Mass Index; CVD, Cardiovascular disease; GDM, Gestational diabetes mellitus; GWG, Gestational weight gain; GWL, Gestational weight loss; HTA, Hypertension; IGR, Intra-uterine growth retardation; IOM, Institute of Medicine; LGA, Large for gestational age; NCD, Non-communicable disease; OB, Obesity; OW, Overweight; PA, Physical activity; PPWL, Post-partum weight loss; PPWR, Post-partum weight retention; PTB, Preterm birth; RCT, Randomized controlled trial; SGA, Small for gestational age; WHO, World Health Organization.

Gestational Weight Gain, Maternal and Neonatal Outcomes

Ideally, total GWG is calculated as the difference between body weight at the first trimester and last antenatal visit prior delivery (37). Gestational weight gain differs between individual women (38), and is associated with several factors such as pre-pregnancy BMI, maternal age, parity, ethnicity, GDM, hypertension, edema and smoking (39). Both over- or under-nutrition during gestation, particularly during the first two trimesters, are related to childhood obesity (33, 40, 41). Gestational weight gain is closely associated with the infant's birth weight and every additional kilogram of GWG can increase birth weight by 7.35g (42). Excessive GWG is related to overweight in early, middle and late childhood (43), and later life (40 years) in daughters (44).

Excessive GWG is a known risk factor for multiple adverse outcomes such as GDM, pregnancy-induced hypertension, preeclampsia, stillbirth, macrosomia, and post-partum hemorrhage (45, 46). It also contributes to long-term PPWR in childbearing women, and related disease outcomes, thus elevating the risks for subsequent pregnancies (47, 48). Pre-pregnancy BMI is a strong predictor of excessive GWG (49–51). Baseline overweight combined with excessive GWG results in an increased risk of fetal complications, and a higher long-term likelihood of retaining excessive weight (50, 51). In women with low socio-economic status, high early pregnancy BMI, nulliparity, and discordant clinician advice are directly associated with excessive GWG (52). A meta-analysis of 17 observational studies showed a significant relationship between excessive GWG and higher PPWR risk (OR 2.08; 95% CI: 1.60 −2.70) (53), however mean PPWR decreased with increasing BMI classes. Authors suggested that GWG, rather than pre-pregnancy BMI, determines the shorter or longer PPWR.

Inter-pregnancy weight gain, which may be due to PPWR or additional weight gain between gestations, is also associated with

adverse pregnancy outcomes (54), and long-term obesity, type 2 diabetes and risk factors for CVD (55). The average PPWR ranges from 0.5 kg (56) to 4 kg (57); however, 14–25% of women who gain significant amounts of weight during pregnancy will retain more than 4.5 kg after birth (55, 57). Woman in child-bearing years (25–34 years) have the highest risk of weight gain compared with men or women in other age groups (58). In Sweden, two-thirds of women weigh more than their pre-pregnancy weight at 6 months postpartum (59) and, in the U.S.A., up to 75% of low-income postpartum mothers are heavier at 1 year postpartum compared with their pre-pregnancy weight (60). In a longitudinal study of 2055 postpartum women in Australia, a greater postnatal increase in BMI was reported for women defined as having excessive GWG (odds ratio 3.72; 95% CI: 3.12–4.31) than for women with adequate GWG. Those who gained excess weight during pregnancy had increased odds of being overweight (2.15; 95% CI: 1.64–2.82) or to have obesity (4.49; 95% CI; 3.42–5.89) 21 years after the index pregnancy (49). So, failure to lose excessive GWG after delivery can contribute to obesity in midlife, and to an intergenerational cycle of obesity within the female population and offspring.

Current Maternal Weight Policies

Despite the growing evidence that maternal obesity and excessive GWG are risk factors for major obstetrical complications, poor subsequent maternal and child health, and for the transmission of obesity to the next generations (61, 62), there is inconsistency in maternal weight gain policies across the world (63). This may be explained by the fact that there is some evidence that women with obesity may have better outcomes if they gain only small amounts of weight, or even lose weight during pregnancy, while there is conflicting evidence that insufficient GWG may result in increased risk of intra-uterine growth retardation (IGR) and small for gestational age (SGA) (64–68). Coherence in guidelines internationally is important to address both inadequate and excessive GWG, including all obesity classes.

In 1990, the U.S.A. IOM produced guidelines for GWG which were been updated in 2009 (**Table 2**) (69). These guidelines are widely considered to be the international gold standard, however they do not provide recommendations for different classes of obesity (I, II, and III), as defined by WHO. Having a pre-pregnancy BMI in the normal range (18.5–24.9 $kg.m^{-2}$), a GWG within the IOM 2009 guidelines, and losing the excessive weight gain during the postpartum period are associated with better short- and long-term health for the mother and the child (47, 70–73). The accurate knowledge of GWG recommendations by pregnant women is associated with appropriate GWG, as is the correct classification of pre-pregnancy BMI (74). However, there is limited evidence that regular weighting, without a concomitant lifestyle intervention, can control GWG. A recent systematic review and meta-analysis including only two RCTs reported no effect of self-weighting or clinician weighting on GWG per week, or excessive GWG, or other pregnancy, birth and infant outcomes (75).

Pregnant women with obesity often lack knowledge about related complications during pregnancy, and communication with healthcare providers is often experienced as stressful, confusing and judgmental (76). Although health care professionals are well positioned to discuss GWG and healthy behaviors during pregnancy, there are many barriers to patient-provider communication such as lack of clinical guidelines, insufficient training, lack of time, concern about the sensitivity of the topic, negative attitudes and the perception that the advice is ineffective (77). A recent American study has shown that only 52% of pregnant women reported provider's advice on weight gain, 63% on physical activity and 56% on nutrition, though health care professionals can influence women's weight related intentions during pregnancy (76). Women who were less educated, had lower income, were non-White, multiparous and reported lower perceived health, were less likely to report physical activity advice.

TABLE 1 | Pre-pregnancy obesity-related risks to women and offspring.

Period	Women	Offspring
Before conception	Menstrual cycle dysregulation, anovulation and infertility (11)	–
Pregnancy	Miscarriage (12) Gestational diabetes mellitus (13) Pregnancy-induced hypertension (14, 15) Preeclampsia (16) Thrombo-embolism (17)	Congenital defects (18, 19) Premature birth (20) Large for gestational age Macrosomia (>4,000 g) (21)
Delivery	Cesarean sections Labor induction, surgical complications and failures of epidural analgesia (3, 22–26)	Stillbirth (24, 27) Neonatal trauma (assisted vaginal delivery and head trauma, shoulder dystocia) (7, 28) Low umbilical arterial pH<7.1 and low Apgar score at 5 and 10 min (7)
Postpartum	Difficulties in initiating and sustaining breastfeeding (29)	Systematic transfer to monitoring in case of GDM (risk of hypoglycemia) (7) Increased admission rate in the intensive care unit (30)
Long-term	Postpartum weight retention and inter-pregnancy obesity (31, 32) Type 2 diabetes Long-term vascular dysfunction (14, 15)	Childhood obesity and premature metabolic syndrome (33) Premature death from cardiovascular disease (22)

TABLE 2 | The United States of America Institute of Medicine Recommendations (2009) for total weight gain during pregnancy, by pre-pregnancy body mass index.

Pre-pregnancy BMI	BMI ($kg.m^{-2}$)	Total weight gain range in kg (lb)
Underweight	<18.5	12.7–18 (28–40)
Normal weight	18.5–24.9	11.3–15.9 (25–35)
Overweight	25–29.9	6.8–11.3 (15–25)
Obesity (classes I, II, III)	>30	5–9 (11–20)

The aim of the following overview of systematic reviews and meta-analyses was to identify lifestyle interventions that have shown to be effective in controlling GWG, PPWR and thus improve maternal and child outcomes, in order to inform health care professionals and policy makers.

MATERIALS AND METHODS

Study Design

A review of international systematic reviews and meta-analyses published in all languages between 1st January 2009 (year of publication of the revised IOM guidelines) (69) and 31st January 2018 was used to identify effective lifestyle interventions to control GWG and/or PPWR.

Participants, Interventions, Comparators

Systematic reviews or meta-analyses that evaluated dietary, physical activity, well-being or a multi-component interventions in pregnancy or postpartum were included. They selected only randomized controlled trials (RCT), or provided a separate analysis for RCTs. The main outcome measures were GWG, or GWG above the IOM guidelines, PPWG or postpartum weight loss (PPWL). Studies could include healthy women from any BMI class or parity, with a singleton pregnancy. Comparators are standard care or minimal care or no intervention.

Search Strategy

The Medline, Pubmed, Web of Science and the Cochrane Library databases were used with a combination of the following keywords: ("obesity" OR "overweight" OR "weight gain" OR "weight retention" OR "weight loss" OR "weight management" OR "weight control") AND ("pregnan*" OR "gestation" OR "obstetrics" OR "post-partum" OR "postpartum" OR "postnatal" OR "post pregnancy" OR "post childbirth" OR "following pregnancy" OR "following childbirth") AND ("lifestyle" OR "behavior" OR "exercise" OR "physical activity" OR "fitness" OR "diet*" OR "nutrition" OR "food" OR "well-being" OR "mental health" OR "psychological health").

Finally, the keywords ("outcome*" OR "complication*" OR "co-morbidities" OR "gestational diabetes" OR "hypertension" OR "pre-eclampsia" OR preeclampsia" OR "hemorrhage" OR "hemorrhage" OR "prematurity" OR "stillbirth" OR "macrosomia" OR "large for gestational age" OR "small for gestational age" OR "dystocia" OR "congenital defect*" OR "neonatal complication*") were combined to the primary search to assess effects of interventions on maternal or fetal/neonatal outcomes.

The search was performed by one person (NFL) in the context of a Master's project in Global Health Policy at the London School of Hygiene and Tropical Medicine, University of London. The specificity of the search was increased using search filters for systematic reviews or meta-analyses (Pubmed). The references obtained in the articles were scanned to ensure a complete collection of the relevant systematic reviews and meta-analyses, however no additional articles were found.

Data Extraction

The following data were extracted from the selected systematic reviews and meta-analyses: "a priori" design, search strategy and data, inclusion criteria for selected studies, included studies, countries, participant's characteristics, recruitment, type of interventions, methods of delivery, comparator, outcome measures, quality assessment, analysis, methods used to combine findings, weighted mean difference or risk ratio (and 95% confidence interval), reported quality of evidence, conclusions, source of support and conflicts of interest (for either the review and included primary study authors).

Quality Assessment

Reviews had to report an objective assessment of the methodological quality of studies to assess the risk of bias, as well as heterogeneity and sensitivity analysis (Guidelines of the US National Heart, Lung and Blood Institute) (78). The quality of the selected studies was examined separately by two investigators (NFL and LJE) using the R-AMSTAR checklist—Revised Assessment of Multiple Systematic Reviews for grading of clinical relevance (79). This instrument contains 11 questions (each with 3 or 5 items) rated from 1 to 4 (total score range from 11 to 44). When there was disagreement in the assessment, a consensus was reached through discussion.

Ethical Considerations

The Ethics Committee of the London School of Hygiene and Tropical Medicine of the University of London considered that this research did not require approval.

RESULTS

Study Selection and Characteristics

The search from the standardized computer databases yielded 3,116 articles (**Figure 1**). Publications which were not a review were excluded and 1,559 studies were extracted. Titles and abstract were reviewed to identify relevant articles. After removing 1,241 animal studies and 2 duplicates, 316 reviews were identified, of these 32 systematic reviews and/or meta-analyses. The full text for each article was obtained and assessed against the inclusion criteria. Seventeen systematic reviews were excluded due to: inappropriate study design ($n = 12$) (80–91); inappropriate population ($n = 1$) (92); inappropriate outcome ($n = 3$) (93–95) or absence of quality assessment ($n = 1$) (96). Data from the remaining 15 systematic reviews and/or meta-analyses were analyzed to identify effective interventions to control GWG and PPWR/PPWL, and any related impact on maternal and infant outcomes, and contributors to success.

Twelve antenatal and six postpartum reviews of lifestyle interventions on GWG or PPWR were identified (**Table 3**). Nine reviews also examined the effects of interventions on maternal or fetal/neonatal outcomes (**Table 4**). The majority of trials were conducted in upper-middle and high-income countries (Australia, Austria, Belgium, Brazil, Canada, China, Colombia, Denmark, Egypt, Finland, Germany, Iran, Italy, Japan, Kosovo, Norway, Sweden, the Netherlands, Spain, Taiwan, Thailand, U.S.A, U.K.). Two studies recruited women with low income

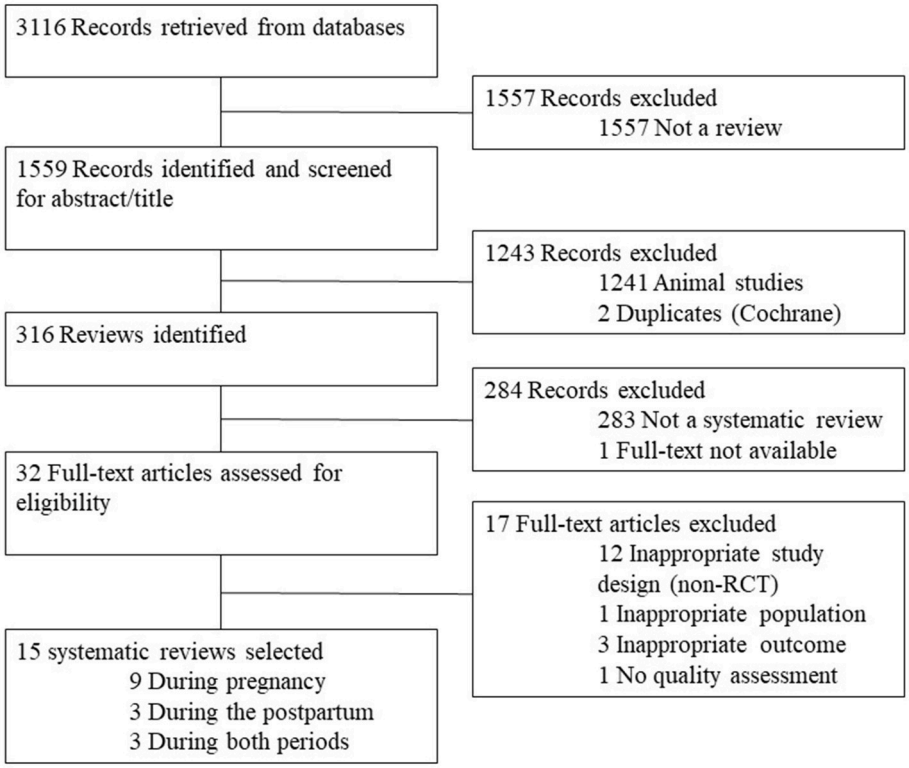

FIGURE 1 | Flow diagram of the systematic reviews retrieved for the review.

in the U.S.A. and Canada. Three reviews conducted a subgroup analysis of antenatal diet, physical activity or multi-component interventions (101, 102, 106); one study examined antenatal and postpartum physical activity interventions (98); and two studies reviewed antenatal and postpartum multi-component interventions (105, 106).

Participants

Participants were healthy women with a singleton pregnancy or postpartum. Both nulliparous and multiparous women were included. Most antenatal studies recruited participants at less than 20 weeks' gestation. The 15 reviews comprised of five to 65 RCTs, and involved 251 to 11,410 women. Three reviews in pregnancy (105–107) and two reviews in postpartum (105, 106) recruited only women with overweight or obesity. The remaining reviews included women from the general population irrespective of weight status, and the proportion of women with a normal BMI varied widely across trials. Only two reviews reported results for women with overweight/obesity, and those with low-risk (normal BMI) separately (101, 102). One review included women with diabetes, but conducted a subgroup analysis after excluding women with pre-existing diabetes or GDM (102). When ethnicity was examined, most participants were Caucasian or there was insufficient information provided to assess ethnicity.

Interventions

Twelve reviews examined the effectiveness of interventions that aimed to change lifestyle (diet, physical activity or both) in pregnant women with any BMI ($n = 9$) or with overweight/obesity ($n = 3$). Antenatal dietary interventions typically included a balanced diet consisting of proteins (15–20%), fat (maximum 30%), and carbohydrates (50–55%) with low glycemic load (high fiber: beans, lentils and vegetables, fruits, unprocessed whole grains). Two RCTs provided energy targets by weight (18–24 kcal/kg).

Antenatal physical activity interventions generally consisted of 20–70 min of exercise per day at light to moderate intensity, 2–5 days per week. Selected trials included supervised ($n = 25$, 35–60 min of aerobic and/or resistance training, weight-bearing exercises) or unsupervised ($n = 8$, counseling) physical activity. Pedometers were used in some studies. The multi-component approach included counseling or exercise sessions, education and feedback on weight gain using behavioral change techniques.

Six reviews examined the effectiveness of interventions that aimed to improve lifestyle (diet, physical activity or both) in postpartum women with any BMI ($n = 4$) or with overweight or obesity ($n = 2$). Post-partum interventions were conducted in community, primary care or secondary care settings. Two physical activity only trials included supervised exercise: 45 min of aerobic activity (brisk walking) at 60–70% of maximal heart rate 4 days per week, or walking 10,000 steps per day, during

TABLE 3 | Summary of effects of lifestyle interventions on gestational weight gain and postpartum weight loss.

Systematic reviews	RCTs (n)	BMI	Participants (n)	R-AMSTAR score	% score	R-AMSTAR Ranking	Weighted mean difference	95% CI	I^2
Physical activity interventions during pregnancy							**GWG**		
Streuling et al. (97)	12	Any BMI	906	33	75	C	−0.61	−1.17 to −0.06	25
Elliot-Sale et al. (98)	3	Any BMI	214	28	64	D	−2.22	−3.14 to −1.30	0
da Silva et al. (99)	18	Any BMI	3,203	30	68	D	−1.1	−1.53 to −0.69	0
Perales et al. (100)	29	Any BMI	Not reported	16	36	D	n.a.		
Multi-component diet and physical activity interventions during pregnancy								**GWG**	
Muktabhant et al. (101)	3	Any BMI	444	40	91	A	−1.8	−3.36 to −0.24	76
Thangaratinam et al. (102)	30	Any BMI	3,140[§]	41	93	A	−1.40	−2.09 to −0.71	80
Shepherd et al. (97)	16	Any BMI	5,052	42	95	A	−0.89	−1.39 to −0.40	43
International Weight Management in Pregnancy Collaborative (103)	33	Any BMI	11,410	33	75	C	−0.7	−0.92 to −0.48	0
O'Brien et al. (104)	4	Any BMI	446	30	68	D	−1.25	−2.39 to 0.11	42
Lau et al. (105)	7	OW/OB	1,652	36	82	B	−0.63	−1.07 to −0.20	14
Choi et al. (106)	7	OW/OB	721	30	68	D	−0.91	−1.76 to −0.06	8
Flynn et al. (107)	13	OW/OB	4,276	27	61	D	NA		
Physical activity interventions during postpartum							**PPWL**		
Elliot-Sale et al. (98)	2	Any BMI	214	28	64	D	−1.74 ($p = 0.06$)	−3.59 to 0.10	0
Multi-component diet and physical activity interventions during postpartum									
Berger et al. (108)	13	Any BMI	1,310	32	73	C	n.a.		
Nascimento et al. (109)	11	Any BMI	769	33	75	C	−2.57	−3.66 to−1.47	66
Lim et al. (110)	32	Any BMI	1,892	29	66	D	−2.3	−3.22 to −1.39	84
Lau et al. (105)[‡]	3	OW/OB	251	36	82	B	−3.6	−6.59 to −0.62	84
Choi et al. (106)	4	OW/OB	547	30	68	D	−1.22	−1.89 to −0.56	25

Results are presented as weighted mean difference and 95% confidence intervals. RCT, randomized controlled trial; BMI, body mass index; OW, overweight; OB, obesity; GWG, gestational weight gain; PPWL, postpartum weight loss; n.s., non-significant; n.a., not applicable (no meta-analysis). [‡] significant effect at 1-2 months postpartum only; [§] Studies on women with pre-existing diabetes or GDM were excluded for this sub-analysis (5 RCTs were excluded).

12 weeks (98). The multi-component diet and physical activity interventions during postpartum comprised of a balanced diet, or a calorie restricted diet, plus supervised (2 trials, walking 3–5 days per week, or general aerobic exercises 5 days per week, or strength training 3 days per week plus walking 10,000 steps per day, during 10–16 weeks) or unsupervised (personalized counseling and skill training, heart rate monitor or pedometer, self-monitoring, feed-back, correspondence programs, text messages, phone calls, Internet) (106, 109, 110). Delivery varied between individual or group sessions, conducted either at home or at a center. The duration of the interventions was 11 days to 36 months.

The comparator in each of the 12 reviews in pregnancy was "usual or standard care." In the six postpartum reviews, "usual or minimal care," true control (no intervention) or an alternative concomitant intervention (information printouts) were used as comparators.

Outcome Measures

Eleven reviews in pregnancy examined the same primary outcome measures (GWG, excessive GWG according to the IOM recommendations); one review selected GDM as the primary outcome measure, but included GWG as a secondary outcome (111). Nine reviews in pregnancy examined maternal or fetal/neonatal outcomes as secondary measures (GDM, Pregnancy-induced hypertension, pre-eclampsia, preterm birth, cesarean delivery, birthweight, macrosomia, LGA, stillbirth, shoulder dystocia, neonatal hypoglycemia, neonatal respiratory distress syndrome, admission to intensive care unit). Six of them assessed adverse events (low GWG, SGA, preterm birth, death).

The six postpartum reviews examined PPWR or PPWL as the primary outcome. Two studies assessed maternal outcomes: cardio-metabolic risks (108), or change in moderate to vigorous physical activity and dietary intake (105). Adverse effects were examined in only one of the six postpartum reviews (108).

Methodological Quality of Included Reviews

The R-AMSTAR assessment results for each review are shown in **Table 3**. Antenatal reviews scored between 16 (very low-quality) and 42 (high-quality) and postpartum reviews between 29 (very-low quality) and 36 (high-quality), out of a possible 44. Areas where the majority of reviews were marked down included not adequately describing excluded studies and statistical tests, and not providing a clinical consensus statement. However, all

TABLE 4 | Summary of effects of lifestyle interventions during pregnancy on relative risks of maternal and neonatal outcomes.

Systematic reviews	RCT (*n*)	BMI	Participants (*n*)	R-AMSTAR Ranking	GDM	Pregnancy-induced HTA	Pre-eclampsia	Caesarian section	Preterm delivery	LGA	Macrosomia	Neonatal RDS
PHYSICAL ACTIVITY INTERVENTIONS DURING PREGNANCY												
Streuling et al. (97)	12	Any BMI	906	C								
Elliot-Sale et al. (98)	3	Any BMI	214	D								
da Silva et al. (99)	18	Any BMI	3,203	D	0.67 (0.49–0.92)					0.51 (0.30–0.87)		
Perales et al. (100)	57	Any BMI	Not reported	D	Reduced risk (4/14 RCTs, aerobic + resistance training), weak.	Reduced risk (1/12 RCTs, aerobic + resistance training), weak.		Reduced risk (3/15 RCTs, aerobic + resistance training), weak.			Reduced risk (3/21 RCTs, aerobic + resistance training), weak.	
MULTI-COMPONENT DIET AND PHYSICAL ACTIVITY INTERVENTIONS DURING PREGNANCY												
Muktabhant et al. (101)	3	Any BMI	444	A		0.70 (0.51–0.96), 5,162 women, low-quality.		0.89 (0.80–1.0, $p = 0.05$), 7,534 women, moderate-quality.			**In OW/OB:** 0.85 (0.73–1.0), moderate-quality	**In OW/OB:** 0.47 (0.26–0.85), moderate-quality.
Thangaratinam et al. (102)	30	Any BMI	Subroup analysis[§], number not reported.	A	Diet* only: In OW/OB: 0.39 (0.23–0.69), low-quality.	Diet* only: 0.30 (0.10–0.88), low-quality; **In OW/OB**, 0.30 (0.10–0.88), low-quality.	Diet* only: 0.82 (0.43–1.42, NS)		Diet* only: 0.26 (0.09–0.74), low-quality.			
Shepherd et al. (111)	16	Any BMI	6,633	A	0.85 (0.71–1.01), 3633 women, ($p = 0.07$); moderate-quality.			0.95 (0.88–1.02), 6,089 women, moderate-quality.			0.89 (0.78–1.01, $p = 0.06$)	0.56 (0.33–0)
International Weight Management in Pregnancy Collaborative (103)	33	Any BMI	11,410	C				0.91 (0.83–0.99), high-quality.				
O'Brien et al. (104)	4	Any BMI	446	D		0.34 (0.13–0.91)						
Lau et al. (105)	7	OW/OB	1,652	B								
Choi et al. (106)	7	OW/OB	721	D								
Flynn et al. (107)	13	OW/OB	4276	D								

*Results are presented as risk ratios, 95% confidence intervals (in brackets) and the reported quality of evidence by authors. BMI, body mass index; OW, overweight; OB, obesity; GDM, gestational diabetes mellitus; HTA, hypertension; LGA, large for gestational age; RDS, respiratory distress syndrome; n.s., not significant. ‡significant effect at 1–2 months postpartum only; §Studies on women with pre-existing diabetes or GDM were excluded for this sub-analysis (5 RCTs were excluded), the remaining number of participants was not reported by authors; *Diet only interventions included of a balanced diet consisting of proteins (15–20%), fat (max. 30%) and carbohydrates (50–55%) including low glycemic load (beans, lentils and vegetables, fruits, unprocessed whole grains). ‡Significant effect at 1-2 months postpartum only.*

reviews reported using the Cochrane Collaboration Risk of Bias tool or the GRADE method.

Risk of Bias

The bias associated with the included trials varied widely across the reviews. Random sequence generation was at low risk of bias for the majority of included studies. Allocation concealment was generally of low or unclear risk of bias. Performance bias was high risk for the majority of the trials, mostly due to the difficulty of blinding study personnel and participants in lifestyle interventions. Detection bias also varied across the reviews, with lower risk of bias for objective outcomes (e.g., body weight). There was an unclear or high risk of attrition bias especially in postpartum trials (drop-out up to 50%). Selective reporting bias was generally unclear or high risk in a large proportion of trials in each review. The proportion of trials with low risk of other biases varied across the reviews.

Quality of Evidence

Four reviews assessed the overall quality of the evidence using the GRADE method (101, 102, 108, 111). Overall the quality of the evidence was high to very low for GWG and moderate to very low for PPWR, and was low for maternal and fetal/neonatal outcomes measured in the reviews and adverse events. There was no data on socioeconomic effects. The reasons for downgrading the evidence included high risk of bias (e.g., attrition), imprecision (wide confidence intervals), and inconsistency (heterogeneity).

Synthesized Findings

Lifestyle Interventions During Pregnancy

Physical activity interventions

The summary of effects of antenatal and postpartum lifestyle interventions on GWG ($n = 12$) or PPWR ($n = 6$) is presented in **Table 3** (detailed description of reviews in **Tables 5–7**). Two studies conducted subgroups analysis to assess the effects of physical activity (supervised or unsupervised) interventions on GWG and maternal or child health outcomes (102, 106).

Physical activity interventions were effective in significantly reducing GWG (mean weighted difference, MD −2.2 to −0.61 kg (97–100, 106). The heterogeneity was low ($I^2 = 0$–25%) and the overall quality of evidence was from low to very low (**Table 3**). No dose-dependent effect could be demonstrated (80), and some difficulties in attending regularly scheduled programs sessions were reported (99).

The summary of effects of antenatal interventions on maternal, fetal or neonatal outcomes is presented in **Table 4** (detailed description of reviews in **Table 6**). In women from all BMI classes, physical activity interventions were effective in reducing the risk of GDM (−33%, very low-quality evidence) (99), cesarean section (very low-quality evidence) (100) and LGA (−49%, very low-quality evidence) (99) compared to standard care. After excluding the three RCTs with high risk of bias, Muktabhant et al. showed also that the likelihood of macrosomia was significantly reduced (RR 0.56, 95% CI 0.36–0.88, 1,274 women, 8 RCTs, $I^2 = 0\%$) in intervention compared to control groups. Combined aerobic and resistance training significantly increased cardiorespiratory fitness and reduced urinary incontinence (moderate-quality evidence) (100). No adverse effect of physical activity (SGA, preterm delivery) could be identified in two reviews (99, 100).

There was no information in women with overweight or obesity. Though one high-quality review showed in a subgroup analysis that physical activity interventions were effective in significantly reducing GWG (weighted MD −1.35, 95% CI −1.80 to −0.89) in the mixed risk group (all BMI) but not in the high-risk group (women with overweight/obesity or at risk of GMD) (101).

Multi-component diet and physical activity interventions

Eight systematic reviews (nine with meta-analysis) included diet and physical activity interventions either as a single or a multi-component program (**Table 3**).

In pregnant women from all BMI classes, multi-component diet and physical activity interventions were effective in reducing GWG (weighted MD −1.8 to −0.7 kg, $I^2 = 0$–80%, high-quality evidence in 3 of 5 reviews) (101–104, 111) and in decreasing the likelihood of excessive GWG (RR 0.72–0.80, moderate-quality evidence) (101, 104). There was also evidence for less PPWR (MD −0.94 kg, 95% CI −1.52 to −0.37; 1,673 women, 6 RCTs) at the latest time reported (from 6 weeks to 12 months postpartum) in the antenatal intervention compared to standard care groups (101). Supervised physical activity, personal counseling, weight monitoring or pre-determined maximal GWG goal, and early intervention contributed to reduced GWG.

In pregnant women with overweight or obesity, a significantly reduced GWG (weighted MD −0.91 to −0.63 kg, $I^2 = 8$–14%, moderate to very low-quality evidence) was also reported in intervention compared to standard care groups (102, 105–107). One high-quality (A) systematic review conducted a subgroup analysis and demonstrated that antenatal diet only interventions (balanced diet, with low glycemic load) had greater effects in reducing GWG in women from all BMI classes (weighted MD −5.53 kg, 95% CI −8.54 to −2.53, $p < 0.001$) or with overweight/obesity (−7.73 kg, 95% CI −9.40 to −6.05 kg, $p < 0.001$, I^2 41%), compared to standard care (102).

Multi-component diet and physical activity interventions were effective in decreasing the risk of pregnancy-induced hypertension (−66 to −30%; low to very low-quality evidence) (101, 104), cesarean delivery (−9 to −5%; high to moderate-quality evidence) (101, 102) and neonatal respiratory distress syndrome (RDS, −44%, high-quality evidence) (101) in women from all BMI classes. There was a non-significant trend toward reduced likelihood of GDM (−15%, moderate-quality evidence) (111) and macrosomia (−11%) (111).

In women with overweight or obesity, multi-component diet and physical activity interventions were effective in decreasing the risks of pregnancy-induced hypertension (−70%, low-quality evidence) (101, 102), macrosomia (−15%, moderate-quality evidence) (101), but not LGA, and neonatal RDS (−53%, moderate-quality evidence) (101). There was no other effect or harm to maternal or infant health reported. Supervised physical activity or personalized prescription of physical activity, e-based platform plus in-person counseling or telephone calls,

TABLE 5 | Systematic reviews and meta-analysis that assessed the effect physical activity interventions in pregnancy.

Quality of reviews*	Systematic reviews	Studies included	Participants (*n*)	Types of intervention	Weighted mean difference or summary risk ratio (95% CI)/findings	Other findings/Contributors
PHYSICAL ACTIVITY INTERVENTIONS						
C (33/44, 75%)	Streuling 2011 (SR+MA) (97)	12 RCTs on GWG.	Any BMI	Light-moderate intensity supervised PA; average frequency 3 days/week; 20–60 min.; aerobic and/or resistance exercises. Duration: from 1st-2nd to 3rd trimester.	Significant reduction of GWG (−0.61 kg; −1.17 to −0.06; I^2 25%; 906 participants; 12 RCTs).	No dose-dependent effect.
D (30/44, 68%)	da Silva 2017 (SR+MA) (99)	18 RCTs on maternal/ infant outcomes (51 cohort studies excluded)	Any BMI	Moderate intensity supervised PA; average frequency 3 days/week; 20–70 min.; aerobic and/or resistance exercises. Duration: from 1st-2nd to 3rd trimester.	Significant reduction of GWG (−1.11 kg; −1.53 to −0.69; I^2 0%; 3,203 participants; 18 RCTs).	Reduced RR of GDM (0.67, 0.49–0.92; I^2 33%; 3,790 participants; 10 RCTs) and LGA (0.51, 95% CI 0.30–0.87; I^2 0%; 1,499 participants; 4 RCTs). No effect on preeclampsia or preterm birth. Difficulties in attending regularly scheduled programs sessions.
D (16/44, 36%)	Perales 2016 (SR) (100)	57 RCTs on maternal health or perinatal outcomes	Any BMI	15 trials aerobic exercises; 4, resistance exercises; 30 combined; 8 counseling. 49 RCTs included supervised PA; 23 of them examined effects of supervised PA on GWG. Duration 12–18 week.	Weak evidence for reduced GWG or for higher likelihood of GWG within IOM guidelines after aerobic or aerobic + resistance exercises or counseling.	Combined aerobic and resistance training: strong evidence for improved cardiorespiratory fitness and reduced urinary incontinence. Weak evidence for reduced GDM, pregnancy-induced HTA, duration of labor or cesarean section, and macrosomia after intervention. No adverse outcome.
D (28/44, 64%)	Elliott-Sale 2015 (SR+MA) (98)	3 RCTs on GWG, from 1990 only	Any BMI	Light-moderate intensity supervised PA; combined aerobic and resistance exercises; frequency 3–5 days/week; 45–60 min. Duration: 12–33 week.	Significant reduction of GWG (−2.22 kg; −3.14 to −1.3, I^2 0%; 214 participants; 3 RCTs).	Methodological quality varied considerably across trials. Small number of RCTs.

*BMI, Body mass index; CI, confidence interval; GWG, gestational weight gain; GDM, gestational diabetes mellitus; HIC, high income countries; IOM, Institute of Medicine; LGA, large for gestational age; LMIC, low and middle income countries; MA, meta-analysis; OW, overweight; OB, obesity; PA, physical activity; PPWR, postpartum weight retention; SGA, small for gestational age; RCT, randomized controlled trial; RR, risk ratio; SR, systematic review; wk, week. *The quality of systematic reviews and meta-analysis was assessed using the R-AMSTAR Checklist (ranking, score). When available, the information on the quality of evidence that was reported by authors is indicated in the findings' columns.*

TABLE 6 | Systematic reviews and meta-analysis that assessed the effect of multi-component interventions in pregnancy.

Quality of reviews*	Systematic reviews	Studies included	Participants (n)	Type of intervention	Weighted mean difference (95% CI)/Findings	Other findings/Contributors
A (40/44, 91%)	Muktabhant 2015 (Cochrane SR+MA) (101)	65 RCTs on GWG	All BMI	Dietary counseling (healthy diet or low-fat or low glycemic load or low-energy diet), supervised or unsupervised exercise, or diet and exercise combined. Duration: from the 1st-2nd to the 3rd trimester.	Reduced risk for excessive GWG (RR 0.80, 0.73–0.87; I^2 52%; 7,096 women; 24 RCTs; high-quality evidence) with PA or combined diet and PA. Five studies reported reduced GWG > 5 kg in intervention vs. control groups. In women with OW/OB, or at risk of diabetes, receiving combined diet and PA interventions, significant reduction of GWG (−0.71 kg, −1.34 to −0.08 kg, I^2 = 57%; 2741 women; 11 RCTs, moderate-quality evidence). Interventions involving low glycemic load, supervised or unsupervised PA, or diet and PA combined all led to similar reductions. Increased likelihood to experience low GWG than those in control groups (RR 1.14, 1.02–1.27; I^2 3%; 4422 women; 11 RCTs; moderate-quality evidence). Largest reduction accounted with supervised diet and PA.	Reduced RR of gestational HTA (0.70, 0.51–0.96; I^2 = 43%; 5,162 women; 11 RCTs; low-quality evidence) and macrosomia (with PA interventions, 0.87, 0.71–1.07, I^2 0%, 2,674 women, 9 RCTs, $p = 0.05$) In women with OW/OB, or at risk of diabetes, reduced RR for macrosomia (0.85, 0.73–1.0; I^2 0%; 3,252 women; 9 RCTs; moderate-quality evidence) and neonatal respiratory distress syndrome (0.47, 95% CI 0.26–0.85; I^2 0%; 2,256 women; 2 RCTs; moderate-quality evidence) after combined diet and PA interventions. No effect on preterm birth, pre-eclampsia, LGA or SGA, or other neonatal outcomes.
A (41/44, 93%)	Thangaratinam 2012 (SR+MA) (102)	34 RCTs on GWG	All BMI (11/34 trials included OW/OB women)	Balanced diet: proteins (15–20%), fat (max. 30%), carbohydrates (50–55%) with low glycemic index; light to moderate intensity PA (resistance training, weight-bearing exercises, walking) or multi-component interventions (using behavioral change techniques and feed-back on weight gain).	Subgroup analysis (excluding women with pre-existing diabetes or GDM; 30 RCTs): Overall reduction of GWG (−1.4 kg, 95% CI −2.09 to −0.71; $p < 0.001$; moderate-quality evidence). Greater effect with diet only interventions (−5.53 kg, −8.54 to −2.53; I^2 41%; 6 RCTs, $p < 0.001$), followed by multi-component approach (−1.06 kg, −1.67 to 0.46; $p < 0.001$) and physical activity (−0.72 kg, −1.2 to −0.25, 14 RCTs, P = 0.003). In OW/OB pregnant women, overall reduction of GWG (−2.1 kg, −3.46 to −0.75; $p < 0.002$; I^2 88%). Diet only interventions, reduction of GWG (−7.73 kg; −6.05 to −9.40; $p < 0.001$; I^2 41%).	Diet only interventions: significant decrease of risk of gestational HTA (RR 0.30, 95% CI 0.10–0.88) and preterm delivery (0.26, 0.09–0.74). Low-quality evidence. There was a trend toward a reduction of RR of pre-eclampsia (0.82, 0.43–1.42, not significant). In OW/OB women, dietary interventions significantly decreased the risk of gestational HTA (RR 0.30, 0.10–0.88). No other effect on maternal or fetal outcomes (no adverse event, no increased risk of SGA, no effect on birth weight).

(Continued)

TABLE 6 | Continued

Quality of reviews*	Systematic reviews	Studies included	Participants (n)	Type of intervention	Weighted mean difference (95% CI)/Findings	Other findings/Contributors
A (42/44, 95%)	Shepherd 2017 (Cochrane SR+MA) (111)	23 RCTs for preventing GDM	All BMI	Combined diet and PA interventions.	Significant reduction of GWG (−0.89 kg, 95% CI −1.39 to −0.40 kg; Tau2 = 0.37; I^2 = 43 %; women = 5,052; RCT = 16).	Reduced risks of cesarean section (0.95, 95% CI 0.88–1.02; 6,089 women; 14 RCTs; moderate-quality evidence) and respiratory distress syndrome (0.56, 0.33–0; 2,411 women, 2 RCTs). Trend toward a reduction of the risk of GDM (RR 0.85; 0.71–1.01; 6,633 women; 19 RCTs; Tau² = 0.05; I^2 42%; $p = 0.07$; moderate-quality evidence) and macrosomia (0.89, 0.78–1.01; 5,368 women, 9 RCTs, $p = 0.06$) in the diet and PA intervention group compared with the standard care group. No difference for pre-eclampsia, pregnancy-induced HTA and/or HTA, perinatal mortality or LGA. No data were reported for infant mortality or morbidity composite.
C (33/44, 75%)	i-WIP 2017 (SR+MA) (103)	36 RCTs on maternal and child outcomes.	All BMI (13/36 trials included OW/OB women)	Diet, physical activity or multi-component interventions.	Significant reduction of GWG (−0.70 kg, −0.92 to −0.48 kg, I^2 = 14%; 9,320 women; 33 RCTs, high- quality evidence).	High-quality evidence that interventions reduced the risk of cesarean section (RR 0.91, 0.83–0.99, I^2 0%; 11,410 women; 32 RCTs), but not for other maternal or child's outcomes.
D (30/44, 68%)	O'Brien 2016 (SR+MA) (104)	12 RCTs on GWG.	All BMI	Written information on diet and physical activity + phone calls or regular weighting; visits with a dietician combined with an exercise program 3–5 days/week in 4 trials. Duration: 14–52 week.	Significant reduction of GWG (−1.25 kg; −2.39 to 0.11; I^2 42%; 446 women; 4 RCTs) and reduced RR for GWG above IOM guidelines (RR 0.72, 0.60–0.8; I^2 0%; 714 women; 5 RCTs).	Wide variation in the type of intervention, the number of contacts, and the intensity. Reduced RR for hypertension (RR 0.34, 0.13–0.91, I² 0%; 243 women; 2 RCTs). No effect on GDM, preeclampsia, preterm birth, macrosomia or SGA.
B (36/44, 82%)	Lau 2017 (SR+MA) (105)	7 RCTs on GWG.	OW/OB	E-based lifestyle interventions (theoretical or conceptual frameworks). Duration: 4 week to 12 months. 7 trials conducted a follow-up up to 12 months.	Significant reduction of GWG (−0.63 kg; −1.07 to −0.20; I^2 14%; 1652 women; 7 RCTs).	Interventions incorporating in-person ($z = 2.02$, $p = 0.04$), phone ($z = 2.07$, $p = 0.04$) or a combination of in-person and phone delivery formats ($z = 2.07$, $p = 0.04$) were found to be more effective for reducing the GWG in comparison with solely e-based platforms ($z = 1.10$, $p = 0.27$). No effect on birth weight.

(Continued)

TABLE 6 | Continued

Quality of reviews*	Systematic reviews	Studies included	Participants (n)	Type of intervention	Weighted mean difference (95% CI)/Findings	Other findings/Contributors
D (30/44, 68%)	Choi 2013 (SR+MA) (106)	7 RCTs	OW/OB	5 RCTs included supervised light-moderate PA activity 3 days/week or multi-component supervised physical activity 1x/week + diet counseling. Duration: from the 1st-2nd to the 3rd trimester.	Significant reduction of GWG (−0.91 kg; −1.76 to −0.06; I^2 8%; 721 women; 7 RCTs) after a PA intervention.	Supervised physical activity plus diet showed a significant greater effect on GWG (−1.17 kg; −2.14 to −0.21; I^2 0%; 372 women; 2 RCTs). Contributors: personalized prescription of PA; goals setting.
D (27/44, 61%)	Flynn 2016 (SR) (107)	13 RCTs on GWG.	OW/OB	Diet only or multi-component diet and PA interventions. National recommendations (energy intake 18–24 kcal/kg in 2 trials); individual feedback and alternative healthy choices. Duration 12–30 weeks.	Multi-component interventions: Significant reduction of GWG in 5 of 10 trials in all women and in one trial including only OB women. Diet only interventions: significant reduction in the 3 trials.	Considerable variation in the methodological design of dietary interventions. No evidenced-based approach for any specific dietary regimen. No effect on maternal or neonatal outcomes (no effect on birth weight).

*BMI, Body mass index; CI, confidence interval; OW, overweight; OB, obesity; GDM, gestational diabetes mellitus; GWG, gestational weight gain; IOM, Institute of Medicine; i-WIP, International Weight Management in Pregnancy Collaborative Group 2017; HTA, hypertension; LGA, large for gestational age; MA, meta-analysis; PA, physical activity; PPWR, postpartum weight retention; QCT, Quasi randomized trial; RCT, randomized controlled trial; RR, risk ratio; SGA, small for gestational age; SR, systematic review. *The quality of systematic reviews was assessed using the R-AMSTAR Checklist (ranking, score, %). When available, information on the quality of evidence reported by authors is indicated in the findings' columns.*

contributed to reduce GWG in women with overweight or obesity (105).

Diet only interventions (balanced diet, with low glycemic load) resulted in significantly greater reductions in the risks of GDM (−61%, low-quality evidence), pregnancy-induced hypertension (−70%, low-quality evidence), and preterm delivery (−74%, low-quality evidence) in women from all BMI classes and in pregnancy-induced hypertension (−70%, low-quality evidence) in women with overweight/obesity (102). There was also a trend toward decreased risk of pre-eclampsia (−18%, low-quality evidence), but no evidence of other maternal or fetal/neonatal effect or harm, especially no evidence for SGA.

Lifestyle Interventions During Postpartum

Six systematic reviews (five with meta-analysis) included physical activity ($n = 1$) or multi-component diet and physical activity interventions ($n = 5$, see **Table 3**);

Physical activity interventions

In women from all BMI classes, one review showed that physical activity interventions (12-week progressive walking protocol in 2 RCTs) resulted in non-significant changes in PPWL (13).

Multi-component diet and physical activity interventions

In women from all BMI classes, combined diet (healthy diet or calorie restricted diet) and physical activity intervention were effective in reducing PPWR (weighted MD −2.6 to −2.3 kg, very low-quality evidence) (109, 110). Use of a heart rate monitor or a pedometer or modern technologies (internet, text messages, emails, phone calls), self-monitoring, and duration less than 6 months contributed to the effects of interventions (109).

In women with overweight or obesity, combined diet and physical interventions (e-based or individual/ group sessions) were effective to reduce PPWL (weighted MD −3.6 to −1.22 kg, moderate to very low-quality evidence) (105, 106). Personalized prescription of PA and goals setting contributed to PPWL (106). No other maternal effect or harm were observed but there is little data. Dewey et al. reported no change in milk volume and composition among women enrolled in an exercise-only intervention compared to usual care (112).

None of the systematic reviews examined effects of lifestyle interventions on quality of life or psychological health during pregnancy or postpartum.

DISCUSSION

Summary of Main Findings

Multi-component dietary and lifestyle interventions are effective in decreasing GWG and the likelihood of weight gain above the IOM guidelines in women of all BMI classes, without any reported maternal or fetal/neonatal adverse effect. Regular light to moderate intensity physical activity during pregnancy reduce GWG, however interventions including a balanced diet with a low glycemic load, are associated with the greatest reduction. Multi-component diet and physical activity interventions decrease the risks of pregnancy-induced hypertension, cesarean section and neonatal respiratory distress

TABLE 7 | Systematic reviews and meta-analysis that assessed the effect of multi-component diet and physical activity interventions in postpartum.

