VEGETARIAN DIETARY PATTERNS IN THE PREVENTION AND TREATMENT OF DISEASE

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VEGETARIAN DIETARY PATTERNS IN THE PREVENTION AND TREATMENT OF DISEASE

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Editorial: Vegetarian Dietary Patterns in the Prevention and Treatment of Disease

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Editorial on the Research Topic

Vegetarian Dietary Patterns in the Prevention and Treatment of Disease

Dietary patterns that emphasize the consumption of vegetables, fruits, whole grains and legumes, are recognized for their health-promoting properties (1). Such diets encompass vegetarian and vegan types, while extending to diverse, plant-predominant dietary patterns (2). Such diets are typically rich in fiber, antioxidants and phytochemicals, but much lower in saturated fat, added sugar, animal protein, and sodium compared to more conventional dietary patterns (3). The current 2015-2020 *Dietary Guidelines for Americans* recommend a vegetarian dietary pattern as one of three healthy dietary patterns, along with the Mediterranean and healthy U.S. style dietary patterns (4). Minimally processed plant foods are emphasized in all three, while only the vegetarian diet excludes meat entirely.

When examining factors that do drive overall, objective measures of diet quality, there are two salient influences: shifts in animal vs. plant food, and shifts from highly processed to unprocessed/minimally processed foods (5). Other things being equal, in modern countries where overt protein or calorie malnutrition is a rare concern, shifts to plant foods correlate consistently and robustly with higher overall diet quality. Other things being equal in nearly any dietary context, shifts from highly processed to less processed foods do the same. Discussions here presuppose the above influences and pertain to high-quality vegetarian diets.

Cardiovascular disease is the leading global cause of mortality, being responsible for 46% of non-communicable disease deaths (6). It has been estimated that about 85.6 million Americans are living with some form of CVD, the prevalence of which continues to rise (7). This increase has been linked to lifestyle factors, particularly low overall diet quality and lack of exercise (8). The systematic review and meta-analysis of prospective cohort studies by Glenn et al. showed that self-reported vegetarian dietary pattern was associated with a 22% lower cardiovascular mortality and a 28% lower incidence of cardiovascular disease, which is an effect comparable to a combination of the most current pharmacotherapies (9). This implies that in the study population, vegetarianism involves not only the avoidance of meat, but a general elevation of diet quality. The benefits associated with high-quality vegetarian diets come without the unwanted side effects and for a much lower market price than standard pharmacotherapy aimed at comparable risk reduction. Furthermore, plant-based eating may also help reduce society's health care costs, such as hospital admissions and doctor's bills, as well as increasing the number of healthy years filled with productivity. Thus, plant-based diets could save billions of dollars in health care costs (7, 10).

It is also important to note that environmental and climate effects of food at scale are relevant to health outcomes- both directly and indirectly. Here, too, there are important implications of vegetarianism. Shifts from animal to plant-food-predominant diets are key recommendations at the interface of human and planetary health (11).

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Allen et al. described a case study of a 54-year old woman who not only reversed her diabetes, but also her systolic dysfunction, increasing her ejection fraction from 21 to 55%, after following a plant-based diet for <6 months. Plant-based diets improve the risk factors for heart failure, but also have direct benefits on heart metabolism and function (12, 13). Given the severe prognosis of heart failure, even limited evidence for benefits from plant-based diets warrants careful attention.

What role does fruit play in weight management? Guyenet performed a systematic review of randomized controlled studies and demonstrated that whole, fresh fruit promotes weight maintenance or modest weight loss and may be therefore used as a part of a healthy diet in the prevention and treatment of excess body weight and adiposity. These findings support the current recommendations by the U.S. Department of Agriculture (4) to increase fruit consumption, which may have a positive impact on public health and obesity control.

Plant-based diets have been shown to be beneficial in a number of different populations. Davis et al. have conducted a randomized controlled trial to test the effectiveness of a plant-based diet and moderate exercise in people with type 2 diabetes in the Republic of the Marshall Islands. The study is still ongoing and it will be exciting to see if diabetes can be significantly improved or even reversed in this part of the world. Singh et al. have shown that plant-based eating is associated with BMI within the recommended range in Hispanic/Latino Seventh-day Adventists. This finding has major implications because currently, diabetes rates are 60% higher among Hispanics/Latinos compared with non-Hispanic Whites (14). Finally, our children and adolescents are the future of our nation. What is the quality of their diet? Would a vegetarian diet promote their health as much as in adults? Are teenagers able to maintain a healthy vegetarian diet? Segovia-Siapco et al. demonstrated that vegetarian adolescents had a more favorable dietary intake profile than non-vegetarians, eating more vegetables, fruits, nuts, legumes and soy products, and much less saturated fat. Such dietary habits likely translate into long-term risk reduction, and prevention of chronic disease.

Alwarith et al. pulled together a review paper on the potential use of plant-based diets for rheumatoid arthritis. This chronic autoimmune disease affects about 1% of the world's population and leads to inflammation, pain, and eventually permanent joint damage (15). The paper presents encouraging evidence that plant-based diets may play an important role in the management and/or remission of rheumatoid arthritis, although some additional trigger foods might need to be eliminated from the diet. The improvements in response to a plant-based diet may be at least partly explained by the changes in gut microbiome.

Tomova et al. described the benefits of vegetarian and vegan diets on gut microbiota. Plant-based diets seem to promote a more diverse and stable gut ecosystem and increase the short-chain fatty acid (SCFA) producing bacteria. The SCFAs improve immune function, blood-brain barrier integrity, increase insulin sensitivity and have cardioprotective effects.

The health benefits of plant-predominant diets have been observed throughout the whole spectrum, from strict vegan diets all the way to much more liberal lacto-ovo-vegetarian and even semi-vegetarian diets. Due to the design of pertinent studies, it is not always possible to distinguish and quantify the health benefits of any of these dietary patterns relative to another at comparable levels of overall quality.

The range of plant-predominant dietary patterns may be recommended for health promotion without population-specific precautions appended. According to the Academy of Nutrition and Dietetics, vegetarian, and vegan diets are appropriate for every stage of life including pregnancy, infancy, and childhood, as well as for athletes. Reliable sources of vitamin B-12 need to be secured (16); these can be food sources, or supplements.

Pesticides and herbicides are still widely used in food plant cultivation. Most pesticides are lipophilic and therefore accumulate in fat, particularly in animals feeding upon these foods for prolonged periods of time (17). Thus, the risks of exposure to these compounds at harmful levels is greater with animal than with plant food consumption. The bioaccumulation of pesticides can have harmful effects on animals, as well as people who consume meat and dairy. Organic foods contain lower levels of pesticides, and also higher levels of certain nutrients (18), but may not be affordable or available for everyone.

Regarding costs, fresh produce is often expensive relative to other available food choices. However, some staple foods in plant-predominant diets, such as rice and other cooking grains, beans, cabbage, and potatoes, are much less expensive than meat, dairy, and most processed foods. Water, of course, is either freely available or inexpensive relative to sugar-sweetened beverages. Beans and lentils are highly nutritious substitutes for animal-food protein; are considerably less expensive; and offer environmental impact benefits as well. Thus, there are many opportunities to adopt a more plant-predominant dietary pattern, and improve overall diet quality, unimpeded by barriers of cost.

Arguments for diverse health benefits of high-quality vegetarian diets are thus varied and robust. They are, as well, timely- as meat consumption is a major consideration in an array of threats to planetary health (19, 20). Loss of rainforest in the Amazon, for instance, is directly attributable to the global appetite for meat. The assembly of papers collected here highlights direct health benefits of well-practiced vegetarianism at a time, and in a context, when the indirect benefits- via effects on aquifers, ecosystems, fires, floods, and droughts- may be even more salient.

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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Vegetables: New Zealand Children Are Not Eating Enough

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We know that eating a variety of vegetables every day is associated with favorable health across the lifecourse. Internationally, food-based dietary guidelines encourage the consumption of a variety of vegetables and fruit but globally,people are not eating enough vegetables to meet the three-or-more-a-day guideline. Vegetables are good sources of vitamins and minerals, fiber, and many bioactive compounds that promote health and provide energy. They also help reduce hidden hunger (micronutrient deficiencies) and support the healthy growth and development of children. New Zealand is a world leader in the production of diverse nutrients and foods yet poverty and other environmental barriers mean only one in two children eats three-or-more servings of vegetables a day. Price and availability are limiting factors. The proliferation of community, school and home vegetable gardens and vegetable cooperatives may improve access. On a macro level, upstream policies such as a "living wage," affordable housing, and land-use planning are required. International dietary solutions include an agricultural shift to intensified horticulture with a focus on vegetables. The consumption of more plant-based foods including vegetables would reduce green-house gases, reduce land clearing, and help prevent diet-related disease if consumed daily across the lifecourse.

Keywords: vegetables, lifecourse health, supply, cost, children, actions

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INTRODUCTION

Lifecourse Health—Plenty of Vegetables Every Day of Life

Recommendations that vegetables and fruit should be consumed every day, from the time solid food starts to complement breast milk, is based on convincing evidence of favorable health, growth, and development associated with their intake (1). For children, this includes the prevention of cardio-metabolic disease (2) and obesity (3). Vegetables are one important part of a lifelong dietary pattern that is lower in energy density and higher in nutrient density. Specifically, vegetables contribute essential fiber, vitamins and minerals and many health-promoting bioactive compounds. Replacing energy-dense foods with more water-rich vegetables (4) not only lowers energy density but increases the volume of food ingested and satiety while improving overall dietary pattern. By volume it is recommended that a variety of colored vegetables make up more than one quarter of all food consumed each day and this is the basis for the MyPlate communication tool (5).

Food-Based Dietary Guidelines

Globally, food-based dietary guidelines (FBDGs, rather than nutrient-based) encourage the consumption of a variety of vegetables and fruit (6) as part of a dietary pattern that provides the required nutrients to the general public. The focus of FBDGs are on food groups rather than

TABLE 1 | Food-based dietary guidelines relating to vegetables and fruit from selected countries.

Country	Message
Sweden	More vegetables and fruit - Eat lots of fruit, vegetables and berries! Ideally, choose high fiber vegs such as root vegetables, cabbage, cauliflower, broccoli, beans and onions.
South Africa	Eat plenty of vegetables and fruit every day.
Mexico	Include vegetables and fresh fruits in each meal. Choose them with peel and in season.
United States	A variety of vegetables from all of the subgroups-dark green, red and orange, legumes (beans and peas), starchy, and others
New Zealand	Eat plenty of vegetables and fruits.
Australia	Plenty of vegetables, including different types and colors, and legumes/beans
Iran	Eat raw and cooked vegetables every day at main meals and snacks
India	Eat plenty of vegetables and fruits
China	Consume plenty of vegetables, milk, and soybeans

Food-based dietary guidelines reported by the FAO (6)

nutrients and they emphasize the need to select a variety of foods within each food group. Foods are grouped by their nutrient profile. For example, there is a focus on foods grouped for their protein content such as fish, lean meat, soy products, eggs, nuts, seeds, and legumes. Another group of mainly carbohydrate foods such as rice, wheat, maize and potatoes make up a large portion of the global energy supply. For some populations, foods that contain calcium such as dairy and bones in fish are a focus. Finally, the food group with arguably the biggest variety, vegetables and fruit, receives much attention in guidelines (**Table 1**) and universal messages which emphasize eating more. Note that the term used currently is vegetables and fruit rather than fruit and vegetables to indicate the importance of vegetables and, now, the recognition that the most nutrient-dense part of a vegetable or fruit is next to the skin (7).

Why Are Vegetables Healthy?

Vegetables are healthy if a variety are consumed as together they provide diverse combinations of essential nutrients and dietary fiber. The dietary diversity score devised by the FAO (8) is one proxy measure of nutrient adequacy and identifies 16 groups of foods. Four of these groups describe vegetable diversity and the importance of each group for diverse and essential nutrients. These four groups are 1. White tubers and roots which are good sources of energy and fiber; 2. "vitamin A rich vegetables and tubers: pumpkin, carrot, squash, or sweet potato that are orange inside, and other locally available vitamin A rich vegetables (e.g., red sweet pepper)"; 3. "dark green leafy vegetables, including wild forms and locally available, vitamin A rich leaves such as amaranth, cassava leaves, kale and spinach" which are also good sources of folate; 4. "other vegetables (e.g., tomato, onion, eggplant) and other locally available vegetables." A higher dietary diversity score is associated with improved growth in children (9) and micronutrient status (10). Vegetables are good sources of the essential nutrients, dietary fiber, vitamins, and minerals. Dietary fiber, broadly described as carbohydrates that are not digested in the small intestine, has benefits to health associated with modifying the rate of transit through the bowel and delaying the absorption of nutrients such as glucose, binding, and excretion of bile acids, fermentation in the large intestine, and the production of butyrate and adding to stool bulk and improving laxation (11). Vegetables are good sources of viscous and non-viscous fibers and diets higher in fiber has been linked to cancer prevention, weight management, lower risk of heart disease and diabetes management and prevention (12).

In addition, beneficial health effects are derived from bioactive compounds such as phenolics, flavonols, flavonoids, capsaicinoids and carotenoids which are found for example in spinach, broccoli, lettuce, onions, pepper, and tomatoes.

There is a renewed focus on hidden hunger which describes micronutrient deficiencies (13) which may be present with obesity, another form of malnutrition. In children this will impact on growth, and development. Whole vegetables and fruits not only enhance consumers' bioactive stores (14) but also improve micronutrient status. Thus, there is a synergistic impact on health derived from the combination of vitamins, minerals, flavonoids, phenolics, pigments, peptides polysaccharides, and fibre (soluble and insoluble) because of the consumption of whole or minimally processed vegetables. Prebiotics such as the oligosaccharides inulin and fructo-oligosaccharides (15) found in vegetables and fruits have been shown to stimulate growth of probiotic bacteria (bifidobacteria and lactic acid bacteria) in the colon which favorably enhances the function of the gastrointestinal and immune systems (16). This unique property of prebiotics is attributed to their ability to resist digestion in the small intestine, eventually undergoing fermentation in the colon. In addition, prebiotics have been shown to increase the absorption of calcium and magnesium, influence blood glucose levels, and improve plasma lipids (16) and laxation.

The need to promote the consumption of vegetables and other plant-based foods compared to animal-based products, such as beef, is warranted due to the more favorable impact of plant rather than animal agriculture on the planet (17). For example, to raise cattle, large quantities of water are required both to produce the grass or feed for the cattle and for the cattle to drink. Cattle emit green-house gases and may pollute the water system. In contrast, vegetable cultivation requires a relatively small amount of water, could be done without pesticides, absorbs carbon dioxide from the atmosphere, and produces oxygen. An intensification of vegetable production is a sustainable way to support populations to meet dietary guidelines, reduce food insecurity, improve health and provide some amelioration to adverse climate change (18).

Consumption—Are Children Eating Their Vegetables?

Worldwide the consumption of fruit and vegetables is low (19) and dependent on household income. Miller and colleagues calculated, with data from 18 countries and 143,305 participants, that in low-income countries three servings of vegetables and two servings of fruit each day for each household member would cost 52% of the household income while in upper-middle income countries the cost was 2%. Vegetables and fruits were more expensive for rural households and consumption decreased as cost increased.

NZ as a Case Study

New Zealand is a world leader in the production of diverse nutrients and foods (20). In New Zealand from 2012 to 2017 the hectares devoted to growing onions increased by 5% but potatoes, buttercup/squash, peas and sweetcorn reduced by 18, 15, 40, and 17%. The number of dairy cattle increased 1%. Currently, 27% of New Zealand children are living in income poverty and 12% of children are living in material hardship which means among other factors reduced access to vegetables and fruit and/or going to school hungry. Food security which includes having time, money and resources to access and prepare food is a substantial and complex problem. From a study that measured the relative change in price of healthier and less healthy foods over 10 years in New Zealand, the authors reported that the price of wholesome healthy foods and minimally processed foods were cheaper in summer compared to less healthy foods and processed foods (21). At the same time the vegetable price index rose from 700 to 900 between 2006 and 2018- an increase of 35%; fruit rose 60% in the same time (22). In the same period, bread and cereals rose 19% and overall the food price index rose 33%.

Over the last 16 years only slightly more than half the children in New Zealand are consuming 3 or more serves of vegetables each day (**Table 2**). At the same time, more than half are consuming sugary drinks and fast foods at least once a week (24). Some groups, such as Pacific Island children, are eating less vegetables than the general population of children. In New Zealand, the most commonly consumed vegetables are potato, potato fries, kumara (sweet potato), carrots, broccoli, peas, lettuce, cauliflower, cabbage, tomatoes, and corn(23). Onions are frequently added to recipes (30). Overall, approximately 25% of the daily dietary fiber intake (median 19g/day) is derived from vegetables (23).

Children depend on their parents and caregivers for food and in low income families there is a tendency to buy more energy dense food, to get the most calories for the money, rather than nutrient dense foods such as vegetables and fruit (31). Food, especially healthier food, is the first essential that low income families compromise on in times of hardship, exacerbating existing nutritional deficiencies resulting from general lack of money (32). As children grow, the overall volume of food intake increases but there is a fall in the quantity of fruit and vegetables and an increase in other foods such as—energy-dense and low cost breakfast cereal and/or convenience foods. We demonstrated this in the longitudinal Pacific Islands Families study between child ages 4 and 6 years (27). In New Zealand, for families living in more highly deprived areas increases in fruits and vegetable prices especially around their off-season compel them to substitute the purchase of healthier whole fruit and vegetables with cheap energy-dense nutrient-poor products. The weight of vegetables required each week for a family of 6 is 12.6 kg (3 serves × 100g × 7 days × 6 people) and provides one fifth to one third of the energy that uncooked rice or pasta which in addition are more robust to transport and have a longer storage time.

TABLE 2 | Proportions of New Zealand children meeting the vegetable guideline of three or more serves of vegetables a day.

Year	Age, y	% meeting vegetable guideline	References
NATIONAL SURVEYS			
2002	5-14	57%	(23)
2011-2014	0 to 14	57%	(24)
2014-2017	0 to 14	53%	(24)
METFORMIN IN GESTATIONAL DIABETES FOLLOW UP STUDY			
2008	2	76 % (n = 147)	(25)
2014	7 to 9	37% (n = 99)	Unpublished data
PACIFIC ISLANDS FAMILIES STUDY			
2004	4	35% (n = 739)	(26)
2006	6	35% (n = 646)	(27)
2009	9.4	49% (n = 976)*	(28)
2014	14	32% (n = 931)	Unpublished data

NB, Adequate vegetable intake is defined for children aged 2–4 years as eating at least two servings of vegetables each day and for children aged 5–14 years as eating at least three servings of vegetables each day (29)—all surveys were food frequency questionnaires except for 2009 which was a dietary habits questionnaire. CNS Children's Nutrition Survey; MIGTOFU Metformin in gestational diabetes: the follow-up study; PIF Pacific Islands Families study.*

Health Promotion and the Way Forward

Messages and actions concerning vegetable consumption within New Zealand and globally are consistent but the barriers are persistent. In addition to cost, the perishability and therefore waste of most fresh whole fruits and vegetables discourages purchase. Frozen and canned vegetables may be a better option and should be included more explicitly in health promotion messages. There are other food processing techniques such as chilling, modified atmospheres, edible coatings that enhance the shelf-life of whole vegetables (33) but they add cost and the addition of packaging presents other problems. Drying and freeze drying are also options but require controlled storage conditions.

Locally grown vegetables are the ideal and every city should have dedicated land for market gardens. In addition, community, school and home vegetable gardens and cooperatives are proliferating in New Zealand. New Zealand is fortunate in that it has a temperate climate which allows a continuous source of vegetables throughout the year. In secondary schools (school years 9 to 13), it has been shown that children attending a school with a garden had a lower body mass index and also reported less fast food consumption (34).

More collaboration between industry, retail, government, and not-for-profit organizations promoting public health is required (35). In addition, town planning, land use, reduction in price and increase in availability of vegetables and equitable and culturally targeted actions to improve vegetable intake for children are required. Further, fruits and vegetables could be targeted separately rather than together, and vegetables could be included in cooking education.

To improve conditions on a micro level, upstream policies such as a "living wage" (36), affordable housing, and land-use planning are required. Global dietary solutions, including an agricultural shift to producing more and at the same time consuming more vegetables and plant-based foods throughout the lifecourse, would reduce green-house gases, reduce land clearing, and help prevent diet-related disease. This is a global challenge.

AUTHOR CONTRIBUTIONS

ER led the conception and writing of the manuscript, SJ-M, FS, and IA contributed and critically revised the manuscript. All authors approved the final manuscript.

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Plant-Based Diets Are Associated With Lower Adiposity Levels Among Hispanic/Latino Adults in the Adventist Multi-Ethnic Nutrition (AMEN) Study

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Background: The Hispanic/Latino population in the US is experiencing high rates of obesity and cardio-metabolic disease that may be attributable to a nutrition transition away from traditional diets emphasizing whole plant foods. In the US, plant-based diets have been shown to be effective in preventing and controlling obesity and cardio-metabolic disease in large samples of primarily non-Hispanic subjects. Studying this association in US Hispanic/Latinos could inform culturally tailored interventions.

Objective: To examine whether the plant-based diet pattern that is frequently followed by Hispanic/Latino Seventh-day Adventists is associated with lower levels of adiposity and adiposity-related biomarkers.

Methods: The Adventist Multiethnic Nutrition Study (AMEN) enrolled 74 Seventh-day Adventists from five Hispanic/Latino churches within a 20 mile radius of Loma Linda, CA into a cross-sectional study of diet (24 h recalls, surveys) and health (anthropometrics and biomarkers).

Results: Vegetarian diet patterns (Vegan, Lacto-ovo vegetarian, Pesco-vegetarian) were associated with significantly lower BMI (24.5 kg/m^2 vs. 27.9 kg/m^2, $p = 0.006$), waist circumference (34.8 in vs. 37.5 in, $p = 0.01$), and fat mass (18.3 kg vs. 23.9 kg, $p = 0.007$), as compared to non-vegetarians. Adiposity was positively associated with pro-inflammatory cytokines (Interleukin-6) in this sample, but adjusting for this effect did not alter the associations with vegetarian diet.

Conclusions: Plant-based eating as practiced by US-based Hispanic/Latino Seventh-day Adventists is associated with BMI in the recommended range. Further work is needed to characterize this type of diet for use in obesity-related interventions among Hispanic/Latinos in the US.

Keywords: Hispanic/Latino, plant-based diet, vegetarian, obesity, Seventh-day Adventist

BACKGROUND

The rates of obesity and diabetes have sharply increased in the last decade among Hispanics/Latinos in North America (1–4). Currently, diabetes is the fifth leading cause of death in Hispanics/Latinos, and the trend represents a health disparity whereby rates are 60% higher among Hispanics/Latinos as compared to non-Hispanic Whites and increasing faster than in other major ethnic groups (3, 5). In a large sample of Hispanic/Latino adults in four regions of United States (n = 16,400), the Hispanic Community Health Study/Study of Latinos found that about 1 in 3 individuals had pre-diabetes, and that only about half of individuals with type 2 diabetes (T2D) had it under control (6).

One potential contributor to this burden of obesity and diabetes is the occurrence of a "nutrition transition" occurring in most people of Mexican origin but accelerated in US Hispanic/Latinos as they acculturate to consuming higher intake of meats and processed foods, and lower intake of whole plant foods commonly found in the traditional diet (i.e., squash, maize, beans) (7–9). For example, the analysis of a nationally representative sample of US Mexican adults found higher rates of overweight/obesity in those who had "transitioned" from traditional eating patterns emphasizing maize to patterns emphasizing red meat, processed meat, and processed foods (10–12). These emerging data support that a plant-based dietary intervention could be effective in primary and secondary prevention of obesity and obesity-related diabetes in the Hispanic/Latino population.

Overall, in a systematic review of 15 intervention studies (17 intervention groups among them) in multi-ethnic samples, Barnard et al. has shown that interventions with the vegetarian diet contributed to an average of more than 3 kg of weight loss (13). For diabetics, a similar systematic review of vegetarian diet interventions indicated significant improvement in glycemic control (14).

A recent pilot study at Loma Linda University has shown that a 5-week plant-based, culturally-tailored diet intervention implemented through community clinics and a church significantly improved hemoglobin A1C levels over a 6 month follow-up in 32 Latino diabetics living in a medically underserved area. At Loma Linda University, more than 60 years of NIH-funded prospective cohort studies [1960 Adventist Mortality Study (15), 1976 Adventist Health Study-1 (16), 2002 Adventist Health Study-2 (17)] have shown that the vegetarian diet was associated with lower risk of weight gain (18, 19), stroke (20), and diabetes (21, 22), and on this causal pathway, a longer life expectancy. These cohorts enrolled Seventh-day Adventists (most recently a nationwide cohort of 96,000 multi-ethnic adults in AHS-2) who provide a unique insight into diet since, due to faith-based recommendations, about 50% are vegetarian, and virtually all avoid smoking and alcohol (23). Faith-based counsels on diet also encourage the consumption of specific plant foods (i.e., legumes, nuts) in place of animal products (19). Hypotheses generated from these cohorts have been tested in landmark intervention trials.

The *A*dventist *M*ulti-*e*thnic *N*utrition (AMEN) Study is a new pilot study from Loma Linda University involving Hispanic/Latino Seventh-day Adventist adults for the purpose of studying the association between their cultural tailoring of a plant-based diet and selected health outcomes. To date, we have observed beneficial associations between health outcomes and culturally-tailored plant-based diet choices in Black Adventists (24, 25) and in Asian Adventists (20), respectively. The aim of this report is to test the hypothesis that the culturally-tailored plant-based dietary pattern practiced by Hispanic/Latino Seventh-day Adventists is associated with lower levels of adiposity and adiposity-related biomarkers. Findings from this work can potentially inform the cultural tailoring of plant-based diet choices for dietary interventions in the broader population of US Hispanic/Latinos.

METHODS

Study Population

The AMEN study is a cross-sectional investigation of diet and cardio-metabolic markers conducted during 2013–2016 in churches within a 20 mile radius of Loma Linda University. Our target congregations were predominantly of Hispanic/Latino background. The inclusion criteria included: (1) age 18 years or older, (2) baptized into the Seventh-day Adventist church, (3) self-identify as Hispanic, and (4) no self-report of dementia, pregnancy, or breastfeeding.

Recruitment efforts were initiated by assembling a community advisory board of influential individuals from the Hispanic and Asian Adventist churches in Southern California. Based on the advice of this board, we selected five Hispanic congregations. An additional criterion was that the congregations be meeting in facilities that could accommodate a weekend health clinic. Recruitment from these congregations was done by: (1) linking recruitment to informative health and lifestyle presentations done by Loma Linda University lifestyle medicine faculty, (2) linking recruitment to health and wellness clinics done on the church premises over a weekend, and (3) notices on church bulletin boards, social media, and through e-mail campaigns.

Through these efforts, we recruited 141 subjects from the five congregations that had been invited to participate, of whom 101 subjects were eligible for enrollment. Due to missing data for pertinent diet and anthropometric variables, our analytic sample consisted of 74 subjects. All data collection forms were translated into English and Spanish, and a bi-lingual translator was available for assistance.

Outcome Measures

Eligible subjects were enrolled if they attended a health assessment clinic that occurred either as part of a health fair at their church or by appointment at Loma Linda University. A 12 h fasting blood sample drawn by a certified phlebotomist and first void urine sample were collected from each participant. For

Abbreviations: AMEN, Adventist Multi-*e*thnic Nutrition Study; US, United States; T2D, Type 2 Diabetes; HbA1c, Hemoglobin A1c; WC, Waist circumference; BMI, Body mass index; BP, blood pressure; AHS-2, Adventist Health Study 2; Veg, Vegetarian; Non-veg, Non-vegetarian.

this analysis, biospecimens were analyzed for a comprehensive metabolic panel, and also tests of inflammatory markers (interleukin-6, C reactive protein) using a previously described protocol by Jaceldo-Siegl et al. (26). Briefly, collection of blood was done after an overnight fast, and serum was separated from the cells within 30 min of collection, placed on wet ice, and then shipped or transported to the central study laboratory at Loma Linda, CA for further processing. Blood was aliquoted to 0.5 mL samples and immediately stored in liquid nitrogen until analysis. Concentrations of CRP and IL-6 were measured in duplicate samples using enzyme linked immunosorbent assay (ELISA) kits from R & D Systems (Minneapolis, MN, USA) for IL-6, Thermo Fisher Scientific (Waltham, MA, USA), and Assaypro (St. Charles, MO, USA) for CRP. For IL-6, the detectable limit was 0.039 pg/mL, and intra- and inter-assay CV were 6.9 and 7.2%; and for CRP, these were 100 pg/mL, 5.5 and 7.6%, respectively.

Blood pressure (BP), weight, height, body mass index (BMI), waist circumference (WC), and body composition were assessed on-site. Height was measured to the nearest quarter inch (0.64 cm) using the Seca 214 Portable Height Rod (Seca Corp, Hamburg, Germany). Weight (measured to the nearest half pound or 0.23 kg) and body composition were assessed using a body composition analyzer (Tanita, Model TBF-300A, Arlington Heights, IL, USA). Waist circumference was obtained using an anthropometric tape placed around the waist just above the hipbone.

Blood pressure and resting pulse were assessed each as the average of three measurements using the OMRON Digital Blood Pressure Monitor HEM-7471C (Omron Healthcare Inc., Vernon Hills, IL, USA). All subjects were subject to a standard protocol, including a 5 min quiet relaxation period prior to the first reading of BP and resting pulse, and 1 min rest between consecutive measurements.

Classification of Vegetarian Diet

Dietary intake was assessed using two methods: (1) A single 24 h recall using standard methodology that has been discussed previously (27), (2) Survey items adapted from EPIC Oxford where subjects were also asked about their consumption (in the past 30 days) of four animal-based products (meat, fish, dairy, and eggs). Using the latter method to survey usual intake, participants were classified as non-vegetarian if they currently eat meat; pescatarian if they eat no meat but eat fish; lacto-ovo vegetarian if they eat dairy and eggs but not meat or fish; and strict vegetarian if they do not consume meat, eggs, fish and dairy. Due to the limited sample size, strict vegetarians, lacto-ovo vegetarians, and pescatarians were considered as following a plant-based diet and collapsed into a "vegetarian" group. Survey items measuring meat intake among Seventh-day Adventists have been validated against multiple 24 h recalls with correlations that exceed 0.80 (27).

Assessment of Demographic, Religiosity, and Culture-Specific Data

We obtained demographic, spoken language, nativity and religiosity data by questionnaire. Religiosity was assessed by asking the question, "How religious are you?" where we defined "religious" as having "to do with a personal experience and not just a behavior, like going to church." Responses were elicited using a 10-point semantic differential scale where 1 represents not religious at all and 10 the most religious.

Statistics

Descriptive analyses comparing vegetarians and non-vegetarians on selected subject characteristics were conducted using t-tests for continuous, and χ^2 or Fisher test for categorical variables. Continuous outcome variables (anthropometric, body composition, pulse, and blood pressure measures) were compared in vegetarians and non-vegetarians using generalized linear models adjusted for age, sex, and education. Ninety-five percent confidence intervals for the difference between these adjusted means were computed using the standard errors of the regression coefficients in the models.

RESULTS

In **Table 1**, we examined demographics characteristics of Hispanic/Latino subjects in the AMEN study. We found that vegetarians tended to be older ($p = 0.006$) and more educated ($p = 0.02$) as compared to non-vegetarians (**Table 1**). There was no significant difference in religiosity between the vegetarians and non-vegetarians.

Vegetarian Diet and Adiposity

In linear regression models (**Table 2**), we tested the association between measures of adiposity (BMI, waist circumference (WC), fat mass, and percent body fat) as outcomes and vegetarian diet status as a main exposure. We found that BMI was lower among the vegetarians than the non-vegetarians (24.5 kg/m^2 vs. 27.9 kg/m^2, $p = 0.006$) after adjusting for age, sex, and education. Vegetarians also had significantly lower waist circumference (34.8 in vs. 37.5 in), fat mass (18.3 kg vs. 23.9 kg), and percent body fat (28.4% vs. 32%) as compared to non-vegetarians. Pulse rate, systolic and diastolic blood pressure values among vegetarians were not significantly different from those of non-vegetarians.

Vegetarian Diet, Adiposity, and Inflammatory Markers

We found that log-transformed interleukin-6 (IL-6) was positively associated with the following adiposity outcomes: BMI ($\beta = 1.64$, $p = 0.04$), waist circumference ($\beta = 1.32$, $p = 0.08$), fat mass ($\beta = 1.32$, $p = 0.05$), and fat percent ($\beta = 4.22$, $p = 0.004$). Adding the main exposure for vegetarian status to the model did not appreciably reduce these associations. For log transformed C-reactive protein (CRP), positive associations were found for fat mass ($\beta = 2.38$, $p = 0.03$) and fat percent ($\beta = 3.13$, $p = 0.004$). Similarly, adding the main exposure did not change CRP associations with measures of adiposity. We note that the power to detect mediation was low, making type 1 errors more likely.

There were no significant differences between vegetarians and non-vegetarians for log-transformed CRP ($\beta = -0.06562$, $p = 0.78$) or log-transformed IL-6 ($\beta = -0.19132$, $p = 0.29$).

TABLE 1 | Selected characteristics among Hispanic/Latino vegetarians and non-vegetarians in the Adventist Multi-ethnic Nutrition (AMEN) Study.

		Vegetarian ($n = 23$)		Non-vegetarian ($n = 51$)		*p*-value
Age (y)[a]	Mean	54.3		47.6		0.03
	SD	14.1		10.6		
Religiosity	Mean	8.22		7.78		0.22
	SD	1.24		1.46		
		n	%	*n*	%	
Sex[a]						0.15
	Male	4	17.4	17	34.0	
	Female	19	82.6	33	66.0	
Income[a]						
	<$50,000	12	57.1	38	77.6	0.08
	≥$51,000	9	42.9	11	22.4	
Education						
	High school or Less	6	26.1	28	54.9	0.02
	College or more	17	73.9	23	45.1	
Marital status						
	Married	17	73.9	39	76.5	0.81
	Not Married	6	26.1	12	23.5	
Language						
	English	2	8.7	7	13.7	0.31
	Spanish	19	82.6	43	84.3	
	Other	2	8.7	1	2.0	
Nativity						
	U.S. Born	3	13.0	4	7.8	0.67
	Foreign Born	20	87.0	47	92.2	

[a]*% Missing data: 1% Age, 1% Sex, 2.08% Income.*

TABLE 2 | Comparison of obesity measures, body composition, pulse, and blood pressure in vegetarians and non-vegetarians in the AMEN Study.