Quality of reviews*	Systematic review	Studies included	Participants	Types of intervention	Weighted mean difference (95% CI)/Findings	Other findings/ Contributors
PHYSICAL ACTIVITY INTERVENTION						
D (28/44, 64%)	Elliott-Sale 2015 (SR+MA)[79]	2 RCTs	All BMI	Individual walking; frequency 4–7 days/week; 45 min; duration 12 weeks.	No significant effect on PPWL (−1.74 kg; 95% CI −3.59 to 0.10, I^2 0%; 128 women; 2 RCTs).	
MULTI-COMPONENT DIET AND PHYSICAL ACTIVITY						
B (36/44, 82%)	Lau 2017 (SR + MA)[80]	5 RCTs on PPWL	OW/ OB	E-based lifestyle interventions (diet, physical activity and weight management components; theoretical or conceptual frameworks); behavioral goals, counseling and skill training, self-monitoring, feed-back. Duration 4 weeks to 12 months.	Significant PPWL (−3.60 kg; 95% CI 6.59–0.62; I^2 84%; 251 women; 3 RCTs) during the 1–2 months postpartum. No significant effect in the 6–12 months postpartum.	Significant increase of MVPA at 6 and 13 weeks, and 12 months postpartum (via subjective measures). Significant reduction of caloric intake at 12–20 weeks and 12 months postpartum using the diet-related software measures. No effect on maternal or neonatal complications.
C (32/44, 73%)	Berger 2014 (SR)[85]	13 RCTs on PPWR (1 diet, 3 PA, 9 combined)	All BMI	Nutrition, exercise or combined diet and PA interventions. Individual counseling, informational pamphlets, telephone calls, text messages, pedometer. Duration 3 to 9 months.	No effect in the 4 good quality RCTs (combined diet and PA). The 4 fair to good quality RCTs reported greater weight loss (from−4.9 to−0.17 kg) in the combined intervention group vs standard care. No effect of diet of PA alone.	No effect on metabolic risk factors or inflammatory biomarkers. Significant reduction of waist-to-hip ratio in one PA trial.
C (33/44, 75%)	Nascimento 2014 (SR+MA)[82]	11 RCTs on PPWL	All BMI (8/11 RCTs with OW/OB women).	Supervised (4 trials) or unsupervised PA (7 trials; heart rate monitor or pedometer, personalized counseling, correspondence programs, text messages, phone calls, web). Walking or general aerobic exercises were recommended. Resistance exercises combined with walking in one trial. Healthy diet or calorie restricted diet. Duration: 10 to 52 weeks.	Significant PPWL (−2.57 kg; 95% CI −3.66 to −1.47; I^2 66%; 769 women; 11 RCTs; 4 high-quality trials).	Contributors: Heart rate monitor or pedometer (−4.09 kg; 95% CI −4.94 to −3.25; I^2 0%; 238 women; 6 RCTs) and exercise combined with intensive dietary intervention (−4.34 kg; 95% CI −5.15 to −3.52; I^2 0%; 314 women; 6 RCTs).
D (29/44, 66%)	Lim 2015 (SR+MA)[83]	46 studies on PPWL (32 RCTs/ 14 observational studies).	All BMI (7/32 RCTs with OW/OB women)	Diet, PA or both. 22 RCTs had only a PA component. In-person participation, self-monitoring, individual or group setting, use of technology, home- or center-based intervention. Duration: 11 days to 36 months.	Significant PPWL (−2.30 kg; 95% CI−3.22 to−1.39, I^2 84%; 1892 women; 32 RCTs).	Contributors: Combined diet and PA intervention versus PA only (−2.59 kg; 95% CI −3.54 to −1.64; I^2 79%; 1,359 women; 17 RCTs); Self-monitoring (−2.59 kg, −3.54 to−1.64; I^2 85%; 1356 women; 17 RCTs); Duration 6 months or less (−3.11 kg; 95% CI −3.54 to −1.64 vs. −1.01 kg; −2.10 to 0.08, $p = 0.01$).

(Continued)

TABLE 7 | Continued

Quality of reviews*	Systematic review	Studies included	Participants	Types of intervention	Weighted mean difference (95% CI)/Findings	Other findings/ Contributors
D (30/44, 68%)	Choi 2013 (SR+MA)[77]	4 RCTs on PPWL	OW/OB	Individual or group sessions on diet and PA; goals setting, self-monitoring, pedometer, telephone call. Restriction of energy intake in 3 trials. Walking; moderate-vigorous intensity; frequency 4–5 times/weeks 30–45 min.; duration 10 to 13 weeks. Supervised in 1 trial.	Significant PPWL (−1.22 kg; 95% CI −1.89 to −0.56; I^2 25%; 547 women; 4 RCTs)	Contributors: personalized prescription of PA; goals setting.

*BMI, Body mass index; CI, confidence interval; MVPA, moderate to vigorous physical activity; OW, overweight; OB, obesity; PA, physical activity; PPWR, postpartum weight retention; PPWL, postpartum weight loss; RCT, randomized controlled trial. *The quality of evidence of systematic reviews and meta-analysis was assessed using the R-AMSTAR Checklist (ranking, score, %). When available, the information on the quality of evidence reported by authors is indicated in the findings' columns.*

syndrome. Diet in particular is associated with greater reduction of the risk of GDM, pregnancy-induced hypertension and preterm delivery, compared with any other intervention. There is no evidence for effects on outcomes related to fetal weight, morbidity and mortality.

In women with overweight and obesity, multi-component diet and physical activity interventions are effective in reducing the risks of pregnancy-induced hypertension, macrosomia and neonatal respiratory distress syndrome. In addition, diet only interventions are effective in decreasing the risks of GDM and pregnancy-induced hypertension in this population. After delivery, multi-component diet and physical activity interventions are effective in reducing PPWR in women of all BMI classes, but no other effect on maternal or infant outcomes are reported.

Effective Interventions to Reduce Gestational Weight Gain

Pregnancy is a time when women may be motivated to change their health behaviors. A healthy diet and regular physical activity are currently recommended during pregnancy in healthy weight pregnant women (69, 113, 114), and women with a BMI over 35 are encouraged to obtain advice from a dietician (115). Our findings support the current recommendations, even if the dietary regimen or the optimal dose of physical activity has not been determined yet. Several components appear to contribute to the control of GWG, such as early intervention implementation, supervised physical activity, personal counseling, weight monitoring combined with a lifestyle intervention, or pre-determined maximal GWG goal.

An increased energy intake during the 2nd and the 3rd trimester is usually recommended (116). Our evidence review shows that diet-based interventions (counseling, balanced diet, low glycemic load, energy target by weight 18–24 kcal/kg, food diary) are associated with the greatest reduction in GWG. Therefore, caution should be taken in women with overweight and obesity in telling them to increase their energy intake in the 2nd and 3rd trimesters.

The epigenetic profile of the developing fetus is sensitive to environmental influence. Maternal diet has been shown to influence DNA methylation patterns in offspring, but research in humans is limited (117). Recently, findings from the ROLO study (Randomized control trial of Low glycaemic index diet to prevent macrosomia) suggested that low glycemic index dietary intervention during pregnancy was associated with subtle, yet widespread differential DNA methylation at regions across the offspring's genome (118). These data imply that exposure to a dietary intervention may impact the neonatal epigenome and therefore their risk of obesity and NCDs during fetal development, though larger studies are required to fully explore interventions in pregnancy.

Women typically reduce their physical activity level during pregnancy (119). This evidence review shows that light to moderate intensity physical activity, including aerobic and resistance exercises, should be encouraged 3 −5 times per weeks for a duration of 30–60 min without adverse effects in healthy

pregnant women. Our findings are in line with the American College of Obstetricians and Gynecologists (ACOG) who recommends that pregnant women should engage in moderate exercise for 30 min per day on most days of the week, with the exception of women with compromising health conditions (e.g., pre-eclampsia) (114). The anatomic and physiological changes, absolute and relative contraindications should be considered. Activities that increase the risk of falls or those that may result in excessive joint stress, should include cautionary advice for most pregnant women, but evaluated on an individual basis with consideration for individual abilities. The major challenge remains how best to engage pregnant women in regular physical activity and sustain changes during the perinatal period. Lau et al. has shown that a combination of in-person, e-based and phone interventions is more effective to reduce GWG, in comparison with an e-based platform alone.(80) It is possible that women with overweight or obesity need a higher dose of physical activity to influence GWG or other pregnancy outcomes compared to normal weight women. Evidence supports moderate intensity physical activity between 150 and 250 min per week to be effective to prevent weight gain in adults with overweight or obesity (120), however there is to date no information in pregnant women.

The effects of antenatal interventions on maternal and fetal morbidities and mortality remained unclear. This review of reviews demonstrates that there is low-quality evidence that multi-component diet and physical activity interventions decrease the likelihood of pregnancy-induced hypertension, cesarean section and neonatal respiratory distress syndrome in women from all BMI classes. Furthermore, diet-based interventions were shown to be effective in decreasing the risks of GDM and pregnancy-induced hypertension in women with overweight and obesity. These findings are of particular importance to primary care providers, as pre-pregnancy obesity is an independent risk factor for serious maternal complications (3, 13–16, 23, 24).

Although the quality of evidence remain low, the available evidence suggests that antenatal multi-component lifestyle interventions are also effective in reducing the risk of macrosomia and neonatal respiratory distress syndrome in women with overweight and obesity (3). As maternal pre-pregnancy obesity is associated with an increased risk for the offspring developing childhood obesity and NCDs in the long term, these findings suggest that health care providers should pay particular attention to this high-risk population, in order to prevent the vicious intergenerational cycle of obesity (3, 33, 40).

Combined diet and physical activity lifestyle interventions are effective to reduce GWG, with evidence from this review showing a decrease by 20–28% in the risk for GWG above the IOM guidelines, although the magnitude of effects on weight is small (−1.8 to −0.7 kg). So weight loss prior pregnancy is probably needed to achieve both GWG goals and optimal pregnancy outcomes (36, 115, 121). The current European Association for the Study of Obesity (EASO) guidelines for the management of adult obesity provide useful information for primary care providers (122). This treatment should be undertaken by a multidisciplinary obesity team with the ability to tackle the different aspects of obesity and its co-morbidities. Weight loss objectives should be realistic (5–10% over a period of 6 months) and individualized. Structured intensive programs using cognitive-behavioral techniques in individual or group setting are effective in achieving realistic goals in an adequate time frame (123).

Whilst bariatric surgery is currently the most cost-effective treatment resulting in substantial weight loss in carefully selected patients, it should only be considered for those patients with severe obesity, or help with co-morbidity management (124). Available data suggests that pregnancy following bariatric surgery is associated with improved maternal and fetal outcomes, compared to women with untreated obesity, however it is also related to premature delivery and increased risk of SGA (125). Pregnancy is therefore not recommended 12–18 months after surgery, and the antenatal care of women how have undergone bariatric surgery should be undertaken at a specialized center (126).

Effective Interventions to Reduce Postpartum Weight Retention

The postpartum period is also a window of opportunity to encourage women to lose excessive weight at a time when they are usually motivated. This evidence review shows that interventions that include both diet and physical activity components, and comprise individualized support and self-monitoring are more likely to be successful in reducing PPWR in all BMI categories. However the optimal approach to reduce PPWR remains uncertain. Ostbye et al. observed that home-based interventions provided a less burdensome and more practical approach than clinic-based attendance (127). Interventions delivered via email, mail/post, telephone, text messaging or the Internet appear to be more practical for postpartum women than traditional face-to-face methods. These methods of delivery for weight loss management have also been successful in the general population (128). Web-based weight management programs have also been found to be as successful as traditional face-to-face counseling for short-term weight loss (129). As women from lower socioeconomic backgrounds are at higher risk of developing obesity after birth, they should be targeted for PPWL interventions (130).

The ACOG recommends a gradual return to physical activity 4–6 weeks after Childbirth (131), however, several studies observed that a high proportion of women are not as active as recommendations advise during the year following childbirth (132). This current review demonstrates that physical activity–based interventions have no effect on PPWR (98), compared to multi-component diet and physical activity approaches (105, 106, 108–110). Dewey et al. (112) suggested that in absence of a proper dietary intervention, women tend to increase their caloric intake as their energy expenditure through exercise increases, thus the calorie deficit required for weight loss cannot be reached resulting in an ineffective intervention. Light to moderate physical activity itself may not be sufficient to induce weight loss after birth weight. However, physical activity during the post-partum period can induce other beneficial effects on health, such as increased

cardiovascular fitness (133) or reduced depression symptoms after child birth (134).

Limitations

Our review of systematic reviews and meta-analyses provides a comprehensive and up to date (up to January 2018) overview of the current reviewed evidence. A rigorous quality assessment was undertaken by two independent reviewers for each review (R-AMSTAR). The quality assessed in each systematic review ranged between high to very low for the benefit observed with GWG and PWWR, but low for other important maternal or neonatal outcomes. The low evidence rating was explained by significant heterogeneity observed in the effect size, risk of publication and related biases, and deficiencies in the quality of the study. There were large differences in the types of interventions and participants, mode of delivery, timing of the measurements and implementation of intervention, dose of intervention, and how it was monitored and supervised. Most included studies were also carried out in middle-high and high-income countries and it is not clear whether these findings are applicable to low income settings.

Implication for Research

Future research should focus on: the optimal dose (type, frequency, intensity and duration) as well as the level of supervision in interventions that aim to reduce GWG and PPWR; measurements of psychosocial determinants of GWG and PPWR; interventions in various groups based on BMI, age, ethnicity, socioeconomic status, parity, and risk status in pregnancy; the sustainability and long term effects of the interventions on the mother and child; the cost-effectiveness of the interventions and their feasibility in terms of incorporation into clinical settings; and strategies to improve the adherence and compliance of lifestyle interventions. Trials should be more systematically designed evaluated and reported. The optimal amount of weight gain or loss during pregnancy that would minimize maternal and fetal complications remains a topic of discussion (64–68), and there is a need to examine the tailoring of interventions to the severity of maternal obesity.

CONCLUSIONS

The burden of obesity not only threatens global health care systems but also the potential to cripple national economies and global development (135). The perinatal period seems to be a critical windows of opportunity to influence long-term obesity and NCDs risk for women and their child, as well as maternal weight status for subsequent pregnancies. Adopting healthy behaviors may also contribute to a healthier lifestyle for the whole family and to the prevention of early childhood obesity (136). Evidence-based recommendations and training programs for health care professionals are urgently needed to increase the proportion who discuss these topics, and who do so accurately (131). Despite the above limitations, some clear conclusions can be made concerning the type and dose of interventions.

A multi-component approach including a balanced diet, with low glycemic load, and light to moderate physical activity, 30–60 min per day 3–5 days per week, should be recommended from the first trimester of pregnancy and maintained during the postpartum. As there is no evidence that the interventions evaluated in this review are associated with adverse maternal or fetal outcomes, we conclude that that desirable outcomes of lifestyle interventions outweigh possible harms. Dietary interventions appear to be most effective in reducing GWG and co-morbidities such as gestational hypertension and preterm birth in the general population, but also pregnancy-induced hypertension, GDM and macrosomia in women with obesity. We hope that this evidence review will serve as a basis to inform new policies on maternal and child health to halt the intergenerational cycle of obesity.

AUTHOR CONTRIBUTIONS

NF-L contributed to the conception and design of the study, conducted the review of reviews, assessed the quality of studies, drafted and revised the manuscript. CS and LE provided input into the conduct of the study, the grading of evidence and the interpretation of data. BM contributed to the review of maternal and fetal complications associated with obesity and excessive GWG and to the interpretation of data. All authors contributed to the manuscript revision, read and approved the submitted version.

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GENERAL COMMENTARY
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Commentary: Obesity and Weight Gain in Pregnancy and Postpartum: an Evidence Review of Lifestyle Interventions to Inform Maternal and Child Health Policies

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A Commentary on

Obesity and Weight Gain in Pregnancy and Postpartum: an Evidence Review of Lifestyle Interventions to Inform Maternal and Child Health Policies
by Farpour-Lambert NJ, Ells LL, Matinez de Tejada B, and Scott C. (2018). Front. Endocrinol. 9:546. doi: 10.3389/fendo.2018.00546

We read with interest the recent review published in *Frontiers in Endocrinology* that was focused on obesity and weight gain in pregnancy and postpartum. The review of systematic reviews and meta-analyses, investigating the effects of lifestyle interventions on gestational weight gain (GWG) and postpartum weight retention (PPWR), provides evidence showing that lifestyle interventions can reduce excess weight gain and associated risk factors. We agree unconditionally that the burden of maternal and childhood obesity needs to be reduced urgently.

There is a clear policy mandate internationally to prevent maternal obesity given the adverse impact on maternal and child health, and the challenges of treating obesity, which are intensive, costly, largely ineffective, and unsustainable at a population level. The World Health Organization (1), National Institute for Health and Care Excellence (2), the Australian Medical Association (3), the Australian National Health and Medical Research Council Obesity Translation Committee (4) and the US Institute of Medicine (5), have unanimously called for targeted efforts to improve

lifestyle behaviors during pregnancy to optimize gestational weight gain, prevent postpartum weight retention and improve short- and long-term maternal health and long-term child health outcomes.

In concordance with the findings of Farpour-Lambert et al. (6), we have reported lifestyle intervention during pregnancy reduces GWG, gestational diabetes, and cesarean births (7, 8), and in postpartum is effective for weight loss, weight gain prevention, and improving metabolic and reproductive outcomes (9, 10). With demonstrated efficacy and an extensive evidence base now established, the consolidation of current evidence and identification of specific gaps is underway, which is vital to inform further trials that address these gaps and advance, rather than simply expand, the field. Furthermore, effective low-intensity and low-cost lifestyle interventions in pregnancy and postpartum need to be implemented at scale to prevent excessive weight gain and to promote healthy lifestyle (11).

Whilst we agree with Farpour-Lambert et al. (6) that multicomponent lifestyle strategies should be offered to women in pregnancy and postpartum, we emphasize that the preconception period is just as important and cannot be ignored in systems level approaches to preventing maternal and childhood obesity (12, 13). It is imperative to improve women's health status before pregnancy. A high body mass index (BMI) in the preconception period reduces fertility and increases complications when pregnancy does occur including gestational diabetes, gestational hypertension, preeclampsia, early pregnancy loss, congenital fetal anomalies, large-for-gestational-age infants, preterm birth and still birth (14) as well as cesarean section (15, 16) and newborn morbidity from shoulder dystocia (17) and increases the risk of subsequent offspring obesity (18). Clear preconception health promotion priorities, related to healthy diet, weight management, dietary supplementation, physical activity, substance use and more, await implementation given the intergenerational effects of sub-optimal lifestyle behaviors from conception.

As a direct response to the growing prevalence of overweight and obesity among women before, during and after pregnancy, a team of Australian researchers formed the Health in Preconception, Pregnancy and Post Birth (HiPPP) Collaborative in 2013 (13). HiPPP encompassed multidisciplinary expertise and engaged stakeholders across community, government, private and public health services, workplaces, primary care, and consumer advocates/patient representatives. HiPPP is a network with the primary aim of improving lifestyle and preventing maternal obesity. The HiPPP network is strengthened by partnership, research, capacity building, knowledge translation and collaboration.

Despite the vast body of research to date, there is currently no international consensus on guidelines around preconception, pregnancy and postpartum healthy behaviors and prevention of weight gain, with only 42% addressing preconception and 13% addressing postpartum phases (19). Furthermore, no country has implemented systems level practice and policy evidence-based strategies targeting preconception, pregnancy and postpartum life stages to prevent obesity. The HiPPP network is focused on addressing the few clear remaining gaps in evidence around efficacy, yet primarily we are progressing implementation research and translation of existing evidence into policy and practice. In this context, we are seeking to collaborate on areas including innovative and generationally relevant electronic health strategies (20, 21). Evidence synthesis, guideline development, strategic prioritized implementation research, translation, capacity building and collaboration are now crucial to drive evidence into practice, improve lifestyles, reduce the obesity epidemic and deliver health impact for the benefit of today's and future generations. We are responding to this need.

HiPPP has now expanded internationally. In 2018, as an alliance of global leaders in the area of preconception and pregnancy health, including early career researchers and consumer advocates, we (the authors on this paper) came together with a vision for improving the health of all women of reproductive age, expectant mothers and their children. Together we are focused on stakeholder and consumer engagement, evidence synthesis, guideline development, workforce capacity building, priority setting, implementation research, translation and scale up for health impact. We are developing a consumer and community involvement (CCI) framework to guide our HiPPP global alliance program of research and our evidence synthesis and guideline appraisal are well advanced.

There is a now a clear and imperative call to action to consolidate and advance current evidence into practice and policy; Farpour-Lambert et al.'s (6) findings support this call to action. Efficacy is established and now is an opportune time to pool existing study data internationally, explore core components, delivery methods, and implementation strategies to demonstrate broader effectiveness of lifestyle interventions in preconception, pregnancy, and postpartum. This includes implementation research, behavior change taxonomy and health economic analyses of value (cost and quality). These activities are well underway and ultimately will enable the international community to implement effective interventions at scale to reduce the prevalence of maternal obesity and improve related health outcomes for both women and future generations.

AUTHOR CONTRIBUTIONS

All authors were part of an inaugural HiPPP global alliance that met in Prato, Italy, 27–28th September, 2018. HS, HT, and JA-B led the writing of this paper and all other authors read drafts and provided comments.

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Analysis of Predictive Equations for Estimating Resting Energy Expenditure in a Large Cohort of Morbidly Obese Patients

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The treatment of obesity requires creating an energy deficit through caloric restriction and physical activity. Energy needs are estimated assessing the resting energy expenditure (REE) that in the clinical practice is estimated using predictive equations. In the present cross sectional study, we compared, in a large cohort of morbidly obese patients, the accuracy of REE predictive equations recommended by current obesity guidelines [Harris-Benedict, WHO/FAO/ONU and Mifflin-St Jeor (MJ)] and/or developed for obese patients (Muller, Muller BC, Lazzer, Lazzer BC), focusing on the effect of comorbidities on the accuracy of the equations. Data on REE measured by indirect calorimetry and body composition were collected in 4,247 obese patients (69% women, mean age 48 ± 19 years, mean BMI 44 ± 7 Kg/m^2) admitted to the Istituto Auxologico Italiano from 1999 to 2014. The performance of the equations was assessed in the whole cohort, in 4 groups with 0, 1, 2, or ≥ 3 comorbidities and in a subgroup of 1,598 patients with 1 comorbidity (47.1% hypertension, 16.7% psychiatric disorders, 13.3% binge eating disorders, 6.1% endocrine disorders, 6.4% type 2 diabetes, 3.5% sleep apnoea, 3.1% dyslipidemia, 2.5% coronary disease). In the whole cohort of obese patients, as well as in each stratum of comorbidity number, the MJ equation had the highest performance for agreement measures and bias. The MJ equation had the best performance in obese patients with ≥3 comorbidities (accuracy of 61.1%, bias of −89.87) and in patients with type 2 diabetes and sleep apnoea (accuracy/bias 69%/−19.17 and 66%/−21.67 respectively), who also have the highest levels of measured REE. In conclusion, MJ equation should be preferred to other equations to estimate the energy needs of Caucasian morbidly obese patients when measurement of the REE cannot be performed. As even MJ equation does not precisely predict REE, it should be better to plan the diet intervention by measuring rather

than estimating REE. Future studies focusing on the clinical differences that determine the high inter-individual variability of the precision of the REE predictive equations (e.g., on the organ-tissue metabolic rate), could help to develop predictive equations with a better performance.

Keywords: resting energy expenditure, indirect calorimetry, comorbidities, REE predictive equations, obesity

INTRODUCTION

The treatment of obesity requires creating an energy deficit through caloric restriction, physical activity, or both (1). The energy needs are based on the resting energy expenditure (REE) which is the major component of the total daily energy expenditure and reflects the energy required to maintain the vital functions at resting state. The major determinant of the REE is the fat- free mass (FFM), which is composed by the sum of two moieties, one with a high metabolic rate (skeletal muscle and visceral organs accounts for 16 and 84% of REE respectively) and one with a low metabolic rate (bone and extracellular mass) (2).

Early studies suggested that obesity is due to a predisposition to a lower REE that contributes with sedentary lifestyles to a positive energy balance (3). Conversely, subsequent studies demonstrated that REE levels are high in non-sarcopenic obese individuals because the body weight increase is associated with a concomitant increase in fat mass (FM) and FFM, and the FM also is an independent predictor of REE with greater effect in subjects with higher FM amount (3). Although it has not been established with certainty what the FFM compartment is the main predictor of REE in obese subjects, some studies support a greater role for the visceral component than muscular mass (2). The REE is commonly measured with indirect calorimetry (4), which however it is not used in the majority of outpatients because it is expensive and time consuming. Thus, in the clinical practice energy needs are estimated using REE predictive equations based on body weight, height, age and sex and/or body composition parameters. Several predictive equations have been proposed in the last century, however current guidelines for the management of obesity still recommend equations developed more than 30 years ago (Harris Benedict 1984, the FAO/WHO/ONU 1985) on populations definitely different from the nutritional point of view from the current ones (5, 6). Since the evidence that the application of Harris Benedict 1984 and FAO/WHO/ONU 1985 for determining REE in overweight/obese subjects tends to overestimate the true metabolic rate, the Academy of Nutrition and Dietetics recommends the use of the Mifflin-St Jeor equation in obese individuals (7–9). In a large cohort of obese subjects free of metabolic or endocrine diseases such as diabetes, hypertension, and hypothyroidism, Lazzer et al developed predictive equations based on age, sex and FFM finding that the measured REE was correctly predicted in 56% of adult subjects. The authors also reported that the accuracy of the predictive equation was not improved by the inclusion of FM in the formula (10).

A very recent external validation of REE predictive equations reported that the accuracy of the formulas decreases going from normal-weight to class 3 obesity (11). This suggests that in morbid obesity, there are factors affecting the REE that are not captured by the available equations. Considering that the probability of having multiple comorbidities increases with the degree of obesity, it could be hypothesized that one of the factors that affects the predictive capacity of the equations is linked to the altered metabolic rate of organs compromised by specific pathologies. In this regard, the REE is altered in patients with cardio-metabolic diseases such as type 2 diabetes, hypertension and sleep apnoea (12–14). For this reason, Huang et al. included a correction factor in a predictive equation specific for patients with type 2 diabetes (14).

In the present cross sectional study, we compared in a large cohort of morbidly obese patients, the accuracy of REE predictive equations recommended by current guidelines and/or developed for obese patients, focusing on the presence and type of comorbidity.

MATERIALS AND METHODS

Study Design and Participants

A cross sectional study was carried out on 4,247 adult Caucasian obese and morbidly obese patients admitted for a weight loss intervention from 1999 to 2014 at the IRCCS Istituto Auxologico Italiano, Piancavallo (Verbania, Italy). Patients were selected for being admitted for a weight loss intervention, being free of acute diseases and having a physiologic Respiratory Quotient (RQ between 0.71 and 1.0) during the indirect calorimetry measurement performed before the weight loss intervention. The following parameters were collected: age, sex, height, weight, FM, FFM, and REE assessed by indirect calorimetry, and the presence comorbidities (hypertension, type 2 diabetes, coronary disease, dyslipidemia, sleep apnoea, endocrine disorders psychiatric disorders and binge eating disorder). The IRCCS Istituto Auxologico Italiano Ethics Committee (https://www.auxologico.it/ricerca-formazione/comitato-etico, Via Ariosto 13, Milano, Italy, e-mail: comitato.etico@auxologico.it) approved the study and all subjects involved were informed and gave their signed consent to use data for research purposes.

Indirect Calorimetry and Body Composition

The REE was measured in the morning between 8 and 9 a.m. after a fasting period of 12 h in thermoneutral conditions (in a 22–25°C room) by an open-circuit, indirect computerized calorimetry (Vmax 29, Sensor Medics, Yorba Linda, CA, USA) which is periodically subjected to quality controls to ensure the reliability of the measures. The flow sensor calibration was completed daily after at least 30 min of warm up of

TABLE 1 | REE predictive equations used in the present study.

Harris Benedict 1984 (kcal/day)	*Male* 88.362 + 4.799*height (cm) + 13.397*weight (kg) − 5.677*age *Female* 447.593 + 3.098*height (cm) +9.247*weight (kg) − 4.330*age
Huang (kcal/day)	71.767 − 2.337*age + 257.293*sex (M = 1; F = 0) + 9.996*weight (Kg) + 4.132*height (cm) + 145.950*DM (non-diabetic = 0; diabetic = 1)
Mifflin-St Jeor (Kcal/day)	9.99*weight (Kg) + 6.25*height (cm) − 4.92*age + 166*sex (M = 1; F = 0) −161
Lazzer 2010 (kcal/day)	11*kg − 3*age + 272*sex (M = 1; F = 0) + 777
Lazzer BC 2010 (Kcal/day)	20*FFM (kg) − 2*age − 11*sex (F = 0; M = 1) + 841
Muller (BMI ≥ 30) (MJoule/day)	0.05*weight (kg) + 1.103*sex (F = 0; M = 1) − 0.01586*age + 2.924
Muller BC (BMI ≥ 30) (MJoule/day)	0.05685*FFM (kg) + 0.04022*FM (kg) + 0.808*sex − 0.01402*age + 2.818
FAO/WHO/ONU (Kcal/day)	*Male* 18–30 yrs 15.4*weight (Kg)−27*height (m) + 717 30–60 yrs 11.3*weight (Kg) + 16*height (m) + 901 ≥60 yrs 8.8 *weight (Kg) + 1,128*height (m) − 1,071 *Female* 18–30 yrs 13.3*weight + 334*height (m) + 35 30–60 yrs 8.7*weight − 25*height (m) + 865 ≥60 yrs 9.2*weight + 637*height (m) − 302

TABLE 2 | Sociodemographic and clinical characteristics of the whole sample of 4,247 obese patients.

Variable	
Age, years	47.66 ± 13.85
Male, *n (%)*	1,300 (31%)
Weight, kg	117.80 ± 24.03
Height, cm	162.79 ± 9.83
BMI, kg/m^2	44.33 ± 7.46
BMI classes, *n (%)*:	
30–35	319 (8%)
35–40	868 (20%)
40–45	1,302 (31%)
≥45	1,758 (41%)
Fat free mass, kg	63.6 ± 18.44
Fat mass, kg	56.3 ± 18.17
Concomitant diseases, *n (%)*	3,242 (76)
Hypertension, *n (%)*	2,095 (49)
Type 2 diabetes, *n (%)*	939 (22)
Coronary disease, *n (%)*	260 (6)
Dyslipidemia, *n (%)*	638 (15)
Sleep apnoea, *n (%)*	576 (14)
Endocrine disorders, *n (%)*	262 (6)
Psychiatric disorders, *n (%)*	597 (14)
Binge eating disorder, *n (%)*	438 (10)
REE measured, kcal/day	1875.41 ± 430.16
FAO/WHO/ONU, kcal/day	1989.8 ± 402.75
Harris Benedict 1984, kcal/day	1986.61 ± 412.97
Huang, kcal/day	1921.63 ± 362.92
Lazzer 2010, kcal/day	2013.14 ± 348.87
Lazzer BC 2010, kcal/day	2015.08 ± 371.84
Mifflin-St Jeor, kcal/day	1849.61 ± 346.88
Muller, kcal/day	2006.7 ± 370.96
Muller BC, kcal/day	1979.3 ± 431.03

Data are expressed as mean ± SD or percentage.

the Vmax calorimeter prior to measurement session. On each testing day, the calorimeter performed two calibration points using two reference gas mixtures (15% O_2/5%CO_2 and 26% O_2/0%CO_2) and the calibration of the environmental gases, allowing measuring the physiological range of the inspired and exhaled volumes. Subjects were physically inactive for at least 12 h and not smoking from at least 8 h. Subjects were awake and in supine position with the head placed in a rigid, transparent ventilated canopy. The respiratory exchange was measured for 30 min or until the steady state (defined as no variations higher than 5% during 5 consecutive min) had been reached. Data of the 10 min acclimation period were discarded. Minute-by-minute measurements of CO_2 (mL/min) expired and, O_2 consumed (mL/min) and RQ (VCO_2/VO_2) were recorded. The REE was calculated using the Weir equation [Kcal/d=1.44*(3.94VO_2+1.11VCO_2)].

The body composition was assessed by the bioelectrical impedance analysis (BIA 101 Anniversary, Akern, Florence Italy), in the morning after an overnight fast and no more than 2 days later the execution of the indirect calorimetry.

REE Predictive Equations

The REE predictive equations used in this study are reported in **Table 1**. We choose the equations recommended for overweight and obese subjects by national and international guidelines and/or developed in large cohorts of obese individuals (5–10, 14–16).

Statistical Analyses

Sociodemographic and clinical continuous variables were expressed as means ± standard deviation (SD) and categorical data as frequencies and proportions. The agreement between measured REE (reference) and REE obtained by the predictive equations was analyzed both graphical visualizations (limits of agreement, LOA) in the Bland-Altman plot (17) and several indices (accuracy, bias, concordance correlation coefficient, CCC, and Mean squared Deviation, MSD) (18). In brief, for each predictive equation, the Bland-Altman plot compares the predicted with the measured REE. Specifically, the comparison consists to plot the differences between the predicted REE and the measured REE on y-axis and the reference on x-axis (19). The LOA reported in the Bland-Altman plot give the range of discordance between predicted and reference that, with a

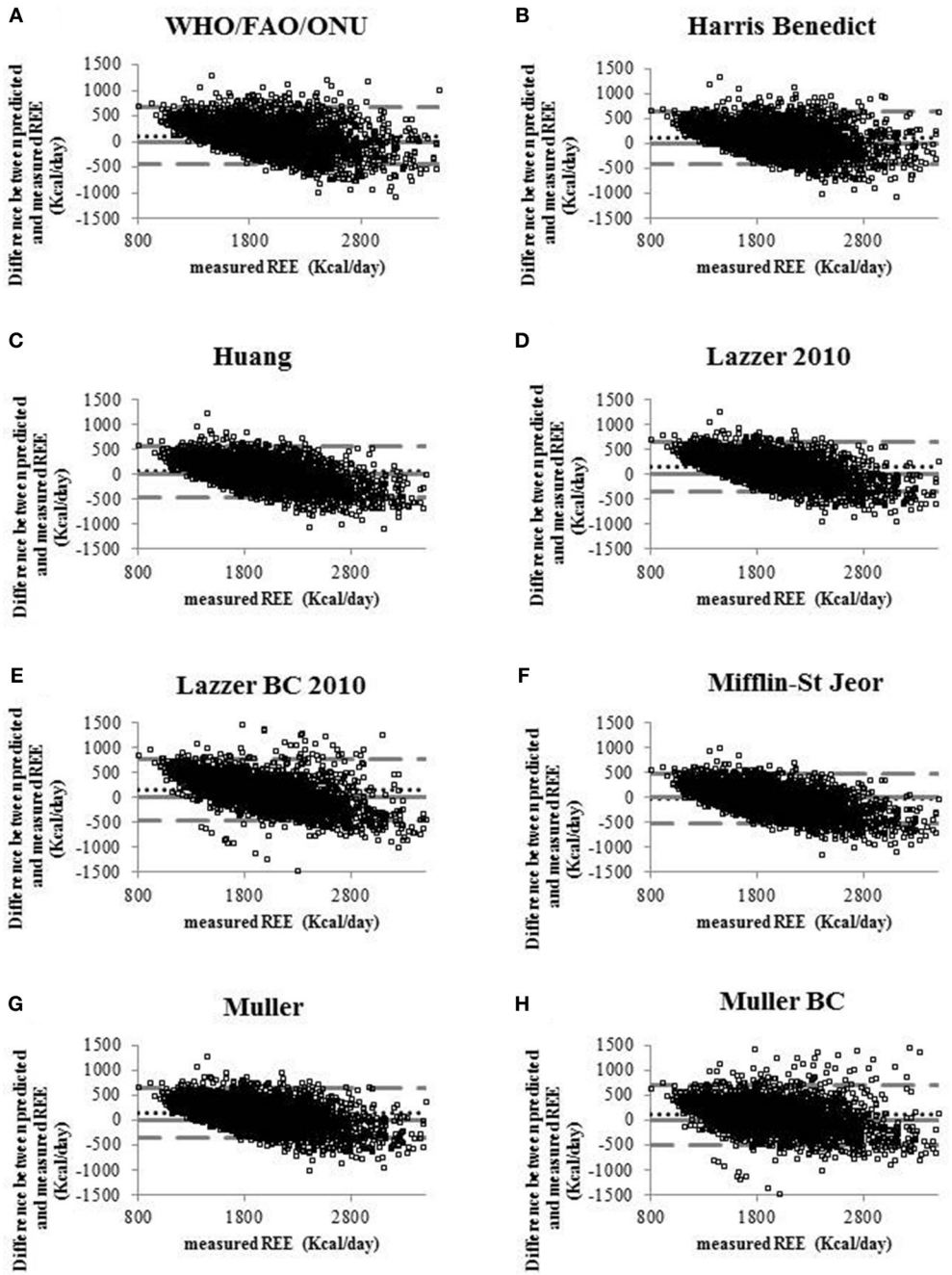

FIGURE 1 | Bland-Altman plots displaying the agreement between measured REE and the REE predicted by eight predictive equations **(A)** WHO/FAO/ONU, **(B)** Harris Benedict 1984, **(C)** Huang, **(D)** Lazzer 2010, **(E)** Lazzer BC 2010, **(F)** Mifflin-St Jeor, **(G)** Muller, **(H)** Muller BC equations. Continuous lines indicate the value of the difference equal to 0 that means that the REE predicted coincides with REE measured. Dotted lines indicate the level of agreement of predicted and measured REE. Pointed lines indicate the mean of the differences between predicted and measured REE (bias).

confidence of 1-α, comprises the true value of discordance. The accuracy is the proportion of patients in which the predicted REE falls in the range of acceptability (±10% of the measured REE).

The bias is the mean of the differences between predicted and measured REE; positive values suggest overestimates of the measurements obtained by the considered specific predictive equation and negative values suggest the opposite. The Concordance Correlation Coefficient (CCC), varying between 0 and 1, measures the concordance between the predicted and measured REE. Finally the Mean Squared Deviation (MSD) represents the expectation of the squared difference between predicted and measured REE, with low values suggesting good

agreement. All analyses were performed in the entire sample and in specific subgroups defined by number and type of comorbidities. Chi square and trend tests were used to test the differences between subgroups. All analyses were performed using SAS version 9.4 software (SAS Institute, Cary, NC, USA).

RESULTS

The **Table 2** describes the clinical characteristics of the cohort of 4,247 obese patients considered in the sample. Seventy eight percent of patients had a concomitant disease. Psychiatric diseases included personality disorders, anxiety and obsessive-compulsive and phobic disorders. Most of endocrine diseases (70%) were represented by hypothyroidism, on L-thyroxin treatment, or subclinical hypothyroidism.

Figure 1 shows the Bland-Altman plots for all predictive equations. The Mifflin-St Jeor equation had the lowest value of bias index which provides a mean measure of the overestimation or the underestimation of the specific equation compared to the measured REE (−25.80 kcal/day). These results fitted with the other agreement measures (accuracy, CCC and MSD), as reported in **Table 3**. The CCC of Mifflin-St Jeor equation was close to 80% with lowest value of MSD and the higher value of accuracy (56.18%). The accuracy of the Mifflin-St Jeor equation increases significantly in the tertiles of BMI (51.47, 57.93, and 58.08% in BMI <40, 40–45.9, and >46 kg/m^2 respectively, $p < 0.001$ for trend). Separation by sex and age tertiles did not affect the accuracy of Mifflin-St Jeor equation. When we stratified the sample by the number of comorbidities, 4 groups were considered (0, 1, 2, or ≥ 3 comorbidities). The proportion of patients of each group was 24, 38, 22, and 16%. Body mass index, measured REE and age (41.7 ± 13.7, 46.5 ± 13.9, 50.5 ± 12.6, and 55.3 ± 10.7 years, $p < 0.001$) increased from the group with 0 to the group with ≥3 comorbidities (**Table 4**). This finding was similar in the two sexes (data not shown). The accuracy of each predictive equation significantly improved with the increase in the number of comorbidities ($p < 0.0001$ for trend for each equation except for the Huang equation which was not significant). In each stratum, the Mifflin-St Jeor equation showed the higher performance for both agreement measures and bias (**Table 5**). In the more complicated obese patients, the Muller BC equation had a performance similar to the Mifflin-St Jeor equation (**Table 5**).

To verify whether the performance of the predictive equations was comorbidity-dependent, we measured the accuracy of the equations in 1,598 patients with only one comorbidity. Forty seven percent of them had hypertension, 16.7% psychiatric disorders, 13.3% binge eating disorders, 6.1% endocrine disorders, 6.4% type 2 diabetes, 3.5% sleep apnoea, 3.1% dyslipidaemia and 2.5% coronary disease (**Table 4**). The Mifflin-St Jeor equation had the highest performance values in patients with type 2 diabetes and sleep apnoea (accuracy/bias 69%/−19.17 and 66%/−21.67 respectively) (**Table 6**) who were also those with the highest levels of measured REE (**Table 4**).

The accuracy of Mifflin-St Jeor equation was significantly higher in diabetic than in non-diabetic patients (68.93 vs. 53.11%, $p < 0.01$) after adjustment for BMI, age and sex obtained by log-binomial regression model. This was observed also for patients with sleep apnoea where the accuracy was 66.07% compared to 33.93% in patients without sleep apnoea ($p = 0.0597$ after adjustment for BMI, age and sex).

TABLE 3 | Comparison of predicted with measured REE.

	Accuracy (%)	Bias		CCC	MSD
		(P-M)	%*		
FAO/WHO/ONU	47.12	114.39	6.1	0.75	89042.44
Harris Benedict 1984	49.28	111.20	5.9	0.78	79953.23
Huang	53.83	46.22	2.5	0.79	65021.93
Lazzer 2010	46.08	137.73	7.3	0.75	80354.97
Lazzer BC 2010	44.69	139.67	7.4	0.67	112628.42
Mifflin-St Jeor	56.18	−25.80	−1.4	0.79	63113.78
Muller	46.86	131.29	7.0	0.77	78981.30
Muller BC	49.12	103.89	5.5	0.74	97616.05

*CCC, Concordance Correlation Coefficient; MSD, Mean Squared Deviation; BC, Body composition; P, REE predicted; M, REE measured; *Mean difference percentage calculated as the mean difference between REE predicted and REE measured divided by the mean of REE measured.*

TABLE 4 | Values of measured REE and BMI according to the number and type of comorbidity.

Number of comorbidities	*N* subjects	Measured REE (kcal.day^{-1})	BMI (kg/m^2)
0	1,005	1782.2 ± 405.09	42.1 ± 6.7
1	1,598	1845.7 ± 420.98	43.9 ± 7.7
2	955	1940.1 ± 434.55	45.9 ± 7.5
≥3	689	1990.7 ± 442.06	46.5 ± 6.9
p-value trend		<0.0001	<0.001
Type of comorbidity for patients with only 1 comorbidity			
Hypertension	754	1841.6 ± 427.26	44.41 ± 7.90
Psychiatric disorders	267	1791.6 ± 383.11	42.45 ± 7.77
Binge eating disorders	213	1821.1 ± 388.96	42.59 ± 6.88
Endocrine disorders	114	1681.0 ± 328.20	42.40 ± 6.65
Type 2 Diabetes	103	2059.4 ± 413.07	46.30 ± 5.91
Sleep Apnoea	56	2194.9 ± 469.10	49.20 ± 8.87
Dyslipidemia	50	1879.5 ± 453.43	42.71 ± 6.27
Coronary disease	41	1803.4 ± 418.85	41.32 ± 7.63

DISCUSSION

Results of our study suggest that Mifflin-St Jeor equation has the highest performance for both accuracy and bias, particularly in patients with more severe and complicated obesity. The best accuracy of the Mifflin-St Jeor equation was recorded in patients with type 2 diabetes and sleep apnoea, whereas this formula has a very low accuracy in patients with hypertension who represent the largest percentage of the obese population. It must

TABLE 5 | Comparison of predicted with measured REE according to the number of comorbidity.

Number of comorbidities		Accuracy (%)	Bias		CCC	MSD
			(P-M)	%*		
0 (*N* = 1,005)	FAO/WHO/ONU	39.60	181.47	11.84	0.72	101766.30
	Harris Benedict 1984	43.28	154.77	10.34	0.75	85548.99
	Huang	51.74	49.07	4.58	0.79	59539.87
	Lazzer 2010	40.70	170.55	11.74	0.72	85803.17
	Lazzer BC 2010	36.12	202.58	13.82	0.62	134676.82
	Mifflin-St Jeor	51.74	28.22	3.34	0.79	59325.23
	Muller	41.69	163.38	11.10	0.73	84119.36
	Muller BC	41.19	143.42	9.36	0.71	111088.29
1 (*N* = 1,598)	FAO/WHO/ONU	44.49	125.90	8.75	0.72	95909.49
	Harris Benedict 1984	47.37	114.46	7.96	0.76	83854.69
	Huang	53.13	30.11	3.55	0.77	66744.29
	Lazzer 2010	43.99	143.20	10.03	0.73	85078.00
	Lazzer BC 2010	41.43	174.45	11.97	0.63	131743.88
	Mifflin-St Jeor	54.13	−12.12	1.20	0.77	65840.58
	Muller	44.49	136.66	9.45	0.74	83757.19
	Muller BC	44.81	135.80	8.91	0.71	113600.30
2 (*N* = 955)	FAO/WHO/ONU	52.98	78.37	5.45	0.79	77409.69
	Harris Benedict 1984	53.61	86.04	5.56	0.81	73905.39
	Huang	56.34	37.98	3.49	0.81	64472.79
	Lazzer 2010	50.58	116.40	7.79	0.79	72064.48
	Lazzer BC 2010	51.20	84.33	6.30	0.75	85019.39
	Mifflin-St Jeor	60.73	−59.30	−1.72	0.81	60849.02
	Muller	50.68	111.66	7.32	0.80	71655.95
	Muller BC	55.71	66.97	4.71	0.79	80940.12
≥3 (*N* = 689)	FAO/WHO/ONU	56.02	39.75	3.49	0.80	70990.02
	Harris Benedict 1984	56.46	74.98	4.96	0.82	71411.12
	Huang	55.01	90.85	6.26	0.79	70053.12
	Lazzer 2010	52.54	106.74	7.27	0.78	73206.67
	Lazzer BC 2010	55.73	43.98	4.30	0.76	74767.81
	Mifflin-St Jeor	61.10	−89.87	−3.15	0.80	65711.27
	Muller	54.57	99.22	6.67	0.80	70826.07
	Muller BC	61.54	23.37	2.85	0.80	64353.62

**Mean difference percentage calculated as the mean difference between REE predicted and REE measured divided by the mean of REE measured. CCC, Concordance Correlation Coefficient; MSD, Mean Squared Deviation; BC, Body composition; P, REE predicted; M, REE measured.*

be emphasized, however, that the accuracy of the Mifflin-St Jeor equation is far from being an ideal tool to correctly predict REE, leading to the conclusion that in obese individuals, it is better to plan a diet intervention by measuring rather than estimating the REE. In this regard, a dietary program set on the REE measured with the indirect calorimetry was shown to be more effective than based on the Harris Benedict equation in promoting weight loss in overweight or obese subjects (20). It would be interesting in the future to verify the efficacy of nutrition plans based on the Mifflin-St Jeor equation. Our results support the use of the Mifflin-St Jeor equation when the indirect calorimeter is unavailable, as recommended by the Academy of Nutrition and Dietetics for obese individuals (8, 9). This equation allows reducing the error brought by using the Harris-Benedict and FAO/WHO/ONU formulas that are still the most used formulas in the clinical practice regardless of the subject's phenotype. In agreement, a recent systematic literature reviews concluded that the Mifflin-St Jeor equation gives the most acceptable REE prediction in obese subjects (21). We observed that the performance of the formulas and in particular of Mifflin-St Jeor equation improves with the increase in the number of comorbidities, which was also associated with the increase in BMI and energy needs. The more likely hypothesis for the good performance of Mifflin St-Jeor equation is that the RENO Diet-Heart cohort of obese individuals in whom this equation was developed, though described as healthy (7), probably had

TABLE 6 | Accuracy (%) and Bias (kcal) of the REE predictive equations according to the single comorbidity in 1,598 patients with only one comorbidity.