	Adjusted for age, sex, and education		
	Mean	**Difference (95% CI)**	***p*-value**
BODY MASS INDEX (kg/m^2)			
Non-veg[a]	27.9	3.3 (1.0, 5.7)	0.006
Veg	24.5		
WAIST CIRCUMFERENCE (in)			
Non-veg	37.5	2.8 (0.6, 4.9)	0.01
Veg	34.8		
FAT MASS (kg)			
Non-veg	23.9	5.5 (1.6, 9.5)	0.007
Veg	18.3		
PERCENT BODY FAT			
Non-veg	32.0	3.6 (0.5, 6.7)	0.025
Veg	28.4		
PULSE RATE			
Non-veg	67.5	2.8 (−2.4, 8.1)	0.28
Veg	64.6		
SYSTOLIC BLOOD PRESSURE			
Non-veg	117.6	−3.0 (−11.1, 5.1)	0.46
Veg	120.6		
DIASTOLIC BLOOD PRESSURE			
Non-veg	77.1	1.1 (−3.6, 5.9)	0.64
Veg	75.9		

[a]*Non-veg, non-vegetarian; Veg, vegetarian (combining vegan, lacto-ovo, and pesco-vegetarian).*

DISCUSSION

In the AMEN study, we found that in a sample of Seventh-day Adventist Hispanic/Latino adults, those following a vegetarian dietary pattern had a BMI that was lower (24.5 kg/m^2 vs. 27.9 kg/m^2, $p = 0.006$) and within federally-recommended limits as compared to non-vegetarians. These findings were confirmed by similar decreases in other measures of adiposity [fat mass (18.3 kg vs. 23.9 kg), and percent body fat (28.4% vs. 32%)] and abdominal adiposity [waist circumference (34.8 in vs. 37.5 in)].

These findings are concordant with findings from non-Hispanic white Adventists (18, 19, 22), Black/African American Adventists (22, 25), and Asian Adventists (20). For Black Adventists, Akbar et al. has reported a "cultural tailoring" of the vegetarian diet chosen whereby Southern and Caribbean influenced dietary patterns introduced a wide range of plant foods into the diet (24). In US-based Asian Indian Adventists, Singh reported the predominance of nuts as a major source of protein in vegetarians who were at lower BMI (20).

Our next step in the AMEN study was to investigate how the protective patterns of plant-based eating in Hispanic/Latino adults were influenced by cultural preference for specific plant foods. These data can potentially inform culturally-tailored plant-based dietary interventions for the broader population of US Hispanic/Latinos who have been experiencing a nutrition transition away from simpler, more traditional diets rich in whole plant foods.

Plant-Based Eating in US Hispanic/Latinos

Our data indicates that more plant-based eating in US-based Hispanic/Latino Adventist adults was associated on average with adiposity indices falling within the federally-recommended limits. If this association holds true also for non-Adventist Hispanics/Latinos, how feasible and scalable is it to have Adventist and non-Adventist Hispanics/Latinos increase their plant-based dietary choices as a means of preventing the obesity and cardiometabolic diseases that are disproportionately prevalent in their community?

When considering this question it is noteworthy that data from Latin America indicate a rich cultural tradition of eating and preparing a diverse range of regional plant foods. For example, the Tarahumara Indians of Mexico traditionally adhered to a diet consisting of beans, corn, and squash and very little meat, and this pattern has been associated with lower risk of cardiometabolic disease (28) and can be significantly compromised by plying the Tarahumara with a typical U.S. diet (29). Studies from South America report that vegan and semi-vegetarian Peruvian and Brazilian subjects (following traditional cultural choices involving plant foods) do have lower rates of hypertension, dyslipidemia, and obesity as compared to omnivores (30). These small studies provide clues that increasing plant-based diet choices based on long-held cultural traditions of eating whole plant foods can potentially be an attractive target for high impact interventions. When designing dietary interventions we note that the strict vegetarian diet pattern is not commonly practiced by US Hispanic/Latinos (31), and further research is needed in this community to examine whether increasing plant food intake to the point of semi-vegetarian or pescatarian pattern can improve health outcomes.

Plant-Based Eating, Pro-Inflammatory Cytokines, and Obesity in Hispanic/Latinos

Among the Hispanic/Latino adults of the AMEN study, we did find that measures of adiposity were associated with pro-inflammatory cytokines such as interleukin-6. Compared to non-vegetarian dietary patterns, vegetarian dietary patterns generate increased microbially-generated short chain fatty acids, which reduce systemic inflammation (e.g., IL-6), increase hormonal satiety-signaling (e.g., glucagon-like peptide-1) and reduce insulin resistance (32, 33). Among Hispanic/Latino subjects, recent studies suggest that IL-6 may be elevated from a young age, and the link across the lifespan to higher rates of cardiometabolic disease may be due to specific IL-6 polymorphisms in Latino youth (34). Interestingly, adding IL-6 to our models examining association with vegetarian diet exposure did not alter the protective effect of the diet on obesity.

Limitations

The AMEN study has important limitations. The cross-sectional and observational design precludes inferring a causal effect from the association of diet with obesity and obesity-related disease. The purpose of our pilot study was to generate culturally-specific hypotheses for testing this association in longitudinal and experimental designs with novel culturally-tailored interventions.

The relatively small sample size limited our statistical power to test more complex food and nutrient models, and for testing interaction and mediation. We note, however, the significant and cross-validating associations between plant-based eating and measures of adiposity that persist despite the limited power to detect these associations.

Religiosity could also be a factor since we are studying a faith-based group. It is noteworthy, however, that we observed no difference in religiosity between the vegetarians and non-vegetarians we studied. Measurement error in dietary assessment is always present in observational work, but we note a decades-long track record by our research group validating diet and vegetarian diet survey measures in Adventists (16, 17, 35).

CONCLUSIONS

Our findings from Hispanic/Latino Seventh-day Adventists indicate that plant-based diet patterns were associated with adiposity in the recommended range, and effects were consistent across multiple measures of adiposity and obesity-related inflammatory markers. Further studies of how Hispanics/Latinos have culturally tailored the Adventist plant-based eating pattern could potentially inform interventions for the broader population of Hispanics/Latinos.

ETHICS STATEMENT

The study protocol was approved by the institutional review board of Loma Linda University and performed in accordance with the ethical standards as laid down in the 1975 Declaration of Helsinki as revised in 2000. All participants provided signed informed consent.

AUTHOR CONTRIBUTIONS

PS: designed the study, supervised data collection and analysis, wrote the final version of the manuscript and obtained funding; KJ-S: designed the study, supervised data collection and analysis, wrote a draft of the manuscript; NC: analyzed data; WS: analyzed data and edited manuscript; LL: data collection coordinator and preliminary analysis; KS: analyzed data and edited manuscript; DE: analyzed data; MJ, HF, DH-B, and WM: edited manuscript.

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CORRECTION
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Corrigendum: Plant-Based Diets Are Associated With Lower Adiposity Levels Among Hispanic/Latino Adults in the Adventist Multi-Ethnic Nutrition (AMEN) Study

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A Corrigendum on

Plant-Based Diets Are Associated With Lower Adiposity Levels Among Hispanic/Latino Adults in the Adventist Multi-Ethnic Nutrition (AMEN) Study
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In the original article, there was a mistake in **Table 2** as published. The waist circumference results were provided in cm and should in fact be in inches. The corrected **Table 2** appears below.

Additionally, throughout the article the waist circumference was listed in centimeters and should in fact be in inches.

Corrections have therefore been made to the following section:

The **Abstract, paragraph four**:

"**Results:** Vegetarian diet patterns (Vegan, Lacto-ovo vegetarian, Pesco-vegetarian) were associated with significantly lower BMI (24.5 kg/m^2 vs. 27.9 kg/m^2, $p = 0.006$), waist circumference (34.8 in vs. 37.5 in, $p = 0.01$), and fat mass (18.3 kg vs. 23.9 kg, $p = 0.007$), as compared to non-vegetarians. Adiposity was positively associated with pro-inflammatory cytokines (Interleukin-6) in this sample, but adjusting for this effect did not alter the associations with vegetarian diet."

The **Results**, subsection **Vegetarian Diet and Adiposity**:

"In linear regression models (Table 2), we tested the association between measures of adiposity (BMI, waist circumference (WC), fat mass, and percent body fat) as outcomes

TABLE 2 | Comparison of obesity measures, body composition, pulse, and blood pressure in vegetarians and non-vegetarians in the AMEN Study.

	Adjusted for age, sex, and education		
	Mean	**Difference (95% CI)**	***p*-value**
BODY MASS INDEX (kg/m^2)			
Non-veg[a]	27.9	3.3 (1.0, 5.7)	0.006
Veg	24.5		
WAIST CIRCUMFERENCE (in)			
Non-veg	37.5	2.8 (0.6, 4.9)	0.01
Veg	34.8		
FAT MASS (kg)			
Non-veg	23.9	5.5 (1.6, 9.5)	0.007
Veg	18.3		
PERCENT BODY FAT			
Non-veg	32.0	3.6 (0.5, 6.7)	0.025
Veg	28.4		
PULSE RATE			
Non-veg	67.5	2.8 (−2.4, 8.1)	0.28
Veg	64.6		
SYSTOLIC BLOOD PRESSURE			
Non-veg	117.6	−3.0 (−11.1, 5.1)	0.46
Veg	120.6		
DIASTOLIC BLOOD PRESSURE			
Non-veg	77.1	1.1 (−3.6, 5.9)	0.64
Veg	75.9		

[a]*Non-veg, non-vegetarian; Veg, vegetarian (combining vegan, lacto-ovo, and pesco-vegetarian).*

and vegetarian diet status as a main exposure. We found that BMI was lower among the vegetarians than the non-vegetarians (24.5 kg/m^2 vs. 27.9 kg/m^2, $p = 0.006$) after adjusting for age, sex, and education. Vegetarians also had significantly lower waist circumference (34.8 in vs. 37.5 in), fat mass (18.3 kg vs. 23.9 kg), and percent body fat (28.4% vs. 32%) as compared to non-vegetarians. Pulse rate, systolic and diastolic blood pressure values among vegetarians were not significantly different from those of non-vegetarians."

The **Discussion, paragraph one**:

"In the AMEN study, we found that in a sample of Seventh-day Adventist Hispanic/Latino adults, those following a vegetarian dietary pattern had a BMI that was lower (24.5 kg/m^2 vs. 27.9 kg/m^2, $p = 0.006$) and within federally-recommended limits as compared to non-vegetarians. These findings were confirmed by similar decreases in other measures of adiposity [fat mass (18.3 kg vs. 23.9 kg), and percent body fat (28.4% vs. 32%)] and abdominal adiposity [waist circumference (34.8 in vs. 37.5 in)]."

The authors apologize for these errors and state that they do not change the scientific conclusions of the article in any way. The original article has been updated.

frontiers in Nutrition

REVIEW
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The Effects of Vegetarian and Vegan Diets on Gut Microbiota

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The difference in gut microbiota composition between individuals following vegan or vegetarian diets and those following omnivorous diets is well documented. A plant-based diet appears to be beneficial for human health by promoting the development of more diverse and stable microbial systems. Additionally, vegans and vegetarians have significantly higher counts of certain *Bacteroidetes*-related operational taxonomic units compared to omnivores. Fibers (that is, non-digestible carbohydrates, found exclusively in plants) most consistently increase lactic acid bacteria, such as *Ruminococcus*, *E. rectale*, and *Roseburia*, and reduce *Clostridium* and *Enterococcus* species. Polyphenols, also abundant in plant foods, increase *Bifidobacterium* and *Lactobacillus*, which provide anti-pathogenic and anti-inflammatory effects and cardiovascular protection. High fiber intake also encourages the growth of species that ferment fiber into metabolites as short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate. The positive health effects of SCFAs are myriad, including improved immunity against pathogens, blood–brain barrier integrity, provision of energy substrates, and regulation of critical functions of the intestine. In conclusion, the available literature suggests that a vegetarian/vegan diet is effective in promoting a diverse ecosystem of beneficial bacteria to support both human gut microbiome and overall health. This review will focus on effects of different diets and nutrient contents, particularly plant-based diets, on the gut microbiota composition and production of microbial metabolites affecting the host health.

Keywords: gut microbiota, fiber, nutrition, plant-based diet, postbiotics

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INTRODUCTION

Recent studies of the human microbiome have emerged as an area of popular interest. For decades, many investigations have elucidated the impact of the human gut microbiota on the physiology of the host, with new and unexpectedly broad implications for health and disease.

The human microbiota, defined as the total of all microbial taxa associated with human beings (bacteria, viruses, fungi, protozoa, archaea), consists of a newly estimated 3×10^{13} (trillion) microbes harbored by each person (1). The term *microbiome* is often incorrectly used interchangeably with the term microbiota. However, microbiome refers to the catalog of these microbes and their genes. The human gut microbiome represents ∼3.3 million non-redundant microbial genes, which outnumbers the human genome of some 21,000 genes in the ratio of ∼150:1 (2). Interestingly, the diversity among the microbiomes of two different individuals is vast compared to their human genomic variation; humans are about 99.9% identical to each other in terms of their genome (3), but their gut microbiome can be up to 80–90% different (4).

Recent advancements in laboratory techniques have revealed functions of the human gut microbiota related to immunity and the gastrointestinal, brain, and cardiovascular systems. Research has also suggested a profound effect of the human gut microbiota on host cells and genes. This extensive interaction has suggested that the microbiome functions effectively as a separate "organ."

Several studies have suggested that there are three basic bacterial enterotypes (5) (1) genus *Prevotella* (considered to be mostly anti-inflammatory and otherwise protective), (2) genus *Bacteroides* (more pro-inflammatory and possibly related to the heightened risk of metabolic syndrome and other pathological conditions), and (3) genus *Ruminococcus* (of which the biological significance is less evident) (6).

An imbalance of the gut microbiota has been linked with gastrointestinal conditions such as reflux, peptic ulcers, irritable bowel syndrome, non-alcoholic steatohepatitis, and inflammatory bowel disease. Additionally, some systemic conditions such as obesity, atherosclerosis, type 2 diabetes, cancer, Alzheimer's and Parkinson's disease, amyotrophic lateral sclerosis, autism spectrum disorder, atopy etc., also appear to be linked to unfavorable changes in gut microbiota composition (7–17). An accumulating body of evidence points to the gut microbiota as a mediator of dietary impact on the host metabolic status. Current research is focusing on the establishment of causal relationships in people and the development of therapeutic interventions such as personalized nutrition (18).

Dietary composition appears to have long-term and acute effects on the gut microbiota ecosystem (19, 20). Different long-term dietary patterns, such as vegetarian/vegan vs. omnivorous diets, have significant influence on gut microbiota composition. The different gut microbiota content is shown to provide different food nutrients metabolites, termed postbiotics. For instance, SCFAs, phytoestrogens, or isothiocyanates are more linked with the plant-based food, while TMAO and secondary bile acids with the meat-based diet. These and other postbiotics take part in the metabolism of the host in different ways. This review will focus on some general as well as specific aspects of this dynamic field of research.

GUT MICROBIOTA: GENERAL ASPECTS

In addition to bacteria, the gut is host to multiple kingdoms: archaea, viruses, and eukaryotes, including fungal species. The gut microbiota is represented by more than 1,000 microbial species, belonging primary to just two phyla: *Bacteroidetes* and *Firmicutes* (21). Based on human stool samples, overall, the genera *Bacteroides*, *Prevotella, Bifidobacterium*, *Eubacterium*, *Clostridium*, *Streptococcus,* and *Enterobacteriaceae* are most commonly found. It should be noted that stool samples provide reasonable estimations of the gut microbiota rather than a complete representation (22). This is because anaerobic species often attach to the gut mucosa, making it difficult to identify all bacterial species present in the large intestine. Also, it is probable that the biological significance of any genera or species is not given by its relative proportion in the whole ecosystem. Rather, its significance is observed through its metabolism/postbiotics and effects on the enteric nervous system, local immunity, brain, and genes.

EFFECT OF DIET ON GUT MICROBIOTA COMPOSITION

The difference in gut microbiota composition between individuals consuming a vegan/vegetarian and an omnivorous diet is well documented. Research shows that vegetarian/vegan diets foster different microbiota when compared to omnivores, with only a marginal difference between vegans and vegetarians (23). Changes in microbiota composition might be due to differences in bacteria directly consumed through food, differences in substrates consumed, variations in transit time through the gastrointestinal system, pH, host secretion influenced by dietary patterns, and regulation of gene expression of the host himself and/or his/her microbiota (24).

A plant-based diet appears to be beneficial for human health by promoting the development of a more diverse gut microbial system, or even distribution of different species (25, 26).

Diversity and Richness of the Gut Microbiota

The diversity of the microbiota appears to have an important association with BMI, obesity, and arterial compliance; and a majority of the research suggests that a plant-based diet fosters a greater microbial diversity. Klimenko et al. found a positive association between alpha-diversity, or local microbial richness, and long-term fruit and vegetable intake ($p < 0.05$) (27). Likewise, Martinez et al. observed that adding whole-grain barley, brown rice, or a mixture of the first two to the diet of volunteers resulted in an increase in microbial diversity ($n = 28$) (28). Klimenko et al. also found a negative association between alpha-diversity and BMI ($p < 0.05$) (27).

However, a short-term dietary intervention advising increased fiber consumption resulted in a slight but significant decrease in diversity ($p < 0.001$). The researchers suggest this reduction in diversity might be the result of a rapid dietary change resulting in a temporary disruption to the microbial composition. This hypothesis of transitory microbial "stress" also explains the slight but significant increase in *Enterobacteriaceae* as a result of the intervention ($p < 0.05$). *Enterobacteriaceae* abundance is typically lower on a vegan diet vs. an omnivorous one ($P < 0.05$) (29). This is likely due to the greater presence of butyrate-producing bacteria on a higher fiber diet, which can lower colonic pH, preventing the growth of pathogenic bacteria, such as *Enterobacteriaceae* (30).

Verdam et al. observed reduced microbial diversity in obese vs. non-obese study participants ($n = 28$). The obese participants also displayed a reduction in the *Bacteriodetes*:*Firmicutes* ratio and an increase in *Proteobacteria*, a pro-inflammatory phylum. Likewise, an increase in C-reactive protein was observed ($p < 0.001$) which inversely correlated with the *Bacteriodetes*:*Firmicutes* ratio ($p < 0.05$). These observations suggest a pro-inflammatory effect of obesity-related microbiota (31). On the other hand, participants from the Adventist Health

Study-2 (60,903) following a vegan diet displayed the lowest BMI values when compared with those following a vegetarian or omnivorous diet (32). These findings indicate that a vegan diet, associated with lower body weight, might benefit microbial diversity and protect against inflammation.

Menni et al. observed that carotid-femoral pulse wave velocity, a measure of arterial stiffness, was negatively associated with microbiome diversity ($p = 0.001$) in women ($n = 617$) (33). This correlation remained significant after adjusting for insulin resistance and visceral fat. Arterial stiffness is oftentimes caused by hyperglycemia or hyperinsulinemia (34) and is significantly correlated with inflammatory adipokine levels. The researchers suggest the association between arterial stiffness and microbial diversity can be explained partially by the role of the gut in modulating systemic inflammation. Thus, an increase in microbial diversity might improve systemic inflammation, thereby reducing arterial stiffness.

Additionally, vegans and vegetarians have a significantly greater richness (alpha diversity) compared to omnivores, specifically counts of certain *Bacteroidetes*-related operational taxonomic units (OTUs) (35). It seems likely that many health benefits of vegetarian/vegan diets are, in part, mediated by the gut microbiota—not only through the higher relative abundance of those OTUs that are currently considered to be protective (*Bacteroidetes*, *Prevotella*, *Roseburia*, etc.), but also from postbiotic and epigenetic effects on various risk factors for chronic inflammation and chronic degenerative diseases (36).

Effects of Diet on the Bacteroidetes:Firmicutes Ratio

Despite significant inter-individual differences, a healthy adult intestinal microbiome is characterized by the dominance of these *Bacteroidetes*-related OTUs along with those of the *Firmicutes* phylum (37, 38). Research has shown variability in these phyla concentrations to be heavily affected by diet, specifically the ratio between the two when comparing omnivorous diets of the type common in North America, vs. a vegetarian/vegan diet. One study compared the bacterial composition between Indian ($n = 11$) and Chinese ($n = 5$) adults (39). While both populations ate diets centered around carbohydrates and vegetables, the Chinese diet was heavier in animal fat and protein than the Indian diet of whole grains and plant-based vegetarian foods. The percentage of *Bacteroidetes* within the microbiomes of Indian participants was nearly four times greater than in the Chinese, 16.39% vs. 4.27%, respectively ($p = 0.001$). The higher abundance of *Bacteroidetes* in Indians was hypothesized to be due to their lower consumption of animal products; indicating a diet lower in animal products to be associated with greater *Bacteroidetes* counts.

Another study compared the fecal microbiota of Italian children ($n = 15$) vs. the fecal microbiota of children living in a rural western Africa, specifically in Burkina Faso ($n = 14$) (40). The Italian children typically consumed a Western diet, high in animal protein, sugar, starch, and fat and low in fiber. The African children of Burkina Faso consumed a diet low in fat and animal protein and rich in starch, fiber, and plant protein. The abundance of *Firmicutes* was twice as much in the Italian children than in the Burkina Faso children (63.7 vs. 27.3%, respectively). The abundance of *Bacteroidetes* in the Italian children was less than half of that seen than in the Burkina Faso children (22.4 vs. 57.7%, respectively). A decrease in *Firmicutes* levels, usually occurring in favor of *Bacteroidetes* and *Bifidobacteria*, as seen in response to an increase in resistant starches, may be beneficial in preventing and treating obesity (41). While these correlations between diet and microbiota composition are observed among different populations, it is important to consider other factors that may play a role, such as ethnicity, host genotypes, environmental factors, etc.

Research has shown that the balance of *Bacteroidetes* and *Firmicutes* is an important marker for obesity and higher BMI. Specifically, a decreased *Bacteroidetes*:*Firmicutes* ratio has a strong negative correlation with BMI ($r_s = 0.59$, $P < 0.001$) (31). A possible explanation for this correlation may be found in the observation that a 20% increase in *Firmicutes* and a corresponding decrease in *Bacteroidetes* abundance is associated with a 150 kcal/day increase in energy harvest, resulting in weight gain overtime. Therefore, an increased *Bacteroidetes*:*Firmicutes* ratio, as seen on a high fiber, plant-based diet, may result in weight loss by reducing the amount of energy extracted from the diet. Further research is needed to determine whether the increase in energy harvest due is due to the *Bacteroidetes*:*Firmicutes* ratio promoting adiposity or representing a host-mediated adaptive response to limit energy uptake (42).

Studies have also shown opposite trends in *Firmicutes* and *Bacteroidetes*. One study compared US children eating a Western diet to Bangladeshi children consuming a plant-based diet of rice, bread, and lentils. The *Bacteroidetes*:*Firmicutes* ratio was three times higher in the US children consuming the Western diet (43). This opposes the previous prediction of a Western diet resulting in a decreased *Bacteroidetes*:*Firmicutes* ratio. The researchers noted age and geographical differences as potential explanation for this departure from the expected ratio, as well as inter-subject variability. Another study asked participants to increase their fiber consumption and avoid Western diet foods. While prior studies would have suggested an increase in *Bacteroidetes*:*Firmicutes*, the ratio decreased (0.13 ± 0.2 to 0.03 ± 0.09, Wilcoxon paired test $p < 0.0001$, $n = 430$) (27). Another study analyzed the microbial composition of lean and obese subjects ($n = 98$) and observed that, when compared to lean subjects, overweight and obese volunteers presented a higher ratio *Bacteroidetes* to *Firmicutes* ($P = 0.001$ and $P = 0.005$, respectively) (44). Likewise, comparison of bacterial phyla did not show a significant difference in the *Bacteroidetes* to *Firmicutes* ratio between obese and lean volunteers ($n = 20$) (45). These examples reflect the difficulties in broadly linking certain phyla to particular diets. The primary challenge in analyzing specific microbiota is the need to consider the state and interaction dynamic of microbes encompassing the whole microbiome.

Effects of Diet on Enterotypes

As mentioned above, there are three main enterotypes observed in human microbiomes: *Prevotella, Bacteroides,* *and Ruminococcus*. *Prevotella*, a genus of the *Bacteroidetes* phyla, appears to be significantly richer in response to a vegan diet. In the previously mentioned study by De Filippo et al., *Prevotella* was exclusively present in the children of Burkina Faso consuming a diet low in fat and animal protein and rich in starch, fiber, and plant protein when compared to children living in Italy consuming a Western diet, high in animal protein, sugar, starch, and fat, and low in fiber (40). Another study compared the diets of 178 elderly residents living in either the community or in long-term residential care (46). The community group was found to consume a low to medium fat, high fiber diet; while the residents in long-term care consumed a moderate to high in fat, and low fiber diet. The study found that those in the community, eating a profile more reflective of a plant-based diet, more frequently had gut communities of the *Prevotella* enterotype.

The study comparing Indian and Chinese adults shows similar results (39). As expected, the Indians who were consuming less animal products and more plant-based foods than the Chinese had a significantly greater percentage of *Prevotella* (13.07 vs. 0.58%, respectively). When the abundance of *Prevotella* was analyzed in Thai vegetarians vs. non-vegetarians, the vegetarians were found to have significantly higher numbers of *Prevotella* ($p = 0.005$) (47). Other studies have shown vegan/vegetarian diets, high in plant-based foods, to be associated with high abundances of *Prevotella* (48, 49). This suggests additional support for greater *Prevotella* presence in those whom consume less animal products and more plant-based food. While mice studies suggest *Prevotella* to improve glucose metabolism by improving glycogen storage (50), the current lack of any additional research makes *Prevotella* merely a genus to describe an overall ecosystem of human gut bacteria, primarily under a plant-based diet.

Bacteroides, another main enterotype and genus of the *Bacteroidetes* phyla, also appears to be affected by diet but in a different way to *Prevotella*. *Bacteroides* has been positively correlated with long-term diets rich in animal protein and saturated fat (20, 27). This is likely due to their ability to tolerate bile, which is common in gut environments of those who consume animal products. In the study mentioned earlier comparing children in the US eating a Western diet vs. children in Bangladesh consuming a plant based diet, *Bacteroides* was the major genus in the US children's microbiota. High proportions of *Bacteroides* are found in the gut of humans consuming a Western diet and the opposite is found in those consuming a high fiber diet of fruits and legumes (27, 37, 43, 47, 48).

Ruminococcus is the third major enterotype and is associated with long term fruit and vegetable consumption. Species of this genus are specialized in degrading complex carbohydrates, such as cellulose and resistant starch, found in plant based foods (51). These microbes degrade dietary fibers, producing butyrate, which acts as an anti-inflammatory compound. *Ruminococcus* is positively associated with low BMI and negatively associated with poor lipid profile (27). Likewise, abundance of *Rumminococcus* has been linked to lower endotoxemia and lower arterial stiffness, a predictor of cardiovascular risk (33). Walnut consumption has been significantly associated with enrichment of *Ruminococcus* as well (38). However, *Ruminococcus* has also been linked to low dietary fiber consumption in college students. While these microbes degrade complex carbohydrates, they also break down the resistant starches found in refined grain products (52). *Ruminococcus* might also play a role in the conversion of animal-derived choline to trimethylamine (TMA) (53). Thus, the abundance of *Ruminococcus* is influenced by both animal and plant based diets.

Effects of Diet on Additional Bacteria

While *Bacteroides* can be pro-inflammatory and their concentration is associated with long term consumption of animal products, a study analyzing 11 vegetarians, 20 vegans, and 29 omnivores (49) found a discrepancy in this generalization. In addition to finding *Clostridium clostridioforme* and *Faecalibacterium prausnitzii*, both considered to be health protective, in higher relative abundance in the vegetarians/vegans compared to the omnivores, *Bacteroides thetaiotaomicron* was also observed in higher abundance in these groups. This discrepancy in categorizing bacteria abundance under a plant-based diet vs. animal-based diet is not uncommon. *Clostridium* cluster XIVa was found in lower ratio in the vegetarian/vegans, contrary to a study showing *Clostridium* cluster XIVa bacteria to be a major component of gut microbiota in vegetarian women (103). Therefore, while generalizations can be made, some genus subtypes will be outliers. This discrepancy in some bacterial phyla in response to diet has been acknowledged by previous review papers and has been attributed to various reasons, such as different microbiome profiling methodologies, different host genetics, body mass index, and red wine and aspartame consumption (54, 55). These are all factors that have been shown to possibly modify our microbiota. Therefore, further studies are warranted in order to isolate their effects from those due to a plant based vs. omnivorous diet.

Taken together, dietary habits influence the composition of the intestinal microbiota. While dietary changes have a relatively fast impact (51) (within a week) on the microbial composition and consequently on its metabolites, these effects are modest and reversible (24). For example, changes of microbiota and immune parameters after a 3-month vegetarian diet are significant, but do not reflect the degree of change that occur with a long-term vegetarian diet (56).

HOW PLANT FOOD COMPONENTS INFLUENCE GUT MICROBIOTA.

Nutrient Bioavailability

Consuming food nutrients with low bioavailability has recently been found to be important. Lower nutrient bioavailability, found in larger food particles, intact plant cell walls, and food without thermal treatment, means that more nutrients reach lower in the gastrointestinal system, thus enriching nutrient delivery to the gut microbiota (57). This helps support normal gut microbiota development and function (57). Modern westernized diets contain more ultra-processed foods and acellular nutrients, or nutrients not containing cells. These components are more easily absorbed in the small intestine, depriving the colon of important nutrients, which may alter the composition and metabolism of the gut microbiota (58). Acellular food, e.g., sugar, has been shown to induce inflammation in young infants, adolescents, women of child-bearing age, and older adults. Whole plant foods have protective effects, favoring the growth of beneficial fiber-degrading bacteria in the colon (58).

Carbohydrates

Unlike digestible carbohydrates, non-digestible carbohydrates, such as resistance starch, and some sugars, reach the large intestine where they can be fermented by the gut microbiota to provide energy or produce postbiotics. However, both digestible and non-digestible carbohydrates may influence the gut microbiota. Digestible carbohydrates from fruits (e.g., glucose, sucrose, and fructose) have been shown to reduce *Bacteroides* and *Clostridia* (54). Non-digestible carbohydrates most consistently increase lactic acid bacteria, *Ruminococcus*, *E. rectale*, and *Roseburia*, and reduce *Clostridium* and *Enterococcus* species (54). Both digestible and non-digestible carbohydrates have been shown to increase *Bifidobacteria*, genus of the *Actinobacteria* phylum.

A study compared the *Bifidobacteria* levels in response to a randomized, double-blind, crossover trail. Participants consumed both a standard enteral formula and a formula supplemented with fructooligiosaccharides (FOS) and fiber (59) as a sole source of nutrition for 14 days. FOS and fiber are both forms of carbohydrates found naturally and abundantly in plant foods–bananas, artichokes, onion, etc. While the volume of formula prescribed was based on individual energy expenditures, a benchmark of 2,000 calories of the FOS/fiber formula provided 10.2 g of FOS and 17.8 g of fiber. The average daily intake of fermentable non-digestible carbohydrates is estimated to be 10 g from inulin and FOS (60). This amount does not include meals and products supplemented with inulin and FOS, which typically add an additional 3–10 g/portion. Therefore, 10.2 g of FOS in the formula is realistic for human consumption. 17.8 g of fiber in the formula is also realistic for human consumption as the average US male and female intake is 18 g and 15 g, respectively [1].

Bifidobacterium is a butyrate-producing genus known to play a protective role in the human gut barrier by providing defense against pathogens and diseases. When participants were given formulas with FOS and fiber, their *Bifidobacteria* increased from 5.1 to 26.6% ($P = 0.003$) after 14 days. When formula was given without FOS and fiber, *Bifidobacteria* only increased from 3.3 to 8.6% ($P = 0.073$). A negative correlation between baseline *Bifidobacteria* and magnitude of the bifidogenic effect was observed, indicating that those with lower initial amounts of *Bifidobacteria* benefit most from fructooligiosaccharides and fiber intake. In contrast, high consumption of cholesterol from animal products, was strongly associated with a lower abundance of *Bifidobacteria* (adj. $p = 0.008$).

While these studies suggest that *Bifidobacterium* increase in response to a fiber-rich, high carbohydrate diet, other studies have shown conflicting results. One important confounding factor may be alcohol intake, which has been strongly associated with a lower abundance of *Bifidobacteria* (adj. $p = 0.006$). Researchers comparing *Bifidobacteria* levels in vegans, vegetarians, and controls, found *Bifidobacteria* to be significantly lower ($p = 0.002$) in vegan samples than in controls eating a standard omnivorous diet. No difference between vegans and vegetarians was observed (29). Another study observed higher *Bifidobacteria* levels in meat eaters compared to participants who switched to a vegetarian diet for 4 weeks after eating a mixed Western diet, high in fat and meat (58). The relative decrease of *Bifidobacterium* in vegetarians and vegans may be explained by a relative abundance of other protective bacteria species, such as *Prevotella*. *Prevotella* has been observed confers anti-inflammatory effects (40) and can decrease the growth of other bacteria by competing for fiber as an energy substrate (61).