	Hypertension		Psychiatric disorders		Binge eating disorders		Endocrine disorders		Type 2 Diabetes		Sleep Apnoea		Dyslipidemia		Coronary disease	
	Accuracy	Bias	Accuracy	Bias	Accuracy	Bias	Accuracy	Bias	Accuracy	Bias	Accuracy	Bias	Accuracy	Bias	Accuracy	Bias
FAO/WHO/ONU	48.01	87.39	39.33	141.50	39.91	180.75	38.60	177.60	49.51	156.89	42.86	191.28	46.00	137.35	41.46	122.67
Harris Benedict 1984	48.67	90.62	44.19	109.77	46.48	143.09	46.49	155.68	57.28	154.51	42.86	203.47	42.00	121.28	39.02	89.48
Huang	51.99	12.45	54.31	12.97	57.28	34.50	47.37	65.85	56.31	164.58	57.14	28.89	56.00	24.40	43.90	15.29
Lazzer 2010	43.63	133.85	39.70	136.02	44.13	156.96	43.86	192.41	56.31	143.45	48.21	161.71	42.00	143.14	43.90	127.94
Lazzer BC 2010	40.45	189.14	38.95	179.33	39.91	190.87	33.33	222.50	64.08	54.61	51.79	47.81	40.00	153.86	36.59	152.53
Mifflin-St Jeor	51.72	−43.03	54.31	16.57	57.28	39.78	50.00	55.14	68.93	−19.17	66.07	−21.67	52.00	−24.84	41.46	−41.03
Muller	45.09	121.51	37.83	133.86	43.66	157.98	44.74	183.90	59.22	149.52	46.43	175.71	42.00	133.32	43.90	109.77
Muller BC	43.77	150.07	42.32	135.32	44.60	143.24	37.72	151.33	37.72	151.33	48.21	75.63	50.00	112.68	39.02	68.14

similar, but under evaluated, chronic comorbidities than our cohort.

In conditions with highest energy needs such as obese individuals with ≥3 comorbidities and type 2 diabetes, the Muller BC equation had an accuracy similar to that of Mifflin-St Jeor equation likely because it takes into account the FM, whose inflammation may increase energy requirements.

Though BMI may be the major determinant of energy needs, specific pathologies seem to influence the measured REE at similar levels of BMI. This finding suggests that specific comorbidities may be accompanied by alterations of the organ metabolic rate/functional mass that modify the energy needs in a way not currently predicted by the equations. It is difficult to ascertain how much a greater measured REE contributes to improvement in the accuracy of the predictive equations, because REE, BMI and clinical severity are parameters highly interrelated. Alternatively, as all equations are based on a linear regression model, it is possible that mathematical reasons justify the parallel increase in the accuracy of the formulas and the measured REE. Since even the application of an artificial neural network did not allow to substantially improve the REE prediction in obese subjects (22), it would be more appropriate to change the approach when prescribing a diet, considering also the ability to mobilize the energy stores over the time rather than the energy demand at rest (23).

The strength of our study is that it was conducted in a large sample of unselected obese patients characterized for type and number of comorbidities representing the more frequent condition in which energy requirements are estimated. Several limitations of the study must be underlined. First, the sample was composed by a homogeneous ethnic group of Caucasian origin and whether formulas perform similarly in other ethnic groups should be verified. Second, we did not collect information on the ongoing pharmacological therapy, and thus we cannot distinguish the effect of comorbidities from that of the associated pharmacological therapy. This should be assessed in future studies.

In conclusion, the Mifflin-St Jeor equation, though far from being an ideal tool to precisely predict the REE, should be preferred to other equations to estimate the energy needs of Caucasian morbidly obese patients when the measurement of REE cannot be implemented.

Future studies focusing on the clinical differences that determine the high inter-individual variability of the precision of the REE predictive equations (e.g., on the organ-tissue metabolic rate), could help to develop predictive equations with a better performance.

AUTHOR CONTRIBUTIONS

RC: data analysis and interpretation, drafting the article, final approval of the version to be published. DS: statistical analysis and data discussion, final approval of the version to be published. AB: data collection and critical revision of data, final approval of the version to be published. MS: data collection, final approval of the version to be published. AT:

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REVIEW
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No Guts, No Loss: Toward the Ideal Treatment for Obesity in the Twenty-First Century

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Over the last century, our knowledge of the processes which control appetite and weight regulation has developed significantly. The understanding of where gut hormones fit into the control of energy homeostasis in addition to the rapid advancement of pharmacotherapeutics has paved the way for the development of novel gut hormone analogs to target weight loss. Currently, bariatric surgery remains the most efficacious treatment for obesity. The emergence of gut hormone analogs may provide a useful non-surgical addition to the armamentarium in treating obesity. Simply targeting single gut hormone pathways may be insufficiently efficacious, and combination/multiple-agonist approaches may be necessary to obtain the results required for clear clinical impact.

Keywords: gut hormones, obesity, GLP-1, Oxyntomodulin, glucagon, gastric bypass surgery, diabetes mellitus

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Obesity is the accumulation of excessive fat that may impair health. It is the driver for several serious non-communicable diseases, including type 2 diabetes, cardiovascular disease, hypertension, dyslipidaemia, cancer, obstructive sleep apnoea and musculoskeletal problems. Aetiologically, obesity derives from the interaction of multiple genetic factors that bias metabolism toward the accumulation of fat, combined with environmental factors including the supply of easily available energy-dense food and reduced opportunities for physical activity. The prevalence of obesity is increasing year on year. Global estimates for obesity prevalence have tripled from 4.3% (1975) to 13.2% (2016) (1).

CURRENT OPTIONS FOR OBESITY

Lifestyle modifications remain the first line treatment for obesity, with evidence from large studies demonstrating only a modest benefit in outcomes. For example, meta-analysis of studies with a minimum of 1 year follow-up demonstrate 5–9% short term weight loss and 3–6% long term weight loss (2). It is possible to lose much more weight with incentivized high-intensity lifestyle changes, however the weight loss is often not sustainable due to counter-regulatory changes after such weight loss. This was highlighted by the long-term follow up of participants who participated in a weight loss competition, losing an average of 58.3 ± 24.9 kg at the end of the competition (3). Participants subsequently exhibited significant metabolic adaptations to counteract weight loss efforts, in particular a sustained reduction in energy expenditure up to 6 years following initial weight loss. This led to weight regain despite continued lifestyle changes. Strikingly, this reduction in energy expenditure persisted despite weight regain. This is a clear (if somewhat extreme) example of how the body defends itself against weight loss. Current lifestyle recommendations are not a

sufficiently effective and/or durable strategy for weight loss and patients require other treatment options.

The choices for the pharmacological treatment of obesity have until recently been limited to orlistat, a pancreatic lipase inhibitor preventing fat absorption within the gut. Average weight loss is around 3% over 1 year, but patient perseverance with treatment is limited due to undesirable gastrointestinal side effects (4). Treatments such as sibutramine and rimonabant have been withdrawn due to psychiatric and cardiovascular side effects respectively (5, 6). Lorcaserin (Belviq®) and phentermine/topiramate (Qsymia®) are both licensed in the US but are not currently licensed in Europe. Bupropion/naltrexone has been approved in the US under the trade name Contrave® and was more recently approved in the European Union in 2015 under the trade name Mysimba®. Overall, these pharmacological treatments are characterized by relatively weak efficacies (5–10% weight loss) and potential side effects, including the possible exacerbation of anxiety or depression, which are common co-morbidities in patients with obesity.

Bariatric treatment of obesity is established as the most effective method of obtaining sustained weight loss. A recent long-term (12-year follow up) study of patients with severe obesity undergoing Roux-en-Y gastric bypass, showed an average weight loss from baseline of 45 kg at 2 years, 36.3 kg at 6 years, and 35 kg at 12 years (7). The data from the Swedish Obesity Study shows that bariatric surgery improves mortality, and reduces the rate of cardiovascular events, cancer and diabetes (8). Long-term follow-up data also show that for obese patients with diabetes, bariatric surgery is more effective than medical therapy alone in improving glycaemic control, lipid profile and quality of life, with up to 3 in 10 patients obtaining a long-term remission, defined as a HbA1c of $\leq$6.5% without anti-diabetic medication (9). Furthermore, mortality rates associated with bariatric surgery are low, similar to a laparoscopic cholecystectomy operation. Despite the highly effective outcomes with bariatric surgery, there are associated post-surgical complications such as post-prandial hypoglycaemia, nutritional deficiencies, and psychosocial issues which may cause long-term morbidity and should not be overlooked (10). In addition, surgery does require considerable resources in terms of specialist surgeons, facilities and follow-up; these limit the deliverability of bariatric surgery to all patients with obesity. Lastly, surgery is a "one size" solution where patients obtain varying levels of weight loss ranging from the inadequate to the excessive from the same procedure, i.e., "one size does not fit all."

Over the last 20 years, a gap has emerged between relatively ineffective pharmacological therapy and effective (but non-titratable and non-reversible) surgical treatments (**Figure 1**). Within this gap, there is a need for pharmacotherapies capable of inducing significant weight loss, sustaining weight loss and which are proven in reducing comorbidities such as the risk of developing overt diabetes and cardiovascular disease. In the following review, we examine the state-of-the art in gut hormone research, and the current considerable efforts to turn these into practical and safe therapies for obesity.

APPETITE AND ENERGY HOMEOSTASIS

The long-term control of body weight is tightly regulated through various homeostatic physiological processes to allow the body to conserve energy. Traditionally, food consumption is thought of in terms of short and long-term signals. In the acute setting, food consumption is regulated by sensory stimuli, gut mechanoreceptors, nutrient concentrations in the plasma, and changes in gut hormones secreted by specialized enteroendocrine cells. Longer term adiposity signals, for example, leptin and insulin, also influence feeding behavior. These homeostatic mechanisms rely on gut-neuronal circuits or the "gut-brain axis" allowing signaling between the periphery and central nervous system to coordinate systemic changes in our physiology (**Figure 2**). Both peripheral circulating peptides and vagal afferents are important in the signaling pathway controlling appetite. The main central regions where "anorexic" or "orexigenic" signals converge include brainstem regions including the dorsal vagal complex (DVC) in the medulla and the hypothalamic nuclei, most importantly the arcuate nucleus. Of the neuronal cell populations within the arcuate nucleus, two leptin-sensitive neuronal cell subtypes are characterized by the expression of specific weight regulating neuropeptides. The first contains Neuropeptide Y (NPY) and Agouti-related peptide (AgRP). The second contains alpha-melanocyte stimulating hormone (alpha-MSH) and cocaine- and amphetamine regulated transcript (CART). Activation of the orexigenic NPY/AgRP neurons leads to food intake, partly by NPY activation of neuropeptide Y1R receptors and partly by AgRP inverse agonism of the MC4R receptor. On the other hand, activation of the anorexigenic alpha-MSH/CART neurons inhibits food intake via activation of MC4R by alpha-MSH. Both pathways operate exclusively and receive signals via the nucleus of the solitary tract (NTS) and the dorsal vagal complex (DVC) of the brainstem, relaying important afferent vagal signals from peripheral gut hormone receptors, for example in the vagal innervation of the hepatic portal vein. The circumventricular organs, such as the median eminence, subfornical organs and area postrema, act as points that allow access for peripheral peptides through the blood-brain barrier or may themselves have receptors for the gut hormones, transducing these peripheral signals into the CNS (e.g., area postrema to the NTS). In addition to homeostatic mechanisms, food intake is controlled by regions of the brain regulating hedonistic pathways, circadian rhythms and cultural/learned experiences and therefore the hypothalamic nuclei also receive important signals from the cortex and mesolimbic system.

GUT HORMONES

The impact of gut hormones on the regulation of appetite was discovered early on when N.F. Maclagan in 1937 investigated the effect of a crude preparation of intestinal mucosa, called enterogastrone which was administered to rabbits at a dose of 2–3 mg/kg (11). He found a transient reduction in food intake in the rabbits and reported this extract was not active

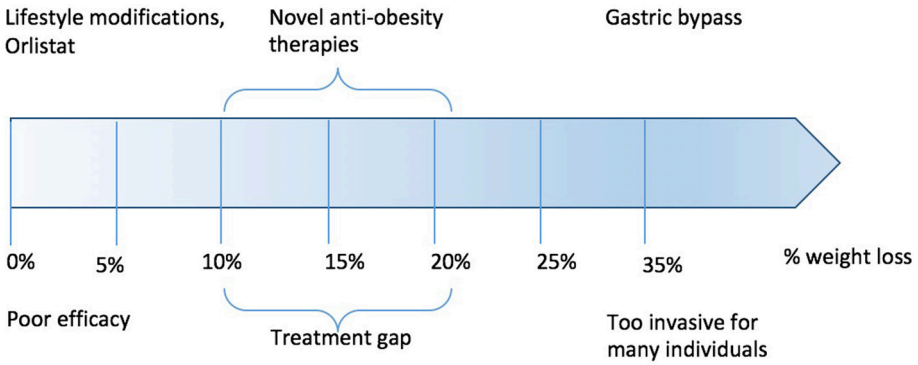

FIGURE 1 | Treatment gap in obesity therapy.

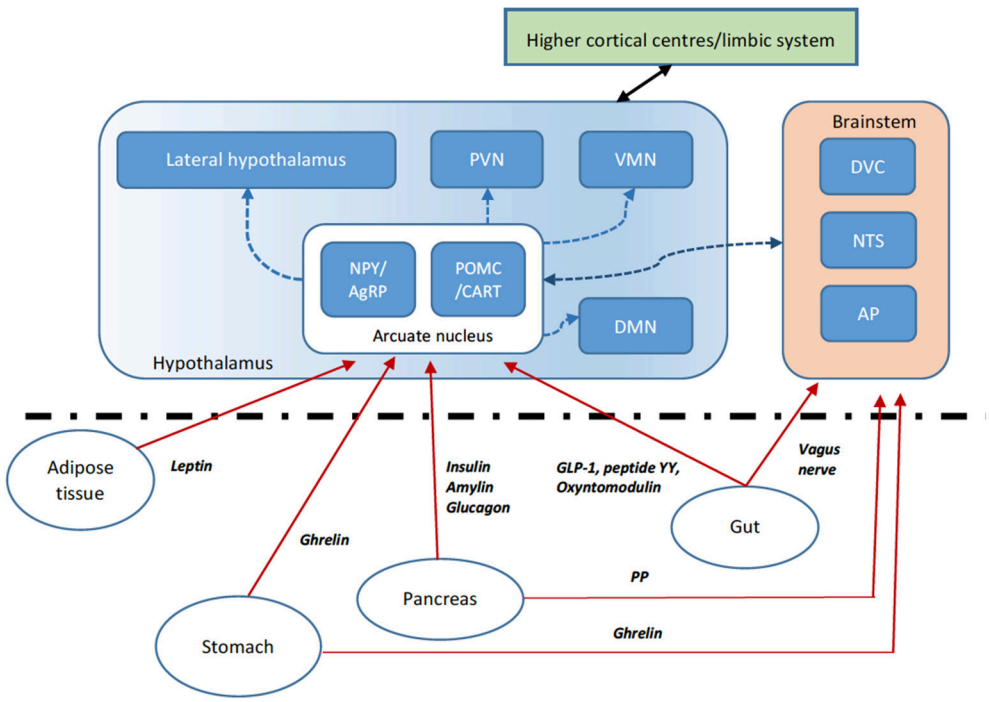

FIGURE 2 | Gut brain axis schematic. The hypothalamus integrates anorexigenic and orexigenic signals. Peripheral signals such as gut peptides, leptin from adipose tissue and pancreatic signals are able to cross the blood-brain barrier at the median eminence (a circumventricular organ) or activate their cognate receptors at other circumventricular organs such as the area postrema (AP). These signals relay to the hypothalamic arcuate nucleus (ARC) via direct activation or indirectly via brainstem regions including the dorsal vagal complex (DVC) and nucleus tractus solitarus (NTS). Within the ARC, two key populations of neurons include those that express the orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP—an inverse agonist of the melanocortin receptor MC4R) and those expressing the anorexigenic pro-opiomelanocortin (POMC), processed to the MC4R agonist alpha-MSH, and cocaine- and amphetamine regulated transcript (CART). The ARC signals to second-order neurons in various hypothalamic nuclei including the paraventricular nucleus (PVN), dorsomedial nucleus (DMN), ventromedial nucleus (VMN) and lateral hypothalamic area (LHA). Second-order neurons express anorexigenic and orexigenic neuropeptides further modulating appetite and energy homeostasis. Vagal afferents also signal directly to brainstem regions. Higher central nervous system centers including the mesolimbic pathways also signal to the hypothalamic nuclei.

by mouth. This observation is now understood to be due to the rapid degradation of gut peptides in the stomach, a process which to this day limits the widespread use of gut hormone therapy in obesity pharmacotherapy. Since Maclagan's early discovery, several gut hormones have been isolated and described (**Table 1**).

CCK—THE FIRST "SATIETY SIGNAL"

The first isolated gut hormone to be investigated was cholecystokinin (CCK), a peptide released by the neuroendocrine I-cells in the duodenum and jejunum in response to fat and protein rich meals (12). The "enterogastrone" preparation used

TABLE 1 | Gastrointestinal hormones regulating food intake.

Gastrointestinal hormone	Peptide family	Site of release	Stimulated by	Receptor(s)	Role in weight regulation
Cholecystokinin (CCK)	Gastrin/CCK	Intestinal I-cells	Fat and protein rich meals	CCK1R/CCK2R	Increased satiety.
Glucagon-like peptide-1 (GLP-1)	Preproglucagon	Intestinal L-cells	Macronutrient intake	GLP-1R	Increased satiety, glucose stimulated insulin secretion, reduced gastric emptying.
Oxyntomodulin	Preproglucagon	Intestinal L-cells	Macronutrient intake	GLP-1R/GCGR	Increased satiety, glucose stimulated insulin secretion, increased energy expenditure.
Glucagon	Preproglucagon	Pancreatic alpha cells	Protein intake, stress, fasting	GCGR	Increased satiety, lipolysis, gluconeogenesis, glycogenolysis.
Peptide tyrosine tyrosine (PYY)	PP-fold	Intestinal L-cells	Macronutrient intake	Neuropeptide Y2R (PYY_{3-36}). Y1R and Y5R (PYY_{1-36})	Increased satiety.
Pancreatic Polypeptide (PP)	PP-fold	Pancreatic PP cells	Macronutrient intake	Neuropeptide Y4R	Increased satiety.
Amylin	–	Pancreatic beta cells	Macronutrient intake	AMY1a, AMY2a and AMY3a	Increased satiety, reduced gastric emptying, modified food reward.
Ghrelin	Ghrelin	Stomach X/A-like cells	Fasting	GHSR	Increased food intake.

by Maclagan in his experiments contained CCK (13). Gerard Smith and colleagues showed that intraperitoneal injection of CCK into fasted rats inhibited food intake by 50% (14). To further understand the physiological role of CCK, the feeding behavior of rats with chronic gastric fistulas were studied. In a series of these experiments, CCK was proposed as the first "satiety signal" (15). CCK, in addition to inhibition of food intake, delays gastric emptying, stimulates pancreatic enzyme secretion and gall bladder contraction. The finding that CCK is a satiety signal led to research into the peptide's potential as an obesity therapy. However, CCK receptor agonists such as loxiglumide did not apparently show any anorectic activity in human volunteers and therefore their development has been abandoned (16).

GLP-1 AND ITS ANALOGUES AS CURRENT CLINICAL TREATMENTS FOR DIABETES AND OBESITY

The most well-known therapeutic gut hormone is GLP-1 which has been developed into analogs for the treatment of type 2 diabetes mellitus over the past 20 years. GLP-1 is released from the neuroendocrine L-cells of the gut. It is the product of post-translational processing of preproglucagon, by prohormone convertase 1 (PC-1) and the peptidyl-glycine alpha-amidating monooxygenase (PHM) which adds the C-terminal amide (**Figure 3**). A variety of GLP-1 variants are secreted *in vivo* including the inactive forms GLP-1_{1-37} and GLP-$1_{1-36}NH_2$, in addition to the active forms, GLP-1_{7-37} and GLP-$1_{7-36}NH_2$ (18). GLP-1's role in mediating the "incretin" effect, whereby insulin secretion is enhanced when enteral glucose is given in comparison to parenteral glucose was observed early on. Wolfgang Schmidt and colleagues first postulated that GLP-1 stimulated insulin secretion and demonstrated that $GLP_{1-36}NH_2$ was able to stimulate glucose-dependent insulin release from isolated rat pancreatic islets, albeit at high concentrations (19). Not long after this, GLP-$1_{7-36}NH_2$ was shown to be a much more potent incretin in humans (20). The interest in this peptide as an obesity therapy followed *in vivo* experiments whereby GLP-$1_{7-36}NH_2$ was administered intracerebroventricularly (ICV) in rats, leading to a dose-dependent reduction in food intake (21). Following on from this, continuous subcutaneous infusion of GLP-$1_{7-36}NH_2$ in both lean and obese humans was shown to cause an increase in satiety and reduction of food intake (22, 23). In contrast to CCK, GLP-$1_{7-36}NH_2$ retained its anorectic effects during continuous infusion and when administered at a dose of 4.8 pmol/kg for 6 weeks led to an average weight loss of 2% (24).

One of the major breakthroughs in this area came when Buckley and Lundquist described the degradation of GLP-1 by cleavage of the N-terminal 2 residues from the peptide (25). It was later found that dipeptidyl peptidase-IV (DPP4) was responsible for this inactivation (26). Around the same time, the GLP-1R agonist exendin-4/exenatide was isolated from the saliva of the Gila monster lizard *(Heloderma suspectum*) by Eng et al. (27). This was developed into the first marketed GLP-1 analog (Byetta®) due to its resistance to DPP4 degradation, extending its half-life and making it practicable as a treatment. Since these discoveries, several GLP-1 analogs have been developed with different structural modifications to implement DPP4 resistance and prolong their half-lives. These GLP-1 analogs are mostly licensed for the treatment of type 2 diabetes, including liraglutide (Victoza®), exenatide (Byetta®), and lixisenatide (Lyxumia®). Longer acting preparations are available and include exenatide LAR (Bydureon®), albiglutide (Tanzeum®), semaglutide (Ozempic®), and dulaglutide (Trulicity®).

The advantage of the GLP-1 analogs over other types of diabetes treatment is the associated weight loss. For example,

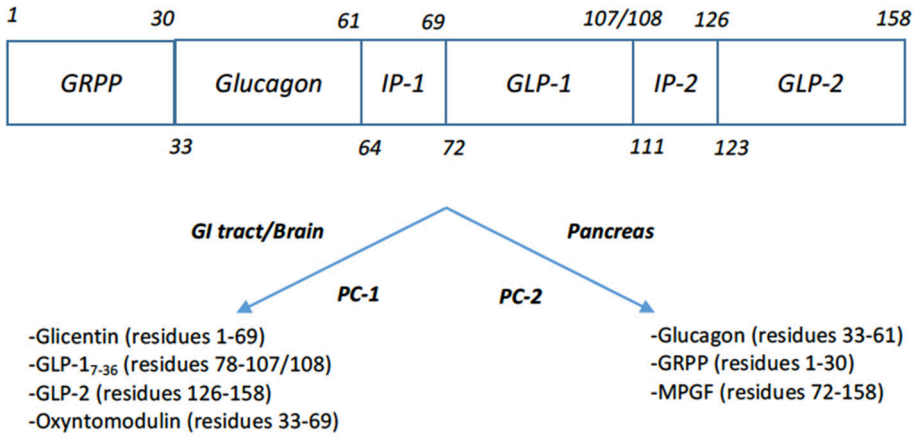

FIGURE 3 | Post-translational products of Preproglucagon (17). Tissue specific processing by proprotein convertase 1 (PC-1) in the intestine and brain of proglucagon leads to formation of glicentin, GLP-1, GLP-2, oxyntomodulin. Note that GLP-1's C-terminal residue 108 is processed to the C-terminal amide. Processing of proglucagon by proprotein convertase 2 (PC-2) in the pancreas leads to the formation of glucagon, glicentin-related polypeptide (GRPP), and major proglucagon fragment (MPGF).

exenatide and its longer acting preparation exenatide LAR (encapsulated in poly(lactic-co-glycolic) microspheres), lead to mean weight reductions of 3.7 and 3.6 kg, respectively, over 30 weeks in a comparative study (28). When given via an annually renewed osmotic mini-pump, exenatide can reduce weight by 2.4–4.2 kg in obese type 2 diabetic patients (29). Liraglutide has a longer half-life (13 h) than exenatide, owing to the linkage of a palmitate fatty acid group by a glutamic acid spacer at position 26, therefore increasing binding to circulating albumin. Licensed doses used in clinical practice range from 0.6 to 1.8 mg daily. Liraglutide 1.8 mg has been shown to have a similar effect on weight loss to exenatide, with weight loss of 3.24 kg over 26 weeks (30). The weekly analog semaglutide, at doses of 0.5 or 1 mg weekly, has also been shown to cause mean weight reductions of 3.5-6.1 kg in trials in obese type 2 diabetics (31).

GLP-1 analogs have also been investigated as a treatment for obesity in non-diabetic patients. Astrup et al. initially showed that liraglutide at doses up to 3 mg daily reduced weight by 7.2 kg on average in a double-blind randomized placebo controlled 20-week proof of concept trial, however there was a dose-related nausea as the main side-effect (32). This proof of concept led to a 56-week randomized placebo-controlled trial in 3731 patients, where it was shown that 3 mg liraglutide led to a reduction in body weight by an average of 8.4 kg, versus the placebo group who lost an average of 2.6 kg (33). As a result, liraglutide 3 mg is now licensed as a treatment in non-diabetic obese patients (Saxenda®). Semaglutide has also been tested in non-diabetic obese subjects in a small double-blinded randomized cross-over study (at a dose of 1.0 mg weekly) and shown to reduce weight on average by 5 kg over 12 weeks in comparison with a 1 kg weight gain in the placebo arm (34). In a Phase II dose-ranging study in non-diabetic obese subjects, semaglutide was given at doses of up to 0.4 mg daily for 52 weeks and compared to liraglutide 3 mg daily: it was shown to reduce weight on average by up to 13.8% at the highest dose of 0.4 mg, compared to 7.8% with liraglutide (35).

Despite the modest weight loss effects described with the GLP-1 analogs, there are a significant proportion of patients who do not respond adequately to GLP-1 analogs in terms of weight loss: the SCALE study showed, for example, that 37% of patients assigned to liraglutide 3 mg either lost <5% baseline weight or even gained weight, in other words they were non-responders (33). Even with semaglutide 0.4 mg daily, the non-response rate was 17% (35). Another issue with GLP-1 analog therapy for obesity are the common gastrointestinal side effects (nausea, vomiting, diarrhea, constipation) which occur in up to 40% of patients, thus limiting the maximal doses that can be given.

THE PP-FOLD ANORECTIC GUT HORMONES: PEPTIDE YY, PANCREATIC POLYPEPTIDE

Peptide tyrosine tyrosine (PYY) is a peptide co-secreted with GLP-1 from the neuroendocrine L-cells of the intestine and is a member of the pancreatic polypeptide (PP) family of proteins. The PP family of proteins contains pancreatic polypeptide (PP), PYY, and the neurotransmitter neuropeptide Y (NPY), which have a common structural hair-pin fold. Full length PYY_{1-36} is processed by DPP4 to PYY_{3-36} which then selectively binds to Y2 neuropeptide Y receptors. PYY is secreted post-prandially and acts as a satiety signal (36). Administration of PYY by infusion in human volunteers leads to reduced food intake and this effect is retained in patients with obesity (37, 38). PYY has a short half-life, limiting its therapeutic practicability. Specific alterations to the PYY_{3-36} sequence can be made to obtain an analog with a prolonged duration of action. Long-acting analogs of PYY which enable weekly injections are being tested in Phase I (ClinicalTrials.gov Identifier NCT01515319). Another analog, with an alpha-helix stabilizing sequence and histidine residues, demonstrated efficacy in reducing food intake for up to 24 h in animal studies (39). Reversible PEGylation of PYY(3–36)

maintains its functional activity and significantly prolongs its half-life *in vivo* (40). An oral preparation of PYY_{3-36} has been tested and does reduce food intake during an *ad libitum* test meal, but did not affect total 24 h food intake after a single dose, suggesting that the effect of a single dose is short-lived (41).

Pancreatic polypeptide (PP) is a 36-amino acid peptide hormone released by the PP cells of the pancreas after a nutrient stimulus. It is secreted in proportion to the calories ingested and remains elevated for up to 6 h (42). Intraperitoneal injections of PP reduce food intake over a 4 h period in both lean and obese mice (43). A short 90-min infusion of PP significantly reduced food intake in healthy human volunteers, and this effect seems to last for 24 h, reducing cumulative food intake by a mean of 25% (44). A stable analog of pancreatic polypeptide, PP 1420, has undergone Phase I trials to confirm the tolerability of single ascending subcutaneous doses of the peptide, PP 1420, in healthy subjects (45). The 7TM Pharma PP analog did not lower body weight in Phase II trials and development of the drug appears to have been terminated.

AMYLIN AND ITS SYNERGISTIC EFFECTS

Amylin is a peptide hormone co-secreted with insulin by the pancreatic beta cells in response to nutrient stimulus. It is a 37-amino acid peptide, derived from an 89 amino-acid precursor protein, referred to as preProIAPP. PreProIAPP is cleaved at the N-terminus resulting in ProIAPP and which is subsequently processed by PC-2. It has various roles including inhibiting glucagon secretion, delaying gastric emptying and acting as a satiety signal. Initial *in vivo* studies showed that intrahypothalamic injection of rat amylin dose dependently reduced feeding in rats for up to 8 h (46). There is also some evidence that amylin is capable of influencing food reward pathways (47). Pramlintide, an analog of Amylin, has been modified to prevent the formation of fibrils preserving stability and solubility of the peptide and has been approved by the FDA for diabetes treatment but not specifically for obesity. In a double-blind placebo-controlled study over 4 months, patients lost approximately 3 kg on average (48). Interestingly, amylin appears to have a synergistic effect with leptin: pre-treatment with amylin reduces the leptin resistance observed in obesity and leads to a mean weight loss of 12.7% when given in combination with recombinant leptin in a proof of concept trial (49, 50). Newer amylin analogs such as davalintide, PEGylated amylin and dual amylin/calcitonin agonists are in pre-clinical development for diabetes/obesity treatment, as yet there are no published clinical trials (47).

INHIBITING GHRELIN TO REDUCE APPETITE

Ghrelin, a 28-amino acid peptide derived from preproghrelin, is secreted by the X/A like cells of the stomach and to a lesser extent the small intestine (51, 52). Through the action of ghrelin-O-transferase (GOAT), the peptide undergoes posttranslational acylation at the serine-3 residue linking it to octanoic acid (53). This modification allows the peptide to cross the blood-brain barrier and bind to its cognate receptor, the growth hormone secretagogue (GHS) receptor. Ghrelin levels rise to a maximum when fasting and fall after feeding (54). Ghrelin, unlike the other gut hormones, increases food intake when given to human volunteers (55), i.e., it is a "hunger hormone" that drives appetite. Consequently, there has been considerable interest in targeting the GHS receptor or inhibiting GOAT as an anti-obesity therapy, for example, utilizing antagonists, inverse agonists, ghrelin vaccines and GOAT enzyme inhibitors (56, 57). There has been varied success. For example, an *in vivo* study using a small molecule ghrelin receptor antagonist in diet-induced obese mice led to reduced food intake and weight loss of up to 15% (58). The ligand-independent constitutive activity of GHS receptor can be inhibited by inverse agonists to the receptor and one such drug has been trialed in Phase I. Interestingly this trial reports that the most common side effect is that of somnolence as well as a positive chronotropic effect on heart rate; these may limit the tolerability and long-term safety of the treatment, however this may open up the possibility that the drug is useful for insomnia (59).

OXYNTOMODULIN AND THE DUAL GLP-1/GLUCAGON AGONIST CONCEPT

Due to the limitations of using single gut hormones (for example GLP-1 alone), researchers have investigated the idea of using the synergism between certain gut hormones to obtain benefits in metabolism and weight regulation. The most well-developed example of this is the dual agonism of GLP-1 and glucagon. Glucagon is secreted by alpha-cells of the pancreatic islets after PC-2 processing of proglucagon (60). In contrast to GLP-1, glucagon's classical effects are to increase hepatic glucose output by stimulating gluconeogenesis and glycogenolysis (61), although knockout of the glucagon receptor in mice only modestly reduces glucose levels by 1–2 mmol/l (62). Glucagon inhibits food intake when given to humans (63).

The interest in glucagon as a partner for GLP-1 comes from three other observations. Glucagon increases energy expenditure, unlike GLP-1, and this effect is retained when the two are co-infused (64, 65). Although animal studies suggest that this thermogenesis is via the activation of brown adipose tissue (BAT), studies in human subjects show that glucagon does not increase BAT activation as assessed by infrared thermometry nor ^{18}F-FDG uptake (66). This BAT-independent effect of glucagon on energy expenditure may be due to substrate cycling (67), however direct evidence for this effect in humans is currently lacking. The second observation is that the acute hyperglycaemia from glucagon can be counter-balanced by GLP-1 co-administration (64). The third observation is that the co-infusion of GLP-1 and glucagon leads to a synergistic suppression of food intake (65). Therefore, dual agonism of glucagon and GLP-1 receptors promises to deliver weight loss efficacy beyond that seen with GLP-1 alone, via synergistic anorectic effects, increased energy expenditure and without any hyperglycaemia arising from the glucagon component.

The prototypical dual GLP-1/glucagon agonist is oxyntomodulin (OXM), which is a peptide hormone secreted by the neuroendocrine L-cells of the intestine. Like glucagon and GLP-1, it is a product of preproglucagon post translational processing by PC1 in the gut. It was first discovered following early experiments showing that intestinal extracts contained an activity similar to pancreatic glucagon. An isolated fraction with glucagon properties was termed "enteroglucagon," later found to include two peptides containing the entire glucagon sequence, glicentin and OXM (68). In addition to containing the glucagon sequence, OXM has an additional C-terminal 8-amino acid octapeptide (labeled IP-1 in **Figure 3**) (69). *In vitro* experiments show that OXM is a full agonist at both the GLP-1 and glucagon receptors, albeit with reduced binding affinities of 10- and 100-fold respectively compared to the cognate peptides (70, 71). Consistent with these receptor binding actions, research has shown that OXM potentiates glucose stimulated insulin secretion via the GLP-1 receptor and is an incretin in its own right (72, 73). Dakin and colleagues showed that ICV injection of OXM into rats inhibits feeding. Importantly, comparison with a "pair-fed group" given the mean food intake of the OXM-treated group showed that OXM treatment led to enhanced weight loss relative to the pair-fed group, implying that the peptide leads to an increase in energy expenditure (74). GLP-1 receptor activity mediates the anorectic action of OXM, but the additional weight loss effect requires glucagon receptor activity (71, 75–77). Thus, OXM represents a dual agonist at the GLP-1 and glucagon receptors, with each receptor respectively mediating anorectic and energy expenditure effects.

In a double-blind, placebo-controlled, cross over study, Cohen and colleagues showed that an intravenous infusion of OXM at 3 pmol/kg/min reduced mean food intake by 19% in healthy human volunteers (78). Wynne and colleagues carried out a double-blind, placebo-controlled, parallel group study, showing preprandial subcutaneous injection of 400 nmol OXM in healthy overweight or obese volunteers for 4 weeks resulted in a mean reduction of body weight by 2.4% (79). This was associated with a 19% reduction in energy intake. OXM, when given as subcutaneous injections for 4 days, does not alter resting energy expenditure, but increased activity-related energy expenditure and total energy expenditure (80).

Due to the intrinsic dual receptor activity of OXM, analogs can therefore be used as unimolecular GLP-1/glucagon dual agonists (81, 82). Structural modifications to OXM to stabilize it have been developed including PEGylation (83), fatty-acid acylation (84, 85), amino acid substitution and other modifications (84). Furthermore, structure-activity studies have identified analogs with superior potency compared to the native peptide (84). Day and colleagues described a PEGylated co-agonist, which when administered once a week, normalized adiposity and glucose tolerance in diet-induced obese mice (86). Another PEGylated long acting dual GLP-1/glucagon receptor agonist was tested *in vivo* in diet-induced obese mice and was shown to improve glycaemia, reduce food intake and induce substantial weight loss (87). Given the positive findings from these studies, several clinical trials of GLP-1/glucagon co-agonists are being carried out by a variety of pharmaceutical companies (82). One of the earliest candidates in this class, MEDI0382, has recently reported Phase I/II results with promising efficacy in improving glycaemia and reducing weight in obese diabetic patients when given for up to 41 days (88).

OTHER DUAL/TRIPLE AGONIST COMBINATIONS (PYY/GLP-1, PYY/OXM, GLP-1/GIP, GLP-1/GIP/GLUCAGON)

Other combinations of analogs with putative synergistic effects on reduction in food intake have been explored. PYY(3-36) has been shown to possess synergistic anorectic effects when combined with GLP-1 or OXM in infusion studies (89, 90). Beglinger's group also showed that an oral combination of PYY/GLP-1 can reduce food intake during an *ad libitum* test meal to a greater extent than either of the two hormones alone (41). However, not all gut hormone combinations give additive/synergistic effects on food intake, for example PYY(3-36) and PP (91).

Another interesting "dual agonist" concept that is being explored is the glucose-dependent insulinotropic polypeptide GIP/GLP-1 co-agonist. GIP is an insulinotropic peptide released by the K-cells of the small intestine in response to nutrient ingestion (18). Therefore, GIP/GLP-1 co-agonists were explored as a means of enhancing the incretin effect. Finan and co-workers engineered peptides with balanced activity at the GLP-1 and GIP receptors, and these were modified with fatty-acid acylation or PEGylation to improve their pharmacokinetics. These GIP/GLP-1 dual agonists produced enhanced weight loss and improvements in glucose levels compared to GLP-1 agonists alone in animal studies. In a Phase I study in diabetic volunteers, they showed that one of the dual agonists can reduce HbA1c with minimal gastrointestinal side effects (92). This dual agonist (NNC0090-2746) went on to Phase II studies in obese diabetic patients over 12 weeks where it was shown to reduce HbA1c by 0.96% compared to placebo and to reduce weight by up to 1.8% relative to placebo, albeit its efficacy was not demonstrably superior to liraglutide (93). Extending this work on dual agonism, Finan and colleagues have also devised triple agonists of the GLP-1, GIP and glucagon receptors. In pre-clinical models, these new agents demonstrate better weight loss than the GLP-1/GIP dual agonist mentioned above (94). Clinical study results are awaited with the GLP-1/GIP/glucagon triple agonists.

TRIPLE AGONISM WITH GLP-1/OXM/PYY—THE PATHWAY TO THE "MEDICAL BYPASS"?

In our continuing efforts to devise a better therapy for obesity, we have taken a tack which is based on the known effects of bariatric surgery on gut hormone physiology. Patients who have undergone RYGB have much higher post-prandial levels of PYY, GLP-1, and OXM (95–98), secretion being triggered by direct and early exposure of jejeunal L-cells to nutrients through the bypass. It is postulated that the release of these hormones leads to a synergistic effect on food intake (via all three hormones)

and increased insulin secretion (via GLP-1 and OXM). The latter process could account for the rapid improvements in glycaemia which are observed early on after surgery, although other factors such as the very low-calorie liquid diet imposed after surgery are also likely to contribute. To test the hypothesis that the elevation of these three hormones were responsible for the benefits of RYGB directly, our group devised a study in which a triple infusion of GLP-1, OXM, and PYY (the "GOP infusion") was given to recreate the post-prandial levels observed in RYGB patients. This led to a significant mean reduction in food intake by 32% when given for 10 h or so to healthy volunteers even though the individual doses of each hormone were calibrated to be sub-anorectic (98). No significant issues with nausea were observed. Continuing studies are underway to examine the effects of GOP infusion when this is given for up to 28 days to obese diabetic patients. Regardless, the GOP infusion study suggests that triple hormone/agonist therapy is feasible and capable of synergistically reducing food intake to achieve better weight loss than single or even dual-agonism, perhaps recapitulating the effect of the RYGB without surgery: the "medical bypass."

CONCLUSIONS

GLP-1 therapy for obesity represents the first generation of gut hormone-based therapies for obesity. In the search for better efficacy than presently available, it seems that approaches targeting multiple satiety and metabolic pathways for the optimal effects on food intake and metabolism will be required, and there is much pharmaceutical development in this sector at present. It is hoped that these efforts will pay off in the form of an efficacious, well-tolerated, safe and even beneficial tool for fighting the global pandemic of obesity.

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Exacting Responses: Lack of Endocrine Cephalic Phase Responses Upon Oro-Sensory Exposure

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Oro-sensory exposure (OSE) to food plays an important role in the regulation of food intake. One proposed underlying mechanism is the occurrence of cephalic phase responses (CPRs). CPRs include the pre-digestive endocrine responses induced by food-related sensory input. Yet, whether OSE duration or sweetness intensity affects CPRs is unknown. The objective of this study was to determine the independent and interactive effects of oro-sensory duration (chewing) and stimulation intensity (sweetness) on endocrine CPRs and satiation. Eighteen males (22 ± 2 years, BMI 22 ± 2 kg/m^2) participated in a 2 × 2 randomized study with a control condition. Each session participants performed modified sham feeding (MSF) with one of the four gel-based model foods. During the control session no MSF was performed. Model foods differed in chewing duration (hard or soft texture) and sweetness (low or high intensity). During each session, eight blood samples were collected up till 25 min after MSF onset. Subsequently, food intake from an *ad libitum* lunch was measured. No typical CPR was found for insulin, pancreatic polypeptide (PP), and ghrelin. However, the overall PP response was 1.1 times greater for the hard sweet MSF condition compared to control ($p = 0.02$). Overall ghrelin responses were 1.1 times greater for the hard model food compared to the soft model food conditions ($p = 0.003$). These differences in endocrine response were not associated with differences in food intake at the subsequent meal. Exploratory sub-analysis of the responsive insulin curves showed that after 2.5 min of MSF the hard texture model foods insulin concentrations were 1.2 greater compared to the soft texture. These findings indicate that texture hardness and sweetness increase the overall PP response and that MSF on hard texture increases the overall ghrelin response compared to soft texture model foods. However, MSF on model foods does not lead to a typical CPR. This study, among others, shows that there are major dissimilarities in the endocrine responses to food stimulation between individuals. This emphasizes the importance of considering cephalic responders and non-responders. More research is needed to understand CPRs in relation to food texture and taste properties.

Keywords: texture, taste, insulin, pancreatic polypeptide, ghrelin

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INTRODUCTION

The current food environment drives a positive energy balance leading to a growing number of obese and overweight people worldwide (1, 2). Knowledge about the mechanisms by which food intake is controlled is key in finding solutions to this problem (3).

Food intake starts with the breakdown of food in the mouth; this process depends on the structure, the flavor, and the palatability of the food (4–7). Besides food qualities, oral processing is determined by the individuals' anatomy, such as mouth size, strength of the jaw muscle (masseter), and automated eating behavior characteristics such as bite size and chewing rate (8–10). Intake of a food is inhibited when the aforementioned food and individuals' characteristics lead to a slow ingestion rate (11). Several physiological mechanisms have been suggested to cause this effect, among which the oro-sensory exposure (OSE) to food (12, 13).

Oro-sensory exposure can be defined as the release of nutrients, odor, and taste molecules from the food matrix in the mouth during oral processing (14). Foods that do not require chewing before the bolus is ready to swallow induce only limited OSE, whereas foods that do require chewing enhance OSE (7). Beverages, for example, can be ingested in a relatively short time and therefore the oral-residence time and thus OSE is limited. This may be a reason why energy-containing beverages suppress appetite and energy intake less compared to equicaloric solid foods (15, 16).

Oro-sensory exposure is necessary for the initiation of cephalic phase responses (CPRs) (16). The cephalic phase is the first phase of digestion, including all physiological, endocrine, and autonomic responses stimulated by cephalic phase sensory input such as taste, smell (OSE), and the sight of food (17, 18). The putative function of these anticipatory responses to food is to optimize digestion and to minimize the impact of meals on homeostasis (19–22). Initial cephalic responses signal stimulation of food intake, while continued sensory stimulation may induce satiation (18). Cephalic phase signals include the production of saliva and endocrine responses in insulin, pancreatic polypeptide (PP), and ghrelin.

A lack of, or diminished cephalic phase responsivity as a result of decreased OSE through rapid food consumption, has been suggested to affect physiological and psychological processes, such as glucose homeostasis, metabolism, and food reward and satiety systems in the brain (12). In both animal (23, 24) and human studies (25), it has been shown that these changes are related to decreased appetite responses and weight gain (26). OSE may therefore play a key role in the regulation of food intake through the induction of CPRs (10, 27, 28).

However, the importance of the duration and intensity of the OSE in order to induce CPRs is unknown. OSE duration affects the CPR as shown by studies of Teff et al. where cephalic responses of insulin and PP were found to mixed nutrient foods and solid foods but not to liquids (29, 30). CPR could also be enhanced through taste (31). This is shown by a study of Just et al. where an increase in insulin plasma concentration was found upon oral cavity stimulation with a sweet sucrose and saccharine solution but not for any of the other taste qualities (32). In addition, Teff found a PP response to mixed nutrient sweet and salty foods with a higher magnitude of the response found for the sweet foods (29). This indicates that sweet taste may have a specific role for nutrient signaling that aids in controlling food intake and food digestion through CPRs (33).

Taken together, OSE can be varied in duration and intensity which may affect CPRs and consequently food intake. However, it is not known if, and to what extent OSE duration and taste intensity induce cephalic responses. Therefore, the main objective of this study was to determine the independent and interactive effects of oro-sensory duration (chewing) and stimulation intensity (sweetness) on the endocrine CPRs and subsequent food intake. To investigate this, we performed a 2×2 factorial randomized crossover study with control condition. Endocrine responses were measured under conditions in which OSE duration (hard and soft texture) and taste intensity (low sweetness and high sweetness) were varied. We expected a higher peak of the endocrine responses (insulin, PP, and ghrelin) for increased OSE magnitudes (i.e. hard texture, long chewing duration, and high sweet intensity) and a consequently lower food intake.

MATERIALS AND METHODS

Subjects

The study was performed at Wageningen University, The Netherlands. Subjects were recruited from the surroundings of Wageningen using flyers and posters. In addition, emails were sent to persons in a database of volunteers who previously had expressed an interest in participating in nutrition studies. Healthy male subjects between 18 and 35 years old with a BMI between 18.5 and 25 kg/m^2 were recruited. Subjects had to eat three meals a day around the same time, and were excluded if they followed an energy-restricted diet or if they had gained or lost >5 kg of body weight during the past 2 months. Subjects were also excluded if they had dental pathologies, chewing, swallowing, or eating difficulties, self-reported taste or smell problems, braces or dentures, and when they used medication. In addition, they were not allowed to participate if they were high-restrained eaters according to the Dutch Eating Behavior Questionnaire (DEBQ): score >2.9 (34). Personnel and thesis students of the Division of Human Nutrition were omitted from participation. During the information meeting, subjects rated the model foods that were used in this study on liking. Subjects were excluded from participation if they disliked one of the model foods (defined as score <4 on a nine point hedonic Likert scale) or had a stronger preference for one of the model foods compared to the others (defined as >2 point difference on a nine point hedonic Likert scale).