A recent *in vitro* study elucidated the specific mechanism of action of carbohydrates, specifically selected dietary fibers, on gut microbiota. The study found the following fibers to have differing prebiotic effects: inulin, alpha-linked galacto-oligosaccharides, beta-linked galacto-oligosaccharides, xylo-oligosaccharides from corn cobs and high-fiber sugar cane, and beta-glucan from oats (62). Beta-glucan induced the growth of *Prevotella* and *Roseburia* with a concomitant increase in SCFA propionate production. Inulin and all oligosaccharides had a strong bifidogenic effect (62). This study also showed that all natural sugars, most notably non-digestible forms like inulin and oligosaccharides, increase SCFA levels (62). The prebiotic effects differ due to the type of bacteria that each fiber is broken down by. This is determined through bacterial specificity in which specific gene clusters within the bacterial genome dictate the saccharolytic enzymes that the bacteria can produce and, therefore, whether they can metabolize the prebiotic substrate (63). Non-digestible carbohydrates not only act as prebiotics by promoting the growth of beneficial microorganisms, but also reduce proinflammatory cytokine production, concentrations of serum triglycerides, total cholesterol, and LDL-cholesterol (54). Thus, non-digestible carbohydrates might confer protective effects against cardiovascular disease and central nervous system disorders.

Proteins

A majority of the studies have noted that protein consumption correlates positively with microbial diversity (54). However, animal and plant-proteins influence the gut microbiota in different ways. For instance, individuals consuming a high animal protein diet, from beef which is also high in fat, displayed lower abundances of bacteria, such as *Roseburia*, *Eubacterium rectale*, and *Ruminococcus bromii*, that metabolize dietary plant polysaccharides (51). Populations of bacteria that increase in response to a high animal protein diet when compared to subjects consuming a meatless diet are typically bile-tolerant microorganisms, such as *Bacteroides* and *Clostridia* (64). Additionally, a high-protein diet typically limits carbohydrate intake, which may lead to a decrease in butyrate-producing bacteria, and thereby to a proinflammatory state and an increased risk of colorectal cancer (65).

Individuals consuming pea protein exhibit increases in beneficial *Bifidobacterium* and *Lactobacillus* and decreases in pathogenic *Bacteroides fragilis* and *Clostridium perfringens* and,

[1] https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/dbrief/12_fiber_intake_0910.pdf. (Accessed February 12, 2019)

consequently increases in intestinal SCFA levels (54). Likewise, plant-derived proteins have been associated with lower mortality than animal-derived proteins (54).

Fats

Current evidence suggests that both the quantity and the quality of consumed fat significantly impact the gut microbiota composition (65).

A plant-based diet is generally naturally low in fat, which favors beneficial *Bifidobacteria* in human gut microbiota. The fat that does come from a vegan/vegetarian diet is made up of predominantly mono and polyunsaturated fats, which increase the *Bacteroidetes*:*Firmicutes* ratio, and on the genera level, increase lactic acid bacteria, *Bifidobacteria* and *Akkermansia muciniphila* (54). Nuts, particularly walnuts, have been shown to improve the gut microbiota by increasing *Ruminococcaceae* and *Bifidobacteria,* and decreasing *Clostridium* sp. cluster XIVa species (38).

On the other hand, saturated fat, found almost exclusively in animal sources, increases *Bilophila* and *Faecalibacterium prausnitzii,* and decreases *Bifidobacterium* (54). Some studies report that this change activates inflammation (induces pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α) and leads to metabolic disorders (66). High consumption of saturated and trans fat, predominately found in a Western diet, increases the risk of cardiovascular disease and reduces *Bacteroidetes*, *Bacteroides*, *Prevotella*, *Lactobacillus* ssp. and *Bifidobacterium* spp, and increases *Firmicutes* (40, 67).

N-3 polyunsaturated fatty acids, have been found to result in either no change to the microbiota, or beneficial increases in *Bifidobacterium*, *Adlercreutzia*, *Lactobacillus, Streptococcus, Desulfovibrio,* and *Verrucomicrobia* (*Akkermansia muciniphila*) (54, 67).

Polyphenols

Polyphenols, or naturally occurring plant metabolites (68), in plant foods increase *Bifidobacterium* and *Lactobacillus* abundance, which provide anti-pathogenic and anti-inflammatory effects and cardiovascular protection (54). Common polyphenol-rich foods include fruits, seeds, vegetables, tea, cocoa products, and wine. For example, polyphenol extracts from tea generate an increase in *Bifidobacterium* and *Lactobacillus–Enterococcus* spp., which then yields an increased SCFA production on human microbiota *in vitro* (69).

INFLUENCE OF MICROBIOME POSTBIOTICS ON HUMAN HEALTH

Research on the gut-brain, gut-lung, and gut-liver axes highlights the importance of the microbiome on systemic human health. Studies note changes in central neural chemistry, inflammatory lung conditions, and non-alcoholic fatty liver disease pathogenesis with changes to microbial composition (70–72). The mechanism of communication among these organs stems from the microbial products and microbial metabolites of ingested nutrients. These products can be diet-independent (such as lipopolysaccharides, ribosomally synthesized post-translationally modified peptides etc.), but here we would like to describe a few examples of well-known diet-dependent metabolites, such as SCFA and others. Depending on the bacteria and location along the intestinal tract, different bioactive molecules can be produced from different prebiotics and nutrients (70, 73). Microbial metabolites can have diverse positive health effects including local anti-inflammatory and immunomodulatory effects, and systemic anti-obesogenic, antihypertensive, hypocholesterolemic, anti-proliferative, and antioxidant effects (74). These postbiotic effects result from modulation of gene expression, metabolism, and intestinal functioning and depend on microbiota composition and substrates, largely dependent on diet.

Short-Chain Fatty Acids

SCFAs act as a substrate to maintain colonic epithelium, and are correlated with plant based food consumption (56). Maintenance of the intestinal barrier prevents endotoxemia and the subsequent inflammatory effects (75, 76). SCFAs acetate, propionate, and butyrate, are mostly microbial metabolites of fermented fiber and other carbohydrates, although a small fraction can derive from proteins. The fecal levels of these metabolites (and the corresponding esters) positively correlate with the consumption of fruits, vegetables, and legumes. Thus, their levels significantly increase in people who begin a plant-based diet. Interestingly, an increase in SCFAs is observed when omnivores consume a Mediterranean diet rich in fruit, legumes and vegetables (77).

While specific gut microbes are predisposed for SCFA production, different bacteria are known to produce different SCFAs. For example, enteric bacteria, such as *Akkermansia muciniphila*, *Bifidobacterium* spp., *Prevotella* spp., and *Bacteroides* spp. produce acetate; *Bacteroides* spp. produce propionate; and *Coprococcus* produces butyrate (78). The most butyrate producing bacteria are in *Clostridium* Cluster XIVa, IV, and XVI. These species are positively correlated with consumption of plant foods, and produce SCFAs that yield several health benefits.

The protective role of acetate, propionate and butyrate against different types of disease, such as type 2 diabetes, inflammatory bowel disease, and immune diseases, is well documented. For example, it has been shown that SCFAs promote immunity against pathogens (78), and are important components for microglia function and maturation and control of the blood–brain barrier integrity (79). Other effect of SCFAs is to increase thermogenesis, preventing/treating obesity (80, 81). SCFAs serve as energy substrates for colonocytes, as well as for the body generally. For example, propionate serves as a gluconeogenic substrate in the liver and in the intestine (78).

Microbial interactions with dietary polysaccharides and the resulting SCFAs are important energy and signaling molecules. It is becoming increasingly accepted that butyrate-producing bacteria and butyrate, *per se*, may be beneficial for human health (78). Butyrate has been shown to play a key role in gut physiology as a major carbon source for colonocytes. It helps regulate critical functions of the intestine, such as intestinal motility, mucus production, visceral sensitivity, the epithelial barrier, immune homeostasis, and the mucosal oxygen gradient (82, 83). Thus, dietary fiber and carbohydrates can affect SCFA degradation while altering the abundance of the associated microbes. Taking together, diets rich in fiber might provide benefits to the intestine, as well as overall health.

Phytoestrogens

Phytoestrogens are plant-derived polyphenols that interact with estrogen receptors with either agonist or antagonist actions. A large majority of polyphenols are delivered to the gut, given their 1% bioavailability (57). The protective effects of plant polyphenols, particularly their anti-cancer, anti-inflammatory, and antioxidant effects, and their association with decreased risks of cardiovascular disease, obesity, diabetes, osteoporosis, and amyloid formation have been observed in humans (84–86). Increasing evidence shows that these effects are reached after bioactivation of the polyphenols by the gut microbiota (87, 88). Even though plant polyphenols have protective effects on human health, especially in the bioactive form, there is still a possibility of adverse effects due to their complexity of action and potential inter-individual variability (89).

While not all types of microbes participating in polyphenol metabolism are yet known, it has recently been shown that *Bifidobacterium*, *Lactobacillus* sp., *Coriobacteriaceae*, *Clostridium* sp., *Bacteroides*, and *Saccharomyces* yeast, are involved in the process of converting polyphenols to equol, urolithins, and enterolignans (74, 88). The qualitative and quantitative proportions of urolithins and equol produced correlate positively with the effects of phytoestrogens (88). Other bacterial species, such as *Coriobacteriaceae* and *Eubacterium,* are responsible for different polyphenol transformations (88).

The interaction of polyphenols and gut microbiota is bidirectional (90, 91). The gut bacteria produce microbial metabolites from polyphenols, which in turn serve as prebiotics for the gut bacteria. These metabolites, particularly urolithins, promote the growth of *Lactobacillus* and *Bifidobacterium*(88).

Vitamins

Gut microbiota are crucial for adequate vitamin levels in the human body. Menaquinone, folate, cobalamin, and riboflavin (ie: vitamins K, B9, and B2) are produced by gut microbes (25). Different bacteria have biosynthetic properties for different vitamins, such as *Bifidobacterium* for vitamins K, B_{12}, biotin, folate, thiamine, *Bacillus subtilis* and *Escherichia coli* for riboflavin (92), and *Lactobacillus* for cobalamin and other B vitamins (93). The pathway analysis of the predicted metagenomes showed an enrichment of folate biosynthesis in vegans compared with omnivores (77).

Isothiocyanates

Isothiocyanates are compounds from glucosinolates, mainly found in plants, like cruciferous vegetables. *Escherichia coli*, certain *Bacteroides*, some *Enterococcus*, *Lactobacillus agilis*, certain *Peptostreptococcus* spp. and *Bifidobacterium* spp. metabolize glucosinolates to isothiocyanates, secreting their own myrosinase enzyme (94). These metabolites have cytoprotective and anti-oxidative effects through regulation of gene expression relating toneoplastic, atherosclerotic, and neurodegenerative processes (25).

Aryl-Hydrocarbon Receptor Ligands

Intestinal aryl-hydrocarbon receptor ligands are predominantly diet derived from plant food, specifically cruciferous vegetables. Through aryl-hydrocarbon receptors, the ligands act to promote intestinal immune function and gut homeostasis (95). Since aryl-hydrocarbon receptor ligands are gut microbiota-derived, any impairment to the gut microbiota, such as from a high-fat diet, can decrease aryl-hydrocarbon receptor ligands. In turn, this can cause gut inflammation and permeability and promote the development of metabolic syndrome, which can be improved by supplementation with a *Lactobacillus* strain (96). Additionally, a decrease in aryl-hydrocarbon receptors or ligands compromises the maintenance of intraepithelial lymphocytes and the control of the microbial load and composition, resulting in heightened immune activation and epithelial damage (95).

Secondary Bile Acids and Coprostanol

A separate group of postbiotics are cholesterol metabolites. Several bacterial strains, isolated from intestine or feces, are described to convert dietary or synthesized *de novo* cholesterol into coprostanol (97, 98), which is poorly absorbed by the human intestine. Thus, serum cholesterol in host is reduced, which decreases the risk of cardiovascular diseases. On the other hand, bile acids synthetized from cholesterol are converted by microbiota into secondary bile acids, found in different tissues and in feces. It is believed secondary bile acids are involved in the equilibrium of health/disease (73, 97). For example, they are associated with inflammatory bowel disease, liver and colon cancer.

Trimethylamine N-Oxide (TMAO)

Trimethylamine N-Oxide is a microbial metabolite believed to be associated with cardiovascular and neurological disorders. Carnitine and choline are the precursors of TMAO and are primarily found in foods of animal origin (eggs, beef, pork), with lower amounts found in beans and fish (99). Several microbial genera, like L-*Ruminococcus*, have been linked to the intake of animal proteins and fat and have been associated with TMAO levels (77). In general, meat intake appears to proliferate species of *Bacteroides*, *Alistipes, Ruminococcus, Clostridia,* and *Bilophila,* and decease *Bifidobacterium*. Higher TMAO levels have also been observed with red meat intake, increasing risk for cardiovascular disease and inflammatory bowel disease (54, 66). Vegetarians have a different gut microbiota composition than omnivores with a diminished capacity to produce trimethylamine (TMA), the precursor to TMAO. The plasma concentrations of TMAO appear to be similar in vegans and lacto-ovo-vegetarians (99, 100).

Lowering TMAO levels may be achieved through greater adherence to the Mediterranean diet, particularly a vegetarian one rich in fruits and vegetables (77, 100). Increased vegetable consumption reduces TMAO levels by reducing the enzymes responsible for converting TMA to TMAO and by remodeling the gut microbiota. The studies have shown TMAO production to decrease in vegetarians, which decreases their cardiovascular risk. To be objective, we have to mention a recent study, leaving a room for further analyses. Vegan fecal microbiota transplantation in metabolic syndrome patients resulted in significant changes in intestinal microbiota composition but failed to show changes in TMAO production. Authors explained that the 2-week follow-up was not a sufficient length of time to observe changes in TMAO production (101).

On average, twenty five percent of plasma metabolites are different between omnivores and vegans, suggesting a significant direct effect of diet on the host metabolome. No unique bacterial taxa have been significantly associated with individual metabolite levels after adjustment for multiple comparisons (102). These findings suggest that while inter-individual variability exists, dietary patterns significantly influence the microbial composition.

CONCLUSION

Current research indicates that diet is the essential factor for human gut microbiota composition, what in its turn is crucial for metabolizing nutrients into active for the host postbiotics. Up to date knowledge suggests that a plant-based diet may be an effective way to promote a diverse ecosystem of beneficial microbes that support overall health. Nonetheless, due to the complexity and inter-individual differences, further research is required to fully characterize the interactions between diet, the microbiome, and health outcomes.

AUTHOR CONTRIBUTIONS

AT and IB contributed to conception and writing of the manuscript, ER, WY, JA, NB, and HK contributed and critically revised the manuscript. All authors approved the final manuscript.

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SYSTEMATIC REVIEW
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Impact of Whole, Fresh Fruit Consumption on Energy Intake and Adiposity: A Systematic Review

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Background: The energy content of whole, fresh fruit derives primarily from simple sugars, which are currently under heightened scrutiny for their potential contribution to obesity and chronic disease risk. Yet fruit also has a relatively low energy density, moderate palatability/reward value, and high fiber content, which together may limit energy intake. Although reasoned arguments can be made that fruit is fattening or slimming, the question is best resolved empirically.

Methods: Methods were preregistered with PROSPERO (CRD42018111830). The primary outcome is the impact of whole, fresh fruit consumption on measures of adiposity including body weight in randomized controlled trials (RCTs). Secondary outcomes are the impact of whole, fresh fruit consumption on energy intake in RCTs, and the association between whole, fresh fruit consumption and changes in measures of adiposity in prospective observational studies. CENTRAL and PubMed databases were searched through October 2018. Cochrane risk of bias tool was used to assess risk of bias in RCTs, and the GRADE method was used to judge and convey the certainty of conclusions. Reporting follows PRISMA guidelines.

Results: RCTs, and particularly those of higher quality, suggest that increasing whole, fresh fruit consumption promotes weight maintenance or modest weight loss over periods of 3–24 weeks (moderate certainty), with limited evidence suggesting that a high intake of fruit favors weight loss among people with overweight or obesity. Consistent with this, single-meal RCTs suggest that consuming whole, fresh fruit tends to decrease energy intake, particularly when consumed prior to a meal or when displacing more energy-dense foods (moderate certainty). Prospective observational studies suggest that habitually higher fruit intake is not associated with weight change, or is associated with modest protection against weight gain, over five or more years.

Conclusions: Current evidence suggests that whole, fresh fruit consumption is unlikely to contribute to excess energy intake and adiposity, but rather has little effect on these outcomes or constrains them modestly. Single-meal RCTs, RCTs lasting 3–24 weeks, and long-term observational studies are relatively consistent in supporting this conclusion. Whole, fresh fruit probably does not contribute to obesity and may have a place in the prevention and management of excess adiposity.

Keywords: fruit, adiposity, body weight, obesity, energy intake, sugar

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INTRODUCTION

Rationale

Worldwide, the total *per capita* burden of disease continues to decline, but it does not do so uniformly. Technological and economic progress have substantially relieved the ancient burdens of starvation, infectious disease, and accidents, yet they have ushered in a new era of non-communicable disorders such as obesity, diabetes, and coronary heart disease (1, 2). A key contributor to these disorders is the overconsumption of energy and accumulation of excess adipose tissue (3–6).

Sucrose and other simple sugars with a sweet taste, henceforth "sugar," have long been suspected as a culprit in energy overconsumption and excess adiposity. In 1980, the United States Department of Agriculture Dietary Guidelines advised the public to "avoid too much sugar" and, as part of a four-point plan for reducing weight, "eat less sugar and sweets" (7). Yet scrutiny of sugar has intensified recently, both within the scientific community and outside it, with certain researchers and popular writers arguing that sugar is a particularly potent driver of obesity and non-communicable disease risk (8–10). Observational and experimental findings indeed suggest that in sufficient quantity, refined sugar can increase energy intake and adiposity (11, 12). The energy content of sweet fruits is primarily in the form of sugar. If sugar is a particularly potent driver of obesity and non-communicable disease risk, this raises the possibility that even whole, fresh fruit may have similar effects, and that conventional advice to increase fruit consumption may be misguided.

On the other hand, fruit differs from refined sugar-containing foods in important respects, such as its lower energy density, lower palatability/reward value, higher fiber content, and higher concentration of essential and non-essential micronutrients. Some of these properties are expected to limit energy intake and adiposity, and together they may render fruit slimming relative to other commonly-eaten foods. Furthermore, the human evolutionary lineage has likely consumed substantial quantities of fruit for tens of millions of years prior to the emergence of obesity and cardiometabolic disease as common health problems, suggesting that it is unlikely to be a major contributor to these conditions (13).

Although reasoned arguments can be made that fruit is fattening or slimming, the question is best resolved empirically. Previous reviews have addressed similar topics (14–17), concluding that fruit aids in the prevention of excess energy intake, is unlikely to increase adiposity, and may reduce adiposity. However, the current review is the most recent to comprehensively review the randomized controlled trial (RCT) and prospective observational literature on whole, fresh fruit intake specifically. Further, it employs best-practice systematic review methods including detailed preregistration of methods with a well-defined search strategy, adherence to PRISMA reporting guidelines, assessment of study bias using the Cochrane risk of bias tool, and assessment of certainty of conclusions using the GRADE method.

Objectives

The objective of this review is to systematically review the randomized controlled trial and prospective observational research literature on the impact of whole, fresh fruit consumption on energy intake and measures of adiposity, and synthesize available studies to form overall conclusions. All studies involving human subjects are considered, and RCTs must report between-group comparisons that isolate differences in whole, fresh fruit consumption as a variable.

Research Question

What is the impact of whole, fresh fruit consumption on energy intake and adiposity?

METHODS

Protocol and Registration

A protocol for this review was preregistered in the PROSPERO systematic review registry prior to initiating literature searches (CRD42018111830)[1], with the exception of brief preliminary searches used to develop the search method. The primary outcome is the impact of whole, fresh fruit consumption on measures of adiposity, as measured by RCTs. A secondary outcome is the impact of whole, fresh fruit consumption on energy intake, as measured by RCTs. An additional secondary outcome is the association of whole, fresh fruit intake with changes of measures of adiposity, as measured by prospective observational studies.

Eligibility Criteria

"Fruit" is defined in the common/culinary sense of a sweet, seed-bearing plant tissue. Fruit varieties that lack seeds are included. "Whole" and "fresh" denote fruit that has not been significantly processed, i.e., raw and whole rather than cooked, pureed, dried, juiced, or powdered. Raw fruit that has been peeled or cut into bite-sized pieces is included. "Change in adiposity" is defined as change in body weight and/or other direct or indirect measures of body fatness, including but not limited to body fat percentage, body mass index, and waist circumference. "RCT" is defined as a study that assigns subjects to different intervention conditions using randomization or pseudorandomization, such that outcomes can be compared between randomized groups. "Prospective observational study" is defined as a non-intervention study that collects data on exposure variables at an earlier time point, and outcomes at a later time point, and reports the association between the two.

Studies were required to be (1) RCTs on the impact of whole, fresh fruit consumption on measures of adiposity including body weight, (2) RCTs on the impact of whole, fresh fruit consumption on energy intake, or (3) prospective observational studies on the association between fruit consumption and body weight and/or adiposity. Only published studies were considered. No language restrictions were applied. All RCTs and prospective observational studies conducted in humans and published through October 2018 were considered.

To be eligible, RCTs must include a between-group difference in fruit intake. The experimental design must isolate between-group differences in fruit intake as a variable, without substantial concurrent between-group differences in other variables such

[1] https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=111830

as vegetable intake. An equivalent non-fruit intervention in a comparison group, such as increased nut intake, is permitted. Energy intake of diets must not be strictly controlled to permit differences in energy intake and adiposity to arise. RCTs must report differences in measures of adiposity, or energy intake, between groups, or such differences must be calculable from data available in the manuscript. Changes in weight and/or adiposity must be measured with a minimum of 2 weeks between baseline and end line to allow meaningful differences in adiposity to emerge, while energy intake RCTs can represent any duration.

For observational outcomes, studies must be prospective observational studies that report the association between the consumption of fresh, whole fruit consumption and subsequent changes in measures of adiposity including body weight. During the course of study selection, the author felt it was appropriate to add one exclusion criterion that was not prespecified: observational studies were excluded if they were potentially confounded by a concurrent intervention. For example, an observational study that reports the association between fruit intake and weight change may be confounded if it is conducted in subjects that received advice to increase fruit intake as part of a weight loss intervention. This criterion excluded four studies, whose findings are broadly similar to those that met inclusion criteria (18–21).

Search Strategy

The search strategy was designed in collaboration with Ben Harnke, Education and Reference Librarian, the University of Colorado Health Sciences Library. RCT searches were conducted in the Cochrane controlled register of trials (CENTRAL), and prospective observational study searches were conducted in PubMed. CENTRAL compiles RCTs from multiple sources

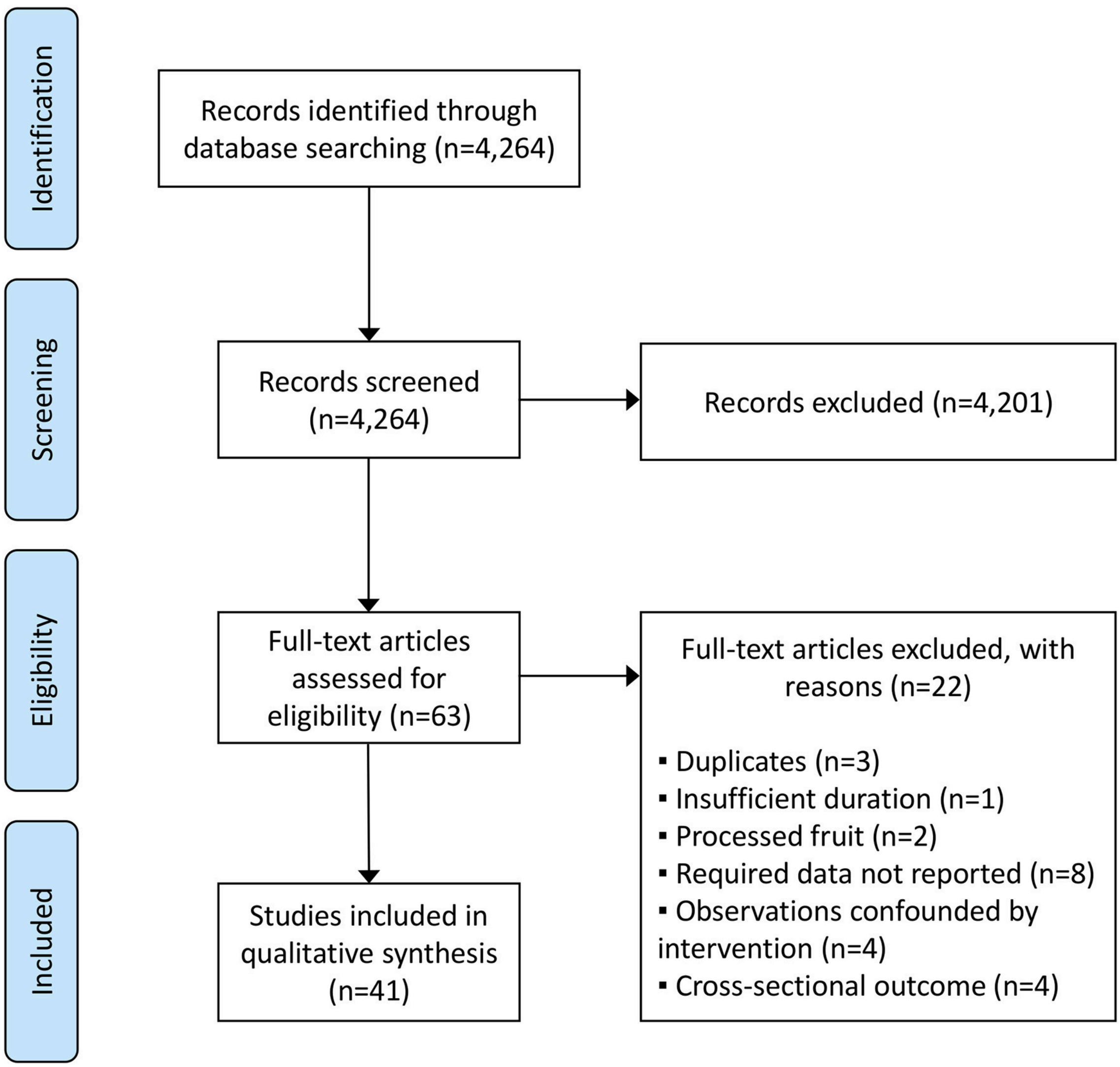

FIGURE 1 | PRISMA flow diagram summarizing the study identification and selection process.

TABLE 1 | Primary outcome: impact of whole, fresh fruit consumption on measures of adiposity in RCTs.

Trial	Subjects	Intervention	Duration	Weight difference	Adiposity difference	Notes
Singh et al. (24)	Men and women with essential hypertension. 131 randomized; 66 guava, 65 control.	0.5–1.0 kg of fresh guava fruit per day before meals vs. no intervention	12 weeks	−0.4 kg (p = NS)	Not reported	No power calculation. Not preregistered. Subjects were asked to maintain weight.
Singh et al. (25)	Men and women with essential hypertension and mild hypercholesterolemia. 101 randomized; 52 guava; 49 control.	0.5–1.0 kg of fresh guava fruit per day before meals vs. no intervention	24 weeks	−0.1 kg (p = NS)	Not reported	No power calculation. Not preregistered.
de Oliveira et al. (26)	Hypercholesterolemic, overweight, non-smoking women, 30–50 years of age. 49 randomized; number in each group unclear.	3 apples, pears, or oat cookies per day. Hypocaloric diet for all subjects.	12 weeks	−0.33 kg ($p = 0.003$)	Not reported	No power calculation. Not preregistered. May have pooled two fruit groups that were individually randomized.
Rodriguez et al. (27)	Women with obesity. 15 randomized; 8 low-fruit; 7 high-fruit.	Low-fruit diet vs. high-fruit diet (goal of 5 vs. 15% of kcal from fructose). Hypocaloric diet for all subjects.	8 weeks	+0.3 kg ($p = 0.781$)	−0.4% BF ($p = 0.231$); −3.1 cm WC ($p = 0.048$); 0.01 WHR ($p = 0.395$)	No power calculation. Not preregistered. Very small sample size.
Fujioka et al. (28)	Men and women with obesity. 91 randomized; 24 grapefruit capsules + apple juice; 22 placebo + apple juice; 21 placebo + grapefruit juice; 24 placebo + fresh grapefruit.	3x a day prior to meals: Group A, Grapefruit capsules + 7 oz apple juice; Group B, placebo capsules + 7 oz apple juice; Group C placebo capsules + 8 oz grapefruit juice; Group D placebo capsules + half a fresh grapefruit. 20–30 min of walking 3–4X per week for all groups.	12 weeks	−1.4 kg vs. group B ($p = 0.048$); −0.1 kg vs. group C (p = NS)	NS difference in % BF (impedance)	Performed a power calculation. Not preregistered.
Rush et al. (29)	Healthy men and women. 12 randomized; 6 kiwi; 6 control.	One kiwi fruit per 30 kg body weight vs. no kiwi fruit. General diet advice and a pedometer for all subjects.	3 weeks (fruit intervention)	+0.9 kg (p = NS)	Not reported	No power calculation. Not preregistered. Very small sample size.
Madero et al. (30)	Men and women with overweight or obesity. 131 randomized; 66 low-fructose diet; 65 natural fructose diet.	Low-fructose diet (<20 g/d) vs. moderate natural-fructose diet (50–70 g/d mostly from fruit). Hypocaloric diet for all subjects.	6 weeks	−1.36 ($p = 0.02$)	−0.8% BF ($p = 0.1$); −0.15 WHR ($p = 0.41$)	Performed a power calculation. Preregistered with anthropometric changes as primary outcome (NCT00868673).
Dow et al. (31)	Men and premenopausal women with overweight or obesity. 74 randomized; 32 control; 42 grapefruit.	Half a fresh grapefruit with each meal (3X per d) vs. no intervention. All subjects were assigned a baseline diet low in fruit and vegetables.	6 weeks	−0.5 ($p = 0.119$)	0.55% BF ($p = 0.337$, impedance); −1.22 cm WC ($p = 0.062$); 0.0 WHR ($p = 0.470$)	Performed a power calculation. Preregistered with weight change as primary outcome (NCT01452841).

(Continued)

TABLE 1 | Continued

Trial	Subjects	Intervention	Duration	Weight difference	Adiposity difference	Notes
Ravn-Haren et al. (32)	Healthy men and women. 34 randomized; number per group unclear.	550 g/d whole apple; 22 mg/d apple pomace; 500 mL/d cloudy apple juice; 500 mL/d clear apple juice; no intervention. Baseline diet was low in polyphenols and pectin.	4 weeks	+0.15 (p = NS) vs. no intervention; −0.07 vs. pomace (p = NS); 0.42 vs. cloudy juice (p = NS); −0.36 vs. clear juice (p = NS).	0.0 WHR vs. no intervention (p = NS); 0.003 WHR vs. pomace (p = NS); −0.008 WHR vs. cloudy juice (p = NS); −0.005 WHR vs. clear juice (p = NS).	No power calculation. Not preregistered. Very small sample size for number of groups. High dropout rate.
Agebratt et al. (33)	Healthy non-obese men and women. 30 randomized; 15 fruit; 15 nuts.	Diet supplementation with 7 kcal/kg body weight/d of fruit vs. nuts.	8 weeks	+0.03 kg (p = 0.95)	−0.2 cm SAD (p = 0.91); −0.08 l VF (p = 0.16)	Performed a power calculation but based on a hepatic fat outcome. Preregistered but not for anthropometric outcomes (NCT02227511). Registry page is sparse.
Kumari et al. (34)	Men and women 18–25 years old. 45 randomized; 15 guava with peel; 15 peeled guava; 15 no intervention.	400 g guava with skin per day (group A) vs. 400 g peeled guava per day (group B) vs. no intervention (group C)	6 weeks	−2.0 kg (A vs. C; p = NR); −2.6 kg (B vs. C; p = NR)	Not reported	No power calculation. Not preregistered.

Randomized controlled trials reporting the impact of whole, fresh fruit consumption on body weight and adiposity. Weight and adiposity differences represent changes in the fruit intervention group relative to the comparator group. Additional strengths and limitations of study design are listed in the "notes" column. NS, not statistically significant ($p > 0.05$). NR, not reported; BF, body fat; WC, waist circumference; WHR, waist-to-hip ratio; SAD, sagittal abdominal diameter; VF, visceral fat.

including PubMed, Embase, Clinicaltrials.gov, handsearches, and other biomedical resources. Brief preliminary searches were used to develop the search method by verifying that studies identified in previous review papers were present (14–16). Formal searches were conducted in November 2018, following preregistration.

The adiposity RCT search employed the following search terms in CENTRAL: ((Fruit OR fruits):ti,ab OR [mh Fruit]) AND ((weight OR "body mass index" OR BMI OR "waist circumference" OR "body fat" OR adiposity OR overweight OR obes* OR leanness OR Overnutrition OR "over nutrition"):ti,ab OR [mh "Body Weight"] OR [mh "Body Weight Changes"] OR [mh "Overweight"] OR [mh "Thinness"] OR [mh "Body Fat Distribution"]).

The energy intake RCT search employed the following search terms in CENTRAL: ((Fruit OR fruits):ti,ab OR [mh Fruit]) AND (("energy" OR calorie* OR caloric OR kilocalorie* OR kcal OR "joule" OR kilojoule OR kJ):tiab) AND ((intake OR consum* OR ingest* OR ate OR eat OR eating):ti,ab OR [mh "Energy Intake"] OR [mh Eating]).

The prospective observational study search employed the following search terms in PubMed, in addition to the "observational study" publication type filter: (Fruit[tiab] OR fruits[tiab] OR "Fruit"[Mesh:NoExp]) AND (weight[tiab] OR "body mass index"[tiab] OR BMI[tiab] OR "waist circumference"[tiab] OR "body fat"[tiab] OR adiposity[tiab] OR overweight[tiab] OR obes*[tiab] OR leanness[tiab] OR Overnutrition[tiab] OR "over nutrition"[tiab] OR "Body Weight"[Mesh:NoExp] OR "Body Weight Changes"[Mesh] OR "Overweight"[Mesh] OR "Thinness"[Mesh] OR "Body Fat Distribution"[Mesh]) AND (prospective*[tiab] OR cohort[tiab] OR longitudinal[tiab] OR follow up stud*[tiab] OR followup stud*[tiab] OR incidence stud*[tiab] OR "Cohort Studies"[Mesh]).

In addition, previous review papers on fruit, energy intake, and adiposity were hand searched for relevant studies (14–16).