Potential subjects were invited to a training visit at Wageningen University. During the training subjects practiced with modified sham feeding (MSF) by repeatedly chewing and spitting out the entire model food bolus upon the moment they would normally swallow. In addition, height and body weight were measured and a research nurse measured the subjects' Hb value (finger prick) and judged the forearm veins for suitability to place an intravenous cannula. Subjects were excluded after the training

session if their Hb value was not within 8.1–11 mmol/l or when the research nurse decided that the veins of the forearm were not suitable for placement of the intravenous cannula. In addition, participants were excluded if they had a recovery rate <85% of the dry weight (see Modified Sham Feeding) of the model food. This 85% cut-off point has been used in previous studies (35).

The study was approved by the Medical Ethical Committee of Wageningen University, The Netherlands (ABR: NL5682408116) and registered in the Dutch trial register under NTR5870 (http://www.trialregister.nl). All subjects signed informed consent prior to the training. Subjects received a financial compensation for their time and effort.

Prior to the study, sample size calculations showed that a minimum of 18 subjects was needed to show an effect of 10% between treatments. For this calculation, we assumed a variation coefficient of 15% incremental area under the curves (IAUC) for the control condition and 20% IAUC for the experimental conditions. In addition, a correlation of within person measures of $r = 0.6$ was assumed (22, 36). Power was set at $1\text{-}\beta = 0.80$ at a significant concentration of $\alpha = 0.05$. In total, 74 subjects joined the information meeting of which 42 subjects were found eligible; however, seven were lost to follow-up. Finally, 35 subjects joined the training session of which 22 subjects were eligible to be included in the test sessions, see **Figure 1**. Finally, 18 were invited for the study.

The subjects that participated in the study were on average 22 ± 2 years old, had a mean (±SD) body mass index of 22 ± 2 kg/m^2 and an average DEBQ restraint score of 1.9 ± 0.5 (34).

Experimental Design

The study had a 2 × 2 factorial randomized crossover-design with a control condition. During the four treatment visits subjects sham fed one of the four types of gel-like models foods. Model foods were formulated to have two levels of oral processing time (soft or hard texture), and two concentrations of sweetness (low or high) (see Model Foods). During the control visit, subjects did not sham feed or eat anything. During each visit, blood samples were taken at fixed time points and after the last blood sample collection, subjects received an *ad libitum* lunch meal. Conditions were randomized, following a Latin square Williams design with 1-week washout in-between visits.

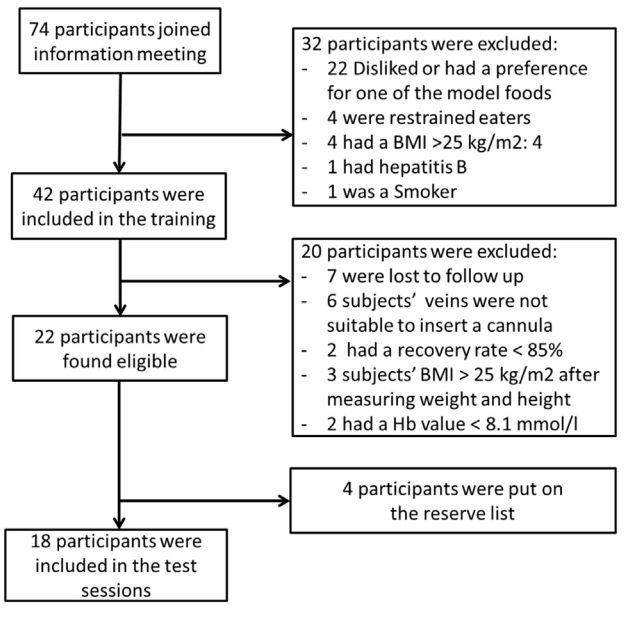

FIGURE 1 | Flow chart of the in- and exclusion of subjects.

Experimental Procedures

The evening preceding the test session subjects were instructed to eat the same meal of their choosing to avoid confounding effects of previous food consumption. Subjects were instructed to eat the meal between 18:00 and 20:00 h after which the subject was not allowed to eat or drink anything except water until the next morning. Subjects received two 500 ml cartons of a milk-based breakfast drink (banana, kiwi, strawberry flavored "goedemorgen fruitontbijt" Vifit Wageningen, The Netherlands, Friesland Campina) to drink until fullness 4 h prior to their arrival time. Subjects were instructed not to eat or drink anything after their breakfast drink and to bring back the cartons. The breakfast drink cartons were covertly weighted to determine intake (average intake of the breakfast drink was 662 ± 258 ml). Subjects were also instructed to avoid high-intensity exercise (everything besides regular speed walking and biking) and alcoholic drinks 24 h preceding the test and to use the same mode of transport every time they visited. To control for compliance subjects were asked to keep a diary of their food and beverage consumptions and physical activity the day before the test session.

Half an hour prior to the start of the test sessions, an intravenous cannula was placed in the antecubital vein by a trained research nurse. Blood drawings were done in a quiet room by a trained research nurse.

Subjects were provided with 15 pieces (of 7 g each) of the model foods at $t2.5$ (baseline sample) which they had to smell. After that subjects started chewing on the model foods, starting to chew was defined as $t = 0$. Blood samples were taken at time points t (min), $t2.5$ (smell), $t0$ (start MSF), $t2.5$, $t5$, $t10$, $t15$ (stop MSF), $t20$, and $t25$. Subjects regularly filled in an appetite questionnaire (see Appetite Ratings and Well-Being).

During the sham feeding session, subjects were recorded with use of a webcam to determine the number of chews and chewing duration. After the sham feeding, part of the test session subjects were provided with an *ad libitum* lunch meal to measure intake as a measure of satiation. For an overview of the entire test session procedure, see **Figure 2**.

Model Foods

Strawberry flavored gel-based model foods similar to our previous study (37) were used. Ingredients used were cream (30% fat, AH Basic), sunflower oil, strawberry flavored pudding powder ("Jelly," Dr. Oetker) and water. To manipulate oral processing time, the hardness of the gels was altered by added thickening agents, that is, carrageenan (type CHP-2) and locust-bean gum (LBG). Thickening agents were added in the following amounts: 0.22/0.22 wt% carrageenan/LBG for the soft and 0.66/0.88 wt% carrageenan/LBG for the hard model food.

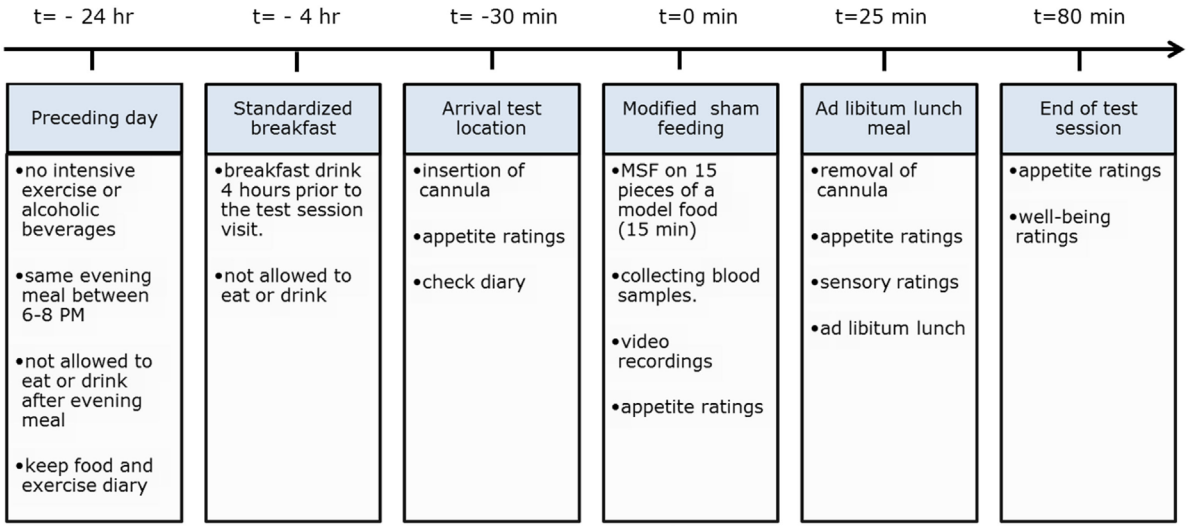

FIGURE 2 | Overview of experimental procedures.

In our previous study, we were unable to increase the difference in perceived sweetness between the low and sweet version of the model foods without changing the liking of the model foods by using a non-caloric sweetener. For the present study, multiple pilot studies were performed to enhance the sweetness difference between the low and high sweet model foods by adding table sugar. We were able to establish a (mean ± SEM) 16 ± 2 mm difference (rated on a 100 mm VAS, $p < 0.001$) in sweetness between the low and high sweet model foods, with no difference ($p = 0.90$) in sweetness between textures (soft and hard) (mean ± SEM) 0.3 ± 2.3. By adding table sugar next to the non-caloric sweetener, we were able to keep palatability of the model foods equal (mean difference ± SE 1.2 ± 2.6, $p = 0.80$). The added calories (table sugar) in the high sweet conditions were thought to be negligible as subjects sham fed the model foods (chew and spit) and did not ingest, in contrast to our previous study (37).

In the final study model, foods significantly differed in sweetness concentration [$F_{(3,51)} = 13.6$, $p < 0.001$] but the different types of model foods were equally liked (mean ± SE, 100-mm VAS: 56 ± 3). Participants did not have a preference for a texture type ($p = 0.38$) or sweetness concentration ($p = 0.23$). The hard and soft texture low sweet variants were significantly less sweet 49 ± 2 compared to the high sweet variants 66 ± 2 ($p < 0.001$). Perceived hardness significantly differed between texture concentrations [$F_{(3,51)} = 56$, $p < 0.001$], mean ± SEM perceived hardness of the hard model food was (58 ± 2) and of the soft model foods (21 ± 2). The average number of chews per model food piece significantly differed between texture variants [$F_{(1,17)} = 138.8$, $p < 0.001$] but not between taste concentrations ($p = 0.64$). Participants chewed on average 31 ± 2 times on the hard model food and 20 ± 2 times on the soft model foods. Chewing duration also differed between texture variants [$F_{(1,17)} = 95.5$, $p < 0.001$] and not between taste concentrations ($p = 0.76$). Average chewing duration on the hard model foods was 22 ± 1 s and on the soft model foods 16 ± 3 s. These model food characteristics are similar to those found in our previous study (37). The ingredients, energy content and macronutrient composition of each of the model foods can be found in **Table 1**.

TABLE 1 | Energy and macronutrient content of the model foods per 100 g.

Model food	Soft low sweet	Soft sweet	Hard low sweet	Hard sweet
Energy (kJ/kcal)	786/188	869/208	803/192	882/211
Protein (g)	0.8	0.8	0.8	0.8
Fat (g)	13.6	13.6	13.6	13.6
Carbohydrate (g)	15.6	20.5	16.5	21.4

Modified Sham Feeding

Subjects were instructed to chew on the model food and to expectorate the entire bolus upon the moment they would normally swallow. This chew and spit method is called MSF and has been used in previous studies (35, 38, 39). With this technique, subjects are orally exposed to the model foods without ingestion. Subjects were trained to MSF during the training session (see Subjects).

Subjects received 15 identical pieces of 7 g of the model foods which they had to "chew and spit." To avoid the confounding effects of "eating" rate rather than number of chews and chew effort on the metabolic and hormone response, subjects received instructions on when to start chewing a new model food piece. After the maximum amount of time had passed (40 s), they were, however, instructed to expectorate the model food if not previously done. The maximum amount of time of 40 s was determined based on a previous study performed with the same model foods (37). Compliance to the instructions for MSF not swallowing the (pieces of) model food was determined by analyzing the dry mass of model food that was spat out. Recovery percentage was calculated by dividing the dry mass of the expectorated boli by the dry mass of the model foods, in line with the method used by Wijlens et al. (35).

In this study, the mean recovery percentage was (mean ± SE) 92.7 ± 0.8%. In grams this means that on average 2.4 ± 0.3 g

of the model foods was swallowed. Per model food type this was 92.0 ± 0.7% (2.2 ± 0.2 g swallowed) for soft low sweet; 89.3 ± 0.8% (3.9 ± 0.3 g swallowed) for soft high sweet; 94.1 ± 0.4% (1.7 ± 0.1 g swallowed) for hard low sweet; and 95.6 ± 0.4% (1.6 ± 0.1 g swallowed) for hard high sweet. This is in line with other studies that also report recovery percentages between 89 and 97% (35, 40–42).

Blood Collection and Plasma Analysis

Glucose concentrations were measured by collecting a blood sample *via* the cannula with a syringe and was directly measured using a blood glucose meter (FreeStyle Freedom Lite).

Insulin samples were collected in 3 ml lithium heparin-coated vacutainer tubes and placed on ice immediately after acquiring. Insulin samples were centrifuged at 1,300 *g* for 10 min at 4°C. Plasma samples for insulin measurements were stored at −25°C until analysis at hospital "De Gelderse vallei" in Ede, The Netherlands. The detection limit of the analysis ranged from 2 to 300 mU/l with an intra assay CV of 2.2% and inter assay CV of 5.7%.

Pancreatic polypeptide blood samples were collected in 2 ml EDTA tubes, stored on ice after acquiring, and centrifuged at 2,500 *g* for 15 min at 4°C. Plasma samples were stored at −80°C until analysis. PP was analyzed by using Human PP Elisa kit (Millipore) with a detection range of 12.3–3,000 pg/ml and a intra assay CV of 3.3% and a inter assay CV of 4.9%.

Ghrelin samples were collected in 2 ml EDTA tubes with added AEBSF blocker (Pefabloc® SC) to a final concentration of 1 mg/ml. Samples were stored on ice after acquiring and centrifuged at 2,500 *g* for 15 min at 4°C. Ghrelin plasma samples were acidified to a final concentration of 0.05 N before storage.

Human ghrelin total ELISA (Millipore) was used to analyze total ghrelin concentrations with a detection range of 50–5,000 pg/ml and a intra assay CV of 1.11% and a inter assay CV of 5.18%. In case of measured values below the detection limit, the lowest detectable concentration of the analysis was used for data analysis.

Oral Processing Characteristics

To measure oral processing characteristics, subjects were video recorded during each session. A webcam (resolution 640 × 280 pixels) was positioned in front of the subject (face-on) where the lower frame was in line with the shoulders, and the upper frame above the top of the cranium and the sides at shoulder width. Subjects were instructed to limit their head movements. Video recordings were analyzed with the use of Observer Noldus XT 11. Behaviors of interest were chewing duration (s) and number of masticatory cycles (number of chews). From these variables, chewing frequency was calculated by dividing the number of chews by the total chewing duration.

Saliva Excretion

Fluid content of the model foods was calculated by subtracting the dry weight of the model food from the total weight of the eaten model foods. In addition, the fluid content of what was spat out was determined by subtracting the dry weight of the spat out bolus from the total amount that was spat out. Saliva content was then calculated by subtracting the water content of the model foods from the fluid content of the expectorated boli (43).

Appetite Ratings and Well-Being

Subjects rated satiety feelings, well-being, and model food characteristics on a 100-mm VAS anchored with "not at all" at 10 mm and "extremely" at 90 mm, see **Figure 3**. Subjects rated hunger, fullness, and desire to eat (DTE) at every time point. Due to time limitations; thirst, DTE, DTE sweet, DTE savory, prospective consumption, and nausea were rated at t = 20, 25, 60 min (**Figure 3**). Subjects rated their well-being after insertion of the cannula, at the same time of each blood sample collection and directly after the *ad libitum* lunch meal. Liking of the model food was rated at $t = 5$ (liking of the taste) and after the sham feeding session at $t = 30$. After the indwelling cannula was removed participants ate a model food piece and rated additional model food specific parameters; liking, DTE the gel, sweetness and hardness.

Food Intake

Directly after the MSF experiment, the cannula was removed and subjects received an *ad libitum* lunch meal. The lunch meal consisted of sandwiches made from two slices of whole bread cut in four small pieces of bread with different types of topping; full fat cheese, ham, apricot jam, or hazelnut spread. The amount of bread toppings was determined in such way that breads with

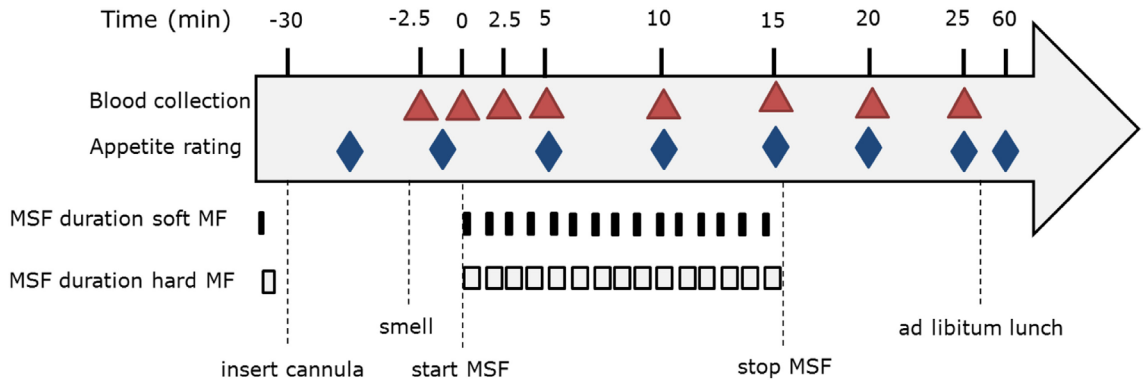

FIGURE 3 | Modified sham feeding (MSF) procedure overview including blood sample collection and appetite ratings.

different toppings were iso-caloric (50–52 kcal per quarter piece). To make sure there was a surplus of sandwiches (*ad libitum*), subjects receive 200% of a normal portion size of bread for each of the different toppings. Based on an average lunch energy intake of 20% of the daily energy need for men (2,500 kcal), subjects should be offered five servings (two slices of bread on top of each other with topping in between) equal to 20 sandwich quarters of each topping.

During these meals, subjects were seated in separate booths and were given 100 ml water with their lunch meal, that they were instructed to drink completely in sips between bites over the course of their lunch meal. They were instructed to eat until pleasantly full, and not to talk to each other. The weight and number of bread rolls was covertly weighed and counted to determine intake.

Statistics

Statistical analyses were performed using SAS (version 9.3; SAS Institute Inc., Cary, NC, USA). Results are presented as mean ± SEM unless otherwise stated. *p*-Values <0.05 were considered statically significant. To test endocrine concentrations and appetite scores, mixed model ANOVA (PROC MIXED) was used. In this model, texture, taste, time, and their interactions were added as fixed factors, the repeated statement was used to indicate the repeated measures over time per subject. Compound symmetry was used as a covariate structure. Normality of the data was checked by visual inspection. Outcome variables that were not normally distributed (insulin, PP, and ghrelin) were log10 transformed before analyses. For these variables, geometric means and ratios with 95% CI are reported.

Outcomes were tested for an order and baseline effect and were found to be significant covariates. Therefore, outcomes (insulin, pp, and ghrelin) were corrected for order and baseline concentrations by adding these two variables as a covariate. Tukey correction was used to compare means between treatments and Dunnett correction to compare means of treatments with control.

In addition, IAUC were calculated for all endocrine outcomes by means of the trapeziual rule and analyzed by means of a mixed model with conditions (texture and taste) as fixed factor, subject as random variable and baseline AUC as covariate. This model without covariate was also used to calculated differences in intake during the *ad libitum* lunch meal and differences in secreted saliva during MSF between treatments.

RESULTS

Cephalic Phase Responses

Glucose

Glucose concentrations remained constant over time [$F_{(7,199)} = 0.78$, $p = 0.61$] with no differences between treatments and the control condition at any of the time points [$F_{(28,476)} = 0.59$, $p = 0.96$], see **Figure 4**.

Insulin

We did not observe an insulin peak during any of the study treatments and we found no significant differences between treatments or control condition at any of the time points [$F_{(28,310)} = 0.53$, $p = 0.97$], see **Figure 5**. Insulin concentrations changed over time [$F_{(7,199)} = 2.6$, $p = 0.02$], insulin levels at 5 min were 1.1 (95% CI: 1.0–1.2) times higher compared to insulin levels at 20 and 25 min. However, these differences in insulin concentrations between the time points could not be attributed to the texture [$F_{(7,119)} = 0.22$, $p = 0.98$] or taste [$F_{(7,119)} = 1.12$, $p = 0.35$] manipulations, see **Figure 5**. IAUC did not differ between treatments [$F_{(1,50)} = 0.16$, $p = 0.69$] or between treatments and control condition [$F_{(4,67)} = 0.72$, $p = 0.58$].

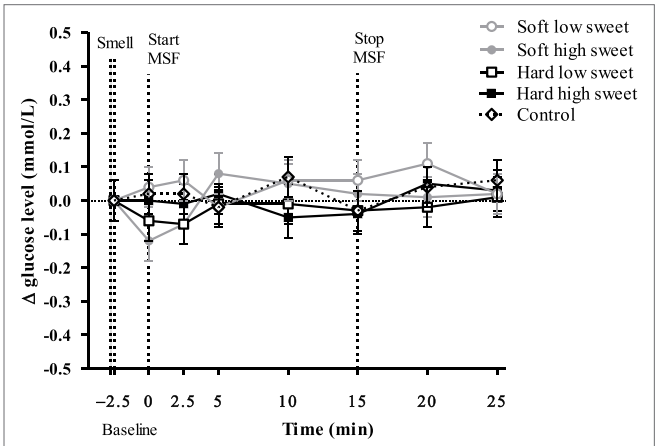

FIGURE 4 | Estimated mean ± SEM Δ glucose concentration (mmol/l) from baseline over time for all treatments and the control condition. No significant differences were found between treatments or control condition at any time point ($p = 0.96$).

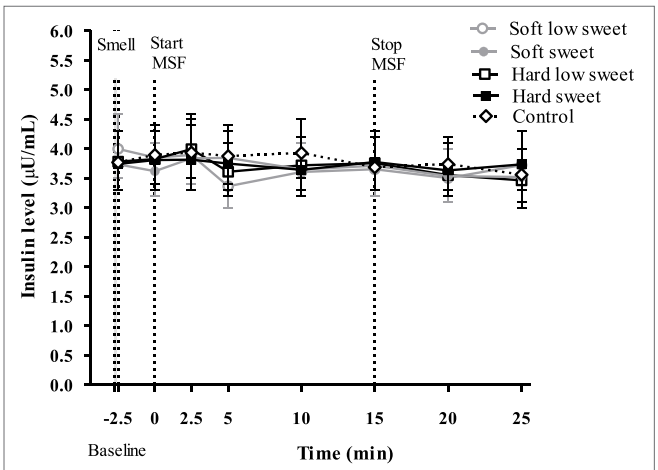

FIGURE 5 | Geometric mean ± 95% CI insulin (μU/ml) concentrations over time per treatment and control condition. No significant differences were found between treatments and control condition at any of the time points ($p = 0.97$).

Pancreatic Polypeptide

Pancreatic polypeptide concentrations did not change over time [$F_{(7,119)} = 1.03$, $p = 0.42$], neither did the PP concentrations differ between treatments nor control condition at any of the individual

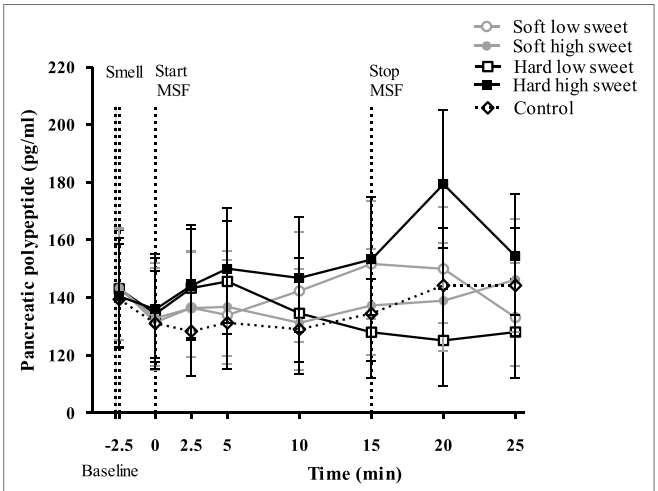

FIGURE 6 | Geometric mean ± 95% CI pancreatic polypeptide concentrations (pg/ml) corrected for baseline concentration over time per treatment and control condition. No significant differences were found between treatments and control condition at any of the time points ($p = 0.72$).

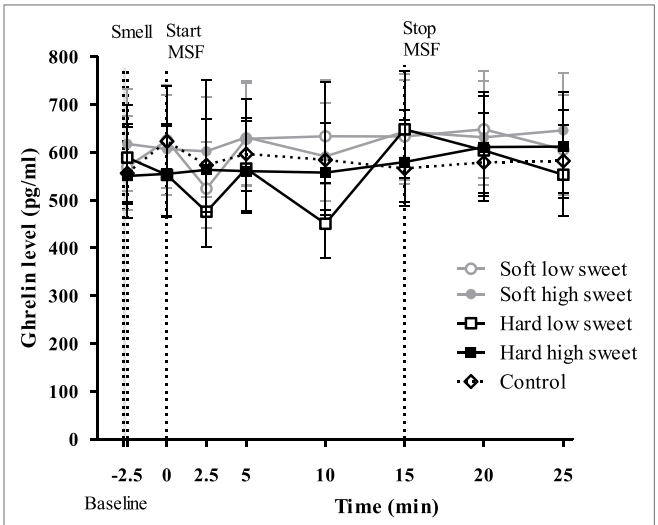

FIGURE 7 | Geometric mean ± 95% CI ghrelin (pg/ml) concentrations corrected for baseline concentration per treatment and control condition. No significant differences were found between treatments and control condition at any time point ($p = 0.63$).

time points [$F_{(28,476)} = 6.18$, $p = 0.72$], see **Figure 6**. However, the total PP response to the hard sweet MSF condition was 1.1 times greater compared to control, which was significant (95% CI = 1.0–1.2, $p = 0.02$). In addition, we found a significant texture/taste interaction effect on PP concentrations [$F_{(1,17)} = 7.53$, $p = 0.01$], the response for the low sweet hard texture model food was 0.9 times lower compared to the sweet hard texture model food curve (95% CI = 0.8–1.0, $p = 0.019$). These effects were, however, too small to show significant differences in IAUC between treatments [$F_{(1,50)} = 2.6$, $p = 0.11$] or between treatments and control condition [$F_{(4,67)} = 1.0$, $p = 0.40$].

Ghrelin

No significant changes over time were found for any of the treatments [$F_{(28,476)} = 0.90$, $p = 0.63$], see **Figure 7**. In addition, we did not find differences between treatments or control condition at any of the individual time points. However, we did find a significant texture effect combining all time points [$F_{(1,17)} = 12.3$, $p = 0.003$]. Ghrelin curves of the hard model food conditions were 1.1 times higher compared to the soft model food conditions (95% CI = 1.0–1.2, $p = 0.003$). However, these differences were too small to result in a difference in IAUC between the treatments [$F_{(1,50)} = 0.27$, $p = 0.60$] and control condition [$F_{(4,67)} = 0.48$, $p = 0.75$].

Saliva Excretion

We found a significant effect of texture [$F_{(1,17)} = 74.6$, $p < 0.001$] and taste [$F_{(1,17)} = 11.3$, $p = 0.004$] but no interaction effect [$F_{(1,17)} = 3.6$, $p = 0.08$] on expectorated saliva, see **Figure 8**. When MSF on hard model foods participants expectorated 12 ± 1.4 g more saliva compared to MSF on the soft model foods ($p < 0.001$). In addition, MSF on a sweet model food led to a 5 ± 1.4 g increased expectoration of saliva compared to MSF on a low-sweet model food ($p = 0.004$).

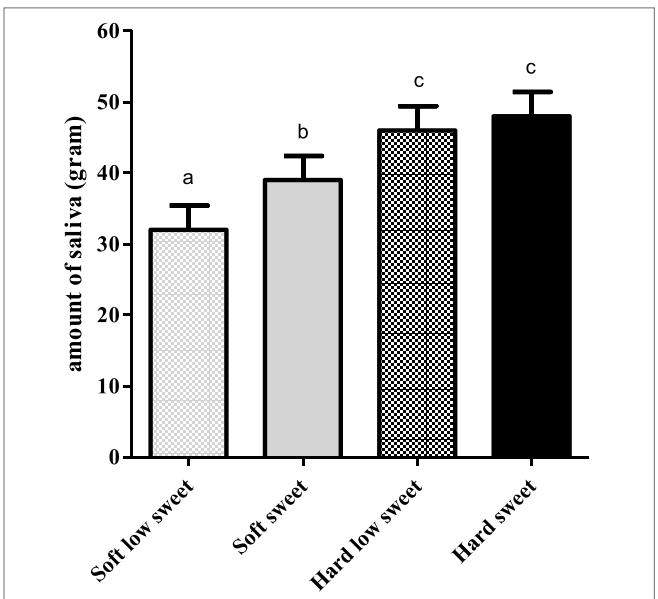

FIGURE 8 | Estimated mean ± SEM saliva content (in grams) of expectorated boli per model food type. Bars with different letters are significantly different.

Exploratory Findings: Cephalic Phase Insulin Responders and Non-Responders

In line with the approach of Dhillon et al. (44) for each condition (including control), curves were considered responsive when insulin concentration increased at $t = 2.5$ from baseline ($t = -2.5$). Based on this, 54% of the curves were classified as responsive and 46% of the curves as non-responsive, see **Table 2**. At a subject level, 33% could be considered responders, defined as having three or four responsive curves out of the four treatments.

For the responsive model, we found a significant texture [$F_{(1,13)} = 4.84$, $p = 0.047$], taste [$F_{(1,17)} = 9.67$, $p = 0.006$], time [$F_{(7,119)} = 2.16$, $p = 0.042$] and texture/taste interaction effect [$F_{(1,5)} = 9.72$, $p = 0.026$] on insulin concentrations over all time points. Analyzing insulin concentrations per time point we found a texture effect [$F_{(1,47)} = 4.09$, $p = 0.049$], 2.5 min after starting sham feeding. Insulin concentrations at 2.5 min were 1.2 times (CI% 1.0–1.4) higher for hard compared to the soft texture conditions ($p = 0.049$). In addition, we found a significant taste effect [$F_{(1,47)} = 8.89$, $p = 0.005$] after 5 min of MSF. Insulin concentrations were 1.2 times (CI% 1.1–1.4) higher for low sweet compared to the sweet model foods ($p = 0.005$), see **Figure 9**.

In addition, a sub-analysis was performed of PP and ghrelin concentrations based on the insulin classification of responder, non-responder curves. For the PP non-responder and responder curves, we did not find any effects (all, $p \geq 0.3$) or differences between treatments and control ($p = 0.06$).

For the ghrelin non-responder and responder curves, we found a significant effect between the hard sweet curve and control curve ($p = 0.009$). For the ghrelin responder curves, we found a texture effect over all time points ($p = 0.007$), hard texture was generally lower compared to the soft texture but not at any specific time point.

TABLE 2 | Percentage of responsive and non-responsive curves per treatment and control condition.

	Soft low sweet	Soft sweet	Hard low sweet	Hard sweet	Control	Total percentage
% responder	15	15	29	20	22	54
% non-responder	25	25	12	20	18	46

Appetite and Well-Being

Hunger feelings over time were significantly lower for the hard high sweet, soft high sweet, and low sweet model foods compared to control, see **Figure 10**. After 10 min of MSF subjects felt less hungry for the sweet compared to the low sweet model foods (mean ± SEM 9 ± 3 difference, $p = 0.003$). Fullness ratings showed a similar trend; fullness over time was scored higher after MSF on the hard sweet and low sweet model foods and the soft sweet model food compared to control (**Figure 11**), significant time points are indicated by the bracket ($p < 0.05$). A small but significant taste effect was found 10 min after the MSF period; subjects felt more full after the high sweet compared to the low sweet MSF treatments (mean difference = 6 ± 4, $p = 0.04$). Prospective consumption ratings post MSF on the sweet soft ($p = 0.03$) and hard ($p < 0.007$) model food differed from control. Prospective consumption ratings were lower (mean ± SEM 8 ± 2) 10 min after MSF comparing the low ($p = 0.03$) and high sweet ($p = 0.012$) soft model food with the control condition. No significant changes (pre to post) were observed between treatments or control condition for DTE sweet or savory. There were no differences in participants' well-being, dizziness, feeling to faint, nausea, and thirst (before, or after MSF) between treatments and the control session (all, $p > 0.32$).

Effect of MSF on Food Intake

There was no effect of texture [$F_{(1,17)} = 0.03$, $p = 0.87$] or taste [$F_{(1,17)} = 0.06$, $p = 0.81$] or an interaction effect of texture and taste on intake [$F_{(3,17)} = 0.06$, $p = 0.81$], see **Figure 12**. In addition, we did not find a significant difference between intake after the MSF sessions and control condition [$F_{(4,68)} = 0.35$, $p = 0.84$]. In addition, no texture, sweetness, or interaction effect on intake of the sweet toppings (jam and hazel nut spread) or savory toppings (ham and cheese) was found.

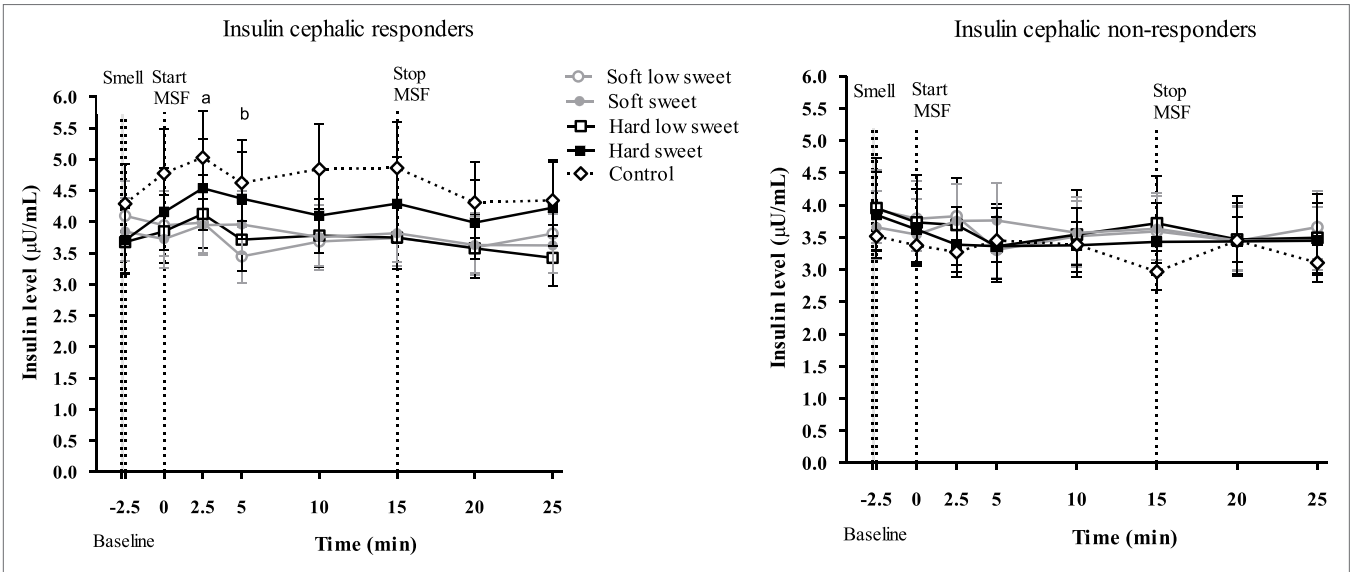

FIGURE 9 | Geometric mean ± 95% CI insulin responder and non-responder curves. Letter "a" indicates a significant texture effect ($p = 0.049$) and "b" indicates a significant sweetness effect ($p = 0.005$).

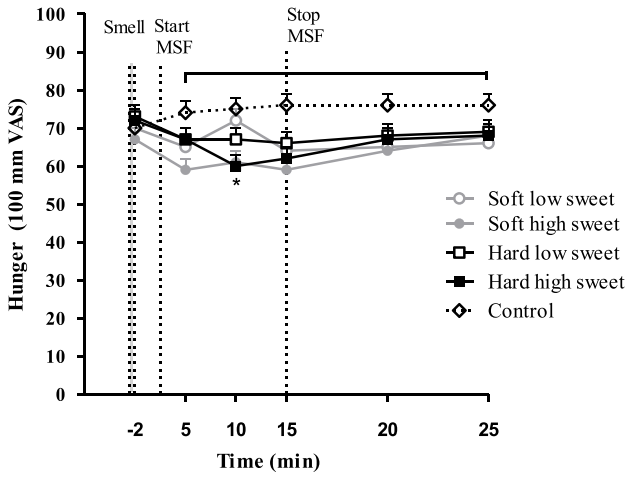

FIGURE 10 | Estimated mean ± SEM hunger changes over time per treatment and control condition. Significant differences were found between treatments and control indicated by the bracket ($p < 0.05$). *Significant taste effect ($p = 0.003$).

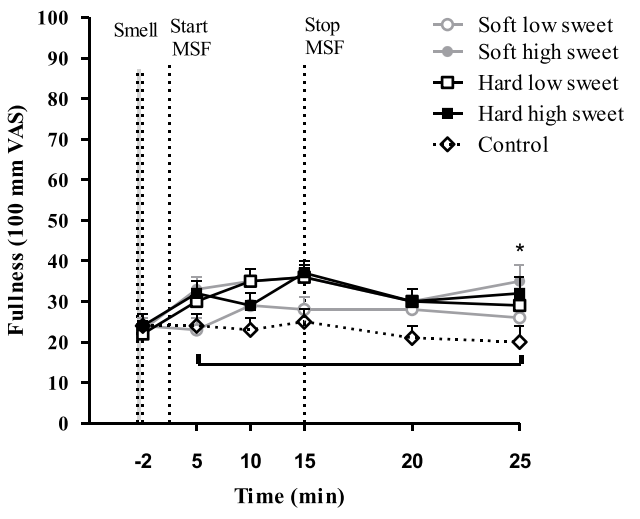

FIGURE 11 | Estimated mean ± SEM fullness changes over time per treatment and control condition. Significant differences were found between treatments and control indicated by the bracket ($p < 0.05$). *Significant taste effect ($p = 0.04$).

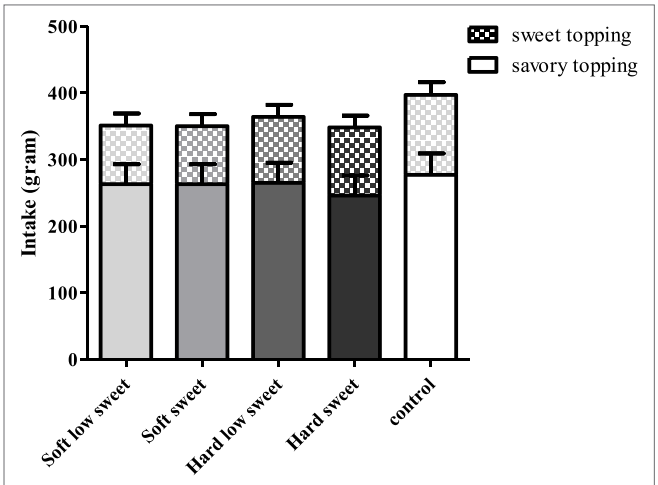

FIGURE 12 | Estimated mean ± SEM post modified sham feeding (MSF) lunch intake (in grams) per model food type. No significant differences were found.

DISCUSSION

We investigated the independent and interactive effects of OSE duration (chewing) and stimulation intensity (sweetness) on endocrine CPRs, and subsequent food intake. In order to study this, we successfully developed a model food system that aid in studying realistic texture and taste manipulations on endocrine responses. The model foods used in this study were equally liked but differed in sweetness level and chewing duration.

The findings of this study show that, insulin levels at 5 min after starting to MSF were 1.1 times higher compared to insulin levels at 20 and 25 min, but these differences between time points could not be attributed to the texture or taste manipulations. In addition, the overall PP response of the hard sweet MSF condition was 1.1 times greater compared to control and a significant texture/taste interaction effect was found. The PP curve of the low sweet hard texture model food was 0.9 times lower compared to the sweet hard texture model food curve. Comparing the total ghrelin curves, we found that the overall ghrelin response of the hard model food conditions was 1.1 times greater compared to the soft model food conditions. No typical cephalic phase peak response was found for any of the endocrine outcome measures. In addition, the found differences are small relative to the variation and would therefore be unlikely to have a physiological effect.

During MSF, participants expectorated 12% more saliva during MSF on the hard compared to the soft model foods. In addition to this texture effect, participants expectorated 5% more saliva when MSF on the sweet compared to the low-sweet model foods. Subjective appetite ratings showed that, in general, subjects felt fuller and less hungry after MSF compared to no MSF. These differences in appetite ratings were, however, not reflected in subsequent food intake during the *ad libitum* lunch meal.

These outcomes are not in line with our hypothesis as we expected to find a typical cephalic peak/spike in the endocrine responses (insulin and PP) and an increase in ghrelin concentrations for increased OSE magnitudes (i.e., hard texture, long chewing duration, and high sweet intensity) and a consequent decrease in food intake.

Our observed lack of a cephalic phase insulin response (CPIR) is in contrast with other studies that did find a CPIR upon sensory stimulation (36, 44–48). Based on conclusions of Teff et al., we expected a typical insulin response of approximately 25% above baseline or 1% of a normal postprandial insulin release within 2–4 min after sensory stimulation, returning back to baseline concentrations by 8–10 min (36, 49). However, we observed no changes from baseline in this study for which there could be various explanations. Characteristics of the stimuli or food used to evoke the cephalic response are of great importance.

The foremost important aspect is palatability; it is assumed that the amplitude of the CPIR depends on the palatability of the stimuli used (50). Studies that have found a CPIR used highly palatable food stimuli such as apple pie (30) while the model foods used in this study were rated as neutral (average liking score was 56 mm on 100-mm VAS).

In addition to palatability, the stimuli should consist of multiple sensory modalities to induce insulin secretion as argued by Zafra et al. (12). This explains the lack of findings in studies where simple nutrient solutions (12, 30, 51) and sweet tablets (52) were used as stimuli. Only one study has shown an increase in insulin plasma concentration upon sucrose and saccharine oral cavity stimulation with a liquid (45 s swirl and spit method) (Just et al.) (32). However, this insulin response was smaller (1.5 μIU/mL rise from baseline for sucrose and 0.9 μIU/mL rise from baseline for saccharine) compared to the 25% above baseline; corresponding to a 2 μIU/mL increase, described by Teff et al. (36, 49). This emphasizes the importance of texture and taste qualities for the induction of detectable cephalic insulin response based upon which we expected to find a cephalic response in this study. Power and Schulkin argues that the association between insulin response and palatability is more important than nutrients, similarly this may also hold for the sensory modalities being more important than nutrients (22).

Besides the stimuli properties, subject characteristics determine the magnitude of the CPIR. The subjects included in this study were healthy and had a low, although healthy, BMI which could possibly explain the lack of CPIR; Teff et al. found that obese subject exhibit a larger CPIR compared to lean subjects (53). Besides BMI, there are several studies that report that there are cephalic phase responders and non-responders (44, 45, 54). In a recent study of Dhillon et al., insulin concentrations were measured during swirling of sweet drinks and MSF of sweet gelatin cubes. In this study, 64 overweight and obese subjects were included and a clear distinction could be made between responsive and non-responsive subjects. Over all treatments, 45% of the measured insulin curves were considered to be responsive (rise in insulin 2 min after stimulation). Insulin responses were mostly observed after exposure to sucrose in solid form (44). In addition, Bellisle et al. also failed to observe a CPIR in 12% of the subjects and Teff et al. found 50–75% of the subjects to be responders (45, 54).

Based on the classification of Dhillon et al. (44) in the present study, 54% of the insulin curves were considered responsive and 46% were considered non-responsive. Sub-analysis of the responsive curves showed an increased insulin concentration at 2.5 min after starting to MSF on the hard model foods, compared to soft. In addition, at 5 min an increased concentration for the sweet compared to the low sweet model foods was found. This indicates the importance of considering insulin responsive and non-responsive subjects when studying CPRs. However, the insulin responder, non-responder classifications were not confirmed by PP and ghrelin curves. This stresses the need of clear responder non-responder criteria for the different cephalic endocrines.

Although CPIR has been most extensively studied, PP is considered to show a more robust endocrine cephalic response that is not influenced by nutrients such as glucose but considered a vagal stimulation marker (36). PP increases 100% above baseline starting 10 min after the onset of a meal or after MSF, and concentrations remain elevated for another 30 min (55). Because PP responses are of a larger magnitude compared to insulin responses they are likely a better measure of graded CPRs to OSE (such as duration and intensity as investigated in this study). However, we also did not find a cephalic PP response. This is not in line with our hypothesis, as several other studies were able to detect a PP response in the absence of a CPIR (56, 57).

However, the lack of a cephalic PP response when MSF model foods is in line with the study findings of Mennella et al. that found no PP response when subjects MSF pudding, which is comparable in texture to the soft model foods used in this study (58). Our findings are also in line with findings of Teff et al. that found no difference in cephalic PP response between sweetness concentrations when subjects sham fed high sweet (unpalatable) and sweet (palatable) cream cheese crackers (29). In light of this finding, it is not surprising that we did not find a difference between sweetness concentrations that were within the palatability range and therefore did not differ largely in sweetness. This suggests that cephalic endocrine responses are not sensitive to small differences in sweetness levels.

Besides the lack of a CPIR and cephalic PP response, we also did not find an increase in ghrelin concentrations comparing treatments to control. Based on other studies we expected an increase in ghrelin concentrations suggesting an initial appetizer effect of MSF (59). In addition, we expected a decrease in ghrelin toward the end of MSF as we hypothesized that continued sensory exposure would lower ghrelin levels (25, 40, 59). The fact that we did not observe either of these ghrelin responses could be due to the mixed macronutrient content of the model foods as it is hypothesized that carbohydrate meals decrease ghrelin concentrations whereas fat and protein stimulate ghrelin secretion (25).

Saliva release is one of the earliest described CPRs, by Pavlov in 1910 (60) and has been documented since by various other studies (61–64). We observed a significant difference in the amount of saliva produced upon MSF the hard and sweet model foods compared to the soft and low sweet model foods. This is in line with previous reports that describe an up to fivefold increase of basal saliva release after 30 min of MSF a steak and French fries meal (65). The sensory receptors that are involved in saliva release are both chemical and mechanical (the movement of chewing stimulates saliva flow) (12). This can explain the differences found in saliva produced between texture and sweetness concentrations in our study. The act of chewing serves as a mechanical stimulant of the sensory receptors stimulating saliva flow. The fact that we found a sweetness effect on saliva production is in contrast with findings of Mattes and Pedersen et al. who showed that the macronutrient composition of the food determines the quality rather than the quantity of saliva. For example, sucrose and fructose stimulate amylase-rich saliva (12).