Study Selection

After search records were identified, SG examined titles and abstracts for studies that met inclusion criteria. Potential candidates were compiled in Excel spreadsheets for examination of full text. SG then examined the full text of each study to verify that inclusion criteria were satisfied, resulting in the exclusion of some studies.

Data Collection

Data were extracted from studies that met inclusion criteria into Excel spreadsheets and Cochrane Review Manager 5.3. Data presented in manuscripts were taken at face value and authors were not contacted for additional information. For RCTs, the following data were extracted: first author, year of publication, number and characteristics of subjects, summary of intervention, duration of intervention, between-group difference in weight with statistical significance, between-group difference in other measures of adiposity with statistical significance, Cochrane risk of bias assessment (based on random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting), additional noteworthy study features. When not directly reported, between-group differences in measures of adiposity and energy intake were calculated based on available data whenever possible.

For observational studies, the following data were extracted: first author, year, number and characteristics of subjects, measure of fruit intake, length of follow-up, covariates accounted for, association between fruit consumption and weight change with statistical significance, association between fruit consumption and other measures of adiposity with statistical significance, additional noteworthy study features. Data from the most adjusted model that does not include energy intake as a covariate were generally preferred, since energy intake is a mechanism by which fruit intake may impact body weight. If an unadjusted or minimally adjusted model was the only one available without energy intake as a covariate, the most adjusted model was selected.

Body mass index measures were only collected if body weight was not reported, since the two measures are redundant in adults who have achieved their final height.

Risk of Bias

Risk of bas was estimated using the Cochrane risk of bias tool for individual studies, according to Cochrane guidelines[2] and using Cochrane Review Manager 5.3, and GRADE was used to judge and communicate the certainty of conclusions for each outcome[3] Risk of bias score was used as a criterion to weight the informativeness of each study when synthesizing conclusions, and GRADE was used to judge and communicate the certainty of each conclusion. Risk of bias graphs and summaries were created using Cochrane Review Manager 5.3.

Synthesis of Results

This review employed a narrative synthesis method whereby study quality was evaluated and conclusions were informally weighted according to study quality (informal, i.e., a quantitative method was not applied to weight the informativeness of individual studies). Among the three outcomes considered, the preregistered primary outcome was assigned the highest weight. Reporting follows PRISMA guidelines (22). Quantitative meta-analysis of RCTs was judged to be suboptimal in this context, given the limited number of studies identified, the fact that the study pool would have been further narrowed by applying more stringent meta-analysis inclusion criteria, and widely varying study methods and quality. Although meta-analyses are commonly performed on fewer than ten studies, typical methods for accomplishing this do not adequately control the false positive rate, particularly in the context of high heterogeneity (23). Finally, given the high risk of bias of most studies identified, the author believes it is more informative to focus on high-quality trials than to pool their findings with less informative trials.

[2]https://handbook-5-1.cochrane.org/chapter_8/8_assessing_risk_of_bias_in_included_studies.htm

[3]https://handbook-5-1.cochrane.org/chapter_12/12_2_1_the_grade_approach.htm.

RESULTS

Study Selection and Characteristics

Figure 1 summarizes the search and study selection process using the PRISMA flow diagram. 4,264 records were identified in database searches, 1,671 for body weight RCTs, 848 for energy intake RCTs, and 1,745 for prospective observational studies. 4,201 records were excluded on the basis of title and abstract contents, leaving 63 potentially eligible studies; 16 body weight RCTs, 10 energy intake RCTs, and 37 observational studies. Many studies were excluded on first pass because they did not isolate whole, fresh fruit intake as a variable; for example, they reported associations between combined fruit/vegetable intake and weight outcomes. Upon inspection of full-text manuscripts, 41 studies met inclusion criteria; 11 body weight RCTs, 5 energy intake RCTs, and 25 observational studies. Reasons for exclusion were that studies were duplicates ($n = 3$), interventions were not long enough to meet inclusion criteria ($n = 1$), interventions involved processed rather than whole, fresh fruit ($n = 2$), studies did not report data that are pertinent to this review ($n = 8$), observational studies were potentially confounded by a concurrent intervention ($n = 4$), and observational studies reported data in a cross-sectional rather than prospective manner ($n = 4$).

RCTs reporting the impact of whole, fresh fruit consumption on body weight and adiposity were published between 1992 and 2016 and are summarized in **Table 1**. Of the 11 RCTs identified, two included fewer than 20 subjects, four included 21–50 subjects, two included 51–100 subjects, and three included more than 100 subjects. Interventions represented a variety of fruit types, but guava ($n = 3$), apple ($n = 2$), and grapefruit ($n = 2$) were named more commonly than other fruit. The quantity of fruit varied widely, with the highest intake representing a goal of 15 percent of energy intake from fruit-derived fructose, which implies ∼30 percent of total energy intake from fruit. Interventions lasted 3–24 weeks, with 6 and 12 weeks being most common. All RCTs reported weight changes, and six also reported changes in other measures of adiposity. Four trials reported performing a power calculation as part of trial design, and three trials were preregistered, of which two were preregistered for an adiposity-related outcome.

RCTs reporting the impact of whole, fresh fruit consumption on energy intake were published between 2003 and 2016 and are summarized in **Table 2**. Of the 5 RCTs identified, two included fewer than 20 subjects, two included 21–50 subjects, and one included more than 50 subjects. Interventions represented several fruit types, but apple ($n = 2$) was named more commonly than other fruit. As in the body weight RCTs (some of which also met inclusion criteria for energy intake), the quantity of fruit varied widely, with the highest intake representing a goal of 15 percent of calorie intake from fruit-derived fructose, which implies ∼30 percent of total energy intake from fruit. Interventions lasted between one meal and 12 weeks, with single-meal and 8-week trials being the most common. Three trials used self-report methods to measure energy intake, two of which used a 3-day weighed food record; food intake in the other two trials was directly measured by investigators. The latter two trials were single-meal studies. Three trials reported performing a power

TABLE 2 | Secondary outcome: impact of whole, fresh fruit consumption on energy intake in RCTs.

Trial	Subjects	Intervention	Duration	Energy intake difference	Measurement method	Notes
de Oliveira et al. (26)	Hypercholesterolemic, overweight, non-smoking women, 30 to 50 years of age. 49 randomized; number in each group unclear.	3 apples, pears, or oat cookies per day. Hypocaloric diet for all subjects.	12 weeks	−22 kcal (p = NR) daily	Food frequency questionnaire and 3-day dietary record	No power calculation. Not preregistered. No between-group p-value provided for EI. May have pooled two fruit groups that were individually randomized.
Rodriguez et al. (27)	Women with obesity. 15 randomized; 8 low-fruit; 7 high-fruit.	Low-fruit diet vs. high-fruit diet (goal of 5 vs. 15% of kcal from fructose). Hypocaloric diet for all subjects.	8 weeks	+47 kcal (p = NS) daily	3-day dietary record	No power calculation. Not preregistered. Very small sample size
Flood-Obbagy and Rolls (35)	Men and women 18–45 years old. 59 randomized; crossover design.	Isocaloric preload with apple, apple sauce, apple juice with added fiber, apple juice, or no preload. Followed by an *ad libitum* test meal.	Single meal	−187 kcal vs. no preload ($p < 0.0001$); −91 kcal vs. apple sauce ($p < 0.02$); −152 kcal vs. apple juice with fiber ($p < 0.02$); −178 kcal vs. apple juice ($p < 0.02$). Figures represent total meal energy intake including preload.	Weighed by investigators	Performed a power calculation. Not preregistered.
James et al. (36)	Healthy pre-menopausal women. 12 randomized; crossover design.	Isocaloric mixed berries vs. confectionary snack, followed by an *ad libitum* test meal.	Single meal	−134 kcal ($p < 0.001$)	Weighed by investigators	Performed a power calculation. Not preregistered.
Agebratt et al. (33)	Healthy non-obese men and women. 30 randomized; 15 fruit; 15 nuts.	Diet supplementation with 7 kcal/kg body weight/d of fruit vs. nuts.	8 weeks	−216 kcal ($p = 0.37$)	3-day weighed dietary record	Performed a power calculation but based on hepatic fat outcome. Preregistered but not for energy intake (NCT02227511). Registry page is sparse.

Randomized controlled trials reporting the impact of whole, fresh fruit consumption on energy intake. Energy intake differences represent the fruit intervention group relative to the comparator group. Additional strengths and limitations of study design are listed in the "notes" column. NS, not statistically significant ($p > 0.05$); NR, not reported.

calculation as part of study design, although one was powered for a hepatic fat outcome. Only one trial was preregistered, also for a hepatic fat outcome.

Prospective observational studies reporting the impact of whole, fresh fruit consumption on body weight and adiposity change were published between 2002 and 2018 and are summarized in **Table 3**. Of the 25 studies identified, seven included fewer than 1,000 subjects, six included 1,000–10,000 subjects, seven included 10,001–50,000 subjects, and five included more than 50,000 subjects. Follow-up length ranged from 6 months to 24 years. Eleven studies reported weight changes, and 18 reported changes in other measures of adiposity. None were preregistered, and only one applied an adjustment for multiple comparisons (Bonferroni correction) to control family-wise error rate when testing several hypotheses using a single data set.

Risk of Bias

Figure 2 presents the Cochrane risk of bias graph, and **Figure 3** presents the Cochrane risk of bias summary for RCTs reporting the impact of whole, fresh fruit consumption on body weight and other measures of adiposity. Six of 11 included trials are at an unclear risk of bias from random sequence generation and allocation concealment due to incomplete description of the randomization process, while the other five are at a low risk of bias. All 11 trials are at a high risk of bias due to lack of blinding of participants, an unavoidable consequence of including whole, fresh fruit consumption as an intervention. This leaves open the possibility that part of the impact of fruit observed in these trials is attributable to placebo effects. The risk of bias due to blinding of outcome assessment is low in all 11 trials, not because investigators were consistently blinded to treatment assignment, but because there is a low risk of bias in measuring body weight due to its simple and objective nature. Nine of 11 trials had a low risk of attrition bias due to low dropout rates, while the other two were at high risk due to high dropout rates. Nine of 11 trials were at an unclear risk of selective reporting bias due to insufficient information about initial study design resulting from a lack of detailed preregistration information, while the other two were at low risk due to detailed preregistration. The risk of other bias was fairly evenly split between low ($n = 3$), unclear ($n = 4$), and high ($n = 4$) risk of bias. The two studies with a low risk of other bias were scored as such because they offered detailed preregistration information with primary outcomes relevant to adiposity.

Body weight RCTs that scored most favorably on the Cochrane risk of bias tool are Madero et al. (30), Dow et al. (31), Agebratt et al. (33), Singh (25), Singh et al. (24), and Kumari et al. (34) (**Figure 3**). Each received a score of "low risk" in four or five of seven domains.

Figure 4 presents the Cochrane risk of bias graph, and **Figure 5** presents the Cochrane risk of bias summary for RCTs reporting the impact of whole, fresh fruit consumption on energy intake. This literature fared more poorly in risk of bias scoring than the body weight RCT literature. All energy intake RCTs are at an unclear risk of bias from random sequence generation, and four of five are at an unclear risk of bias from allocation concealment. This is due to incomplete description of the randomization process. For the same reason as the body weight RCTs, all energy intake RCTs are at a high risk of bias due to lack of blinding of participants, leaving open the possibility of placebo effects. The risk of bias due to blinding of outcome assessment is high in three of five energy intake RCTs due to reliance on self-reported measures of energy intake, and low in two of five due to directly measured energy intake. Four of five trials had a low risk of attrition bias due to low dropout rates. All trials were at an unclear risk of selective reporting bias due to insufficient information about initial study design resulting from a lack of detailed preregistration information. The risk of other bias was unclear in four of five trials due to insufficient information.

Energy intake RCTs that scored most favorably on the Cochrane risk of bias tool are Flood-Obbagy and Rolls (35), James et al. (36), and Agebratt et al. (33) (**Figure 5**), although none received a score of "low risk" in more than two of seven domains.

Although observational studies were not assessed using the Cochrane risk of bias tool, features that are informative of bias risk will nevertheless be considered here. All observational studies included in this review used self-report methods to measure fruit intake, most commonly food frequency questionnaires (**Table 3**). This introduces a substantial source of error that may also introduce an unknown degree of bias. Validation studies suggest that the Pearson correlation coefficient between fruit intake measured by food frequency questionnaires and 7-day weighed food record is 0.50–0.67, implying that 65–75 percent (R^2) of the variability in fruit intake identified by 7-day weighed food record is not captured by food frequency questionnaire (62). Although it has been argued that self-report error is randomly distributed and should not bias associations, the author is uncertain to what extent this argument is correct, and in which contexts.

Observational studies are inherently more limited than RCTs as tools for causal inference because relationships between exposure and outcome variables may be confounded by other variables. For example, between-person variation in fruit and vegetable intake has a genetic component and "individuals genetically predisposed to low fruit and vegetable consumption may be predisposed to higher [body mass index]," suggesting a possible source of bias that could be both important and difficult to correct (63). For this reason among others, observational relationships between fruit intake and body weight changes can be difficult to interpret. Most of the studies represented in **Table 3** adjusted extensively for confounding variables in an attempt to limit confounding bias. However, it is difficult to be certain that the most impactful confounding variables were measured and appropriately incorporated into multivariate models.

In addition, it is difficult to be certain that observational relationships were not overadjusted, attenuating the measured association between fruit intake and body weight/adiposity outcomes. In this regard, it is notable that many studies adjusted for energy intake, which seems suboptimal for the purposes of this review since modified energy intake may be a key intermediate variable between fruit intake and body weight/adiposity outcomes.

TABLE 3 | Secondary outcome: association of whole, fresh fruit intake with changes in measures of adiposity in prospective observational studies.

Study	Subjects	Intake measure	Follow-up length	Weight change	Adiposity change	Covariates	Notes
Schulz et al. (37)	17,369 German non-smoking men and women	Food frequency questionnaire	2 years	Not reported	Men: OR per 100 g daily intake for small wt gain 1.04 (p = NS); OR for large wt gain 0.94 (p = NS); OR for small wt loss 1.05 (p = NS); OR for large wt loss 1.03 (p = NS). Women: OR per 100 g intake for small wt gain, 0.94 (p = NS); OR for large wt gain 0.94 (p = NS); OR for small wt loss 1.01 (p = NS); OR for large wt loss 1.03 (p = NS).	Age, initial body weight and height, education, weight history, medication, menopause, life and health contentment, dietary change, physical activity, prevalent diabetes and thyroid disease	Not adjusted for multiple comparisons. Not preregistered.
Field et al. (38)	14,918 US boys and girls ages 9–14	Food frequency questionnaire	3 years	Not reported	Boys: 0.0 SD BMI z-score (p = NS); Girls: 0.0 SD BMI z-score (p = NS)	Age, age squared, Tanner stage, height change, baseline weight, physical activity, and inactivity	Not adjusted for multiple comparisons. Not preregistered.
Newby et al. (39)	1,379 US boys and girls ages 2–5	Food frequency questionnaire	6–12 months	+0.02 kg/year per daily serving ($p = 0.41$)	Not reported	Age, sex, ethnicity, residence, level of poverty, maternal education, birth weight, food groups	Not adjusted for multiple comparisons. Not preregistered.
Drapeau et al. (40)	248 Canadian men and women	3-day dietary record	6 years	−0.18 kg per 1 percent increase in fruit intake ($p = 0.03$)	−0.16 % BF per 1 percent increase in fruit intake ($p = 0.01$); −0.19 cm waist circumference per 1 percent increase in fruit intake ($p = 0.03$); −1.05 mm sum of 6 skinfold thicknesses per 1 percent increase in fruit intake ($p = 0.01$)	Age, baseline body weight, or adiposity indicators, changes in daily physical activity level	Not adjusted for multiple comparisons. Not preregistered.
He et al. (41)	74,063 US female health professionals ages 38–63	Food frequency questionnaire	12 years	Not reported	OR for obesity 0.76 in highest vs. lowest quintile of fruit intake change ($p = 0.0007$); OR for major weight gain 0.73 in highest vs. lowest quintile of fruit intake change ($p = 0.03$)	Age, year of follow-up, change in physical activity, change in cigarette smoking status, changes in alcohol consumption and caffeine intake, change in use of hormone replacement therapy, changes in energy-adjusted intakes of saturated fat, polyunsaturated fat, monounsaturated fat, trans-unsaturated fatty acid, protein, total energy, baseline BMI	Not adjusted for multiple comparisons. Not preregistered.
Koh-Banerjee et al. (42)	27,082 US male health professionals ages 40–75	Food frequency questionnaire	8 years	−2.51 kg per 20 g/d increase in fruit fiber ($p < 0.001$)	Not reported	Age, baseline fruit intake, smoking, baseline weight, and baseline values and changes in refined grains, calories, total physical activity, alcohol, protein, and trans, saturated, monounsaturated, and polyunsaturated fats	Not adjusted for multiple comparisons. Not preregistered.
Nooyens et al. (43)	288 Dutch men ages 50–65 years	Food frequency questionnaire	5 years	−0.02 kg/year per serving increase of fruit per week ($p = 0.03$)	−0.03 cm/year WC per serving increase of fruit per week ($p < 0.01$)	Retirement status, type of job, interaction between retirement and type of job, age, smoking, baseline fruit intake, physical activity, intake of potatoes, breakfast, sugar-sweetened beverages, fiber density	Not adjusted for multiple comparisons. Not preregistered.
Sanchez-Villegas et al. (44)	6,319 Spanish male and female university graduates	Food frequency questionnaire	28 months	−0.09 kg in highest vs. lowest tertile of fruit intake ($p = 0.46$)	Not reported	Age, sex, baseline BMI, smoking, physical activity, alcohol consumption, energy, intake, change in dietary habits, physical activity, intake of cereals, vegetables, legumes, fish, nuts, meat, full-fat dairy, olive oil, red wine	Not adjusted for multiple comparisons. Not preregistered.