Compared to control, MSF decreased feelings of hunger and prospective consumption and increased fullness over the sham feed period up to 15 min. Differences between treatments and control were approximately 10 mm (100-mm VAS), which is

modest but considered meaningful (66, 67). These findings are in line with previous studies that showed that prolonged oral stimulation alone suppresses hunger and increases fullness (35, 68). The differences in appetite ratings were not reflected in the amount eaten during the consequent meal, although, intake was 11% lower for the MSF conditions compared to the control condition but this was not significant. This in contrast with a study of Wijlens et al. that found a 15–19% decrease in intake after MSF cake for 8 min (35). Possibly, in our case, the total chewing duration was not long enough to affect intake significantly. A study of Mennella et al. also did not report an effect of MSF pudding on intake at a consequent meal (58). Few MSF studies have investigated the effect on subsequent food intake; most studies had a preload design with real food intake where subjects were instructed to chew each bite for a fixed number of time after which they measured intake of a meal (20, 69, 70). For example, a study by Lavin et al. showed that chewing a sweet pastille 10 times before swallowing compared to drinking a sweet liquid or water reduces intake (28). Another reason why we did not find a difference in intake after MSF might be because artificial sweeteners were used to manipulate sweetness of the model foods. It is contentious whether tasting (but not ingesting) artificial sweeteners has the same satiating capacity as glucose and sucrose (71). In addition, the fact that we did not see an effect of MSF on intake could be attributed to the time between MSF and the lunch; lunch was provided approximately 15 min after MSF because of the blood samples that were collected up to 10 min after MSF. This may have been too long to affect intake, which is confirmed by the appetite ratings that returned back to baseline 10 min after MSF.

To be able to manipulate texture and sweetness concentration while keeping all other food properties equal, model foods were used in this study. Although our model food system facilitates a very controlled way of studying the effect of small changes in food properties on physiological responses, the disadvantage of using model foods is that subjects are relatively unfamiliar with them, besides, our model foods were rated neutral for liking. Liking and expectations of food (ingestion) are both important to elicit a cephalic response, this could therefore be the reason why we did not find a cephalic insulin, PP or ghrelin response, in contrast with other studies (12, 30, 50, 72).

Another explanation why we did not find a response could be because larger responses are seen in overweight subjects while this study included healthy, normal weight subjects (54). In addition, 54% of the insulin curves in this study were responsive and 46% were non-responsive and because of that, on average, no effect of treatments could be found and no cephalic insulin response was seen.

Taken together the before mentioned food and subject prerequisites to evoke measurable CPRs, it can be concluded that these are highly exacting responses that do not occur in every person at every eating occasion.

CONCLUSION

Our findings indicate that MSF on model foods does not lead to typical CPRs. Nevertheless, texture hardness and sweetness increases the total PP response, and MSF on hard texture increases the total ghrelin response compared to soft texture model foods. However, these effects are rather small and this study, among others, shows that there are major dissimilarities in cephalic phase endocrine responses to food stimulation between persons. This emphasizes that inter-individual factors need to be taken into account and stresses the importance of taking into consideration that there are cephalic responders and non-responders. These variable responses to food stimuli make it difficult to study the effect of realistic changes in food properties on CPRs. Therefore, more research is needed to further elucidate the effects of food texture and taste properties on CPRs.

ETHICS STATEMENT

This study was carried out in accordance with the Declaration of Helsinki and approved by the Medical Ethical Committee of Wageningen University with written informed consent from all subjects.

AUTHOR CONTRIBUTIONS

ML, PS, MM, and CG designed the research (project conception, development of overall research plan, and study oversight). ML, PS, and MM wrote the manuscript. ML conducted the research. CG, PS, and MM read and approved the final version of the manuscript.

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Hematocrit Values Predict Carotid Intimal-Media Thickness in Obese Patients With Non-Alcoholic Fatty Liver Disease: A Cross-Sectional Study

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Background: Literature data suggest with some criticism that full-fledged cardiovascular (CV) events (acute or chronic) are likely predicted by blood components, which are reported to be associated with the presence/severity of non-alcoholic fatty liver disease (NAFLD). This study was aimed at determining which marker(s) derived from blood count, such as white blood cells, neutrophils, neutrophil/lymphocyte ratio, platelet count, hemoglobin, mean corpuscular volume, hematocrit values were associated with ear or subclinical atherosclerosis, in obese patients of various classes suffering from NAFLD.

Methods: One hundred consecutive obese patients presenting NAFLD at ultrasound, with low prevalence of co-morbidities and no history or instrumental features of CV diseases, underwent carotid intima-media thickness (IMT) assessment by Doppler ultrasonography. All of them were studied taking into account anthropometric parameters, the metabolic profile, and inflammatory markers.

Results: White blood cells and neutrophil count showed no statistical association with IMT, which was predicted by the amount of visceral adiposity, as appreciated by ultrasonography. After adjusting for visceral adiposity and smoking status, only age and hematocrit contextually predicted early atherosclerosis, evaluated as IMT. Visceral adiposity was a confounding factor in foreseeing IMT.

Conclusion: Hematocrit values along with the patient's age suggest an initial atherosclerosis, evaluated as IMT, and if this finding is confirmed in larger cohorts, could be added to other canonical CV risk factors. Inferences can be enhanced by future prospective studies that aim to identify the relationships between incident cardio-metabolic cases and this hematologic parameter.

Keywords: hematocrit, carotid intima-media thickness, obesity-related non-alcoholic fatty liver disease, US, CV risk

INTRODUCTION

Literature data show that both full-fledged cardiovascular (CV) events (acute or chronic) and mortality derived from CV disease are likely predicted by blood components, but there are also dissenting studies.

In fact, while high count of white blood cells is an independent predictor of coronary artery disease risk in patients of both genders (1), also after adjusting for the classical risk factors (2), further research has failed to discern any association between hematological indices and coronary calcification (3). Markers including coronary artery calcification, carotid intima-media thickness (IMT), and ankle-brachial index can be used to assess CV risk, even in patients with no symptoms of heart disease. Indeed, the coronary artery disease risk associated with high white blood cells count was comparable to those of other inflammatory markers (4). Interestingly, higher levels of white blood cells are independently related to the presence of non-alcoholic fatty liver disease (NAFLD) (5), a condition reckoned as a CV risk *per se*.

Not only with total count of white blood cells was an association of the long-term carotid artery disease-related mortality found but also with an its subtype, i.e., neutrophil count (6, 7). In keeping with the subtypes of white blood cells, attention has been directed to predict coronary artery disease using the neutrophil-to-lymphocyte ratio (8, 9). Furthermore, the neutrophil-to-lymphocyte ratio is higher in patients with the most severe form of NAFLD (10, 11), although this finding has not been confirmed (12). Literature offers contrasting data about hemoglobin levels. In fact, lower levels are independent predictor of late mortality in patients undergoing coronary artery bypass grafting (13) while higher levels seem to predict the histological severity of NAFLD (14).

A blood constituent that has captured the interest of many researchers is hematocrit, which is an independent risk factor for CV mortality (15). But, also in this context, there is some disagreement. Whereas patients with chronic cerebral infarctions had hematocrit values significantly related to the carotid IMT (16), a meta-analysis comparing hematocrit and coronary artery disease risk showed a limited prediction in disease-free subjects (17). Similarly, evaluating asymptomatic cerebrovascular damage, no relationship was found between hematocrit and IMT (18). As to NAFLD, there is evidence that hematocrit levels are independently associated with fibrosis in NAFLD patients (19). Another blood cell component showing to play a role in coronary artery disease (20) is the mean volume of platelets, while their total count predicts fibrosis severity, but not steatosis grade in patients with liver-biopsy confirmed NAFLD (21, 22).

Apart data that shows an association between advanced and well-established forms of CV disease and blood components, little evidence exists about the link between hematological parameters and early stages or subclinical CV diseases, characterized by early atherosclerosis (23) in obesity-related NAFLD patients.

As previously stated, carotid IMT is increasingly regarded as a surrogate marker for assessing the initial process of atherosclerosis, its ability relying on predicting future clinical CV end-points (24). Focusing on ultrasonographic (US) evidence of IMT, we aimed at detecting any correlations between this vascular parameter and any component of blood count in a population of obese patients, with ultrasonography (US) feature of NAFLD. We also analyzed canonical and non-canonical cardio-metabolic risk factors, such as increased age, gender, smoking status, blood pressure, body fat distribution, both systemic and hepatic insulin resistance, lipid profile—including triglyceride/high-density lipoprotein (HDL) cholesterol ratio and the atherogenic index of plasma (AIP), markers of acute and chronic inflammation, C-reactive protein (CRP), fibrinogen, ferritin, and spleen volume (25).

MATERIALS AND METHODS

This cross-sectional study was performed enrolling 100 obese patients characterized by low prevalence of co-morbidities, without any history or sign of CV disease but with US findings of NAFLD. The research protocols were approved by the Ethics Committee of the Federico II University Medical School of Naples (protocol number: 231-05). All participants provided their written informed consent to participate in this study.

Exclusion Criteria

Previous or recent CV diseases, such as coronary artery disease, cerebrovascular disease, or peripheral artery disease were excluded on the basis of history and appropriate testing. Any viral, autoimmune, metabolic liver disease (Wilson disease, hemochromatosis, or antitrypsin deficiency) was ruled out. Celiac disease was excluded on the grounds of the IgA anti-tissue transglutaminase antibodies absence. Alcohol abuse tested according to the DSM-IV diagnostic criteria, carrying out screening tests such as MAST (Michigan Alcohol Screening Test) and CAGE (Cut down, Annoyed, Guilty, and Eye opener), as well as random tests for blood alcohol concentration and the use of a surrogate marker, e.g., mean corpuscular volume (MCV). The therapy of patients was maintained according their schedules.

Metabolic Profile

Overweight and the class of obesity (I, II, III) was set by body mass index (BMI) cut-offs, i.e., 25–29.9, 30–34.9, 35–39.9, and >40 kg/m^2, respectively. Abdominal obesity was assessed evaluating waist circumference (WC) measured at the midpoint between the lower border of the rib cage and the iliac crest. Hip circumference was measured around the widest part of the buttocks, with the tape parallel to the floor, and the waist-to-hip ratio (WHR) was calculated.

The canonical Adults Treatment Panel III was chosen to define the metabolic syndrome, considering at least three criteria: plasma glucose concentrations ≥100 mg/dL, WC >102/88 cm (male/female), serum HDL concentration <50 mg/dL for women and <40 mg/dL for men, blood pressure ≥130/85 mm Hg, and serum triglyceride concentration ≥150 mg/dL. Insulin resistance was studied by the HOmeostatic Metabolic Assessment (HOMA) method with the formula: fasting insulin (μU/mL) × fasting glucose (mg/dL)/405 (26). A value of HOMA >2 was introduced as limit of the presence of insulin resistance (25). More than five determinations of HOMA in different situations were taken into

account. Triglyceride values of subjects who had fasted at least 12/14 h before the blood draw were evaluated, averaging the results of at least two determinations, made on different days. Moreover, the triglyceride/HDL cholesterol ratio was evaluated, considering abnormal values ≥1.65 (men) and ≥1.32 (women) (27, 28). Low-density lipoprotein (LDL) was calculated by the following formula: LDL = total cholesterol − HDL − (triglycerides/5). A new index evaluating hepatic insulin resistance, generated through stepwise linear regression, i.e., 2.2607 + 0.427 × log HOMA was computed (29). The AIP was calculated according to the following formula: AIP = log triglycerides/HDL (30).

Ultrasonography Features

The spleen diameter, assessed on longitudinal scan, was chosen to evaluate the spleen volume and was carried out by the posterolateral approach. The classification of hepatic steatosis/NAFLD was based on the following scale of increased echogenity: grade 0 = absent, 1 = light, 2 = moderate, 3 = severe, unraveling the difference between the densities of the liver and the right kidney obtained on the same longitudinal sonographic plane. The levels of brightness of the liver and right kidney were calculated three times directly from the frozen images. Subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) were assessed by transverse scanning. SAT was defined as the thickness between the skin-fat interface and the linea alba, avoiding compression. VAT was defined as the distance between the anterior wall of the aorta and the internal face of the recto-abdominal muscle perpendicular to the aorta, measured 1 cm above the umbilicus. When the aortic walls were obscured by bowel gas, a Doppler scan was used to detect them (25). The common carotid artery, the carotid bulb, and the near and far wall segments of the internal carotid artery were scanned bilaterally. Images were obtained in longitudinal sections with a single lateral angle of insonation, optimizing the image for the far wall. IMT was defined as the distance between the ultrasound interfaces of the lumen-intima and media-adventitia. Six manual measurements were performed, with automatic border detection, at equal distances along 1 cm on the far wall of the common carotid, according to the consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force, endorsed by the Society for Vascular Medicine (31).

Blood Pressure Measurements

Systolic/diastolic blood pressure values were obtained averaging three consecutive measurements taken during usual practice hours, after subjects had rested for 5 min in the sitting position.

Laboratory Data

Blood samples were collected from all participants in the early morning after an overnight fast. An automated hematologic analyzer (Coulter LH750) was used to measure total and differential blood parameters/counts. Testing for serum triglycerides, HDL, fasting insulin, alanine transferase (ALT), gamma-glutamyl transferase, alkaline phosphate, cholinesterase, fasting glucose, fasting insulin, fibrinogen, and ferritin were performed at enrollment by in-house standard procedures. Hs-CRP values, determined by the ELISA test, with reference values between 0.3 and 8.6 mg/L in healthy men and between 0.2 and 9.1 mg/L in healthy women (BioCheck, Inc., CA, USA) were determined in collected and aliquoted samples and frozen at −20°C.

Smoking status was categorized into five classes, i.e., class 1 as non-smokers, class 2 as past smokers, class 3 as social/weekend smokers, class 4 as light/moderate smokers, and finally class 5 as active smokers.

Statistics

The Kolmogorov–Smirnov test was evaluated to detect whether the variables were normally distributed and accordingly were reported as mean plus SD. Variables not normally distributed were expressed as median (25–75 IQR). The difference in medians was assessed by the Mann–Whitney test for independent samples. Chi-square evaluated frequencies. The two-way cross-tabulation was performed to assess the relation between the severity of obesity and gender on the one hand, and hepatic steatosis grade at ultrasonography on the other. As exploratory step, the degree of the association between single parameters was studied using Spearman's coefficient of rank correlation (ρ) for non-uniform intervals. To assess the independent effect of a quantitative variable (predictor or explanatory) on eventual prediction of another one, the linear regression analysis (least squares) was used evaluating the coefficient and the *t* (*t*-value). As multivariate analysis, the multiple regression was adopted (Backward Stepwise Selection), first entering all independent variables if $P = 0.05$ into the model (at univariate analysis), and then removing if $P = 0.1$ the non-significant variables sequentially, with a maximum number of 10 steps. IMT was chosen as dependent variable. To appreciate which variables contribute to any extent to the regression equation, the magnitude of standardized coefficient beta (β) was calculated. To assess multicollinearity, variance inflation factor and tolerance were set at >10 and <0.1, respectively. The effect size was calculated evaluating the R^2 (<0.25: small, from 0.25 to 0.64: medium, ≥0.65: large). The maximum number of variables put in the multiple regression analysis never exceeded the ratio of one variable to 10 patients. When analyzing a dataset in which there was one independent variable that determined an outcome, measured with a dichotomous variable—i.e., no smoking status—the logistic regression was performed (OR and 95% CI or coefficient plus SE).

The concordance correlation coefficient (ρ_c), which measures precision and accuracy, was adopted to evaluate the degree of pair observations at ultrasonography. The statistical analysis was performed operating on Systat 13 (Richmond, CA, USA) and MedCalc Version 15.2.1 ® (Frank Schoonjans) software packages.

RESULTS

Characteristics of the whole population are shown in **Table 1**. The most part of patients (89%) showed obesity of second and third class, without any difference for gender (cross-tabulation $P = 0.4$). Specifically, referring to class I/II/II/of obesity there were 7/26/35 females, respectively, while 4/18/20 were males belonging to the aforementioned classes. Concerning the metabolic syndrome presence, 31 females out of 68 and 26 males out of 32 fulfilled the criteria, chi-square 11.29, $P = 0.0007$. Hepatic

TABLE 1 | Characteristics of obese patients with non-alcoholic fatty liver disease (n = 100), including metabolic parameters.

Gender M/F	32/68	Age	43.5 (32–52)
BMI	40.7 (37.1–45.1)	BM I > 45 (n)	26
WC females (cm)	118.8 ± 16.1	WC males (cm)	132.8 ± 110.6
WHR males	1.02 ± 0.05	WHR females	0.90 ± 0.06
SAT (cm)	2.8 ± 0.06	VAT (cm)	7.3 ± 0.06 2.1
Ferritin females (ng/mL)	38.5 (19.5–70.5)	Ferritin males (ng/mL)	169.7 (140–197)
Fibrinogen (g/L)	323 (285.5–356)	CRP (mg/mL)	0.46 (0.24–0.89)
γ-GT (U/L)	22.5 (14.5–36)	ALT (U/L)	23 (18–33)
Triglycerides (mg/dL)	104.5 (76.5–160.5)	LDL (mg/dL)	169.7 (140.3–1.97)
HDL females (mg/dL)	50.8 ± 11.05	HDL males (mg/mL)	43.7 ± 9.06
Triglyceride/HDL ratio (females)	1.93 (1.38–2.81)	Triglyceride/HDL ratio (males)	3.26 (1.61–4.82)
AIP (females)	0.29 ± 0.23	AIP (males)	0.50 ± 0.36
Hepatic IR	2.4 (2.3–2.5)	HOMA	2.4 (1.32–3.72)
SBP (mm Hg)	130 (120–130)	DBP (mm Hg)	80 (75–100)
IMT > 0.9 mm (yes/no)	35/65	IMT (mm)	0.08 (0.07–0.10)
Hematocrit (females)	39.8 ± 2.99	Hematocrit (males)	44.5 (42.4–46)
White blood cells (mm^3)	7,750 (6,700–8,850)	Neutrophil lymphocyte ratio	2.06 (1.62–2.31)
Hemoglobin (females) (g/dL)	13.5 ± 1.14	Hemoglobin (males) (g/dL)	15.1 (14.5–15.6)
MCV	86.7 (82.7–90.1)	Platelet count (10^9/L)	254 ± 54
Spleen longitudinal diameter (mm)	11.23 ± 0.14	Spleen longitudinal diameter > 12 mm (n)	15

Values were expressed as m ± SD or median (25–75 IQR).

n, number of patients; WC, waist circumference; BMI, body mass index; WHR, waist-to-hip ratio; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; HDL, high-density lipoprotein cholesterol; CRP, C-reactive protein; ALT, aminotransferase; γ-GT, gamma-glutamyl transglutaminase; LDL, low-density lipoprotein cholesterol; HOMA, HOmeostatic Metabolic Assessment; SBP, systolic blood pressure; DBP, diastolic blood pressure; IMT, intima-media thickness; AIP, atherogenic index of plasma; MCV, mean corpuscular volume.

steatosis was related to the degree of obesity (cross-tabulation $P = 0.014$). Hypertension occurred only in 4 obese patients, while type 2 diabetes mellitus in 16, confirming the low prevalence of co-morbidities. Nearly one-third of patients presented an increase in carotid IMT. Interestingly, only 45 subjects were labeled as active smokers versus 35 self-declared no-smokers or past smokers, while the remainders were light or weekend smokers. Insulin resistance, evaluated as HOMA >2 and present in 56 obese patients, was significantly higher in those diagnosed at US with moderate compared to mild grade of hepatic steatosis (median 2.65 versus 1.42, $P = 0.006$ and Mann–Whitney U). Hepatic insulin resistance in males was lower than systemic insulin resistance, expressed as HOMA, i.e., median 2.48 versus 3.49, $P = 0.003$; in females, there was no difference, i.e., median 2.39 versus 2.02, $P = 0.45$, Mann–Whitney U. The hematocrit mean value was in both gender generally at the upper limit of normality.

Associations

The most relevant correlations are shown in **Table 2**.

Furthermore, IMT was not related to white blood cells ($\rho = 0.09$, $P = 0.35$) or neutrophil count ($\rho = 0.12$, $P = 0.24$). There was a trend of significance concerning the relationship between BMI and platelets count ($\rho = 0.19$, $P = 0.056$) and between hepatic steatosis grade at US and IMT ($\rho = 0.18$, $P = 0.07$).

Univariate Analysis

The most relevant predictions at linear regression are shown in **Table 3**.

Intima-media thickness was not predicted by white blood cells count, neutrophils, and neutrophil/lymphocyte ratio, $P = 0.13$, 0.19, and 0.94, respectively. Noticeably, hematocrit was not predicted by age, $P = 0.17$. As collateral finding, MCV was not predicted by WC, $P = 0.8$.

TABLE 2 | The most relevant associations.

IMT	Hematocrit	$\rho = 0.353$	$P = 0.003$
IMT	Hemoglobin	$\rho = 0.28$	$P = 0.004$
IMT	Platelets count	$\rho = 0.30$	$P = 0.0$
Hemoglobin	Mean corpuscular volume	$\rho = 0.4$	$P < 0.001$
Hematocrit	Hepatic steatosis severity	$\rho = 0.23$	$P = 0.021$
Hepatic insulin resistance	Hepatic steatosis severity	$\rho = 0.27$	$P = 0.007$
Triglyceride/HDL ratio	WC	$\rho = 0.325$	$P = 0.007$
γ-GT	HOMA	$\rho = 0.50$	$P = 0.0001$
γ-GT	Ferritin	$\rho = 0.48$	$P = 0.001$
HOMA	Hematocrit	$\rho = 0.29$	$P = 0.029$

IMT, intima-media thickness; HOMA, HOmeostatic Metabolic Assessment; γ-GT, gamma-glutamyl transglutaminase; WC, waist circumference; HDL, high-density lipoprotein cholesterol; ALT, alanine transferase.

Metabolic syndrome occurred much more frequently in those patients who presented an elevation of both ALT and γ-GT serum levels. (Spearman ρ 0.59 with P = 0.001 and 0.41 with P = 0.001, respectively).

TABLE 3 | Predictions at univariate analysis.

		Coefficient	*t*	*P*
IMT	Age	0.0012	6.77	<0.0001
IMT	Hematocrit	0.0026	3.45	0.001
IMT	Platelets count	−0.00000017	−3.09	0.0025
IMT	Mean corpuscular volume	0.0011	3.28	0.0014
HOMA	Hematocrit	0.0045	2.7	0.007

IMT, intima-media thickness; HOMA, HOmeostatic Metabolic Assessment.

As expected, IMT was predicted by smoking status, coefficient 0.0067, $t = 3.51$, $P = 0.001$. In details, non-smoking status was predicted by hematocrit, OR 0.77, 95% CI = 0.67–0.89, $P = 0.0002$.

Multivariate Analysis

First of all, studying classical risk factors for atherosclerosis, IMT was predicted only by LDL among the metabolic parameters, i.e., hepatic insulin resistance, HOMA, LDL, HDL, AIP, triglyceride/

HDL, with coefficient 0.0001, $\beta = 0.25$, $t = 2.58$, $P = 0.011$, $R^2 = 0.06$.

Adding to the model predicting IMT, (i) blood count factors (hematocrit, MCV, and platelets), (ii) age, and (iii) smoking habit, resulted to be predicted by age and hematocrit, coefficient 0.001, $\beta = 0.55$, $t = 6.57$, $P < 0.001$, and coefficient 0.0020, $\beta = 0.30$, $t = 3.199$, $P = 0.0019$, $R^2 = 0.38$, respectively. It is worth underlining that hemoglobin was excluded from the analysis due to collinearity with MCV.

Successively, evaluating the role of anthropometric features, among BMI, WC, WHR, SAT, and VAT, IMT was predicted by VAT alone, with coefficient −0.0085, $\beta = 0.49$, $t = 5.5$, $P < 0.0001$, $R^2 = 0.24$.

Furthermore, age and hematocrit, adjusted for VAT, maintained their predictive value, although there was a reduction of more than 20% of the coefficient, lending credence to the fact that visceral adiposity is a real confounding factor. The results for age were coefficient 0.001, $\beta = 0.47$, $t = 5.3$, $P < 0.0001$; for hematocrit: coefficient 0.001, $\beta = 0.24$, $t = 2.48$, $P = 0.014$, for VAT: coefficient 0.002, $\beta = 0.225$, $t = 2.2$, $P = 0.025$, $R^2 = 0.41$. Finally, when these three variables predicting IMT, i.e., age, hematocrit, and VAT were adjusted for gender, this variable was excluded from the model, evidencing its lack of influence.

Reliability

To substantiate the clinical significance of US features, the inter-intra-observational reproducibility of the sonographic estimations was high, with a ρ_c of 0.93 and 0.89, respectively.

DISCUSSION

In our studied population (obese patients of second and third grade, with low prevalence of co-morbidities but suffering from NAFLD), both age and hematocrit values predicted IMT, also after adjusting for other evidenced predictors of IMT, i.e., amount of the visceral adiposity, expressed as VAT and evaluated by US and smoking habit. Noteworthy, the latter was also predicted by hematocrit in line with a previous study that found an increase in hematocrit values among smokers and in heavy smokers, these changes likely predisposing to greater risk of developing atherosclerotic plaques and coronary heart disease (32).

Concerning other blood count components, white blood cells and neutrophil count did not show any relation to IMT in our population. Considering both these blood components as inflammatory markers, interestingly our data on CRP are in agreement with the latter finding, in the sense that this acute phase protein was not associated with IMT in our population, partially in accordance with other results showing that CRP and carotid IMT levels appear to be directly related in women, but not in men in a population of 2,640 subjects, gender equally represented, differently from our study (33).

Analyzing the possible mechanisms underlying the association between hematocrit levels and early atherosclerosis, blood viscosity is central to explaining this phenomenon. Unfortunately, recent literature offers divergent data. In fact, Carallo et al. demonstrate that blood viscosity seems independent of classical coronary heart disease risk factors and is unrelated to hematocrit and plasma viscosity, suggesting a possible direct effect of aging on red blood cells (34). By contrast, the passage time, another index of blood rheology, correlated better with hematocrit ($r = 0.422$) than white blood cell count ($r = 0.295$) and platelet count ($r = 0.204$) (35).

Confronting the aforementioned data, we hypothesize that red blood cells increase the adhesiveness of platelets by the erythrocyte-derived ADP available for platelet activation and causes the dispersion of platelets toward the sub-endothelial surface due to blood flow (36, 37). Experimental studies have suggested that red blood cells promote thrombin generation and that the thrombin concentration is proportionate to hematocrit levels (38, 39). As a result, hematocrit could promote thrombosis and atherosclerosis, and consequently CV disease. The finding that in humans blood viscosity, evaluated as hematocrit, is involved in the endothelial response to an increase in shear stress confirms the role of hematocrit in hypertension and consequently in the atherosclerotic process (40).

The relationship between obesity-related NAFLD and atherosclerosis *via* thrombotic mechanisms should not be overlooked. In fact, in a rat model of NAFLD, induced by feeding a fat-rich diet for 24 weeks, the imbalance of plasma PGI2 and thromboxane A2 (TXA2) levels played a role in the pathogenesis of NAFLD (41). TXA2, a marker of platelet activation, evaluated as serum levels thromboxane B2, is higher in obese subjects than in lean ones, and this might be a clue to their increased CV risk (42).

To draw further conclusions from our results, it is worth mentioning that hematocrit was positively associated with insulin resistance—evaluated as HOMA—an association considered an epiphenomenon of visceral adiposity (18). The latter finding confirms that an interplay between alterations of adipose tissue distribution/function and hematocrit could be involved determining broad effects on in the induction/maintenance of atherosclerosis. As partially supporting evidence, taking into account that insulin resistance is a major underlying mechanism responsible for the "metabolic syndrome," which is also known as insulin resistance syndrome, authors found that beyond elevated levels of hemoglobin and red blood cells counts hematocrit was significantly associated with clustered components of metabolic syndrome in women (43). It is necessary to highlight that differences in definitions of CV disease risk, as well as ethnic and racial differences may account for the absence of consistency across studies.

We did not assess the prevalence of abnormal blood data in our obese patients, extensively reported elsewhere (44), nor were able to confirm previous data from others concerning the link between hepatic steatosis severity and white blood cells count. Indeed, recent research clearly showed that white blood cells count was a significant factor associated with incident NAFLD in Han Chinese without NAFLD at base line, with a HR of 1.152 in the highest quartile, obtained by Cox proportional hazards regression analysis (45).

The role of NAFLD in predicting IMT was not definitely evident in this study, but we emphasize that the NAFLD severity was not assessed by histology, due to the lack of consent to invasiveness by our obese patients. Supportively, we stress that IMT was predicted by the visceral adiposity, a clear sign of NAFLD presence, directly or indirectly representing a CV risk factor (46).

Accordingly, it should be stressed that visceral adiposity was a real confounding factor in predicting IMT, consequently playing a noticeable role in determining/worsening atherosclerosis. In multiple regression, the lack of retaining of smoking as predictor of IMT is likely due the reduced number of active smokers in our population and multicollinearity with age, according to recent investigation (47).

The apparently surprising negative correlation between IMT and platelets count is likely due to the treatment with anti-platelet drugs and to vitamin B12 and/or folate deficiency, often present in the obese, according to de Ilvar et al. (48).

Coming back to our core finding, when comparing our results with relevant findings from other reports of literature by principal search sources during the last decade, interesting data, although not univocal, was found about the relation between hematocrit levels and CV disease risk factors. In fact, this association has been reported inconsistently. Recent findings suggest that both elevated and decreased hematocrit levels are associated with an increased risk of established CV disease, even though the more significant risk was found in the highest quartile of patients with ischemic stroke and coronary artery disease (49). On the other hand, the study of Arbel et al., found that, in patients with angiographically normal coronary arteries and slow coronary flow, hematocrit levels are associated with slower coronary blood flow (SCF). The phenomenon of SCF in the presence of normal coronary arteries may indicate endothelial dysfunction, which is characteristic of an early stage in the development of atherosclerosis. Despite this association, such patients do not have increased carotid IMT values (50). In line with our results stands a study by Tabara et al. including 1,978 participants from two independent cohorts, which showed that hematocrit was positively associated with insulin resistance and insulin sensitivity. However, this association was lost after further adjustment for visceral fat area and plasma ALT, concluding that this association was epiphenomenon of visceral adiposity and hepatic fat excess (18).

Limitations

First, we acknowledge that the type of study did not allow us to draw conclusions on the direction of the associations.

Second, ours was a single-center study, and large-scale prospective studies are needed to confirm this association and establish whether this new information is sufficient to modify existing clinical practice.

Possible drawbacks were not having evaluated the adipose districts by the more precise magnetic resonance imaging and NAFLD by histology. Anyway, US-detecting NAFLD is a reliable technique in epidemiological studies (51).

Finally, having selected a population with low rate of co-morbidities, which presented relatively few CV risk factors, has its limitations.

CONCLUSION AND FUTURE DIRECTIONS

The combination of hematocrit measurements with one of the "canonical risk factors," e.g., the patients' age, can improve our ability to detect an early atherosclerosis in the obese. Future observational studies in this setting, comparing patients with low and high rate of co-morbidities, will play an important role in the decision-making process providing invaluable information on the reliability of hematocrit determinations at the light that these are widely available, easy to carry out and interpret. Specifically, as implications for current practice, hematocrit values can alert physician to the need of making a quick diagnosis of incident cardio-metabolic cases when facing such patients with low rate of co-morbidities but NAFLD. Obese patients affected by various co-morbidities are likely to have more severe or premature atherosclerosis due to combined mechanisms and it would be extremely interesting whether hematocrits still remain a predictor of carotid IMT. Indeed, such patients requiring more frequent physician visits have greater opportunity to undergo screening or to have early symptoms of CV disease investigated.

As corollary of our finding, hematocrit determinations could have a further significant impact on clinical decision, mainly on cutting back the spending of the health care system and consequently costs in a real-world environment. Finally, this study merits further research in the sense that a better and more nuanced understanding of the molecular basis underlying the association between hematocrit and carotid IMT and its pathogenic pathways may lead to new strategies to improve CV health as well as overall health and increase longevity.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of "the Declaration of Helsinki." The protocol was approved by the "Federico II University Medical School." Oral or when possible written informed consent was obtained from all subjects.

AUTHOR CONTRIBUTIONS

GT conceived the study. All the authors equally contributed to the analysis and interpretation of data, to the draft of manuscript, shared the content, and accepted that GT submitted the paper on their behalf.

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MINI REVIEW
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Depression and Obesity: Integrating the Role of Stress, Neuroendocrine Dysfunction and Inflammatory Pathways

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Literature on depression and obesity describes the relevance of the hypothalamic pituitary adrenal axis dysfunction, sympathetic nervous system (SNS) activation, and inflammatory processes as well as the interaction of genetic and environmental factors. Recent investigation in obesity highlights the involvement of several regulation systems, particularly in white adipose tissue. The hypothalamic pituitary adrenal axis, gonadal, growth hormone, leptin, sympathetic nervous system and adrenergic, dopaminergic, and serotoninergic central pathways, all seem interconnected and involved in obesity. From another perspective, the role of psychosocial chronic stressors, determining poor mental and physical health, is well documented. Empirical data can support biologically conceivable theories describing how perceptions of the external social environment are transduced into cellular inflammation and depression. Although in neurobiological models of depression, stress responses are associated with neuroendocrine and neuro-inflammatory processes, concerning similar pathways to those described in obesity, an integrating model is still lacking. The aim of this mini-review is to offer a reflexion on the interplay between the neuroendocrine dysfunctions related to chronic stress and the nature of the shared biologic mechanisms in the pathophysiology of both clinical entities, depression and obesity. We highlight dysfunctional answers of mind body systems that are usually activated to promote regulation and adaptation. Stress response, as a mediator between different level phenomena, may undertake the role of a plausible link between psychological and biological determinants of disease. Depression and obesity are major public health issues, urging for new insights and novel interventions and this discussion points to the need of a more in-depth approach.

Keywords: depression, obesity, stress, inflammation, neuroendocrine dysfunction

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INTRODUCTION

Depression is diagnosed to almost 4% of the world population, with 16.6% lifetime prevalence rate, having a major impact on social and public health (1, 2).

In neurobiological models of depression, stress responses, although dependent on individual's predisposition, are associated with inflammatory mechanisms promoting the activation of serotoninergic pathways and reducing serotonin availability. The neuroendocrine dysregulation,

the immune response, and the neuro-inflammatory processes also determines changes in monoaminergic systems (serotonin and norepinephrine) classically associated with the etiology of depression and different symptom profiles (3–5).

Recent research aims to describe the common pathways to clarity different features presented in literature, assuming the relevance of the hypothalamic–pituitary–adrenal axis (HPA) activation, with the increase in glucocorticoids production, and Sympathetic Nervous System (SNS) activation (6, 7). New paradigms emerge, such as the immunologic, neuroendocrine, and inflammatory ones, through the investigation of pro-inflammatory cytokines, systemic inflammation in metabolic syndrome, the role of other neuropeptides and receptors as well as the interaction of genetic and environmental factors.

Obesity should not be considered just a disease; from an evolutionary point of view it is perhaps better understood as a product of genetic selection promoting thrifty phenotype individuals, actually living in abundance. In fact, as Bjorntorp stated, the recent obesity epidemic might be due to ancient genetic evolutionary changes becoming apparent in a society of prosperity and longer life expectancy. Also, obesity comorbidities may result more from increasingly stressful environmental factors, rather than from a normal genetic mechanism selected to fight energy deprivation and to promote survival (8). Searching for the reasons of obesity will imply looking for the complex relationships between environment and genes, pre-programmed biological determinants and the external context acting upon them as a vulnerability factor and influenced by individual aspects such as cognition and behavior. New issues arise from the obesity epidemic in developed societies, namely the need to reformulate our clinical approach, through a multidisciplinary and preventive perspective.

The aim of this mini-review is to offer a reflexion on the interplay between the neuroendocrine dysfunctions related to chronic stress and the nature of the common biologic mechanisms in the pathophysiology of both clinical entities, depression and obesity.

THE STRESS RESPONSE AND THE NEUROENDOCRINE DYSFUNCTION

The perception and evaluation of the stressful nature of an event, relies on behavioral and genetic factors, as well as on the previous experiences and individual resources (9, 10).

Acute and chronic stressors are diverse in nature and consequences. Several studies showed mixed findings regarding the nature of stressors, their temporal dimension and the unpredictability of events (11). Psychosocial stressors with a chronic dimension are well documented as determinants of poor mental and physical health, leading to significant burden to health systems, mortality, morbidity and general wellbeing, predominantly in western societies (12–14).

Chronic stressors create a sustained physiological arousal which allows a set of biological and behavioral adaptive responses. However, the evaluation processes and therefore the impact of a stressor on the organism, depends upon cognitive and emotional factors, which modulates the individual experience of a threatening event (10, 11). Stress vulnerability and its role in several diseases is determined by characteristics such as personality (temperament; character) cognitive resources, emotion regulation, coping strategies, and contextual factors such as social support (13, 15).

Chronic psychological or biological stressors, threatening the homeostatic balance, determine an overload state which promotes stability through change—allostasis. The allostatic load represents the cluster of processes allowing an organized and maintained adaptive response from the stressed organism. In this context, the organism exposure to the response mediators (neuroendocrine or immunological ones) can be distressing and disease promoting (16).

The biological adaptive responses are mediated by regulatory mechanisms, globally known as the stress system, which includes Central Nervous System (CNS) coordination of the HPA axis and Autonomous Nervous System (ANS) which are known to be involved in metabolic syndrome, obesity, and depression.

While in normal circumstances the HPA activation suppresses pro-inflammatory and antiviral immune response, in threatening conditions, when exposure to actual or perceived danger is maintained, the HPA axis promotes an increase in inflammatory response. This process, referred as glucocorticoid resistance or glucocorticoid insensitivity, follows the immune cells' loss of sensitivity to the anti-inflammatory effects of glucocorticoids in order to compensate for their persistent secretion. The details for glucocorticoid resistance are not completely understood, but perhaps its purpose represents an adaptive process, as elevations in pro-inflammatory cytokines accelerate wound healing and limit infections, having a protective role (17, 18).

The HPA axis interact with the ANS, contributing to allostasis. Corticotropin-releasing factor activation increases norepinephrine levels, regulating pro-inflammatory cytokine production. Once released, norepinephrine modulates immune response gene transcription mostly via stimulation of β-adrenergic receptors (19). Sympathetic activation determines an increase in blood pressure and heart rate, and also inhibits the parasympathetic branch of the ANS which modulates immune responses through both the efferent and afferent fibers of the vagus nerve, enabling it to prevent excessive inflammation. Slavish and Irwin (18) state that these data can support an empirical basis for biologically conceivable theories describing how perceptions of the external social environment are transduced into cellular inflammation and depression, proposing their integration into a "social signal transduction theory of depression." The authors proposed that social threatening situations are represented in brain regions which process experiences of negative affect and rejection-related distress. The connections to lower level brain regions, including the hypothalamus and brainstem regions do not directly regulate inflammatory activity, but they influence systemic inflammation by modulating the activity of the HPA axis and the SNS. While cortisol suppresses inflammatory activity, epinephrine, and norepinephrine promotes inflammation by interacting with immune systems cells through specific receptors.

STRESS, DEPRESSION, AND INFLAMMATION

It is now evident that major life stressors can result in homeostatic imbalance and abnormal immune responses, increasing inflammatory activity, and resulting in mental disorders namely depression (20).

Findings linking inflammation and depression are well established and described by several authors since the 1990's (21, 22). This evidence comes from the high levels of inflammatory markers in patients with depression even in the absence of other pathologies, the co-occurrence of depression and inflammatory diseases and the increased risk of depression in patients treated with cytokines (23).

The results of a meta-analysis of 24 studies measuring cytokines in depressed patients, found that individuals with Major Depression had significantly higher concentrations of Tumoral Necrosis Factor alpha (TNF-α) and Interleukin 6 (IL-6) compared to controls (24). Increased peripheral inflammatory markers were found among antidepressant non-responders more often than those who responded to treatment. (3, 25, 26). Higher levels of pro-inflammatory cytokines, Interleukin−1 beta (IL-1β), IL-6 and TNF-α were found in the blood or in the brain of these patients (21, 27, 28). Depression is also accompanied by an increase in acute phase proteins such as haptoglobin, α1-antitrypsin, ceruloplasmin, and C-reactive protein (27, 29).

Cytokines are potent modulators of behavior and mood, and play a central role in the immune system and inflammatory response (21). There are several examples of this in animal models research (30) as well as in cancer or Hepatitis C patients treated with interferon-α (IFN) which is associated with a high incidence of depression (31). Over the last years, research attempting to elucidate the mechanisms involved in the serious collateral effects associated to this agent, namely cognitive disorders and depression has increased. IFN induces changes in the endocrine function (hypothalamic-pituitary-adrenal axis) and in neurotransmission activity (especially serotonin and dopamine) (32–34).

The mechanism by which depression is induced by IFN is still being researched and it is, very likely, multifactorial (34, 35). In agreeance with the literature about the relation between inflammatory cytokine and the serotonin pathways (5-HT), evidence shows that IFN can affect the expression of serotonergic 1A receptors (5-HT1A) (33, 36), which is consistent with what is observed in depressed individuals (37). IFN also reduces the levels of peripheral tryptophan, an effect that is correlated to depression (38).

These data suggest that different physiopathological pathways may be connected to the development of specific symptomatic dimensions, including mood/cognitive symptoms *versus* neurovegetative symptoms, in the context of the cytokine activation system. In addition, Schmidt et al. (39) pointed to the need of a biomarker panel for depression which can perhaps allow for the recognition of a biological signature of major depression subtypes.

OBESITY AND DEPRESSION—NEURO-INFLAMMATORY AND ENDOCRINE PATHWAYS

Recent investigation suggests the involvement of several regulatory systems in obesity, particularly in white adipose tissue (WAT). HPA axis, gonadal, growth hormone, leptin, SNS, and adrenergic, dopaminergic, and serotoninergic central pathways, all seem interconnected and involved with obesity. Genetic factors are also relevant, and recent data highlights the glucocorticoids, dopamine or leptin receptors' role (40, 41).

In animal models, stress increases the release of neuropeptide Y, which promotes the growth and differentiation of adipocytes and angiogenesis in the presence of high fat and sugar diet. Prolonged activation of neuropeptide Y and its receptor system (NPY–NPY2R) in adipocytes and endothelial cells is associated to the increase of adipose tissue and metabolic syndrome (42). Adipose tissue, producing, and releasing a variety of hormones and peptides is understood as an endocrine organ, integrating the communication network between peripheral organs and the CNS (43).

Persistent inflammation associated to the increase in adipose tissue can be due to pro-inflammatory cytokines such as TNF-α and IL6 produced by the adipose tissue itself, explaining the neuroendocrine activation and the lipids or glucose metabolism changes observed in obesity (44). The hyperactivation of HPA axis can provoke obesity according to homeostatic and non-homeostatic pathways. The first includes corticotrophin releasing hormone (CRH) suppression, leptin resistance, and increased NPY release. Non-homeostatic pathways include food associated reward and pleasure (dopaminergic and opioidergic pathways) inducing a shift to a hypercaloric diet. In societies presenting high levels of stress and easy available high caloric food, activation of HPA axis might be an important contributing factor to the obesity epidemic (45).

The WAT is a mosaic of adipocytes, nervous tissue, immune cells, connective tissue matrix, and stromovascular cells (46).

Adipokines are proteins specifically secreted from the WAT adipocytes with a local and systemic action. There are pro-inflammatory and anti-inflammatory adipokines. The main examples are leptin, resistin, and adiponectin (47). Leptin is a pro-inflammatory adipokine that regulates dietary intake trough leptin receptors located in the hypothalamus. It promotes the sensation of satisfaction and also increases energy expenditure. Resistin, a pro-inflammatory adipokine increases the secretion of IL-1, IL-6, and TNF-α from macrophages and simultaneously rises its level by the action of these same cytokines (47, 48). Adiponectin, with a predominantly anti-inflammatory role, is reduced in obese persons, inhibiting Th1 responses, polarizating pro-inflamatory M1 macrophages to the anti-inflammatory M2 type, IL-6 and TNF-α production and an increase in cytokine IL-10 secretion (47, 48).

This shift from a physiological toward a dysfunctional expression of adipokines follows obesity where a hypertrophy of the adipose tissue induces hypoxia and an inflammatory

response. An infiltration of macrophages changes the profile of the adipose tissue to a pro-inflammatory one. There is a dampening of the expression of adiponectin, whilst increasing the secretion of leptin, IL-6, TNF-α, and PAI-1 (49).

Beyond its peripheral role, systemic cytokines such as IL-6, TNF-α derived from adipose tissue also have access to the CNS leading to activation of microglia which turns the inflammation a central one (50).

The blood brain barrier (BBB) is not a passive structure and reacts to stimuli, changing its permeability and secreting inflammatory mediators to both the circulation and CNS leading to neuroinflammation (50). The pro-inflammatory cytokines can activate indoleamine 2,3-dioxygenase (IDO) and induce neuroinflammation through the synthesis of neurotoxic tryptophan catabolites (TRYCATS), including kynurenine, 3-hydroxykynurenine and quinolinic acid. It has been suggested that depression is associated with these neurotoxic products' consequences to the brain, in addition to the depletion of serotonin, resulting from the increased tryptophan catabolism linked to the IDO functioning (51).

Obesity also initiates an increased immune response against lipopolysaccharides (LPS) of different commensal gram negative bacteria. This immune response against LPS suggests that bacterial translocation to mesenteric lymph nodes or into the systemic circulation might take place, when a "leaky gut" develops with obesity, with increased permeability of the gut wall and a change of the usual intestinal microbiota (52). This immune response will raise the level of circulating systemic cytokines (49). The mucosal intestinal status is sent to the brain via vagal afferent neurons, another communication path between the CNS and the periphery, leading to brain adaptation to inflammation (53).

INTEGRATING THE MODELS

From the previous review we can conclude that the relationship between chronic stress, depression, obesity, and its autonomic and neuroendocrine mediation is well documented, as schematically presented in **Figure 1**.

However, depression and obesity are heterogeneous disorders/conditions and it seems that current neurobiological models lack sufficient power or specificity to explain the diversity of the clinical presentations and interactions.

In recent research the stress-diathesis model of depression was tested, pointing to a significant interaction between polygenic risk scores and personal life events, contributing to a higher risk of depression (54). Clusters of vulnerability, or different diathesis, emphasize the role of personality and affective traits, such as high anxiety trait, as a key vulnerability phenotype to stressful events in the etiology of depression (55). In fact, a previous affective style can amplify the impact of major or minor repetitive stressors, imposing an overload to stress regulatory systems.

Stress can cause depression and other comorbidities through neurobiological pathways leading to neuroinflammation, but also through behavioral ones. In what concerns these later pathways, the relevance of emotional eating in overweight and obesity, triggered by stressful life events and negative affect, seems to lead to an interesting approach. Human studies on the effects of stress in eating behavior, specifically emotional eating, reveal contradictory results. However, they point to individual differences in stress reactivity and to an interaction between cognitive (attentional bias, escape from threatening stimuli), affective (negative affect, emotion regulation, and coping strategies) and a biologic susceptibility (tryptophan and serotonin levels as well as genetic influences on serotonin transporters) (56). Recent research demonstrates that, in women,

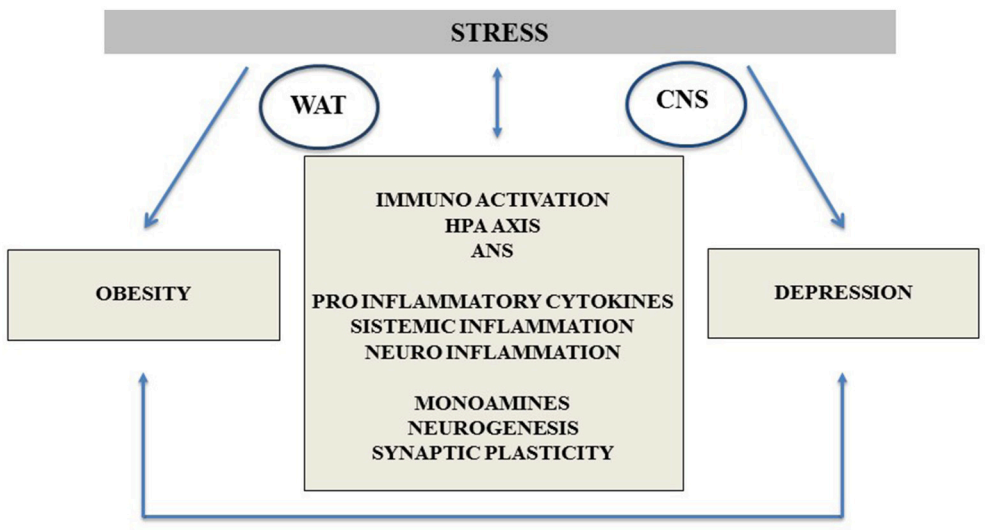

FIGURE 1 | Depression and obesity common neurobiological pathways, highlighting the stress response mechanisms and its impact on the comorbidity between both entities. CNS, central nervous system; WAT, white adipose tissue; HPA Axis, hypothalamic pituitary adrenal Axis; ANS, autonomous nervous system.

emotional eating as a psychological eating style, can act as mediator between depression and weight gain (57).

A positive association was found between depression and obesity in the general population, although more marked among women (58). Another study found that individuals with increased psychological distress or depression and a greater polygenic load for obesity were more likely to become obese (59). A profile of moderate depressive symptoms was differentially more associated with obesity when compared with an acute profile of depressive symptoms; if inflammation was controlled for, this link was attenuated. Distinct symptoms profiles may point toward different pathways to increase risk of obesity (60).

A putative role for leptin, adiponectin, and resistin in the pathophysiology of neuropsychiatric conditions associated with metabolic abnormalities, including major depression has emerged. Nonetheless, there are currently no validated peripheral biomarkers for the diagnosis, treatment selection and response prediction in major depression. Detection of inflammatory adipokines and cytokines, like adiponectin, leptin, and resistin, as well as IL6 and C-reactive protein peripheral levels, might fill this gap (61).

Managing obesity can help reduce the risks of other morbid conditions such as depression by inhibiting inflammatory mechanisms associated with obesity (62). Nevertheless, the link between depression and obesity needs further research. Some studies found conflicting and diverging results with the therapeutic approach of extreme obesity. Both increasing and decreasing depressive symptoms were associated with weight loss (63, 64).

In what concerns antidepressants and the bidirectional link between obesity and depression, a recent review highlight the need to control for several confounders, such as age, gender, hormonal status, profile of symptoms, doses and mechanism of action of different drugs. Although a trend to the association of treatment failure and obesity is showed in several studies, a definitive conclusions is still not possible (65).

Considering the higher risk of depression in patients with severe obesity (66), treatment modalities should be tailored according to the needs imposed by such an association.

From a developmental point of view, genetic dispositions and early life adversity can lead to maladaptive stress responses in adulthood, increasing stress vulnerability and amplifying the impact of negative life events (67, 68), leading to depression and also to a psychoneuroimmune dysregulation, promoting obesity. On the other hand obesity, increasing neuroinflammation, may impair monoaminergic neurotransmission, neurogenesis, and synaptic plasticity, potentiating the risk of depression (30).

The search for biomarkers of major depression, using genomic and transcriptomic methodologies, is a promising avenue for the future. However, studies conducted in the last years, although supporting the inflammatory model and identifying potential markers of depression, does not offer a clear and unequivocal profile (69–71).

In summary, despite different levels of evidence, we argue that comorbidity between obesity and depression relies on the association between neuroendocrine and immunologic regulation in allostatic states, in the context of genetic vulnerabilities and behavioral responses to chronic stress. Translational research, improving our understanding of the underlying mechanisms and their clinical implications may contribute to the development of new treatment and prevention strategies.

CONCLUSIONS

Current research highlights the relevance of neuroendocrine and immunologic disruption in several diseases, and inflammation may represent the common mechanism relating different features of premorbid and pathological states. Stress response as a mediator between different level phenomena assumes the role of a plausible link between psychological and biological determinants of health and disease.

Depression and obesity share alterations in cytokine systemic profiles, activation of inflammatory and immune pathways as well as in neuroinflammation, perpetuating the cycle of central/periphery pathogenic interactions. Medical comorbidities associated to obesity and depression are related to a cluster of risk factors, including the metabolic syndrome, associated to the increased morbidity and mortality caused by diabetes, cardiovascular diseases and some cancers (72), all sharing an inflammatory background. Individual variability may be related to psychosocial variables that can amplify the biologic vulnerability, genetically determined or under developmental constrictions.

In the following decades, depression and obesity are expected to represent major public health issues urging for new insights and integrated interventions, both in pharmacological and psychosocial levels.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Chinese Medicine in the Battle Against Obesity and Metabolic Diseases

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Obesity is a multi-factor chronic disease caused by the mixed influence of genetics, environments and an imbalance of energy intake and expenditure. Due to lifestyle changes, modern society sees a rapid increase in obesity occurrence along with an aggravated risk of metabolic syndromes in the general population, including diabetes, hepatic steatosis, cardiovascular diseases and certain types of cancer. Although obesity has become a serious worldwide public health hazard, effective and safe drugs treating obesity are still missing. Traditional Chinese medicine (TCM) has been implicated in practical use in China for thousands of years and has accumulated substantial front line experience in treating various diseases. Compared to western medicine that features defined composition and clear molecular mechanisms, TCM is consisted with complex ingredients from plants and animals and prescribed based on overall symptoms and collective experience. Because of their fundamental differences, TCM and western medicine were once considered irreconcilable. However, nowadays, sophisticated isolation technologies and deepened molecular understanding of the active ingredients of TCM are gradually bridging the gap between the two, enabling the identification of active TCM components for drug development under the western-style paradigms. Thus, studies on TCM open a new therapeutic avenue and show great potential in the combat against obesity, though challenges exist. In this review, we highlight six key candidate substances derived from TCM, including artemisinin, curcumin, celastrol, capsaicin, berberine and ginsenosides, to review their recent discoveries in the metabolic field, with special focus on their therapeutic efficacy and molecular mechanisms in treating obesity and metabolic diseases. In addition, we discuss the translational challenges and perspectives in implementing modern Chinese medicine into the western pharmaceutical industry.

Keywords: traditional Chinese medicine, artemisinin, curcumin, celastrol, capsaicin, berberine, ginsenosides

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Abbreviations: AMPK, Protein kinase AMP-activated catalytic subunit alpha 1; ATF2, Activating transcription factor 2; C/EBP, CCAAT/enhancer binding protein; HO-1, Heme oxygenase-1; HSF1, Heat shock factor 1; LDLR, Low density lipoprotein receptor; MAPK, Mitogen activated kinase-like protein; NRF2, Muclear factor erythroid 2; PGC1α, Peroxisome proliferative activated receptor gamma coactivator 1 alpha; PPARγ, Peroxisome proliferator activated receptor gamma; PRDM16, PR/SET domain 16; Sirt1, Sirtuin 1; SREBP1, Sterol regulatory element binding protein 1; STAT3, Signal transducer and activator of transcription 3; TPRM8, Transient receptor potential cation channel subfamily M member 8; TRPA1, Transient receptor potential cation channel subfamily A member 1; TRPV1, Transient receptor potential cation channel subfamily V member 1; TRPV4, Transient receptor potential cation channel subfamily V member 4; UCP1, Uncoupling protein 1.

INTRODUCTION

For thousands of years, before the introduction of western medicine, Chinese people relied on traditional Chinese medicine (TCM) to treat diseases and relieve discomfort. TCM is complex formula constituted of mixed medicinal extracts from Chinese herbs and animals, and is prescribed based on the philosophical theory of Chinese medicine, for instance, the theories of "Yin Yang" and "Five Elements." Though mysterious and empirical, TCM has gained front line experience in combating diseases for millennia in Chinese history with effectiveness and low side effects. However, in the modern society, with the prevalence of western medicine, which is developed under strict experimental proofs and feature clear-cut molecular compositions and mechanisms of action, TCM meets serious criticisms and challenges due to undefined compositions and unclear therapeutic mechanisms (Qiu, 2007). Fortunately, nowadays, sophisticated isolation techniques have enabled scientists to identify active ingredients from TCM to elucidate the molecular mechanisms of their therapeutic effects, thus promote the re-discovery of ancient TCM compounds to be potentially developed as drugs under western medical standard. A few promising TCM compounds with successful implications include, but not limit to, artemisinin in treating malaria (Liu et al., 2006) and arsenic trioxide in acute promyelocytic leukemia (Lo-Coco et al., 2013), with more waiting to be added to the list.

Looking back into the history of human diseases, before the enlightenment of modern medicine and vaccinology, infectious disease outbreaks decimated large populations and were once the most threatening events to mankind. In today's society, however, with large scale vaccination and a highly organized disease control system, the biggest challenge to human health has shifted to noninfectious chronic disease (NCD) like obesity, metabolic diseases, cardiovascular diseases, neurodegenerative diseases, inflammatory diseases and various types of cancer. Among them, obesity holds special importance as it is a major risk factor for many other metabolic diseases, i.e., type 2 diabetes mellitus, nonalcoholic fatty liver disease, hyperlipidemia, etc., thus renders it an effective target for disease intervention (Haslam and James, 2005). Briefly, obesity is an excessive deposit of white fat in the body. The abnormal accumulation of fat poses adverse impacts on the metabolic fitness of the body and is positively associated with aberrant metabolic complications including hyperglycemia, insulin resistance, dyslipidemia, hypertension (Bays and Dujovne, 2006) and chronic low-grade inflammation (Saltiel and Olefsky, 2017). Fat tissues are metabolically active organs and are classified as white, brown and beige fat based on their morphology, physiology and functions (Boss and Farmer, 2012; Xu et al., 2018). Together they maintain the energy balance of the body with white fat storing energy in forms of triglycerides, while brown and beige fat dissipating energy for thermogenesis *via* UCP1 activation. The disturbance of energy balance among adipose tissues may lead to increased adiposity and/or obesity.

Considering different therapeutic leads, compounds derived from TCM possess two major advantages over synthetic drugs in treating obesity. First, obesity is a complex disease that shows no clear pathogens and its pathogenesis involves multiple genetic factors and signaling networks. In patients with obesity and metabolic diseases, multiple organs, i.e., fat tissues, liver, muscle, and even brain suffer pathological changes. In this sense, the concept of TCM emphasizes the holistic treatment of a disease. Studies on TCM have demonstrated that their active ingredients target multiple organs and tissues in the body to exert systemic effects, which match the characteristics of obesity. Second, obesity and metabolic diseases are not as immediately crippling or death threatening as infectious diseases. Patients require long-term care and persistent treatment to prevent advance disease progression, which greatly burdens their family and the society as a whole. Thus, it is important to take long-term effectiveness and safety into account when developing drugs for obesity. In this regard, most TCM consists of herbal-based medicinal extracts that have been prescribed and widely used in Chinese people's daily life for purposes ranging from health-promoting to disease treatment for thousands of years, which provide solid though empirical evidence for its safety. These characteristics make TCM a promising source for new drug development for obesity and metabolic diseases. However, putting their appealing efficacies aside, the caveat is that much of TCM has unclear compositions and vague mechanisms, which not only hinders their wide application into modern clinic, but can sometimes be detrimental. One extreme case is the recently reported implication of aristolochic acids and similar compounds from *Aristolochia* and related plant in liver cancers in Asia population for their mutagenesis attribution (Ng et al., 2017). Thus, it is vital to place TCM in the pipeline of standard western drug development: identification, isolation and/or synthesis of the active component, clarification of the molecular target and mechanism, and eventually developed as a pharmaceutical (Corson and Crews, 2007). During this process, various factors like therapeutic mechanism, clinical data, development cost, etc. all play important roles. In recent years, great interests have been focused on TCM, producing a substantial amount of research data. In this review, we highlight recent studies of six promising TCM compounds in the metabolic aspect based upon their clinical, mechanistic properties or current application, including artemisinin, curcumin, celastrol, capsaicin, berberine and ginsenosides.

ACKNOWLEDGED VS. CONTROVERSIAL: ARTEMISININ AND CURCUMIN

Although the interests in TCM's potential in treating obesity and metabolic diseases only start to rise in recent years, many TCM ingredients have already been isolated and tested over the years. Substantial data demonstrates their effectiveness and low adverse effects in various diseases, promoting a few into pre-clinical or clinical trials, though not all trials gave consistent results. Among them, artemisinin and curcumin represent two different extremes, one's efficacy is well acknowledged while the other is shrouded in controversy.

Artemisinin

Artemisinin is derived from the sweet wormwood (*Artemisia annua* L.) that is regularly used as classic TCM. Artemisinin and its semi-synthetic derivatives-based therapy are currently the standard and the most effective treatment for uncomplicated *Plasmodium falciparum* malaria. In 2015, Dr. Youyou Tu was awarded the Nobel Prize for medicine for discovering and isolating artemisinin. This makes artemisinin the most acknowledged and successful TCM compound.

Aside from malaria, in recent years, with the spike in overweight and obesity rate, more and more attentions are focusing on the roles of artemisinin and its derivatives in treating obesity and metabolic diseases. For example, artemisinic acid and artesunate are found to inhibit the development and differentiation of adipocytes by suppressing master regulators C/EBPs and PPARγ in adipogenesis (Lee et al., 2012; Jang, 2016). In addition, since the groundbreaking discovery of brown and beige fat in human adults, activation of brown fat and browning of white fat, two major sources of adaptive thermogenesis and important outputs for energy expenditure, have emerged as potential therapeutic means to treat obesity and metabolic diseases (Cypess and Kahn, 2010; Boss and Farmer, 2012). By high-throughput screening over 3000 compounds in differentiated 3T3-L1 and C3H10T1/2 adipocytes to find small-molecule compounds capable of activating thermogenesis, Lu et al have identified artemether, an artemisinin derivative, as an activator of browning and thermogenesis *in vitro*. Further examination reveals artemisinin and other artemisinin derivatives, dihydroartemisinin, artesunate and arteether, could also promote browning. Importantly, local delivery of artemether into subcutaneous fat or systemic delivery via tail vein in mice effectively reduces high fat diet induced body weight gain, enhances cold tolerance and improves insulin sensitivity. Mechanistic study indicates that p38 MAPK/ATF2 axis and Akt/mTOR pathway are partially responsible for the browning effects of artemether (Lu et al., 2016). In another study, it is reported that leaf extracts of Artemisia annua, the source of artemisinin, attenuates hepatic steatosis and inflammation in diet-induced obese (DIO) mice (Kim et al., 2016). These studies shed first light on the possibility of exploiting artemisinin and its derivatives as an anti-obesity and anti-fatty liver drug *in vivo*, though detailed mechanistic study and clinical data are warranted. As a paradigm of the translational application of TCM, continued characterization of the metabolic properties of artemisinin and its derivatives would bring hope to patients with obesity and metabolic diseases.

Curcumin

In sharp comparison with artemisinin, whose effectiveness is well acknowledged in academia, curcumin may be the most controversial TCM compound in the eyes of western chemists and biologists. Curcumin is the principal curcuminoid in the turmeric of the ginger family. There are thousands of reports and over 120 clinical trials studying the effectiveness of curcumin in various diseases, including erectile dysfunction, hirsutism, baldness, cancer, and Alzheimer's disease, yet the results fail to reconcile with one another. The contradictions are probably due to the poor solubility of curcumin in aqueous solution, thus limiting its bioactivity and stability in many studies (Nelson et al., 2017). When interpreting results from various studies, one has to keep in mind that, on one hand, substantial evidences exist in a large body of literatures about curcumin's biological activities under different circumstances and its effectiveness both *in vitro* and *in vivo* (Heger, 2017). But on the other hand, it is of major concern that curcumin fluoresces naturally, which may interfere with drug screening that relies highly on fluorescence signals, producing screen artifacts and generating false positive results (Baker, 2017). Thus, any attempt at the pharmacological use of curcumin should take these factors into account.

In the metabolic aspects, in adipocytes, consistent reports indicate that curcumin suppresses adipocyte differentiation by affecting classic regulators of adipogenesis (Ejaz et al., 2009; Kim et al., 2011; Sakuma et al., 2017). Curcumin also ameliorates hypoxia-induced insulin resistance and inflammation in 3T3-L1 adipocytes (Priyanka et al., 2017). In the browning process, it has been shown that curcumin treatment induces browning in primary white adipocytes and adipose tissues and augments thermogenic and mitochondrial gene programs in a classic norepinephrine dependent manner (Wang et al., 2015; Lone et al., 2016). In rodents, curcumin treatment enhances energy expenditure in DIO murine models and protects against weight gain and inflammation of adipose tissues (Weisberg et al., 2008; Shao et al., 2012). In a few clinic trials, curcumin has been shown to improve insulin resistance and hyperlipidemia in patients with metabolic syndromes (Mohammadi et al., 2013; Ganjali et al., 2014), while others produce negative results (Nelson et al., 2017).

Importantly, the resolution to the predicament of insolubility and instability of curcumin, which hinders its clinical application, may lie in the researches of curcumin analogs. For example, curcumin-3, 4-dichloro phenyl pyrazole (CDPP) shows significantly improved bioactivity while retains curcumin's capability in suppressing adipocyte differentiation and preventing hyperlipidemia in DIO rodents (Gupta et al., 2017). C66, a novel curcumin derivative, inhibits JNK phosphorylation, reduces high glucose-induced inflammation in cardiomyocytes and prevents the development of diabetic cardiomyopathy in mice (Pan et al., 2014). Alternatively, nano-formulated curcumin generated using nanoparticles has shown improved bioactivity and efficacy compared to native curcumin in various *in vitro* and *in vivo* disease models, indicating its potential implication in metabolic studies (Rahimi et al., 2016). However, despite the metabolic benefits in curcumin-treated adipocytes and mice models, these studies lack detailed mechanisms and target molecules. Further mechanistic investigations are needed to disperse the controversy around curcumin before it can be further implicated in the treatment of obesity and metabolic diseases.

MULTI-TARGETS VS. SINGLE TARGET: CELASTROL AND CAPSAICIN

Mechanistic studies are vital in drug development to prevent potential side effects. Compounds with single targets are favored

by scientists for their balanced efficacy and safeness, although compounds with multiple targets in multiple organs could be of potential interest in treating obesity and metabolic diseases since the patients usually feature pathological changes in not one but multiple organs, i.e., adipose tissues, liver and muscle. As an example, celastrol is a TCM compound targeting multiple metabolic organs with detailed mechanisms of action deciphered (Liu et al., 2015; Ma et al., 2015; Hu et al., 2017). In comparison, capsaicin is well known for functioning through its receptor TRPV1 (Caterina et al., 1997), the mechanism of which has been extensively studied.

Celastrol

Celastrol (tripterine) is a chemical compound isolated from the root extracts of Tripterygium wilfordii (Thunder god vine, TGV). As a TCM, TGV has wide and longtime application in treating inflammatory diseases in patients, i.e., rheumatoid arthritis. As one of the major active ingredients of TGV, celastrol has multiple functions in anti-oxidation, anti-inflammation, anti-neurodegeneration, and anti-cancer, though its anti-inflammation effects are most extensively studied (Cascão et al., 2017). Recently, a series of studies have brought celastrol onto the metabolic stage and highlight it as a versatile regulator of obesity and metabolic diseases with both central and peripheral targets. Centrally, Liu et al. (2015) has reported that celastrol functions as a leptin sensitizer to reduce food intake in DIO mice and possibly treats obesity by both activating leptin receptor-STAT3 pathway and inhibiting NF-κB in the hypothalamus. Peripherally, Zhang's group has demonstrated that under inflammatory conditions, celastrol binds to Nur77, an orphan nuclear receptor and an inducer of mitochondria apoptosis, and promotes its translocation into mitochondrial for inflamed mitochondrial autophagy and clearance, thus reduces inflammation and improves hepatic steatosis in mice (Hu et al., 2017). Celastrol could also induce Sirt1 transcription and alleviate hepatic steatosis by decreasing SREBP1 and increasing AMPKα expression (Zhang et al., 2016). Another report from Luo et al. (2017) reveals that in liver and adipose tissues of DIO mice, celastrol suppresses inflammation by reducing macrophage M1 polarization via its regulation on the Nrf2/HO-1, MAPK, and NF-κB pathways.

Apart from its impact on obesity and metabolic diseases through inflammatory inhibition in multiple organs, celastrol also exerts its beneficial effects by directly promoting energy expenditure. HSF1 is a classic transcription factor induced by multiple stimuli, including heat shock, oxidative and mechanical stresses (Anckar and Sistonen, 2011). It orchestrates the function of heat shock proteins in protein refolding and damage repair, thus is vital for protein homeostasis and cell survival (Gomez-Pastor et al., 2018). Interestingly, HSF1 could promote mitochondrial biogenesis and adaptive thermogenesis via its interaction with and transactivation of PGC1α, the master regulator of energy metabolism that was previously thought to be mainly induced by cold exposure (Ma et al., 2015). Celastrol activates HSF1 as shown by HSF1 phosphorylation and activation of its downstream target genes (Westerheide et al., 2004). Based on these preliminary data and the fact that celastrol is derived from TCM with wide application in humans, Ma et al. (2015) test its metabolic effects and reveal that celastrol prevents weight gain, improves hepatic steatosis and ameliorates insulin resistance in mice fed a high fat diet. Intriguingly, in this study, celastrol blocks obesity progression in mice without reducing food intake or affecting hypothalamus metabolic genes expression, possibly because these mice didn't develop severe leptin resistance as the DIO model used in Liu's paper. Instead, celastrol activates the HSF1-PGC1α axis in peripheral organs to induce browning of white fat and promote mitochondrial function in muscle. Celastrol treatment loses these beneficial effects in HSF1 and PGC1α deficient cells and mice, suggesting that HSF1-PGC1α axis is at least partially responsible for the metabolic function of celastrol in the peripheral organs (Ma et al., 2015).

In patients with obesity and metabolic diseases, multiple organs, i.e., adipose tissues, liver, muscle and brain experience pathological changes. It has been shown that celastrol targets multiple organs and improves their metabolic performances simultaneously, thus rendering it a TCM compound with comprehensive effects in treating obesity and metabolic diseases (Ma et al., 2015). Future work could be focused on its structural modification to obtain celastrol derivatives that preferentially target specific organs. Besides, it is of note that triptolide, another major active component of Tripterygium wilfordii, shows similar anti-inflammatory and anti-cancer effects to celastrol (Li et al., 2014). Combined treatment of triptolide and celastrol shows synergistic effects in suppressing tumor cell growth *in vitro* (Jiang et al., 2015). It would be worthwhile to test their synergistic effects in the treatment of obesity and metabolic diseases. Finally, although celastrol treatment of various concentrations and lengths of time shows no overt side effects or toxicity in rodents, low sperm density and some toxicity are reported in male patients treated with TGV for rheumatoid arthritis (Lopez et al., 2005). Future studies are warranted to elucidate the efficacy and a safe concentration of action for celastrol and other TGV constituents in clinic.

Capsaicin

One hundred years ago, Capsaicin was identified and purified as one of the major active biological components of peppers (Capsicum). Peppers taste spicy by inducing the "hot" sensation to different extents and are widely used in daily life as a seasoning. They also have longtime use in TCM to relieve physical pain and treat gastrosis, detrusor hyperreflexia and rheumatic arthritis. Among active TCM compounds translated for pharmaceutical use, capsaicin is one of the best examples featuring extensively studied molecular targets and mechanisms. Unlike celastrol, which exerts its function through multiple targets in diverse organs, capsaicin functions through a single and clear target, its receptor TRPV1 (Caterina et al., 1997). TRPV1, a Ca^{2+} ion channel highly enriched in a specific subset of peptidergic sensory neuron in human and rodents, was identified and cloned in 1997 (Gunthorpe and Szallasi, 2008). It is a prime target for pain relief upon activation by capsaicin, as well as thermal heat, acidic conditions and allyl isothiocyanate (Gavva, 2008). TRPV1 antagonists reduce pain by blocking receptor signal transduction, but their clinical application is hurdled by the simultaneous

induction of hyperthermia by TRPV1 blockade, potentially due to TRPV1's function in maintaining body temperature in central nervous system (Cui et al., 2006). It is interesting that long term or high dose treatment of TRPV1 agonists, such as capsaicin, cause receptor desensitization or kill the TRPV1 neurons, thus mimicking TRPV1 deficiency and leads to pain alleviation, suppression of TRPV1 mediated inflammation but without the conundrum of TRPV1 antagonists (Knotkova et al., 2008).

With the accumulation of evidence about the regulatory function of TRPV1 in the central nervous system for food intake and body temperature, as well as in peripheral organs for insulin and adipokine secretion, agonist-mediated TRPV1 desensitization begins to attract attention for its potential application in metabolic field. Capsaicin, as the classical TRPV1 agonist, has been shown in human and in rodents to increase satiety, reduce food intake, increase sympathetic nervous system activity (Reinbach et al., 2009; Janssens et al., 2014), and not surprisingly, enhance lipid metabolism (Kang et al., 2010) and activate thermogenesis (Baskaran et al., 2017). Mechanically, these reports consistently demonstrate that the metabolic effects of capsaicin no longer exist when TRPV1 is deficient, though capsaicin may also induce the Sirt1/PRDM16/PPARγ axis for its browning effects on white fat (Reinbach et al., 2009; Kang et al., 2010; Janssens et al., 2014; Baskaran et al., 2017). These findings signify the important potential of capsaicin and TRPV1 in the metabolic aspect. It has to be noted that other TPRV family members, i.e., TRPA1, TPRM8, and TRPV4, could be activated by different agonists/antagonists or sense different stimuli to exert various metabolic regulation, a particular intriguing example being TRPV4, which functions as a negative regulator of thermogenesis (Ye et al., 2012). Thus, aside from capsaicin, TCM would serve as a valuable source to screen more active compounds that target different TRPVs to open new therapeutic avenues in treating obesity and metabolic diseases.

COMMON VS. RARE: BERBERINE AND GINSENOSIDES

Traditional Chinese medicine contains medical extracts from a wide range of plants, animals and mineral products. In the long list, some are common everyday plants while some are rare and hard to cultivate. In drug development, after primary considerations like efficacy and safety are met, low development cost would be an advantage. A good example is Coptis chinensis and its active ingredient berberine, a common OTC drug with high popularity vs. ginseng, a TCM shrouded in mysterious atmosphere because of its effectiveness and rarity, though its development as a modern drug is hindered by its complex ingredients, ginsenosides.

Berberine

Coptis chinensis, a common and easy to cultivate medicinal herb, is one of the most widely used TCM with versatile effects since ancient China. The famous ancient Chinese proverb "a bitter medicine cures the disease," the bitter medicine referring to Coptis chinensis, exemplifies its long history of use and popularity in people. Coptis chinensis is routinely prescribed to treat bacteria induced diarrhea for its antibiotic properties. Today, it has been identified that the major active ingredient in Coptis chinensis is berberine. With the development and refinement of advanced synthesis technology, berberine is produced in large quantities and at low cost, thus becoming a standard collection in the medicine cabinet of most Chinese families. Besides diarrhea, *in vitro* and *in vivo* studies suggest that berberine is a potential drug in the treatment of type 2 diabetes mellitus, hyperlipidemia, and certain types of cancer (Liu et al., 2016). As early as 2004, Jiang's group has found that berberine binds to the 3′UTR of LDLR mRNA, resulting in increased LDLR stability, enhanced hepatic LDL assimilation and reduced cholesterol level. In hypercholesterolemic patients and hyperlipidemic hamsters, berberine treatment significantly lowers total cholesterol, LDL and triglyceride levels with a mechanism of action distinct from Statins, a classic cholesterol-lowering drug targeting HMG-CoA reductase (Kong et al., 2004). Later, Ning's group showed the effectiveness of berberine in lipid and glycemic control in larger cohort of patients with Type 2 diabetes and hyperlipidemia based on comprehensive metabolomics (Zhang et al., 2008; Gu et al., 2010). Furthermore, berberine is shown to activate thermogenesis in adipocytes through the AMPK-PGC1α axis, which leads to increased energy expenditure, reduced weight gain and improved cold tolerance in obese db/db mice (Zhang et al., 2014).

Of note, berberine also exerts anti-aging function by mechanisms inhibiting mTOR/S6 pathway via AMPK activation, as well as reducing the endogenous ROS level and constitutive oxidative DNA damages through NRF2 (Halicka et al., 2012). In Drosophila melanogaster, berberine prolongs life span and stimulates locomotor activity potentially by blocking kynurenine formation from tryptophan, which is associated with aging (Navrotskaya et al., 2012). Since it has been shown that metabolic improvements are one of the major drives for longevity (Canaan et al., 2014; Ma et al., 2014), it would be interesting to assess how much of the extended longevity is contributed by berberine's promotion of metabolic health in the future and whether mammalian lifespan is also affected by berberine treatment.

In marked contrast to its effective in some clinical trials, the plasma level of berberine is found to be fairly low in patients (Hua et al., 2007), indicating that besides the classic pharmacological model, other factors might also in play in exerting berberine's beneficial effects. Promoted by sophisticated sequencing technologies, there has been a rapid growth in gut microbiota researches, spotlighting its critical involvement in the progression of multiple diseases including obesity and metabolic diseases. Due to its antimicrobial activity and poor solubility and absorption in the gut, berberine is put under the spotlight for its impacts on gut microbiota. As demonstrated by several independent groups, berberine improves high fat diet induced obesity and metabolic diseases through intestinal microbiota modulations (Han et al., 2011). Berberine treatment causes a structural change in gut microbial flora, largely reduces its diversity by enriching a few short-chain fatty acid (SCFA) producing bacteria including Blautia and Allobaculum, in turn elevates SCFA levels in the intestine and alleviates host

inflammation (Zhang et al., 2012). Berberine also affects bile acid metabolism via its modulation on intestinal flora, by inhibiting cholic acid 7α-dehydroxylation conversion into deoxycholic acid (Gu et al., 2015). Interestingly, in ApoE$^{-/-}$ atherosclerosis mice model, in addition to inducing a higher Akkermansia abundance in the gut flora, berberine administration is also found to preserve gut barrier integrity by increasing intestinal epithelial tight junction and colon mucus layer thickness in high fat diet-fed mice (Zhu et al., 2018). Last but not least, berberine could potentially promote metabolic health by playing a role in the microbiota-gut-brain axis. For example, berberine increases serum Glucagon-like Peptide-1 (GLP-1) and Neuropeptide Y level while decreases Orexin A level, which are all gut-brain peptides critical in satiety and energy homeostasis (Sun et al., 2016). Of note, GLP1 receptor is found to be elevated in the hypothalamus of berberine-treated mice, suggesting central nervous system might be another target for metabolic regulation by berberine (Sun et al., 2016).

Ginsenosides

Hidden in deep mountains, and difficult to find, ginseng is regarded by Chinese people as a rare and precious diet supplement to strengthen holistic health. Modern studies have shown that ginseng has anti-oxidant and anti-inflammation properties in cardiovascular and central nervous system, thus favoring healthy aging. The active ingredients in ginseng are ginsenosides, a class of steroid glycosides and triterpene saponins. There are more than 30 biologically active ginsenosides in ginseng, i.e., protopanaxadiols and protopanaxatriols, that all retain the beneficial effects of ginseng to some extent, thus it would be interesting to systemically assess the similarities and differences of these ginsenosides.

Numerous studies have demonstrated that ginsenosides are effective in preventing obesity, hyperlipidemia, hyperglycemia, and hepatic steatosis in DIO mice and rats, their action of targets including adipose tissue, liver, muscle and brain. In 3T3-L1 adipocytes, ginsenosides Rb1, Rg3, Rh1, Rf, and Re etc. suppress adipocyte differentiation by inhibiting the classical adipogenic transcription regulators PPARγ and C/EBPs (Lee et al., 2011; Gu et al., 2013; Siraj et al., 2015; Koh et al., 2017). In one independent research, Rb1 is shown to induce browning effects in 3T3-L1 adipocytes by increasing PPARγ activity, which is abrogated by PPARγ antagonist GW9692 treatment (Mu et al., 2015). Ginsenosides Rb1, Rg1, Rg5, and Re also target skeleton muscle for enhanced insulin sensitivity and cardiomyocytes for improved cardiac functions (Xie et al., 2006; Guan et al., 2017; Peng et al., 2017; Xiao et al., 2017b). In liver, Rg1, Rg3, Rg5, and Rb2 prevent hepatic steatosis with AMPK as their possible common target (Shen et al., 2013; Huang et al., 2017; Lee et al., 2017; Xiao et al., 2017a). There are also a few researches report that ginsenosides may target central nervous system in obese mice to improve leptin sensitivity in cortex and relieve central inflammation in hypothalamus (Wu et al., 2014).

Despite their consistent effectiveness in rodents and cellular models, meta-analysis of recent clinical trials assessing ginseng effects in cardiovascular diseases and metabolic diseases reveal that around 65% of the studies show significant improvements in ginseng treated groups, while the rest produce negative results (Kim et al., 2015). For instance, one study has shown that ginseng itself and ginsenoside Re fail to improve β-cell function or insulin sensitivity in overweight and obese population with diabetes (Reeds et al., 2011). These inconsistencies might be due to the vast variants of ginseng species in North America and East Asia and the complexity of biologically active ginsenosides in different ginsengs. It would be worthwhile to invest more energy in isolating and characterizing the function of individual ginsenoside from various ginseng species, based on which more accurate clinical trials could be performed to assess their therapeutic potential in patients. Plus, developing efficient synthetic routes to obtain specific ginsenoside of interest would be of importance since extracting ginsenosides from ginseng might be expensive and low yield.

Perspectives

In this review, we have highlighted six compounds derived from TCM, artemisinin, curcumin, celastrol, capsaicin, berberine and ginsenoside, and evaluated various aspects of their property and their potentials in treating obesity and metabolic diseases (**Figure 1**). There are substantial clinical data on the safeness and side effects of artemisinin during its implication as a standard treatment for malaria. This makes it relatively easy to be transformed into a metabolic drug, although more data have to be obtained on its impact on metabolic parameters. On the other hand, clinical trials using curcumin have produced controversial results, which urge for more randomized, double-blind, parallel controlled, multi-center clinical trials for fair judgment before it could be put into use. Celastrol, capsaicin and berberine are all promising novel therapeutics against obesity and metabolic diseases for their convincing effectiveness on metabolic improvement from *in vitro* and *in vivo* studies. Clinical data is required to access their efficacy and side effects on patients in the future. Ginseng, though famous for its holistic effects, has to be carefully analyzed to identify the detailed functions of individual ginsenosides in metabolism. Then, efforts are needed to find a cheaper way to synthesize the desired active ginsenosides. Besides the six compounds reviewed here, more TCM ingredients are waiting to be re-discovered and developed as novel drugs targeting obesity and metabolic diseases.

Along the history of mankind's everlasting pursuit for health, TCM plays an indispensable role with its unique yet empirical theory in diagnosing and treating diseases, which still holds significance in the modern point of view. Like the increased acceptance and popularity of Chinese ancient philosophy in western world, TCM theory is attracting more and more attention from the western medical establishment. For instance, TCM acknowledges the crosstalk among different organs and considers human body as a whole. Rather than targeting the affected parts alone, TCM strives to fight diseases in an integrated way. Its focus on complex interactions within biological systems coincides with the core idea of "Systems Biology," which is a powerful and fundamental tool for researches on subjects as complicated as human bodies. Secondly, similar to "Precision Medicine," TCM treatment is highly personalized. Ailments as simple as cold or headache are characterized based on different pathogenesis and

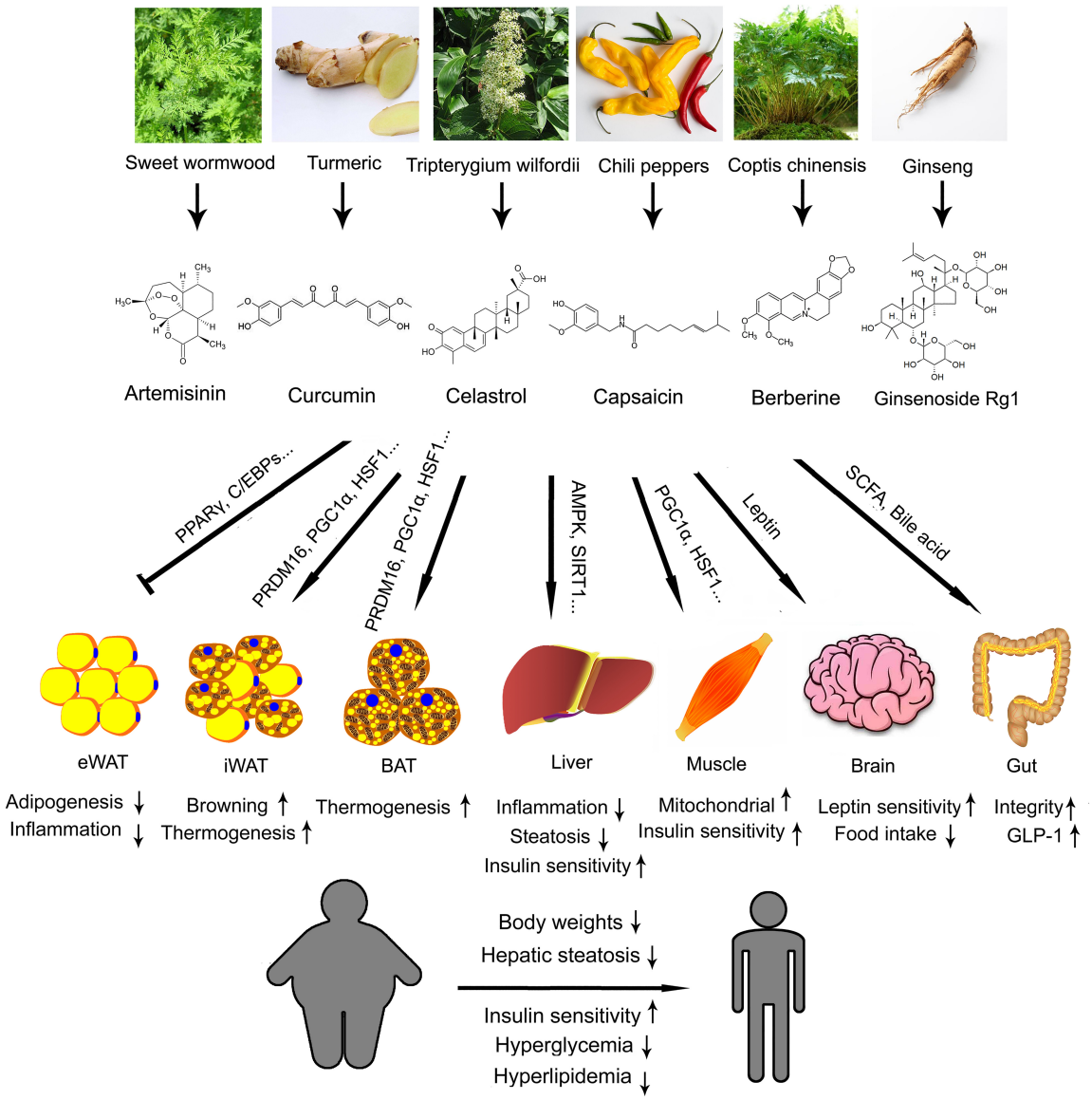

FIGURE 1 | Illustration summary of the original Traditional Chinese medicine (TCM) herbs, coumpound structures, metabolic effects and major mechanisms of artemisinin, curcumin, celastrol, capsaicin, berberine, and ginsenoside. eWAT, epididymal white adipose tissue; iWAT, inguinal white adipose tissue; BAT, brown adipose tissue.

handled accordingly, rather than using a standard symptom-based protocol as commonly practiced in western medicine. Other factors like patients' gender, sex, age, living condition, and lifestyle all play a role when design a prescription in TCM. It may seem chaotic at first impression, but it is clear now that a disease, especially obesity and metabolic diseases, is a highly interactive outcome of one's genetic and environment, thus the diagnostic strategy of TCM is not without solid foundation, although detailed mechanisms behind TCM theory have to be clearly addressed before it could be put into modern use. Thirdly, TCM formula is mostly Fufang (empirically combining multiple medicinal extracts to achieve best efficacy against a disease), which is similar to "Combination Therapy" in modern medicine. Like in the case of celastrol and triptolide, TCM Fufang formula is a rich source to discover synergistic effects of numerous active ingredients to develop new combination therapy paradigm of better curative effects. Of course, similar to western drugs, TCM compounds have to be carefully reviewed and tested to decide the ideal treating strategy and eliminate side effects before they could be used as a therapeutic. To this end, a solid research and development protocol is indispensable to unravel the ancient secrets hidden in TCM to benefit patients in the modern world, at the same time prevents sporadic yet notorious incidence like *Aristolochia* and aristolochic acids in liver cancer (Ng et al., 2017).

Regarding the six candidate compounds discussed in this review, although *in vitro* mechanistic studies are extensive, it is

of note that the effects of artemisinin and celastrol on metabolic parameters are evaluated in cellular and rodent models, which have limited extrapolation in human and require further clinical tests. Clinical studies on capcaisin and berberine produced positive data on metabolic fitness yet more studies are warranted, whereas the inconsistencies within the numerous clinical trials on curcumin and ginsenosides need reconciling. It is important to focus future studies on addressing these points to promote their translation into pharmaceuticals against obesity and metabolic diseases.

In summary, though great efforts are still needed to better understand its mechanisms and clinical relevance, TCM possesses great potential as a vast and readily available source for finding and developing new drugs against obesity and metabolic diseases. With the high prevalence of obesity and metabolic diseases in population and the resulting high cost for diseases care, it would be wise to devote more resources in researching of TCM for new therapeutic inspirations.

AUTHOR CONTRIBUTIONS

LX and XM conceived the review and LX, WZ, DW, and XM wrote the manuscript.

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Circulating and Adipose Tissue mRNA Levels of Zinc-α2-Glycoprotein, Leptin, High-Molecular-Weight Adiponectin, and Tumor Necrosis Factor-Alpha in Colorectal Cancer Patients With or Without Obesity

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Objectives: To explore zinc-α2-glycoprotein (ZAG), leptin, high-molecular-weight adiponectin (HMW-ADPN), and tumor necrosis factor-alpha (TNF-α) levels in serum and subcutaneous and visceral white adipose tissue (sWAT and vWAT) among normal weight (NW) and overweight/obese (OW/OB) patients with colorectal cancer (CRC).

Methods: A total of 76 Chinese CRC patients (42 NW + CRC, 34 OW/OB + CRC) and 40 healthy controls were recruited. Serum levels of the adipokines of interest were measured by an enzyme-linked immunosorbent assay method, and their mRNA levels in sWAT and vWAT were determined by reverse transcription quantitative PCR methods.

Results: Serum ZAG levels in the NW + CRC group were significantly increased by 11.7% compared with the healthy controls. Serum leptin levels in the OW/OB + CRC group were found to be increased by 57.7%, while HMW-ADPN levels were decreased by 23.5% when compared with the NW + CRC group of CRC patients. Additionally, *ZAG* mRNA levels in sWAT were significantly reduced by 78.8% in OB + CRC in comparison with NW + CRC patients. *ZAG* mRNA levels were negatively associated with body mass index (BMI) in sWAT but positively correlated with BMI in vWAT. *TNF-α* mRNA levels in vWAT of OB + CRC patients were significantly increased by 2.8-fold when compared with NW + CRC patients. In particular, CRC was independently associated with serum ZAG levels. The risk of CRC in participants with high tertile serum ZAG levels was 5.84-fold higher than in those with low tertile ZAG levels after adjusting for age, gender, and other confounders [odds ratio (OR) = 6.84, 95% confidence interval (CI) 1.70–27.54, $P = 0.03$]. The CRC risk in participants with high tertile leptin levels was only 10.7% of those with low tertile leptin levels (OR = 0.11, 95% CI 0.01–0.89, $P = 0.04$). The area under the receiver operating characteristic (ROC) curve of ZAG was 0.66 (95% CI 0.54–0.77, $P < 0.05$). At the cutoff value of 1.42 μg/mL serum ZAG, the sensitivity and specificity for differentiating patients with CRC from controls were 62.2 and 69.2%, respectively.

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Conclusion: Serum ZAG levels were significantly increased in CRC patients. Subjects with higher circulating ZAG and lower leptin levels were more likely to have CRC than those with lower ZAG and higher leptin levels. Serum ZAG might be a potential diagnostic biomarker for CRC in the Chinese population.

Keywords: colorectal cancer, adipokines, zinc-α2-glycoprotein, obesity, leptin

INTRODUCTION

Colorectal cancer (CRC) is the third most predominant cancer in men and the second in women around the world (1). Approximately one million new cases of CRC are diagnosed every year, and half a million people die yearly from this cancer worldwide (2). Though the etiology and pathogenesis of CRC is still unclear, a growing body of evidence has shown that obesity, particularly visceral obesity, is a risk factor for CRC (3, 4). It has been reported that the risk of CRC increases by 7 and 4%, respectively, for every 2 kg/m^2 increase in body mass index (BMI) or 2 cm increase in waist circumference (4). Furthermore, increasing adiposity may influence its prognosis, including the recurrence, disease-free survival, and mortality of patients with CRC (5).

Although the mechanisms by which obesity contributes to the occurrence and development of CRC are multifactorial and have not yet been fully elucidated, accumulating evidence has shown that adipose tissue dysfunction in obesity, which causes an alteration of adipokine secretion, may mediate the relationship between obesity and CRC (6–8). Among these adipokines, adiponectin, leptin, and tumor necrosis factor-alpha (TNF-α) have been largely reported to be implicated in the development of CRC. Recent studies reported a significant inverse association of total and high-molecular-weight adiponectin (HMW-ADPN) with colorectal adenoma (9), not only for early CRC but also for advanced CRC patients (10). Studies in Western populations performed by Kumor and Salageanu et al. observed significantly lower serum leptin levels in CRC patients than in controls (11, 12). Additionally, a case-control study performed by Joshi et al. in a South Korean population found a negative association between leptin and CRC risk (13). TNF-α is usually considered to be a powerful anticancer agent because of its ability to induce necrosis of cancers. However, in recent years, accumulating evidence has demonstrated that TNF-α is increased during obesity (14) and may serve as a pro-cancer cytokine that is involved in carcinogenesis and cancer progression (15, 16). Higher serum levels of TNF-α have been shown to be associated with an increased risk of colorectal adenomas (17).

Zinc-α2-glycoprotein (ZAG, also called AZGP1) is a newly identified adipokine that is downregulated in obese patients and obese mice (18, 19). Recent studies have found that ZAG is also expressed in several malignancies, such as prostate, breast, and lung cancer (20–22), and the diagnostic value of serum ZAG in prostate cancer patients has also been reported (23). ZAG production is associated with the histological grade of prostate and breast cancer (24, 25). Thus, it is reasonable for us to wonder whether ZAG has any effect on CRC development and progression.

In the context of a role of ZAG in patients with CRC, so far, only three studies have been published (26–28). Early in 2012, Agesen et al. found high *ZAG* gene expression in the tumor tissue of CRC patients by using exon-level microarrays in a multimedical center, multi-ethnic (Norwegian, USA, and Australia) and large-scale sample study (26). Ji et al. found the elevated ZAG levels in the sera and tumor tissues of CRC patients, and the elevated serum ZAG levels in CRC patients were correlated with an advanced clinical stage and poor prognosis (27). They also showed that the area under the curve (AUC) of the receiver operating characteristic (ROC) curve of ZAG was 0.95, which suggested that ZAG might be used as a potential serum biomarker for the diagnosis and prognosis of CRC patients (27). Studies by Xue et al. further suggested that the predictive diagnostic value of ZAG in serum was higher than carbohydrate 19-9 (CA19-9) but lower than carcinoembryonic antigen (CEA) (28). All these findings suggest that ZAG may play an important role in the development and progression of CRC. However, as we know, obesity alters the expression of ZAG (18, 19) and might affect the pathogenesis of CRC. Thus, studies on the role of ZAG in CRC should be undertaken separately in normal weight (NW) and overweight/obese (OW/OB) CRC patients. In addition, the previous studies mentioned above all focused on ZAG expression in normal and carcinoma tissues. Given that ZAG is an adipokine that can be secreted from adipose tissue, it is necessary to explore the expression of ZAG in subcutaneous and visceral white adipose tissue (sWAT and vWAT) in CRC patients.

Thus, the aim of our present study was (i) to provide serum ZAG profiles in three different groups (NW + CRC patients, OW/OB + CRC patients, and healthy controls); (ii) to investigate the mRNA expressions of *ZAG* in sWAT and vWAT in NW + CRC and OB + CRC patients; and (iii) to assess the association between circulating ZAG concentrations and the risk of CRC. In addition, three other adipokines—HMW-ADPN, leptin, and TNF-α—were also assessed in this study.

MATERIALS AND METHODS

Study Subjects

A total of 76 CRC patients (38 with colon cancer and 38 with rectal cancer) who underwent surgery at the Department of

Abbreviations: CRC, colorectal cancer; sWAT, subcutaneous white adipose tissue; vWAT, visceral white adipose tissue; NW, normal weight; OW/OB, overweight/obese; ZAG, zinc-α2-glycoprotein; HMW-ADPN, high-molecular-weight adiponectin; TNF-α, tumor necrosis factor-alpha; ELISA, enzyme-linked immunosorbent assay; ROC, receiver operating characteristic; BMI, body mass index; AUC, area under the curve; CA19-9, carbohydrate 19-9; CEA, carcinoembryonic antigen; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FBG, fasting blood glucose; RT-qPCR, reverse transcription quantitative PCR; SD, standard deviation; OR, odds ratio; CI, confidence interval.

General Surgery of Peking Union Medical College Hospital from June 2012 to April 2014 were recruited. All included participants were pathologically confirmed with colon/rectal cancer. BMI was calculated as weight (kilograms) divided by height (square meters). Patients with acute inflammatory disease, chronic rheumatic diseases, or other malignant tumors and those with BMI <18 kg/m^2 were excluded from this study. In addition, 40 healthy subjects (18 kg/m^2 < BMI < 25 kg/m^2) were collected from the physical examination center with normal liver, kidney, and heart function and normal routine blood and urine tests, and their systolic blood pressure (SBP) and diastolic blood pressure (DBP) were also in the normal ranges. Informed consent was signed by all participants, and the study was approved by the ethics committee of Peking Union Medical College Hospital (No. S-364).

Blood and Tissue Sample Collection and Processing

Colorectal cancer patients were divided into NW + CRC (18 kg/m^2 < BMI < 25 kg/m^2, $n = 42$) and OW/OB + CRC (BMI ≥ 25 kg/m^2, $n = 34$) groups. All subjects had fasted overnight for at least 12 h, and blood samples were collected before the surgical operation. Serum was separated by centrifugation at 3,000 *g* for 10 min at 4°C and was stored in 1.5 mL Eppendorf tubes at −80°C for further analysis. In addition, sWAT and vWAT were obtained during the surgical procedure in nine OB + CRC patients and nine age-sex matched NW + CRC patients. Samples of adipose tissue were immediately frozen in liquid nitrogen and subsequently stored at −80°C for further study.

Serum Biochemical Parameters and Adipokine Measurements

Serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and fasting blood glucose (FBG) levels were determined by routine automated laboratory methods in our clinical laboratory. Serum adipokines including ZAG, HMW-ADPN, leptin, and TNF-α were measured by commercially available enzyme-linked immunosorbent assay kits (USCN Life Science Inc., Wuhan, China) according to the manufacturer's instructions. The low limits of detection for ZAG, HMW-ADPN, leptin, and TNF-α were 1.80 ng/mL, 0.07 ng/mL, 0.06 ng/mL, and 6.50 pg/mL, respectively. The intra- and inter-assay coefficients of variation were 1.13 and 8.52% for ZAG, 0.59 and 4.60% for HMW-ADPN, 1.72 and 2.32% for leptin, and 2.07 and 2.76% for TNF-α.

Total RNA Preparation and Reverse Transcription Quantitative PCR

Total RNA was extracted from human sWAT and vWAT by using E.Z.N.A Total RNA Kit I (Omega, San Diego, CA, USA) according to the manufacturer's recommendations. Total RNA concentrations were estimated by Nano Drop 2000C (Thermo, Forma, USA). Then, 1.0 μg of total RNA was reverse transcribed into cDNA by using 1.0 μL Omniscript reverse transcriptase (Qiagen, Hilden, Germany), 10 U RNase inhibitor and an Oligo-dT primer (Promega, Madison, WI, USA) at 37°C for 60 min. PCR amplification was performed on an ABI 7500 PCR instrument (Applied Biosystems, CA, USA) with each gene in duplicate. The reaction conditions consisted of an initial denaturation step (10 min at 95°C) and a cycling step (denaturation for 15 s at 95°C and annealing and extending for 1 min at 60°C for 40 cycles). β-Actin was used for normalization, and all the primer sequences used were listed in Table S1 in Supplementary Material. The results are expressed as fold changes of Ct value relative to controls by using the $2^{-\Delta\Delta Ct}$ formula (29).

Statistical Analysis

Data are shown as the mean ± SD or median with interquartile range. Normal distribution of the variables was evaluated using the Shapiro–Wilk *W* test. Comparison of variables between two groups was performed by either the independent sample *t*-test or Mann–Whitney *U* test according to the data distribution. Univariate and multivariate logistic regression analyses were used to estimate the odds ratio (OR) and 95% confidence intervals (CIs) of each variable for CRC. Cutoff point analysis, defined by the largest distance from the diagonal line of the ROC curve [sensitivity × (1 − specificity)], was used to identify the optimal value of serum ZAG levels that differentiated healthy people from patients with CRC. The sensitivity and specificity of the index for the cutoff point were also calculated. Stepwise multiple regression analysis was performed to explore the variables independently related to ZAG levels in serum and WAT. All statistical computations were run on SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS

General Characteristics of Subjects in NW + CRC, OW/OB + CRC, and Control Groups

The characteristics of the CRC patients and healthy controls have been summarized in **Table 1**. Generally, NW + CRC patients have a significantly higher age but lower HDL-C levels when compared with control subjects (all $P < 0.05$). As expected, patients in the OW/OB + CRC group presented with a higher body weight, BMI, SBP, and TG than those in the NW + CRC group (all $P < 0.05$). However, no significant difference was observed with regard to height, DBP, FBG, TC, or LDL-C in these two groups.

Serum Levels of ZAG, Leptin, HMW-ADPN, and TNF-α in CRC Patients and Healthy Controls

As shown in **Figure 1A**, serum ZAG levels in NW + CRC patients were 11.7% higher than in healthy controls (1.53 ± 0.30 vs. 1.37 ± 0.31 μg/mL, $P < 0.05$). In addition, serum levels of leptin in NW + CRC patients had a tendency to be lower compared with healthy controls (1.82 ± 1.85 vs. 2.13 ± 1.36 ng/mL, $P = 0.07$) (**Figure 1B**). No significant difference was found in serum HMW-ADPN and TNF-α levels between NW + CRC patients and healthy controls (**Figures 1C,D**).

TABLE 1 | General characteristics of subjects in NW + CRC, OW/OB + CRC, and control groups.

Characteristics	Controls (n = 40)	Patients (n = 76)		
		All CRC (n = 76)	NW + CRC (n = 42)	OW/OB + CRC (n = 34)
Gender (M:F)	30/10	48/28	29/13	19/15
Age (years)	63.6 ± 7.4	67.5 ± 10.8[a]	67.9 ± 9.4[a]	67.1 ± 12.5
Height (cm)	166.5 ± 8.0	166.3 ± 7.8	166.5 ± 6.3	165.9 ± 9.3
Body weight (kg)	63.2 ± 7.8	69.3 ± 12.8[a]	61.2 ± 6.3	79.3 ± 11.7[b]
BMI (kg/m^2)	22.5 ± 1.9	25.0 ± 3.9[a]	22.0 ± 1.6	28.7 ± 2.5[b]
Hypertension (%)	0	33 (43.4%)	17 (40.5%)	16 (47.1%)
Type 2 DM (%)	0	10 (13.2%)	4 (9.5%)	6 (17.6%)
Cardiovascular disease (%)	0	9 (11.8%)	4 (9.5%)	5 (14.7%)
SBP (mmHg)	121.3 ± 9.8	127.0 ± 14.6[a]	123.4 ± 15.4	131.5 ± 12.4[b]
DBP (mmHg)	74.7 ± 6.9	74.5 ± 9.8	73.5 ± 9.8	75.7 ± 9.7
FBG (mmol/L)	5.04 ± 0.43	5.65 ± 1.62[a]	5.45 ± 1.50	5.89 ± 1.75
TC (mmol/L)	4.79 ± 0.59	4.81 ± 0.97	4.62 ± 0.75	5.04 ± 1.16
TG (mmol/L)	1.23 ± 0.50	1.37 ± 0.57	1.21 ± 0.52	1.59 ± 0.56[b]
HDL-C (mmol/L)	1.37 ± 0.32	1.09 ± 0.23[a]	1.13 ± 0.26[a]	1.04 ± 0.18
LDL-C (mmol/L)	2.90 ± 0.48	2.95 ± 0.72	2.86 ± 0.67	3.07 ± 0.78

Values are mean ± SD.

NW, normal weight; OW/OB, overweight/obese; CRC, colorectal cancer; BMI, body mass index; DM, diabetes mellitus; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

[a]*P < 0.05 compared with control group.*

[b]*P < 0.05 compared with NW + CRC group.*

As shown in **Figures 1E–H**, serum HMW-ADPN levels in OW/OB + CRC patients were significantly decreased by 23.5% (2.02 ± 1.19 vs. 2.64 ± 1.30 μg/mL, $P < 0.05$), while the leptin levels were significantly increased by 57.7% (2.87 ± 1.47 vs. 1.82 ± 1.90 ng/mL, $P < 0.01$) when compared with NW + CRC patients. However, no significant difference was observed in serum ZAG and TNF-α levels between these two groups.

Next, serum levels of the four adipokines were further analyzed in male and female subjects, separately. As shown in Figure S1 in Supplementary Material, for men, serum ZAG levels in NW + CRC patients were 22.2% higher (1.57 ± 0.31 vs. 1.28 ± 0.25 μg/mL, $P < 0.05$), while the leptin levels were 30.1% lower (1.20 ± 1.36 vs. 1.71 ± 1.10 ng/mL, $P < 0.05$) than in healthy controls. Serum HMW-ADPN levels in male OW/OB + CRC patients were significantly decreased by 37.5% (1.67 ± 1.05 vs. 2.68 ± 1.41 μg/mL, $P < 0.05$), while the leptin levels were significantly increased by 86.1% (2.22 ± 1.29 vs. 1.20 ± 1.36 ng/mL, $P < 0.01$) when compared with NW + CRC patients. However, no significant difference was observed in serum ZAG, leptin, HMW-ADPN, and TNF-α levels in women across these three groups (Figure S2 in Supplementary Material).

Expression of *ZAG*, *Leptin*, *HMW-ADPN*, and *TNF*-α in sWAT and vWAT of NW + CRC and OB + CRC Patients

In our present study, the mRNA levels of *ZAG*, *leptin*, *HMW-ADPN*, and *TNF*-α were also measured in sWAT and vWAT from nine NW + CRC and nine OB + CRC patients. Baseline characteristics of the patients have been summarized in Table S2 in Supplementary Material. Our results showed that *ZAG* mRNA levels in sWAT were significantly lower in OB + CRC patients than in NW + CRC patients (reduced by 78.8%, $P < 0.01$) as presented in **Figure 2A**. Additionally, *TNF*-α mRNA levels in vWAT of OB + CRC patients were significantly increased by 2.8-fold when compared with NW + CRC patients ($P < 0.05$) (**Figure 2H**). No significant differences in *HMW-ADPN* or *leptin* mRNA levels in sWAT and vWAT were observed between these two groups (**Figures 2B–G**).

The Relationships Between Clinical Parameters and Serum ZAG Levels and *ZAG* mRNA Levels in sWAT and vWAT in CRC Patients

As shown in **Table 2**, a significant positive correlation between circulating ZAG levels and TNF-α levels was found after adjusting for age and sex in CRC patients ($r = 0.27$, $P = 0.03$). *ZAG* mRNA levels in sWAT were negatively associated with BMI ($r = -0.55$, $P = 0.03$), whereas *ZAG* mRNA levels in vWAT were positively correlated with BMI ($r = 0.60$, $P = 0.01$). In addition, *ZAG* mRNA levels in sWAT were also found to be positively associated with serum HMW-ADPN levels ($r = 0.54$, $P = 0.03$). No significant relationship between serum ZAG levels and BMI was found in CRC patients.

Next, stepwise multivariate linear regression was performed. As displayed in **Table 3**, CRC, TNF-α, and HDL-C were independent factors associated with serum ZAG levels after adjusting for age, gender, BMI, TC, TG, SBP, DBP, FBG, HDL-C, and LDL-C. Among them, the presence of CRC was found to be independently positively associated with serum ZAG levels ($B = 0.24$, $P < 0.01$), which was consistent with the higher serum ZAG levels in CRC patients as displayed in **Figure 1A**. Serum TNF-α levels were also independently positively associated with serum ZAG levels ($B = 0.01$, $P < 0.01$), which was also in accordance with the results shown in **Table 2** by partial correlation analysis. In addition, BMI was independently negatively related to *ZAG* mRNA levels in sWAT but independently positively related to *ZAG* mRNA levels in vWAT, which was also consistent with the results demonstrated in **Table 2**.

Association of ZAG, Leptin, HMW-ADPN, and TNF-α with CRC Risks

Next, all subjects were stratified into trisections according to ZAG tertiles (lowest: <1.30 μg/mL; median: 1.30–1.51 μg/mL; highest: ≥1.51 μg/mL). As shown in **Table 4**, the CRC risk was 2.43-fold higher in subjects with the high ZAG level than those with the low serum ZAG levels (OR = 3.43, 95% CI 1.24–9.49, $P = 0.02$) after adjusting for age and gender (Model 1). This increased probability of CRC risk still remained after further adjusting for BMI, SBP, DBP, and FBG based on Model 1 (Model 2, OR = 3.96, 95% CI 1.28–12.27, $P = 0.04$) and TC, TG, HDL-C, and LDL-C based on Model 2 (Model 3, OR = 6.84, 95% CI 1.70–27.54, $P = 0.03$). In addition, serum leptin levels were also categorized into tertiles (lowest: <1.12 ng/mL; median: 1.12–2.92 ng/mL; highest: ≥2.92 ng/mL). As presented in **Table 4**, the probability of CRC

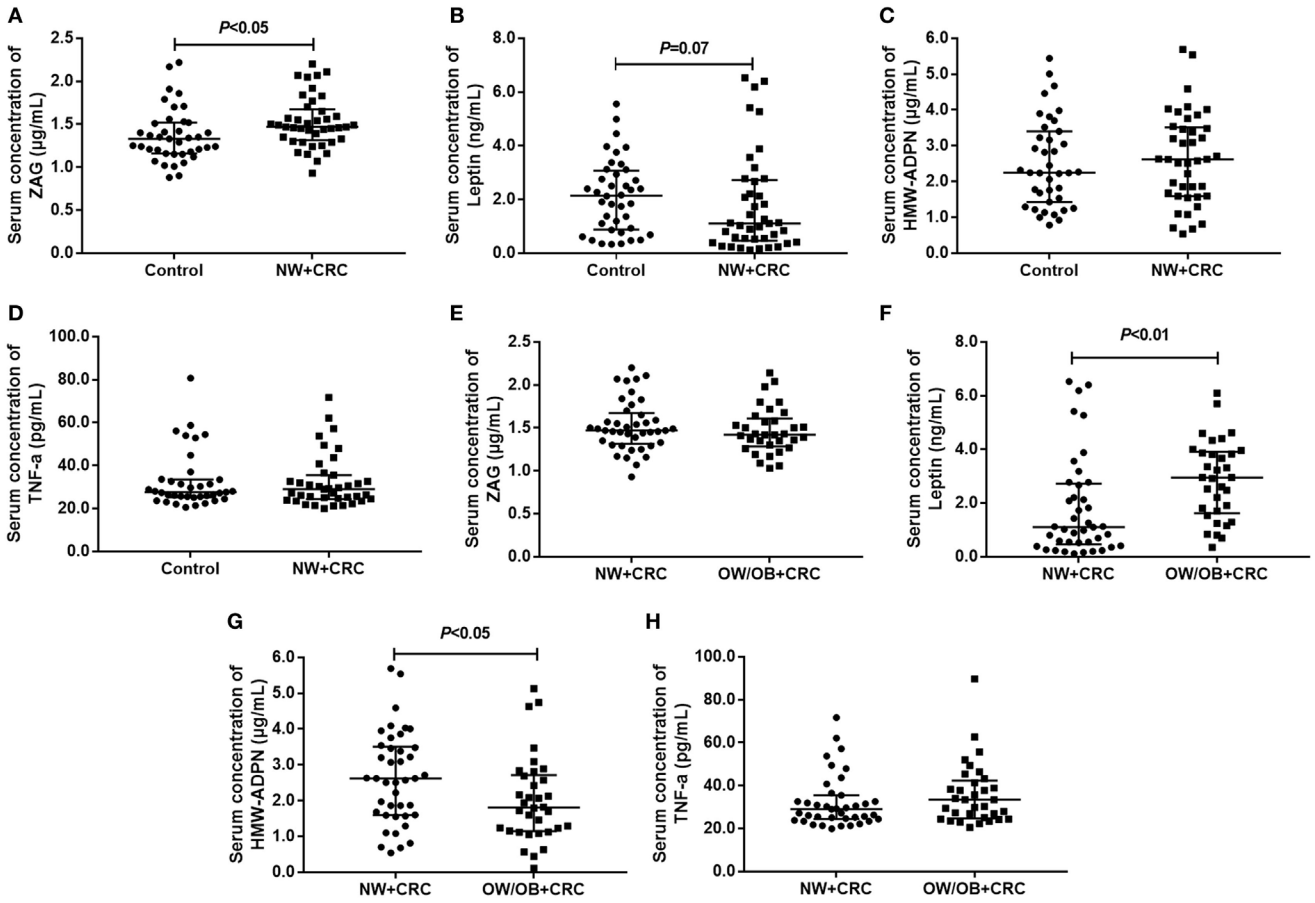

FIGURE 1 | Serum levels of ZAG, leptin, HMW-ADPN, and TNF-α in NW + CRC patients and healthy controls **(A–D)** as well as in NW + CRC and OW/OB + CRC patients **(E–H)**. Abbreviations: ZAG, zinc-α2-glycoprotein; HMW-ADPN, high-molecular-weight- adiponectin; TNF-α, tumor necrosis factor-alpha; NW, normal weight; OW/OB, overweight/obese; CRC, colorectal cancer. All values are expressed as the median with the interquartile range.

risk decreased by 94.0% in participants with the highest leptin level compared with those with the lowest serum leptin levels (OR = 0.06, 95% CI 0.01–0.41, $P = 0.01$) after adjusting for age, gender, BMI, SBP, DBP, and FBG (Model 2). This decreased risk of CRC remained after further adjusting for TC, TG, HDL-C, and LDL-C based on Model 2 (Model 3, OR = 0.11, 95% CI 0.01–0.89, $P = 0.04$). However, no significant differences were found in the OR of CRC risks between the tertiles of HMW-ADPN and TNF-α levels.

Diagnostic Values of Serum ZAG, Leptin, HMW-ADPN, and TNF-α for CRC

Subsequently, ROC curve analysis was used to investigate the potential application of the four serum adipokines for the discrimination between patients with CRC and healthy people. Our results indicate that only ZAG can effectively discriminate between CRC patients and healthy individuals within ROC curve areas of 0.655 (95% CI 0.544–0.767, $P < 0.05$) (**Figure 3A–D**). At the cutoff value of 1.42 μg/mL for ZAG, the sensitivity and specificity for the discrimination of CRC are 62.2 and 69.2%, respectively.

DISCUSSION

Zinc-α2-glycoprotein is a 41-kDa secreted glycoprotein that was first identified in human plasma in 1961 (30). Previous studies have shown that ZAG is expressed at high levels in a variety of malignancies, such as prostate, breast, and lung cancer (20–22). Serum ZAG was found to be a potential biomarker for prostate cancer (23). Early in 2012, Agesen et al. found increased *ZAG* gene expression at the transcriptional levels in CRC tissues from Western populations, including Norwegian, American, and Australian populations (26). Further studies in Chinese populations also reported that ZAG was upregulated at the transcriptional and posttranscriptional levels in fresh colon cancer tissues (27), suggesting that ZAG might be a potential biomarker for CRC in both Western and Eastern populations. Consistent with its elevated expression in cancer tissues, Xue et al. also found higher serum ZAG levels in CRC patients than in healthy controls (28). Further analysis showed that there was a positive association between serum ZAG levels and CRC clinical stages (28). Another study performed by Ji et al. found that serum ZAG was elevated in CRC patients, and CRC patients with higher ZAG levels

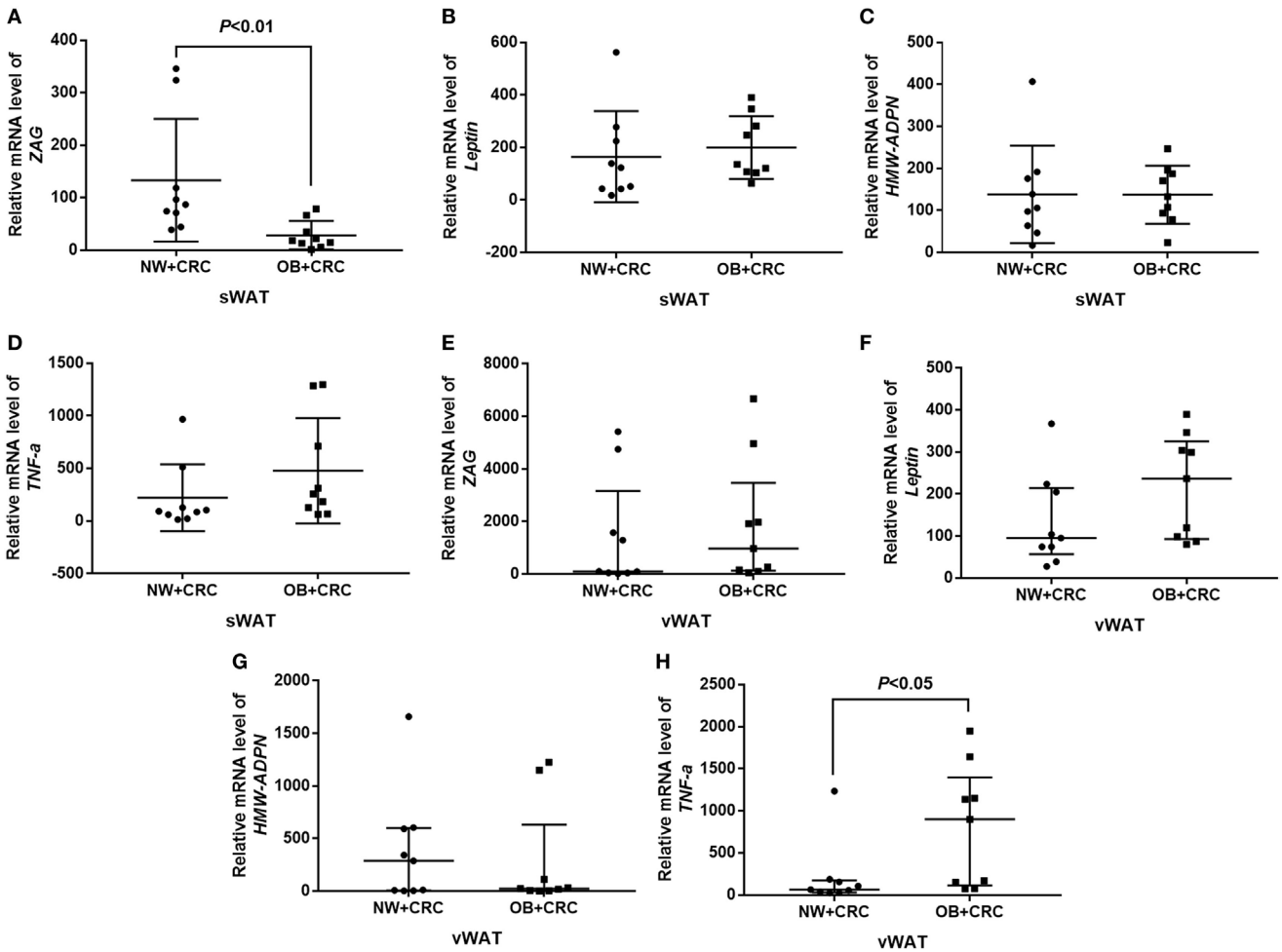

FIGURE 2 | ZAG, leptin, HMW-ADPN, and TNF-α mRNA levels in sWAT **(A–D)** and vWAT **(E–H)** of NW + CRC and OB + CRC patients. Abbreviations: ZAG, zinc-α2-glycoprotein; HMW-ADPN, high-molecular-weight-adiponectin; TNF-α, tumor necrosis factor-alpha; sWAT, subcutaneous white adipose tissue; vWAT, visceral white adipose tissue; NW, normal weight; OB, obese; CRC, colorectal cancer. All values are expressed as the median with the interquartile range.

showed worse clinical outcomes (27). In our present study, we also found that serum ZAG levels were significantly increased in CRC patients with NW and CRC was found to be independently associated with serum ZAG levels. Based on these data, it is thus reasonable to assume that the elevated expression of ZAG in CRC tissues results in high serum ZAG levels, which may further promote CRC development. In addition, our study showed that the significantly higher serum ZAG levels only presented in male CRC patients, which is consistent with the epidemiological findings that men are at a higher risk of CRC.

Next, all subjects were stratified into trisections according to their serum ZAG tertiles. The results showed that the CRC risk was 2.43-fold higher in subjects with the highest ZAG level than those with the lowest serum ZAG levels after adjusting for age and gender (Model 1). This increased probability of CRC remained after further adjusting for BMI, SBP, DBP, and FBG based on Model 1 (Model 2, OR = 3.96) and TC, TG, HDL-C, and LDL-C based on Model 2 (Model 3, OR = 6.84), suggesting that ZAG overexpression is a significant risk factor for CRC, independent of other clinical pathological factors. Further analyses using ROC curves showed that the AUC of ZAG was 0.655. At the cutoff value of 1.42 μg/mL, the diagnostic value of ZAG had 62.2% sensitivity and 69.2% specificity. In accordance with our results, Ji et al. reported that serum ZAG was a useful biomarker for CRC within ROC curve areas of 0.9572 (95% CI 0.9173–0.9971) in a cohort of 534 Chinese individuals (27). Studies conducted by Xue et al. further found that the AUC of ZAG was 0.742 (95% CI 0.656–0.827), which was lower than the AUC of CEA (0.746, 95% CI 0.665–0.827) but higher than the AUC of CA19-9 (0.676, 95% CI 0.578–0.774) in a total of 160 Chinese subjects (28). All of these findings suggest that ZAG could be used as a potential serum biomarker for CRC.

Zinc-α2-glycoprotein is also a novel adipokine that can be secreted by adipose tissue. Decreased ZAG levels in sWAT of obese patients and its negative association with BMI have been previously reported (19, 31–33). In our present study, we observed for the first time that *ZAG* mRNA levels in sWAT of OB + CRC patients were also significantly decreased when compared with

TABLE 2 | Partial correlation analysis between ZAG levels in both serum and WAT and clinical parameters in CRC patients.

	Serum ZAG (n = 76)		sWAT ZAG (n = 18)		vWAT ZAG (n = 18)	
	r	*P*	*r*	*P*	*R*	*P*
Age (years)	−0.15	0.21	−0.03	0.93	−0.25	0.33
BMI (kg/m^2)	−0.15	0.23	−0.55	**0.03**	0.60	**0.01**
SBP (mmHg)	−0.04	0.75	−0.32	0.25	−0.29	0.29
DBP (mmHg)	−0.04	0.77	−0.41	0.13	0.01	0.98
FBG (mmol/L)	0.04	0.77	0.04	0.88	0.03	0.91
TC (mmol/L)	−0.04	0.77	−0.21	0.46	0.26	0.35
TG (mmol/L)	−0.02	0.10	−0.08	0.80	−0.30	0.29
HDL-C (mmol/L)	0.13	0.30	−0.02	0.95	−0.09	0.74
LDL-C (mmol/L)	−0.16	0.17	−0.14	0.63	0.28	0.31
Serum ZAG (μg/mL)	–	–	−0.10	0.73	0.08	0.77
Serum leptin (ng/mL)	−0.02	0.84	−0.52	0.06	0.52	0.06
Serum HMW-ADPN (μg/mL)	0.01	0.97	0.54	**0.03**	−0.46	0.07
Serum TNF-α (pg/mL)	0.27	**0.03**	−0.34	0.24	−0.1	0.68

Values are age-sex-adjusted Spearman partial correlation coefficients.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ZAG, zinc-α2-glycoprotein; HMW-ADPN, high-molecular-weight-adiponectin; TNF-α, tumor necrosis factor-alpha; sWAT, subcutaneous white adipose tissue; vWAT, visceral white adipose tissue.
Bold font means p < 0.05.

TABLE 3 | Multiple regression analysis for the variables independently related to serum ZAG and *ZAG* mRNA in sWAT and vWAT in CRC patients.

	B	SE of *B*	β	*P*
Serum ZAG (R^2 = 0.52)				
CRC (0 or 1)	0.24	0.06	0.34	<0.01
TNF-α	0.01	0.00	0.34	<0.01
HDL-C	0.31	0.10	0.31	<0.01
Constant	0.66	0.15		<0.01
***ZAG* mRNA in sWAT (R^2 = 0.298)**				
BMI	−10.00	4.10	−0.55	0.03
Constant	351.44	112.27		0.01
***ZAG* mRNA in vWAT (R^2 = 0.553)**				
BMI	243.68	66.76	0.70	<0.01
SBP	−83.08	33.38	−0.48	0.03
Constant	4,763.52	4,120.78		0.27

Stepwise multiple regression analysis was used. Variables also entered into multiple regression analysis but not included in the equation: age, gender, BMI, total cholesterol, triglycerides, SBP, diastolic blood pressure, fasting blood glucose, low-density lipoprotein cholesterol, serum high-molecular-weight-adiponectin, and serum leptin.
B, regression coefficient; β, standardized regression coefficient; sWAT, subcutaneous white adipose tissue; vWAT, visceral white adipose tissue; 0; non-cancer; 1; colorectal cancer; ZAG, zinc-α2-glycoprotein; CRC, colorectal cancer; HDL-C, high-density lipoprotein cholesterol; BMI, body mass index; SBP, systolic blood pressure.

NW + CRC patients. Further partial correlation and multiple regression analysis found a negative relationship between *ZAG* mRNA levels in sWAT and BMI, and BMI was independently negatively related to *ZAG* levels in sWAT. These results suggest that the decreased ZAG levels in sWAT and its negative relationship with BMI were observed both in simple OW/OB patients and in CRC patients. By contrast, *ZAG* mRNA levels in vWAT were found to be positively related with BMI in our present study. In contrast with our findings in CRC patients, previous studies in simple obese patients performed by Mracek and Selva et al. found that ZAG expression in vWAT was significantly lower in obese patients and showed a negative correlation with BMI (19, 34). Given these results together, we speculate that *ZAG* mRNA levels in sWAT and vWAT might play a different role in CRC patients. Previous studies by Balaz have shown that ZAG in sWAT, but not in vWAT, is associated with whole-body insulin sensitivity (31). Although the biological mechanisms of the different role of sWAT and vWAT in CRC are still not well known, a possible explanation for this difference may be attributed to the much more severe insulin resistance of vWAT compared to sWAT (31). In addition, *ZAG* mRNA levels in vWAT were for the first time found to be negatively correlated with SBP in our present study. Further studies need to be done to validate this phenomenon.

TABLE 4 | Unconditional logistic regression analysis of colorectal cancer risks according to tertiles of zinc-α2-glycoprotein (ZAG), leptin, high-molecular-weight adiponectin (HMW-ADPN), and tumor necrosis factor-alpha (TNF-α) in all subjects.

	Tertile (number of cases and controls)			
Measurement	**Lowest odds ratio (OR) [95% confidence interval (CI)]**	**Median OR (95% CI)**	**Highest OR (95% CI)**	***P* for trend**
ZAG				
Range (μg/mL)	<1.30	≥1.30 to <1.51	≥1.51	
Cases/controls	18/20	29/9	28/11	
Univariate	1.00 (reference)	3.06 (1.17–8.04)	2.85 (1.08–7.52)	**0.04**
Model 1	1.00 (reference)	3.35 (1.23–9.12)	3.43 (1.24–9.49)	**0.02**
Model 2	1.00 (reference)	2.97 (0.98–9.04)	3.96 (1.28–12.27)	**0.04**
Model 3	1.00 (reference)	2.52 (0.70–9.09)	6.84 (1.70–27.54)	**0.03**
Leptin				
Range (ng/mL)	<1.12	≥1.12 to <2.92	≥2.92	
Cases/controls	27/12	22/17	26/11	
Univariate	1.00 (reference)	0.65 (0.26–1.65)	1.14 (0.42–3.04)	0.46
Model 1	1.00 (reference)	0.60 (0.23–1.57)	0.76 (0.24–2.38)	0.58
Model 2	1.00 (reference)	0.13 (0.03–0.53)	0.06 (0.01–0.41)	**0.01**
Model 3	1.00 (reference)	0.13 (0.03–0.64)	0.11 (0.01–0.89)	**0.04**
HMW-ADPN				
Range (μg/mL)	<1.68	≥1.68 to <2.89	≥2.89	
Cases/controls	26/12	25/13	24/15	
Univariate	1.00 (reference)	0.79 (0.30–2.05)	0.70 (0.26–1.83)	0.76
Model 1	1.00 (reference)	0.61 (0.22–1.69)	0.56 (0.20–1.54)	0.49
Model 2	1.00 (reference)	0.95 (0.30–2.94)	1.17 (0.36–3.81)	0.92
Model 3	1.00 (reference)	0.92 (0.26–3.33)	1.59 (0.41–6.18)	0.65
TNF-α				
Range (pg/mL)	<25.93	≥25.93 to <33.43	≥32.43	
Cases/controls	26/12	21/17	28/11	
Univariate	1.00 (reference)	0.70 (0.27–1.83)	1.35 (0.50–3.68)	0.42
Model 1	1.00 (reference)	0.75 (0.27–2.05)	1.65 (0.58–4.71)	0.32
Model 2	1.00 (reference)	1.16 (0.36–3.80)	1.33 (0.40–4.44)	0.90
Model 3	1.00 (reference)	1.25 (0.31–4.97)	1.210 (0.32–4.54)	0.94

Multivariate ORs and 95% CIs from unconditional logistic regression models were used in the analysis.
Model 1: basic model, adjusted for age and gender.
Model 2: further adjusted for body mass index, systolic blood pressure, diastolic blood pressure, and fasting blood glucose based on the model 1.
Model 3: full model, further adjusted for total cholesterol (<5.18 mmol/L, ≥5.18 mmol/L), triglycerides (<1.7 mmol/L, ≥1.7 mmol/L), high-density lipoprotein cholesterol (<1.04 mmol/L, ≥1.04 mmol/L), and low-density lipoprotein cholesterol (<3.37 mmol/L, ≥3.37 mmol/L) based on the model 2.
Bold font means p < 0.05.

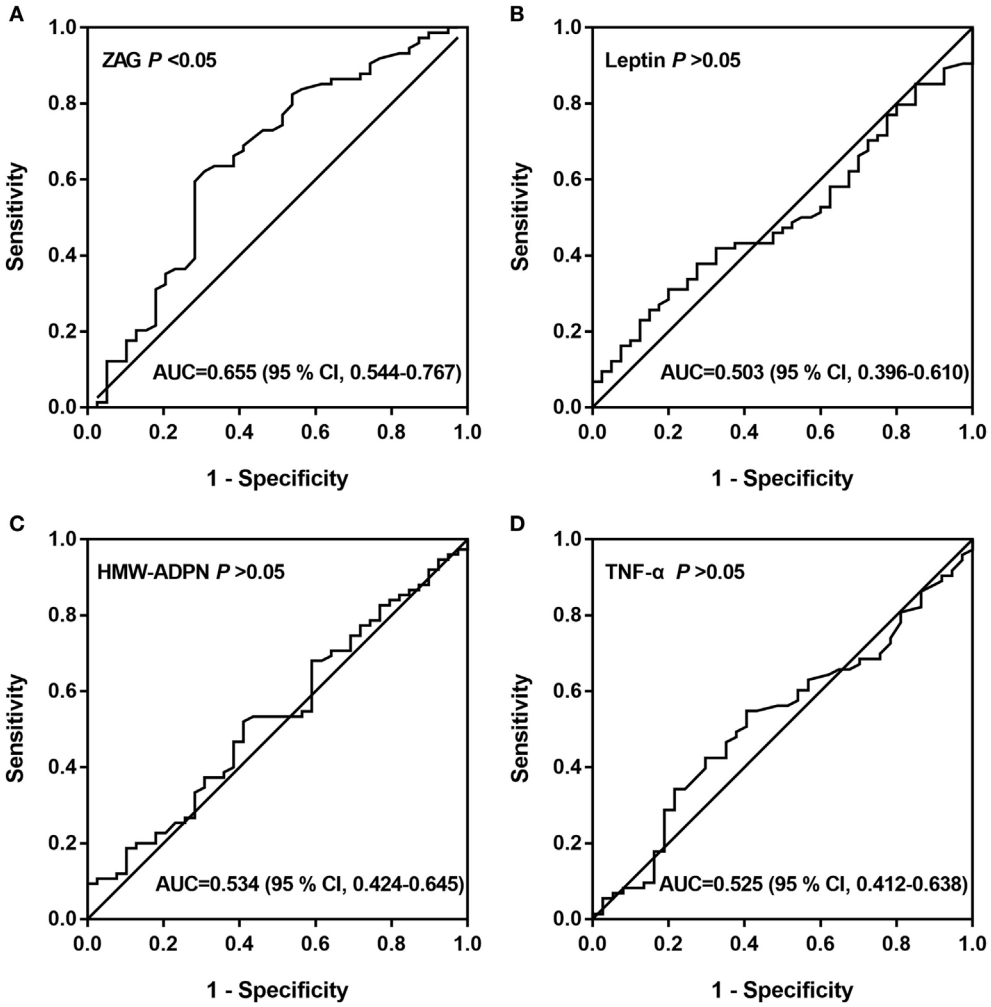

FIGURE 3 | ROC curves of serum ZAG **(A)**, leptin **(B)**, HMW-ADPN **(C)**, and TNF-α **(D)**. ROC curves were derived by plotting the relationship between the specificity and the sensitivity at various cutoff levels. Abbreviations: ZAG, zinc-α2-glycoprotein; HMW-ADPN, high-molecular-weight-adiponectin; TNF-α, tumor necrosis factor-alpha; ROC, receiver operating characteristic; AUC, area under the curve.

After stratifying all subjects into trisections according to serum leptin tertiles, the probability of CRC risk was found to decrease by 94.0% in subjects with the highest leptin level compared to those with the lowest serum leptin levels after adjusting for age, gender, BMI, SBP, DBP, and FBG (Model 2, OR = 0.06). This decreased risk of CRC remained even after further adjusting for TC, TG, HDL-C, and LDL-C based on Model 2 (Model 3, OR = 0.11), suggesting that leptin might be a protective factor against CRC, which is independent of other clinical pathological factors. In line with our results, a case–control study performed by Joshi et al. in a South Korean population found a negative association between leptin and CRC risk (13). Additionally, studies in Western populations performed by Kumor and Salageanu et al. observed significantly lower serum leptin levels in CRC patients than controls (11, 12). Our present study also demonstrated that serum leptin levels have a lower trend in NW + CRC patients than healthy controls ($P = 0.07$).

Next, the CRC patients were further divided into OW/OB or NW groups. Interestingly, a significantly higher serum leptin levels was observed in OW/OB Chinese CRC patients when compared with NW CRC patients. Consistent with our results, studies performed by Stachowicz et al. in a total of 146 Caucasians with CRC also demonstrated that OW/OB CRC patients had statistically higher serum leptin levels than NW patients (35). It is well known that obese patients have markedly increased circulating leptin levels compared with NW controls (36). In our present study, we first found that the OW/OB CRC Chinese patients also had higher serum leptin than NW CRC patients.

ADPN is a 30-kDa protein hormone secreted exclusively from adipose tissue (37), and HMW-ADPN is now considered the most active form of adiponectin (38). ADPN has been shown to be decreased in obese subjects and is supposed to exert anti-inflammatory and anticancerous activity (39). Our present study also observed significantly decreased serum levels of HMW-ADPN

in OW/OB + CRC patients compared to NW + CRC patients, similar to its profile in obese patients (40).

Tumor necrosis factor-alpha is a key pro-inflammatory cytokine produced by macrophages cells and secreted by adipocytes (41). It has been widely accepted that an obesity-associated low grade of chronic inflammation is an important contributing factor in CRC pathogenesis (42). However, in this study, no significant differences were found in the serum TNF-α level between NW + CRC patients and healthy controls, or between NW + CRC and OW/OB + CRC patients. In accordance with our results, Amor et al. measured the plasma levels of TNF-α in lean and obese subjects with and without CRC also found no significant change in plasma TNF-α levels between patients with CRC or obesity (43). Additionally, our study observed that *TNF*-α mRNA levels were upregulated in vWAT from OB + CRC patients compared to NW + CRC patients. Studies performed by Delgado et al. also reported that vWAT, but not sWAT, was an indicator of inflammation (44). These results suggest that TNF-α secreted by vWAT, instead of sWAT, may be involved in obesity-related CRC development.

In conclusion, our study found that serum ZAG levels were significantly increased in CRC patients. *ZAG* mRNA levels in sWAT were found to be significantly reduced in OB + CRC Chinese patients in comparison with NW + CRC patients. The patients with the highest tertile ZAG serum levels were more likely to have CRC. At the cutoff value of 1.42 μg/mL for serum ZAG, the sensitivity and specificity for differentiating patients with CRC from controls were 62.2 and 69.2%, respectively. Additional and more comprehensive studies are needed to explore the detailed mechanisms of the role of ZAG in CRC development.

ETHICS STATEMENT

Informed consent was signed by all participants and the study was approved by the ethics committee of Peking Union Medical College Hospital (No. S-364).

AUTHOR CONTRIBUTIONS

HZ designed the experiments and revised the primary manuscript. ML analyzed the data and wrote the primary manuscript. NZ performed the molecular biological experiments. HP, GL, NL, LW, HY, and KY collected the clinical materials and serum samples and finished the clinical and biochemical parameters measurements. FG designed the experiments, supervised the whole study, and revised the primary manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fendo.2018.00190/full#supplementary-material.

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REVIEW
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Mechanistic Insights Into the Interaction Between Transcription Factors and Epigenetic Modifications and the Contribution to the Development of Obesity

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Objective: The development of obesity is inseparable from genetic and epigenetic factors, and transcription factors (TFs) play an essential role in these two mechanisms. This review analyzes the interaction of TFs with epigenetic modifications and the epigenetic mechanisms underlying peroxisome proliferator-activated receptor (PPAR)γ, an important transcription factor, in the development of obesity.

Methods: We describe the relationship between TFs and different epigenetic modifications and illustrate the several mechanisms described. Next, we summarize the epigenetic mechanisms of PPARs, an important class of transcription factors involved in obesity, that induce obesity with different triggering factors. Finally, we discuss the mechanisms of epigenetic modification of PPAR-related ligands in lipid metabolism and propose future avenues of research.

Results: TFs participate in epigenetic modifications in different forms, causing changes in gene expression. The interactions between the different epigenetic modifications and PPARs are important biological developments that affect fat tissue differentiation, lipogenesis, and lipid metabolism, thereby inducing or inhibiting the development of obesity. We then highlight the need for more research to understand the role of epigenetic modifications and PPARs.

Conclusions: Epigenetic mechanisms involved in the regulation of PPARs may be excellent therapeutic targets for obesity treatment. However, there is a need for a deeper understanding of how PPARs and other obesity-related transcription factors interact with epigenetic modifications.

Keywords: obesity, transcription factors, epigenetics, DNA methylation, histone modifications, chromatin remodeling, non-coding RNA, interaction

INTRODUCTION

Obesity is currently the world's most conspicuous nutritional disease. It is currently considered a serious threat to human health because of its high prevalence (1). The expansion of fat mass, adipocyte size increase, and to a lesser extent cell proliferation (hyperplasia) are important features of obesity (2). The increase in adipose tissue corresponds to increase in size and number of adipocytes (3), i.e., lipogenesis and differentiation. Factors that regulate fat tissue differentiation such as insulin, growth hormone, and glucocorticoids promote differentiation of pre-adipocytes (4, 5) and genetic and transcriptional regulation (6, 7). Among regulatory factors, transcription factors (TFs) play a critical role in the regulation of adipocyte differentiation. Studies have shown that lipogenesis and differentiation are regulated coordinately by several TFs, mainly including the peroxisome proliferator-activated receptor γ (PPARγ), CCAAT enhancer binding proteins (C/EBPs), and the transcription factor sterol regulatory element binding protein-1 (SREBP-1) (8, 9). PPARγ is the most critical TF for the regulation of adipocyte differentiation, being a necessary and sufficient condition for the differentiation of adipose tissue (10). The CAAT/enhancer family has the function of activating CAAT repeats of specific gene DNA enhancers. Its family members, C/EBPα, C/EBPβ, and C/EBPδ, are considered important factors in adipocyte differentiation. C/EBPδ and C/EBPα can induce the expression of the PPARγ gene (11), which is probably achieved through a transcriptional effect of the C/EBP binding site in the PPARγ promoter (12). Activated PPARγ, in turn, increases the expression of the C/EBPα gene. In the absence of C/EBPα, cells can only express PPARγ at low levels and cannot form adipocytes (13). In mammals, SREBP is classified into two types: SREBP1 and SREBP2. SREBP1 is also known as adipocyte determination and differentiation-dependent factor 1 (ADDl), and can act independently as a TF to regulate adipocyte differentiation and transcription of genes related to cholesterol metabolism (14). Studies have shown that SREBP promotes the synthesis of triglycerides by up-regulating the expression of genes such as fatty acid synthase and lipoprotein lipase (14). SREBP can also activate PPAR expression or induce the expression of an endogenous ligand of PPAR to increase its lipogenic activity and promote adipocyte differentiation (15). In addition to the above TFs, cyclic AMP response element binding protein (CREB), nuclear factor of activated T cells (NFAT), kruppel-like factor (KLF) family, and forkhead transcription factor (FOX) proteins have been demonstrated to affect adipogenesis (16, 17).

However, the complex mechanisms of adipocyte differentiation remain to be further elucidated (18). Epigenetics is a likely candidate to elucidate the missing links that lead to obesity. Specific dietary behavior leads to change in epigenetic patterns and regulates gene expression via epigenetic modifications (19, 20). These changes interfere with metabolic homeostasis and result in body adiposity (21, 22). Epigenetic changes in chromatin also occur during adipocyte differentiation (23, 24). Some studies indicated that the epigenetic changes at the gene locus of a specific TF correlate with the body-mass index (BMI) in humans (25, 26). This suggests that the development of obesity may be inseparable from the interaction between TFs and epigenetic modification. However, it is still mostly unclear how cells establish the connection between TFs and epigenetic modifications and how it leads to regulation of gene expression. Several specific mechanisms in biological processes, from epigenetic changes to gene regulation, are still not fully understood. A systematic investigation of the link between TFs and epigenetics will provide a deeper understanding of pathogenesis in the metabolism and thereby provide a basis for more appropriate treatment regimens. This review describes the critical link between epigenetic modifications and TFs and their contribution to the development of obesity.

INTERACTION BETWEEN EPIGENETIC MODIFICATIONS AND TFS

Epigenetics can be described as reversible and heritable changes to the DNA that regulates chromatin structure and gene expression without altering the DNA sequence (27). The genome of each organism contains both DNA sequence information and epigenetic information. The interaction between the two specific factors maintains the function of organs and cells. Research has confirmed that there are specific genes in the body that can confer different susceptibilities to disease. However, owing to the stability of the genomic structure, most environmental factors cannot cause gene mutations or DNA sequence changes (28, 29). Therefore, some authors speculate that the changes in gene expression mediated by epigenetic modifications cause genomic abnormalities in the body during development, or result in increased susceptibility to certain diseases (30, 31). However, this process requires the participation of TFs. The currently most studied epigenetic modifications are DNA methylation, histone modification, chromatin remodeling, and non-coding RNA. The relationship between epigenetic modifications and TFs are described below.

DNA METHYLATION AND TFS

DNA methylation is a process in which cytosine is converted to 5-methylcytosine (5-mC) by DNA methyltransferases (DNMTs) with S-adenosylmethionine (SAM) as a methyl donor (32, 33). In mammals, DNMTs comprise three active enzymes, DNMT1, DNMT3A, and DNMT3B, and one related regulatory protein, DNMT3L (33). DNMTs can promote DNA methylation and cause gene silencing. In contrast, the ten-eleven translocation (TET) enzyme is associated with DNA demethylation by reactivating the expression of the silenced gene (34). In most vertebrates, DNA methylation occurs predominantly at highly aggregated CpG dinucleotides, termed CpG islands (CGI), which overlap with gene promoter regions and can be bound by ubiquitous TFs (35). The association between methylation and TFs can be described in the following four ways.

DNA Methylation Interferes With the Binding of TF to Target Genes

DNA methylation prevents the binding of TFs to their binding sites and influences gene transcription (36, 37). Studies showed that CpG methylation of the cAMP-responsive element (CRE) leads to loss of the binding site and of transcriptional activity of some TFs *in vitro* and *in vivo* (38). However, the methylation of the CRE sequence, which is often associated with promoters of cell type-specific genes, creates a TF binding site for C/EBPα, thereby activating transcription during adipocyte differentiation (39, 40) (**Figure 1A**).

Competition Between TFs and Transcriptional Repressors for mCpG Sites

Transcriptional repressors compete with TFs for binding to methyl-CpG sites in the DNA sequence and block transcription. In mammals, one of the main transcriptional repressors are the methyl-CpG-binding domain (MBD) proteins, which comprise eleven known proteins containing the MBD domain (41, 42). These proteins are recruited to a specific sequence containing methylated cytosines, and prevent TFs and RNA polymerase from forming the initial complex at the corresponding methyl-CpG site (43, 44), thereby inhibiting gene transcription (**Figure 1B**).

Association of (de)Methylation Enzymes and TFs in DNA Methylation

The expression of DNMT and demethylase genes are affected by TFs. TFs modulate promoter methylation levels by recruiting and forming complexes with DNMTs (45). Knocking out some TFs can reduce the recruitment of DNMTs and reduce gene methylation levels in the glioma cell line (46) (**Figure 1C**).

Methylation of TFs

DNA methylation can also modify TFs. Methylation of TFs belongs to non-histone methylation. Previous studies have indicated that non-histones can be methylated at lysine residues (47). For example, the RelA (p65) subunit of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is methylated by lysine methyltransferase Set9 at lysine residues 314 and 315 in cells. It inhibits the activity of NF-κB and prevents it from binding to DNA, thereby causing transcriptional repression (48). In contrast, nuclear receptor-binding SET domain-containing protein 1 (NSD1, a lysine methylase) can methylate RelA at K218 and K221 and active NF-κB transcriptional activity in cell line (49) (**Figure 1D**).

HISTONE MODIFICATION AND TFS

Histone modifications are another essential component of epigenetics. Histones comprise core histones (H3, H4, H2A, and H2B) and linker histones, H1/H5. Two copies of the four core histones are wrapped around ∼165 bp of DNA to constitute nucleosomes and form eukaryotic chromatin with the linker histone H1 (50, 51). In histones, flexible charged tails extend from the core region as N- or C-terminal ends and can be covalently modified by various enzymes. This modification is termed post-translational modification (PTM) and may lead to changes in chromatin structure and further genome regulation. Known modifications of N-terminal tails of histones include methylation, acetylation, phosphorylation, ubiquitination, ADP ribosylation, and sumoylation (52). Histone acetylation and deacetylation are the most studied (52, 53), and are considered reversible processes controlled by histone acetyltransferases (HATs) (54) and histone deacetylases (HDAC) (55), respectively. In addition, histone methylation and demethylation are catalyzed by histone methyltransferases (HMTs) and histone demethylases (HDMs), respectively. The modification on histone tails occurs sequentially or in combination, termed "histone code" (56). This code is read by binding of specific TFs. Associations between histone modifications and TFs are divided into the following categories.

Histone Modification Enzymes Interact With TFs

TFs act as a "porter" to recruit histone modification enzymes to regulate gene expression. For example, during histone acetylation, specific TFs repress or activate gene expression through HDAC or HAT recruitment, respectively (57). The activator or repressor role of these TFs on the promoter may depend on its binding site or time on DNA in the nuclear extracts of murine L929 cells (58) (**Figure 2A**). In some cases, TF recruits HATs or HDACs and then binds to these enzymes to form a complex that increases the enzymatic activity and further regulates the expression of related genes (59, 60). Changes in expression of these genes may result from changes in chromatin structure caused by histone modifications (56). The condensed state of chromatin leads to an inability of TFs to bind to target genes when histone is acetylated (61). Coincidentally, HATs may also act on TFs and induce acetylation of these proteins (62–64). Expression of related genes is regulated by the acetylation levels of these TFs (62, 65, 66) (**Figure 2B**).

Crosstalk of TFs With Histone Modifications and PTMs

Modification of the histone code widely occurs in PTM of histone tails. A modification may either enhance or inhibit another (67–69). For instance, the phosphorylation of H3S10 leads to acetylation of H3K14 (67) but blocks acetylation of H3K9 in cell cycle progression (68). Similarly, this modification pattern may occur in TFs. For example, phosphorylation at serine residues 276 and 536 of the P65 subunit of NF-κB facilitates P65 acetylation at lysine 310 in a mammalian one-hybrid system (70, 71) (**Figure 2C**). On the other hand, phosphorylation of P65 also promotes P65 ubiquitination and leads to the degradation of P65 and repression of transcription in cells. However, this process may require the participation of GCN5 (a HAT) (72) (**Figure 2D**). Post-translational modifications of NF-κB were reviewed previously (73).

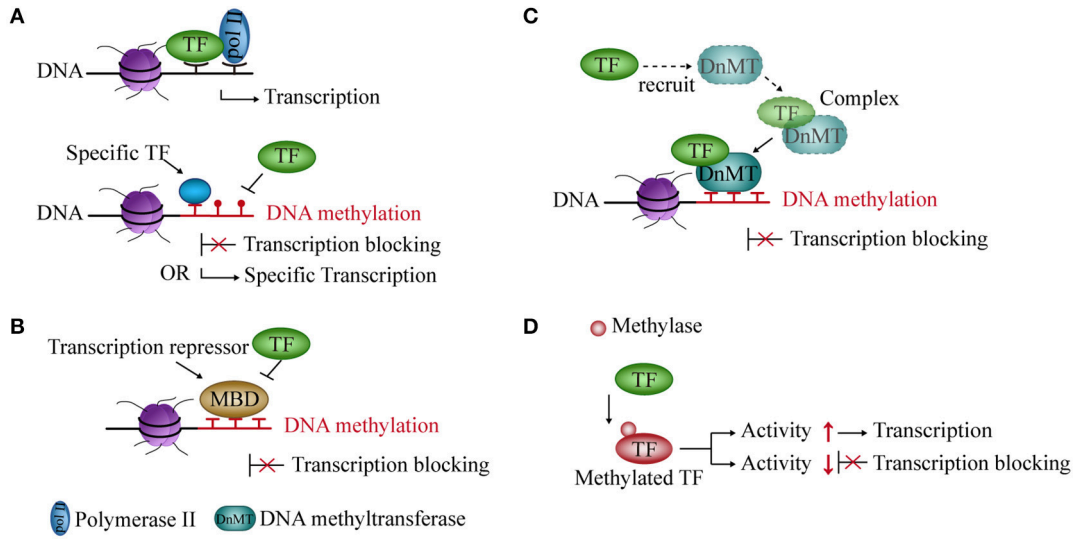

FIGURE 1 | Interaction between DNA methylation and transcription factors (TFs). **(A)** Methylated DNA inhibits the binding of TFs to DNA but also creates binding sites for some specific TFs and activates transcription. **(B)** Transcriptional repressors occupy an mCpG site, thereby blocking transcription. **(C)** TFs recruit DNA methyltransferases to form a complex and repress transcription. **(D)** Methylase acts on TF and regulates its activity and transcription.

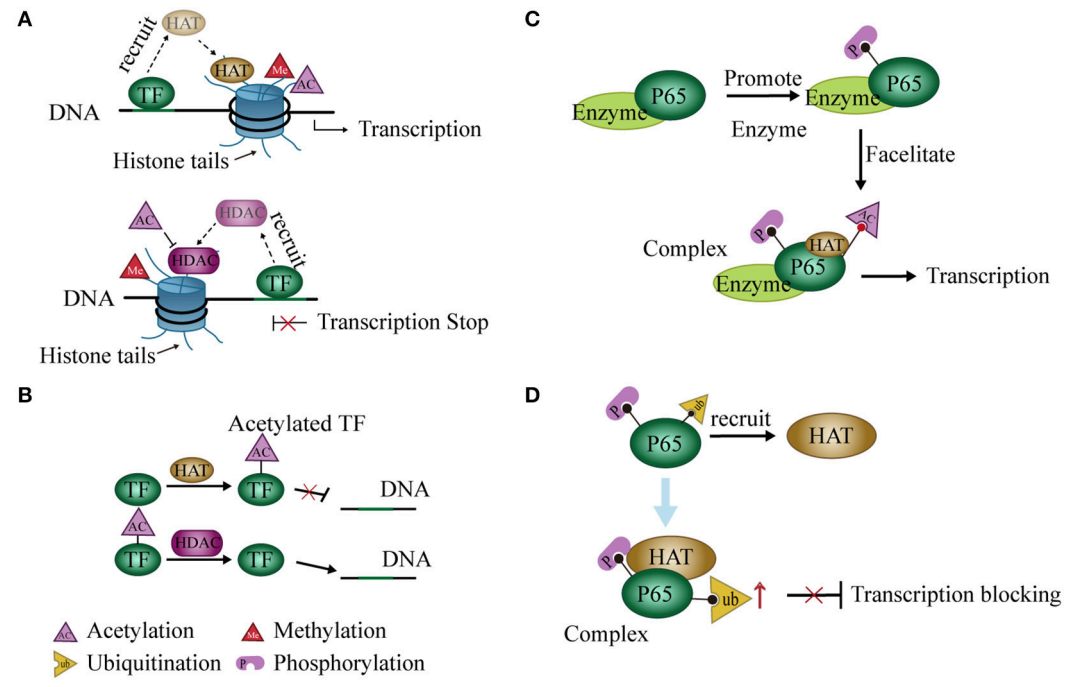

FIGURE 2 | Interaction between histone modification and transcription factors (TFs). **(A)** TFs recruit histone deacetylases (HDACs) or histone acetyltransferases (HATs) and regulate gene expression by promoting histone deacetylation or acetylation, respectively. **(B)** HDACs or HATs can act on TFs and interfere with binding to DNA. **(C)** Phosphorylation at some sites of p65 by related enzymes facilitates the acetylation of other sites, thereby promoting transcription of NF-κB. **(D)** Phosphorylation of P65 recruits HATs to promote P65 ubiquitination and block transcription.

CHROMATIN REMODELING AND TFS

Chromatin remodeling is a dynamic modification of chromatin architecture, which opens the access for regulatory transcription machinery proteins and condenses DNA to control gene expression. Remodeling is accomplished in three ways: (1) covalent histone modifications by enzymes such as HATs, HDACs, and HMTs; (2) ATP-dependent chromatin-remodeling

complexes (74), such as the SWI/SNF family (BAF60a, BAF250, and BAF57), the ISWI family, the Mi-2/CHD family, and the INO80 family; and (3) utilization of histone variants (75). Histone modifications loosen or tighten the DNA wrapped around histones, and thereby allow or prevent binding of TFs to the DNA (75). In contrast, ATP-dependent chromatin-remodeling complexes have a common ATPase domain and utilize the energy of ATP hydrolysis to mobilize nucleosomes along DNA. These complexes can expel histones from DNA or facilitate the exchange of histone variants, which modulate DNA accessibility and alter nucleosome structure (76). From these mechanisms, the connection between chromatin remodeling and TFs is probably reflected in the following aspects.

Role of Pioneer Factors in Opening Chromatin

Genes in eukaryotic cells are packaged in chromatin. TFs must reorganize local nucleosome structure and create an open site for successful interaction with genomic regulatory elements during transcription. TFs with this characteristic are termed "pioneer factors" (77). Examples include the forkhead box (FOX) proteins and the glucocorticoid receptor (GR) (78–80). These TFs recruit SWI/SNF and form a complex to open chromatin (81). The resident time of the complex is short, and the opened chromatin state is maintained for a time (82). The site then becomes accessible to other adjacent TFs to bind after dissociation of the complex (82, 83). (**Figure 3A**).

Binding of TFs to Chromatin Remodeling Complexes

TFs cannot induce chromatin remodeling independently during transcriptional regulation, but need to recruit chromatin remodeling complexes. The PRotein Interactions by Structural Matching (PRISM) algorithm shows that GR binds to several subunits of SWI/SNF such as BAF60a, BAF250, and BAF57 (84). C/EBPα and/or C/EBPβ have also been shown to recruit the SWI/SNF complex to target promoters and regulate the differentiation of adipocytes, neutrophil granulocytes, and hepatocytes in mice (85, 86) (**Figure 3B**).

Histone Modification by TFs Induce Chromatin Remodeling

Chromatin remodeling is closely related to modifications of histone N-terminal tails, and these modifications affect chromatin stability (87). During histone modification, histone variants are inserted into nucleosomes that alter their structure and function, which further affect the highly ordered structure of chromatin (88). In addition, activated TFs can mediate the acetylation of histones and evict nucleosomes, which remodel chromatin and induce transcription initiation or elongation *in vitro* (89) (**Figure 3C**).

NON-CODING RNA AND TFS

It is well known that <2% of transcripts in the mammalian genome have a protein-coding function, with the remaining 98% being non-coding RNA (ncRNA) (90). ncRNA are mainly classified into two types: microRNA (miRNA) and long non-coding RNA (lncRNA). Studies have found that the promoter region of miRNA and lncRNA genes can contain different epigenetic modifications and are involved in many biological processes (91–94) through the interaction with TFs. Any abnormality in these transcriptional processes can lead to disease (95).

Interaction Between miRNA and TFs

TFs and miRNA play an important role in gene transcription and post-transcriptional regulation. Studies found that genes regulated by the same TF and miRNA present a significant co-expressed pattern (96, 97). During regulation, TFs bind to the upstream promoter region of the miRNA gene and exert an influence on transcription. In addition, some miRNAs conversely affect translation of TFs (98). Therefore, TFs and miRNA make up a complex regulatory network termed feed-forward loop (96) in stabilizing gene regulation (9). Ten kinds of interactions have been described between TFs, miRNA, and genes (9, 99) (**Figure 4A**).

Interaction Between lncRNA and TFs

LncRNAs can alter the transcription of genes by interacting with TFs, chromatin-modifying complexes, or mRNA (100, 101). The interaction between lncRNA and TFs can be divided into the following categories.

a LncRNAs act as co-factors or inhibitors to regulate the activity of TFs and control NF-κB signaling (102). LncRNA-Cox2 promotes degradation of IkBa in the cytoplasm (102). Knock out of lncRNA-Cox2 leads to reduced expression of NF-κB (102). However, TFs can also affect lncRNA expression (103). The roles of lncRNAs in cancer metabolism were identified as energy stress-induced lncRNA by FoxO transcription factors (104) (**Figure 4B**).

b TFs and lncRNAs form a binary network and combine with genes to form a ternary complex (105, 106). In a recent study, 530 TF-lncRNA pairs were identified in the cell cycle (107). In a trans-acting network, lncRNAs regulate TF-mediated chromatin remodeling and transcription (108). In addition, lncRNA can recruit one TF to the genomic loci of another TF and inhibit gene transcription (109). The suppressed TF, in turn, inhibits the expression of the lncRNA (109). The TF-lncRNA-gene-network shows that 69 genes and lncRNA are controlled by TFs (100) (**Figure 4C**).

c LncRNA can form complexes with TFs to regulate transcription of target genes (110, 111). For example, Lethe, a lncRNA, binds to RelA and prevents it from binding to the NF-κB target promoter, thereby inhibiting transcription of proinflammatory cytokine genes (112). However, binding of lncRNA-p21 to HIF-1a, a key TF that mediates the response to hypoxia, can disrupt the interaction with the Von Hippel-Lindau (VHL) protein

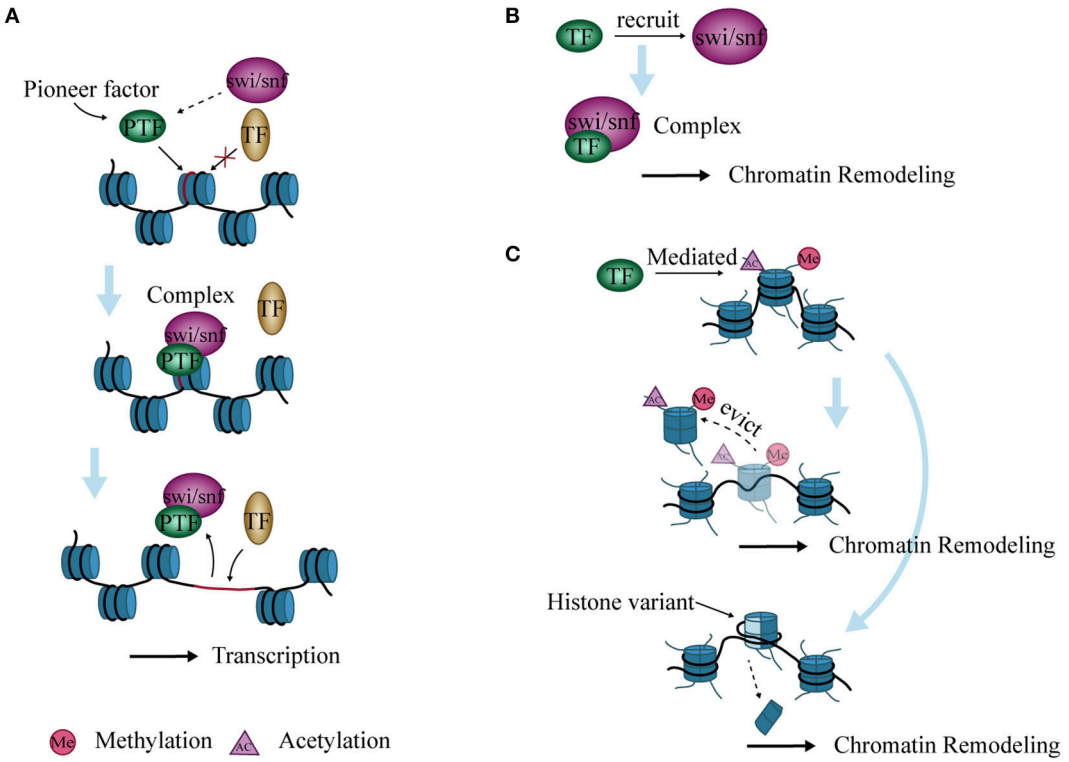

FIGURE 3 | Interaction between chromatin remodeling and transcription factors (TFs). **(A)** Pioneer factors bind to specific DNA sites and form a complex with SWI/SNF to open chromatin. The complex then allows the binding of other TFs, thereby promoting transcription. **(B)** TFs recruit SWI/SNF to form complexes that lead to chromatin remodeling. **(C)** Histone variants are inserted into nucleosomes, or nucleosomes are evicted from DNA after histone modification mediated by TFs, ultimately causing chromatin remodeling.

and cause high levels of HIF-1a expression (113). This is a positive feedback loop between TF and lncRNA (**Figure 4D**).

ROLE OF TFS IN EPIGENETIC REGULATION IN THE DEVELOPMENT OF OBESITY

Role of PPARγ in Obesity

PPARγ is a critical transcriptional regulator of adipogenesis in mammals, is closely related to regulation of lipids and glucose metabolism, and is associated with the control of obesity and related diseases (114, 115). Siersbaek et al. analyzed the PPARγ and C/EBPα binding sites and found that PPARγ and C/EBPα regulate the expression of most genes associated with adipogenesis (116). The BPro12Ala and 6CAC478CAT exon polymorphisms of the PPARγ gene are significantly related to the incidence of severe obesity (117). In the USA, mutations in the PPARγ gene can severely cause certain types of sexual obesity in women (118). Animal studies also provide more direct evidence for research in this area. For example, Japanese researchers reported that, in high-fat diets, mice lacking PPARγ exhibited significant dysplasia of adipocytes, smaller cell morphology, and lower fat content, causing pathological features such as obesity and severe insulin resistance (119). PPARγ not only promotes the proliferation and differentiation of adipocytes but also confers insulin sensitivity to adipocytes (120). An increase in insulin sensitivity can in turn promote the expression of the PPARγ gene in adipose tissue, thereby positively accelerating the differentiation of adipocytes. Both these aspects greatly increase the efficiency of adipocyte synthesis and storage and are important factors for obesity.

In addition, PPARs also play an important role in lipid metabolism. After feeding, carbohydrates and fats are converted into glucose and chylomicrons, respectively, and enter circulation. Most of the glucose is absorbed by the liver. If hepatic glucose storage capacity is reached, the remaining glucose is used for lipogenesis. Studies have shown that rats in the fed state increase the amount of SREBP1, which promotes the conversion of glucose to acetyl-CoA, followed by synthesis of fatty acids. Fatty acids are further converted into triglycerides and very low-density lipoproteins and are stored in the body (120, 121). This may result from the fact that PPARγ is a target gene for SREBP1 (122), which is involved in the production of endogenous PPARγ ligands (possibly fatty acids). SREBP1 stimulates the uptake of glucose and fatty acids, which are subsequently converted to triglycerides (14, 15).

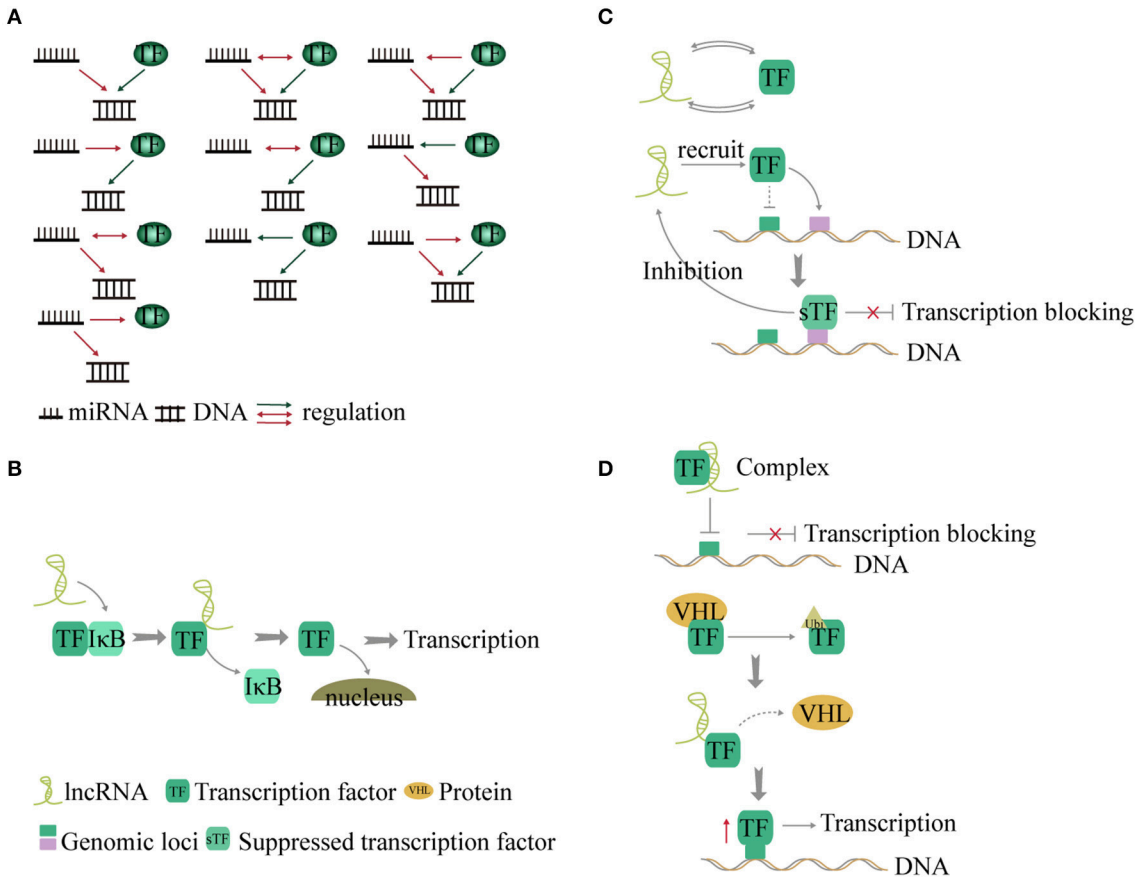

FIGURE 4 | Interaction between non-coding RNA and transcription factors (TFs). **(A)** TF and miRNA present a feed-forward loop pattern. There are 10 modes of interaction between TF, miRNA, and genes. **(B)** LncRNAs act as co-factors or inhibitors to control some transcription factor-related signaling pathways. **(C)** LncRNAs and TFs interact with each other to regulate transcription by forming a binary network. In addition, lncRNA recruits TF to other gene loci and blocks transcription. Conversely the suppressed TF inhibits the expression of the lncRNA. **(D)**. LncRNA forms a complex with TF to suppress gene transcription. Additionally, lncRNA disrupts the interaction between TF and the VHL protein and forms a complex with TF to reduce the levels of TF ubiquitination, thus increasing the expression of TF and promoting gene transcription.

ROLE OF PPARγ IN EPIGENETIC FACTORS IN ADIPOSE TISSUE

DNA Methylation and PPARγ

Studies have shown that DNA methylation regulates the expression of PPARγ. Two studies examined the CpG methylation of the PPARγ gene in 9–year–old overweight children (123, 124), and both confirmed the presence of different levels of methylation. In addition, one group pointed out that the level of CpG methylation is negatively correlated with the expression of PPARγ (124), whereas the other group suggested that PPARγ methylation levels may be related to child body size, with an upward trend with age (123).

Studies on PPARγ in animals have revealed that the PPARγ2 gene promoter is highly methylated in mouse 3T3-L1 pre-adipocytes (125). However, with the differentiation of 3T3-L1 pre-adipocytes, the PPARγ2 promoter is gradually demethylated, and the expression of PPARγ2 mRNA is gradually increased. The DNA methylation inhibitor 5-aza-2′-deoxycytidine interferes with the normal differentiation of 3T3-L1 pre-adipocytes and inhibits lipid accumulation in adipocytes (125). Compared with wild-type mice, methylation levels of the PPARγ2 promoter in visceral adipocytes increase, whereas PPARγ2 mRNA levels decrease in obese diabetic mice (125). This suggests that DNA methylation of the PPARγ promoter can inhibit the expression of the PPARγ gene and is associated with the occurrence of obesity and diabetes. However, no studies mentioning the involvement of DNMT or demethylase in the regulation of PPAR gene during fat differentiation or obesity were found.

Histone Modification and PPARγ

Histone methylation regulates PPARγ gene expression. Several HMTs and HDMs are known to be involved in the regulation of PPARγ expression and lipogenesis (126). Myeloid/lymphoid or mixed-lineage leukemia protein 3 (MLL3) and myeloid/lymphoid or mixed-lineage leukemia protein 4 (MLL4) are major H3K4 methyltransferases in mammalian cells (126). Studies have shown that MLL4 can bind to the PPARγ

locus and increase PPARγ gene expression and lipogenesis. The early adipogenic TF C/EBPβ serves as a pioneer TF and recruits MLL4 to the PPARγ gene loci and induces PPARγ and C/EBPα expression. MLL4 is then recruited to cooperate with other adipogenic TFs for adipocyte gene expression (126). In 3T3-L1 pre-adipocytes and mature adipocytes, the TF tonicity-responsive enhancer binding protein (TonEBP) binds to the PPARγ2 promoter directly, giving rise to H3K9me2 in the PPARγ2 promoter, thereby inhibiting PPARγ2 activity (127). PPARγ2 expression decreases, thereby inhibiting adipogenesis. The binding of TonEBP to the PPARγ2 promoter is associated with blocking C/EBPβ binding to H3K9m2 (127). In addition, PPARγ is also regulated by SET domain family proteins (128). Importantly, SET domain bifurcated 8 (SETD8) is a direct target of PPARγ and is induced during adipocyte differentiation. SETD8 regulates many target genes that depend on PPARγ activation by increasing the monomethylation levels of H4K20 during adipogenesis (128).

Histone acetylation also regulates PPARγ expression. It was found that the epigenetic modification of the PPARγ gene is regulated by C/EBPs and GR *in vitro* in a lipogenic cell model (129). The transient recruitment of GR and C/EBPβ by a complex consisting of MED1 and p300 (HAT) in the enhancer region of PPARγ2 causes a significant increase in the levels of H3K9 acetylation in this region, which enhances the expression of PPARγ2 and becomes a major driver of adipogenesis (130). Analysis at the genome level revealed that, during differentiation of 3T3-L1 cells, H3K9 and H3K27 acetylation at the PPARγ locus increases significantly, and both acetylation at both sites are positively correlated with the expression of the PPARγ gene. However, the roles of H3K9 and H3K27 acetylases in lipogenesis and PPARγ expression have not been determined (131). In contrast to the functions of HATs, HDACs repress PPARγ expression by deacetylating histones (132). Several HDACs are significantly downregulated during adipogenesis (133). SIRT1, a class III NAD-dependent HDAC, is an inhibitor of PPARγ (134). *In vivo*, fasting-induced SIRT1 binds to the PPARγ binding site of a fat-specific gene, enhancing its inhibitory function and thereby blocking adipogenesis (134). Studies have also confirmed that SIRT1 plays a role in the browning of white adipose tissue (135). This may be related to the deacetylation effect of SIRT1 on the regulation of PPARγ, whereas PPARγ promotes the production of brown fat by inducing the transcription-assisted regulator PRDM16 (135).

Chromatin Remodeling and PPARγ

During lipogenesis, the chromatin environment at the PPARγ locus also needs to be correctly remodeled to allow expression of PPARγ target genes. Within a few hours after differentiation of 3T3-L1 preadipocytes, chromatin remodeling occurs in the PPARγ locus leading to an open state (129). This chromatin opening may be related to histone modifications at the PPAR locus and binding of pioneer TFs (127). Studies have shown that remodeling and opening of the PPARγ2 promoter region are dependent on protein kinase A (PKA). Knockout of the PKA gene using shRNA results in decreased chromatin accessibility in the PPARγ2 promoter region (136). In addition, studies have shown that specific binding to and function of the PPARγ promoter in adipocytes requires the participation of G-protein suppressor 2 (GPS2) (137). GPS2 primes a local chromatin environment via inhibition of the ubiquitin ligase RNF8 and stabilization of the H3K9 histone demethylase KDM4A/JMJD2 (137). Moreover, the SWI/SNF chromatin remodeling complex regulates PPARγ2 expression during adipogenesis. PPARγ activity also depends on components of a chromatin remodeling SWI/SNF complex with ATPase BRG1 and BAF60c subunits (138). A dominant mutant of Brg1 inhibits the transdifferentiation of fibroblasts into adipocytes induced by PPARγ, C/EBPα, and C/EBPβ (139). However, it remains unclear how Brg1 is recruited to the PPARγ promoter.

Chromatin remodeling can be regulated by replacement of canonical histones by histone variants. Genome-wide localization studies have shown that the variant H2A.Z, which is mainly involved in the regulation of gene expression, can be preferentially located in the promoter and enhancer regions

TABLE 1 | Regulation of PPARγ by miRNAs during adipogenesis and obesity.

miRNA	Function	Species	Experimental system	References
miR-27a miR-27b	↓adipogenesis	Obese mice	3T3-L1 pre-adipocytes	(145, 146)
		Human	Human multipotent adipose-derived stem cells.	(147)
miR-130 miR-130b	↓adipogenesis	Human	3T3-L1 pre-adipocytes	(148)
		Obese mice	Epididymal adipose tissue	(149)
miR-301a	↓adipogenesis	Obese mice	3T3-L1 pre-adipocytes	(142)
miR-302a	↓adipogenesis	Obese mice	3T3-L1 pre-adipocytes	(150)
miR-548d-5p	↓adipogenesis	Human	Human bone marrow mesenchymal stem cells	(151)
miR-103	↑adipogenesis	Obese mice	3T3-L1 pre-adipocytes	(152)
miR-143	↑adipogenesis	Obese mice	3T3-L1 pre-adipocytes	(152, 153)
miR-200a	↑adipogenesis	Yak	Separate cells from perirenal adipose tissue	(154)
miR-335	↑adipogenesis	Obese mice	3T3-L1 adipocytes	(155)
miR-375	↑adipogenesis	Obese mice	3T3-L1 pre-adipocytes	(156)

↑ *Promotes lipogenesism,* ↓ *Inhibits lipogenesis.*

(140). Studies have shown that, during adipogenesis, the E1A-binding protein p400 complexed with subunit bromo-containing protein 8, the p400/Brd8 complex, influences the expression of PPARγ target genes by inserting the histone variant H2A.Z into the transcriptional regulatory region (141). This is crucial for differentiation of fat tissue.

Non-coding RNA and PPARγ

Non-coding RNA is an important post-transcriptional gene expression regulator that modulates many physiological and pathological processes (142). Multiple obesity-associated miRNAs have been identified in adipose tissue in obese humans, rats, and mice (143, 144). Several of these miRNAs can regulate PPARγ, and are classified into two categories: inhibition and stimulation of PPAR transcription (**Table 1**). Five miRNA can bind to the 3′ UTR of the PPARγ gene mRNA or interact with PPARγ at the cellular level to inhibit PPARγ protein expression and adipocyte differentiation (142, 145–151). In contrast, miR-103, miR-143, miR-200a, miR-335, and miR-375 have a lipogenic effect during adipocyte development resulting from elevated PPARγ expression (152–156). The ectopic expression of miR-103 increases the accumulation of triglycerides in adipocytes and up-regulates the expression of PPARγ2 (152). MiRNA, PPARγ gene, PPARγ, and related transcription factors form a cyclic network that regulates fat tissue formation.

In the process of 3T3-L1 cell differentiation, lncRNA can also affect lipogenesis by affecting PPARγ transcription. It was found that, in 3T3-L1 adipocytes, lncRNA U90926 inhibits the activity of the PPARγ2 promoter, thereby inhibiting PPARγ expression and lipogenesis (157). In contrast, lncRNA NEAT1 plays a regulatory role in alternative splicing events of PPARγ during differentiation of mouse 3T3-L1 cells. Interference with NEAT1 expression up-regulates PPARγ2 expression and promote fat tissue differentiation (158). In addition, overexpression of lncRNA HOTAIR promotes the expression of PPARγ and the differentiation of pre-adipocytes into mature adipocytes, although the mechanism remains unclear (159). The mechanism underlying the role of lncRNA in lipogenesis and obesity still needs to be further investigated.

PARTICIPATION OF PPARS IN EPIGENETIC MODULATION OF LIPID METABOLISM

Fatty acids can be oxidized to CO_2 and H_2O in the presence of oxygen, releasing large amounts of energy and becoming one of the main energy sources for the body. Most of the fatty acids in the human body are exogenous fatty acids derived from foods and can be utilized through transformation and processing. The body can also convert sugars and proteins into endogenous fatty acids and triglycerides to store energy. Fatty acid synthase catalyzes the first step in de novo lipogenesis.

PPARs are ligand-activated receptors that heterodimerize with the retinoid X receptor (RXR), bind reactive elements in target genes, and induce transcription. PPARγ can modulate fatty acid metabolism, promote differentiation of adipocytes, and regulate lipid storage through transcription of a series of lipid-related proteins in the posterior segment (160). In animals, it has been demonstrated that fatty acids act as ligands for PPARs and activate the expression of genes involved in fatty acid metabolisms such as the transmembrane proteins CD36, lipoprotein lipase, fatty acid-binding protein 4, and long-chain acyl-CoA synthetase 1 (161). Studies have found that multiple miRNAs are involved in the regulation of fatty acids. MiR-122 is the first miRNA confirmed to be involved in lipid regulation and is the most abundant miRNA identified in liver tissue (162). MiR-122 plays a prominent role in maintaining the phenotype of liver cells and fatty acid metabolism (162). In mice, inhibition of miR-122 expression can lead to a sustained reduction in plasma cholesterol levels, increased hepatic fatty acid oxidation, and decreased liver fatty acid and cholesterol synthesis rates. Ultimately, high-density lipoprotein and apolipoprotein A1 levels increase, and low-density lipoprotein and apolipoprotein B levels are reduced (163). In addition, inhibition of miR-205-5p expression in genetically improved farmed tilapia liver can increase fatty acid synthase and PPARα mRNA levels and thus regulate hepatic lipid metabolism (164). Increased PPARα levels have been shown to increase liver fatty acid oxidation and reduce circulating triglyceride levels to regulate rodent obesity (165). In addition to miRNA regulation, studies in rodents have shown that decreased PPARα promoter methylation contributes to enhanced expression of carnitine palmitoyl transferase-1, a regulatory enzyme in fatty acid oxidation, and decreased expression of fatty acid synthase (166). However, no PPAR-mediated lipid metabolism modulation by mechanisms such as histone modification and chromatin remodeling has been described. Another important transcription factor, SREBP1, also plays an important role in lipid metabolism. NAD-dependent deacetylase sirtuin-1(SIRT1) can remove acetyl groups from lysine at positions 289 and 309 of SREBP-1c, inhibiting SREBP-1c activity, decreasing its stability, and reducing lipid production (167).

POSSIBLE THERAPEUTIC DIRECTION IN THE FUTURE

PPAR is a pivotal regulator of fat formation and metabolism. Knocking out PPARγ in adipose tissue of mice can prevent high-fat diet-induced obesity and insulin resistance (168). Because of its complex and diverse biological functions, it has grown up to be a therapeutic target for obesity-related diseases (114). For example, PPARγ agonists have been applied to treat hyperlipidemia, hyperglycemia, diabetes (114, 169). However, these drugs, such as Thiazolidinedione (TZD), have reduced insulin resistance but also increased lipid accumulation in skeletal muscle by promoting adipocyte hypertrophy and hyperplasia (170). Therefore, long-term use of PPARγ agonists may trigger unexpected effects on systemic metabolism, including insulin sensitivity. Other more appropriate treatments are needed. With the development of epigenetics, epigenetic markers have become a useful tool to assess the risk of obesity and metabolic disorders (171). Perhaps, in the future, the epigenetic modification of PPARγ may be intervened to

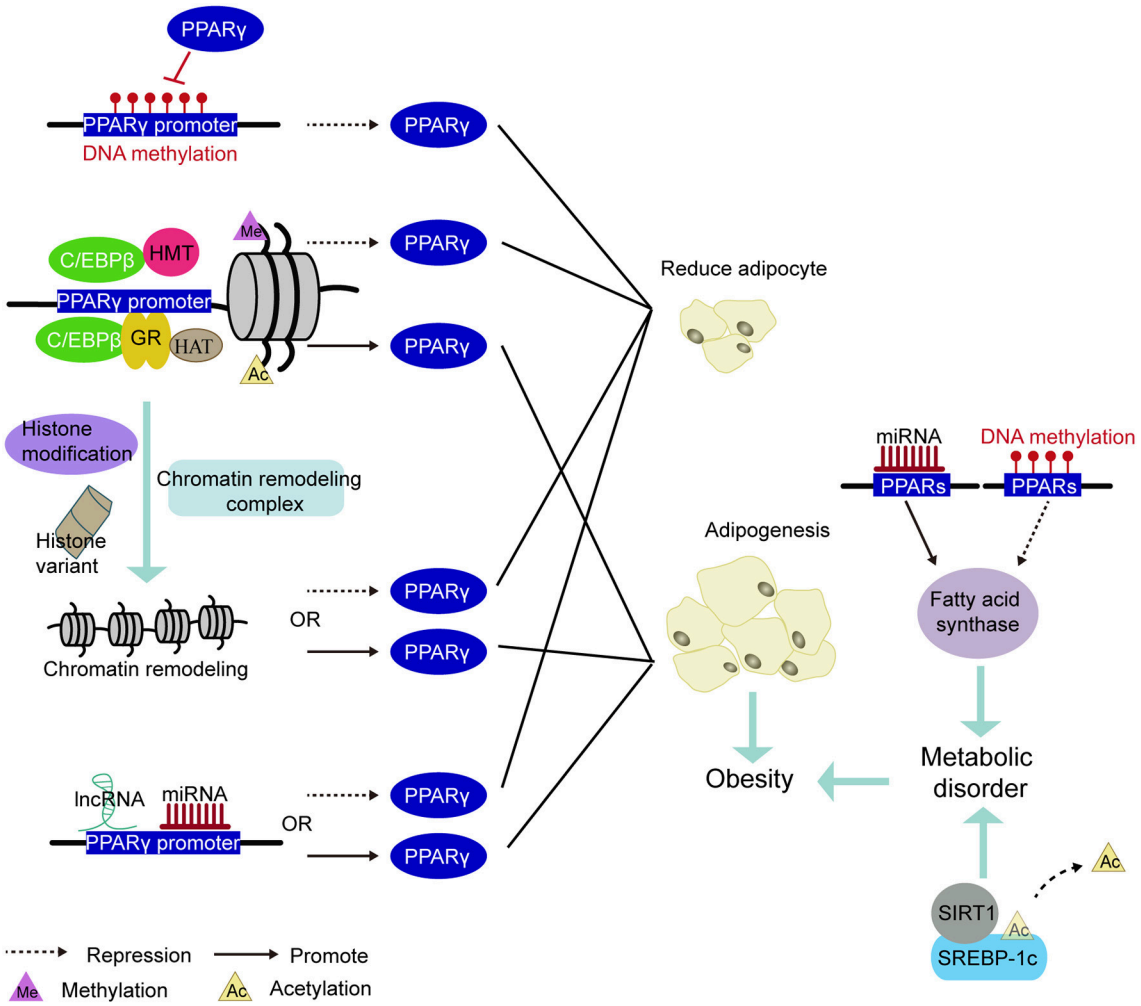

FIGURE 5 | PPARs be involved in the mechanism of epigenetic modification leading to obesity. Methylation of the PPARγ promoter region inhibited PPARγ expression to decrease adipogenesis. The pioneering transcription factor (e.g., C/EBPβ or GR) recruit histone acetyltransferases or histone methyltransferases in the vicinity of the PPARγ promoter and cause histone methylation or acetylation, thereby regulating PPARγ expression. Under various factors such as histone modifications, histone variants, and chromatin remodeling complexes, the chromatin remodeling in the PPARγ promoter induces changes in the expression of PPARγ. The miRNA and lncRNA acting on the PPARγ promoter region also govern the expression of PPARγ. Inhibited expression of PPARγ reduces the production of adiposity. Conversely, promoting the expression of PPARγ increases in adipogenesis and leads to obesity. In addition, methylation and miRNA modification of the PPARs promoter region regulates the expression of fatty acid synthase and participate in lipid metabolism. Deacetylation of the transcription factor SREBP-1c is some other important way in regulating lipid metabolism. Disorders of lipid metabolism can also induce obesity.

regulate the expression of genes involved in lipogenesis without compromising metabolism *in vivo*, thereby reducing the occurrence of obesity and obesity-related diseases (**Figure 5**).

CONCLUSION

A variety of physiological, biochemical, genetic, and behavioral factors can cause obesity. To control the occurrence of this disease, it is necessary to reasonably control the diet and perform a moderate amount of exercise so as to reduce the storage of residual energy within the body. In addition, it is also necessary to control the excessive proliferation and differentiation of adipocytes. Knowledge of epigenetic modifications provides novel insights into the pathogenesis of obesity. Owing to the importance of PPARγ in lipogenesis and lipid metabolism in humans and other animals, the epigenetic mechanisms underlying transcriptional regulation of PPARγ will remain a research focus in the field of lipid biology and medicine and may result in improved treatments for obesity. Although much evidence for epigenetic regulation has been reported in recent years, few studies have shed light on the role of epigenetic modifications, transcription factors, and upstream and downstream mechanisms involved in obesity. In the future, a better understanding of the interrelationship between regulation of obesity-related transcription factors and epigenetics will be needed to explore effective therapeutic

methods for the treatment of obesity using epigenetic modifications.

AUTHOR CONTRIBUTIONS

QH and CM prepared the first draft and wrote the final version of the manuscript. LC and DL were involved in literature searches. FL and RC critically revised the manuscript and gave constructive opinions on articles.

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