(Continued)

TABLE 3 | Continued

Study	Subjects	Intake measure	Follow-up length	Weight change	Adiposity change	Covariates	Notes
te Velde et al. (45)	168 Dutch men and women	Dietary history interview	24 years	Not reported	−0.71 BMI units in highest vs. lowest quartile of fruit intake (p = NS); −0.16 mm sum of 6 skinfolds in highest vs. lowest quartile of fruit intake (p = NS)	Sex, bone age at 13 years, total energy intake, physical activity, tobacco use, fiber intake	Not adjusted for multiple comparisons. Not preregistered.
Vioque et al. (46)	206 Spanish men and women ages 15–80	Food frequency questionnaire	10 years	Not reported	OR 0.62 for weight gain >3.41 kg over 10 years in highest vs. lowest quartile of fruit intake ($p = 0.211$)	Sex, age, educational level, BMI, time spent watching TV, presence of disease, baseline height, energy intake, energy-adjusted intakes of protein, saturated fat, monounsaturated fat, polyunsaturated, fiber, caffeine, alcohol	Not adjusted for multiple comparisons. Not preregistered.
Buijsse et al. (47)	89,432 Danish, German, UK, Italian, and Dutch men and women	Food frequency questionnaire	6.5 years	−0.016 kg/y per additional 100 g fruit intake ($p < 0.05$)	Not reported	Age, sex, cohort, product term UK-Nor X fruit/vegetable intake, years of follow-up, baseline weight, baseline height, change in smoking status, baseline physical activity (dummy variables), education, alcohol intake, postmenopausal status, postmenopausal hormone use	Not adjusted for multiple comparisons. Not preregistered.
Halkjaer et al. (48)	42,696 Danish men and women ages 50–64	Food frequency questionnaire	5 years	Not reported	Men: −0.07 cm WC per additional 60 kcal/d fruit intake ($p < 0.001$). Women: −0.10 cm WC per additional 60 kcal/d fruit intake ($p < 0.07$).	Baseline waist circumference, body mass index, age, smoking, sport, hours of sport, energy intake from wine, beer, and spirits, baseline intake of 21 food and beverage groups	Not adjusted for multiple comparisons. Not preregistered.
Berz et al. (49)	2,327 US girls age 9 years	3-day dietary record	10 years	Not reported	−2.1 BMI units in highest vs. lowest fruit intake ($p < 0.001$)	Race, height, SES, physical activity level, television viewing and video game playing, and total energy	Not adjusted for multiple comparisons. Not preregistered.
Mozaffarian et al. (50)	120,877 US male and female health professionals	Food frequency questionnaire	12–20 years	−0.22 kg per 4-year period per 1-serving increase ($p < 0.001$)	Not reported	Age, baseline BMI, sleep duration, changes in physical activity, alcohol use, television watching, smoking, vegetables, nuts, dairy, potatoes, grains, sugar-sweetened beverages, fruit juice, diet beverages, sweets, meats, trans fat, fried foods	Not adjusted for multiple comparisons. Not preregistered.
Romaguera et al. (51)	48,631 Danish, German, UK, Italian, and Dutch men and women	Food frequency questionnaire	5.5 years	Not reported	−0.04 cm/y WC (adjusted for BMI) per 100 kcal increment of fruit intake ($p < 0.001$)	Energy intake, age, baseline weight, baseline height, baseline WC (adjusted for BMI), smoking, alcohol intake, physical activity, education, follow-up duration, menopausal status, hormone replacement therapy	Not adjusted for multiple comparisons. Not preregistered.
Mirmiran et al. (52)	1,930 Iranian men and women ages 19–70	Food frequency questionnaire	3 years	−0.42 kg in highest vs. lowest quartile of fruit intake ($p = 0.01$)	−0.53 cm WC in highest vs. lowest quartile of fruit intake ($p = 0.006$)	Sex, age at baseline, BMI, education, smoking, physical activity, total energy intake, dietary carbohydrate, fat, protein	Not adjusted for multiple comparisons. Not preregistered.

(Continued)

TABLE 3 | Continued

Study	Subjects	Intake measure	Follow-up length	Weight change	Adiposity change	Covariates	Notes
Vergnaud et al. (53)	373,803 Danish, French, German, Greek, Italian, Dutch, Norwegian, Spanish, Swedish, and UK men and women	Food frequency questionnaire	5 years	Men: −0.001 kg/y per additional 100 g fruit intake ($p = 0.75$). Women: −0.001 kg/y per additional 100 g fruit intake ($p = 0.20$).	Not reported	Age, education, physical activity, change in smoking status, BMI at baseline, follow-up time, energy intake, energy intake from alcohol, plausibility of total energy intake reporting, vegetable intake	Not adjusted for multiple comparisons. Not preregistered. Intake data were calibrated using 24-h dietary recall data.
Bayer et al. (54)	1,252 girls and boys age 6	Parental questionnaire	4 years	Not reported	+0.014 unit BMI z-score in high vs. low fruit consumers (p = NS). +0.033 unit BMI *z*-score for decreased fruit intake ($p = 0.808$). −0.126 unit BMI *z*-score for increased fruit intake ($p = 0.348$).	Physical activity, cluster	Not adjusted for multiple comparisons. Not preregistered.
Bertoia et al. (55)	133,468 US male and female health professionals	Food frequency questionnaire	13–14 years (mean)	−0.24 kg per 4-year period per 1-serving increase ($p < 0.05$)	Not reported	Baseline age, BMI, change in smoking status, physical activity, hours of sitting or watching TV, hours of sleep, fried potatoes, juice, whole grains, refined grains, fried foods, nuts, whole-fat dairy, low-fat dairy, sugar-sweetened beverages, sweets, processed meats, non-processed meats, trans fat, alcohol, seafood	Not adjusted for multiple comparisons. Not preregistered. Similar to Mozaffarian et al. (50).
de Munter et al. (56)	23,108 Swedish men and women ages 18–84	Questionnaire	8 years	Not reported	Men: −0.07 BMI units in "≥ daily" vs. "less than daily" fruit intake group (p = NS); RR 0.89 for overweight incidence (p = NS); RR 0.90 for obesity incidence (p = NS). Women: +0.02 BMI units in "≥ daily" vs. "less than daily" fruit intake group (p = NS); RR 0.94 for overweight incidence (p = NS); RR 0.88 for obesity incidence (p = NS).	Age, education, physical activity, alcohol intake, smoking	Not adjusted for multiple comparisons. Not preregistered.
Rautiainen et al. (57)	18,146 US female health professionals aged ≥45	Food frequency questionnaire	15.9 years	−0.01 kg in highest vs. lowest quintile of fruit intake ($p = 0.46$)	HR 0.87 for obesity or overweight in highest vs. lowest quintile of fruit intake ($p = 0.01$)	"Age, randomization treatment assignment, physical activity, history of hypercholesterolemia or hypertension, smoking status, postmenopausal status, postmenopausal hormone use, alcohol use, multivitamin use, energy intake, baseline BMI"	Not adjusted for multiple comparisons. Not preregistered.
Hur et al. (58)	770 Korean male and female children and adolescents	3-day dietary record	4 years	Not reported	−0.08 unit BMI z-score per g/d fruit sugar ($p < 0.05$). −0.60% BF per g/d fruit sugar (p = NS).		Not adjusted for multiple comparisons. Not preregistered.

(Continued)

TABLE 3 | Continued

Study	Subjects	Intake measure	Follow-up length	Weight change	Adiposity change	Covariates	Notes
Bel-Serrat et al. (59)	2,755 Irish boys and girls ages 6–10	Parental food frequency questionnaire	3 years	Not reported	OR 2.16 for developing overweight/obesity in "sometimes/never" fresh fruit vs. "every day/most days" group ($p < 0.01$). −0.04 unit BMI *z*-score in "sometimes/never" fresh fruit vs. "every day/most days" group (p = NS).	Energy intake, income, sex, age	Not adjusted for multiple comparisons. Not preregistered.
Mumena et al. (60)	336 Caribbean (St. Kitts and Nevis, Trinidad and Tobago) boys and girls ages 6–10	24-h dietary recall	18 months	Not reported	Children who became overweight or obese had a lower fruit intake at baseline than children who did not ($p = 0.017$)	Measurement round, follow-up length, age, sex, baseline *z*-BMI, baseline abdominal obesity status, school socioeconomic status, school location, household ownership	Adjusted for multiple comparisons (Bonferroni). Not preregistered.
Okop et al. (61)	800 South African men and women	Food frequency questionnaire	4.5 years	Not reported	OR 1.47 of weight gain ≥5% in "seldom or no daily fruit" vs. "daily fruit" ($p < 0.05$)	N/A	Not adjusted for multiple comparisons. Not preregistered.

Prospective observational studies reporting the association between whole, fresh fruit consumption and body weight and adiposity changes. Additional strengths and limitations of study design are listed in the "notes" column. NS, not statistically significant ($p > 0.05$); NR, not reported; OR, odds ratio; RR, relative risk; HR, hazard ratio; BMI, body mass index; BF, body fat; WC, waist circumference.

An additional potential source of bias is that none of the observational studies reported preregistration, leaving open the possibility that outcome selection and data analysis methods were (perhaps inadvertently) guided toward preferred outcomes. The great diversity of analytic methods represented in these studies amplifies this concern because it demonstrates that the possible space of analytic methods is vast (64). Preregistration narrows this space *a priori*.

Finally, only one observational study reported adjusting significance tests for multiple comparisons, e.g., Bonferroni correction. The more hypothesis tests that are performed on a single data set, the higher the likelihood of a false positive finding. Since the commonly accepted false positive rate in the biomedical research community is 5 percent, testing ten hypotheses yields a 40 percent risk of that at least one of the ten tests will return a false positive finding. Many of the observational studies identified here tested ten or more hypotheses, and most represent datasets that have been analyzed using many statistical tests in other contexts (64). Overall, the risk of bias in the observational literature considered here appears quite high and must limit the strength of causal inferences drawn from it.

Synthesized Findings

Body Weight RCTs

The primary outcome of this review is the impact of whole, fresh fruit consumption on measures of adiposity including body weight, as measured by RCTs. Given the limited number of studies identified, which would be further reduced by more stringent meta-analysis inclusion criteria, and large variation of methodology and study quality, qualitative synthesis appears most appropriate.

Of the 11 RCTs included, seven reported numerical reductions of body weight as a result of increased fruit consumption relative to a comparison group, while four reported numerical increases of weight (**Table 1**). Among trials that reported numerical reductions of body weight, three comparisons were statistically significant (26, 28, 30), while none of the comparisons suggesting weight increases achieved statistical significance. This is consistent with the possibility that the weight increases result from random chance. In support of this possibility, studies that reported non-significant weight increases from fruit consumption tended to be statistically underpowered, with as few as six subjects per group (**Table 1**).

Among the two trials that reported performing a power calculation *a priori* and were preregistered with measures of adiposity as the primary outcome, Madero et al. (30) reported a significant weight loss of −1.36 kg over 6 weeks of substantially increased fruit consumption (30), while Dow et al. (31) reported a non-significant weight loss of −0.5 kg over 6 weeks of modestly increased fruit consumption (31). In addition, Madero et al. (30) received the lowest risk of bias score of all studies considered, and Dow et al. (31) tied for the second lowest risk of bias. In contrast, three of the four trials reporting non-significant increases of body weight from increased fruit consumption were among those with the highest risk of bias, while the fourth, which reported a non-significant and negligible weight gain of 0.03 kg over 8 weeks, was among those with relatively lower risk of bias

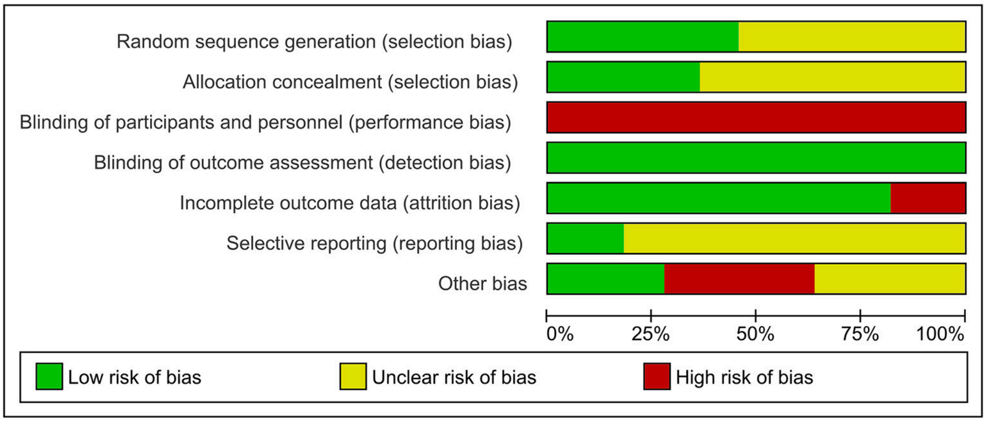

FIGURE 2 | Risk of bias graph for the primary outcome: the impact of whole, fresh fruit consumption on measures of body weight and adiposity in RCTs. Bars illustrate the proportion of trials that received a particular risk of bias score in each risk of bias domain.

(**Figure 3**). It is evident that the trials reporting weight reductions tend to have higher methodological quality than those reporting weight increases, suggesting that the former should be viewed as more informative.

Six trials reported measures of adiposity other than body weight, including body fat percentage, waist circumference, waist-to-hip ratio, sagittal abdominal diameter, and visceral fat volume (**Table 1**). Only one measure achieved statistical significance, a waist circumference reduction of −3.1 cm over 8 weeks of a high-fruit diet (27). However, this finding is questionable due to the study's small sample size, high risk of bias, and apparent lack of correction for multiple comparisons.

Since Madero et al. (30) and Dow et al. (31) appear to surpass the others in methodological quality and relevance to adiposity modification, these will be discussed in detail and will contribute disproportionately to the overall conclusions of this review (30, 31). These two trials are particularly relevant to adiposity modification because all subjects had overweight or obesity. Madero et al. (30) was preregistered with anthropometric changes as the primary outcome, and sample size was selected using a power calculation. Sample size was larger than all but one other trial, which had the same number of subjects. This trial also received the lowest risk of bias score among the 11 trials identified, with low risk in all categories except participant blinding, in which high risk is unavoidable due to the impossibility of blinding subjects, and allocation concealment, which was judged as unclear because it was not described in the manuscript.

One hundred and thirty one men and women with overweight or obesity were randomly assigned to eat a low-fructose diet (<20 g/d) vs. a natural-fructose diet (50–70 g/d) in which most of the fructose was from whole, fresh fruit, for 6 weeks. The latter is approximately equivalent to five to eight whole medium apples per day, or eleven to fifteen whole oranges per day. After 6 weeks, the natural-fructose group had lost 1.36 kg more weight than the low-fructose group ($p = 0.02$). The trial also reported non-significant reductions of body fat percentage and waist-to-hip ratio in the natural-fructose group relative to the low-fructose group.

Dow et al. (31) was preregistered with body weight change as the primary outcome, and sample size was selected using a power calculation (31). This trial tied for the second-lowest risk of bias score among the 11 trials identified, with low risk of bias in four of seven domains (**Figure 3**). Its risk of bias in participant blinding was judged as high, which is unavoidable. Its risk of bias due to randomization and allocation concealment are unclear due to a lack of information in the manuscript.

Seventy-four men and premenopausal women with overweight or obesity were randomly assigned to eat half a fresh grapefruit three times per day prior to each meal, vs. no intervention, for 6 weeks. In addition, all subjects were assigned to a baseline diet low in fruit and vegetables. After 6 weeks, the grapefruit group had lost 0.5 kg more weight than the control group, but this difference was not statistically significant ($p = 0.119$). Between-group differences in body fat percentage (+0.55 percent), waist circumference (−1.22 cm), and waist-to-hip ratio (0.0) were also non-significant.

Informally weighting the strength of findings according to study quality and risk of bias, the overall RCT literature suggests that increasing whole, fresh fruit consumption promotes weight maintenance or modest weight loss over periods of 3–24 weeks, with limited evidence suggesting that high intakes of fruit lead to weight loss among people with overweight or obesity. The overall quality of evidence according to the GRADE method is moderate, indicating that the true effect of fruit consumption on measures of adiposity is probably close to the effect estimated in the RCTs considered here, particularly those of higher quality.

Energy Intake RCTs

A secondary outcome of this review is the impact of whole, fresh fruit consumption on measures of energy intake, as measured by RCTs. Given the small number of studies identified, and large variation of methodology and study quality, qualitative synthesis appears most appropriate.

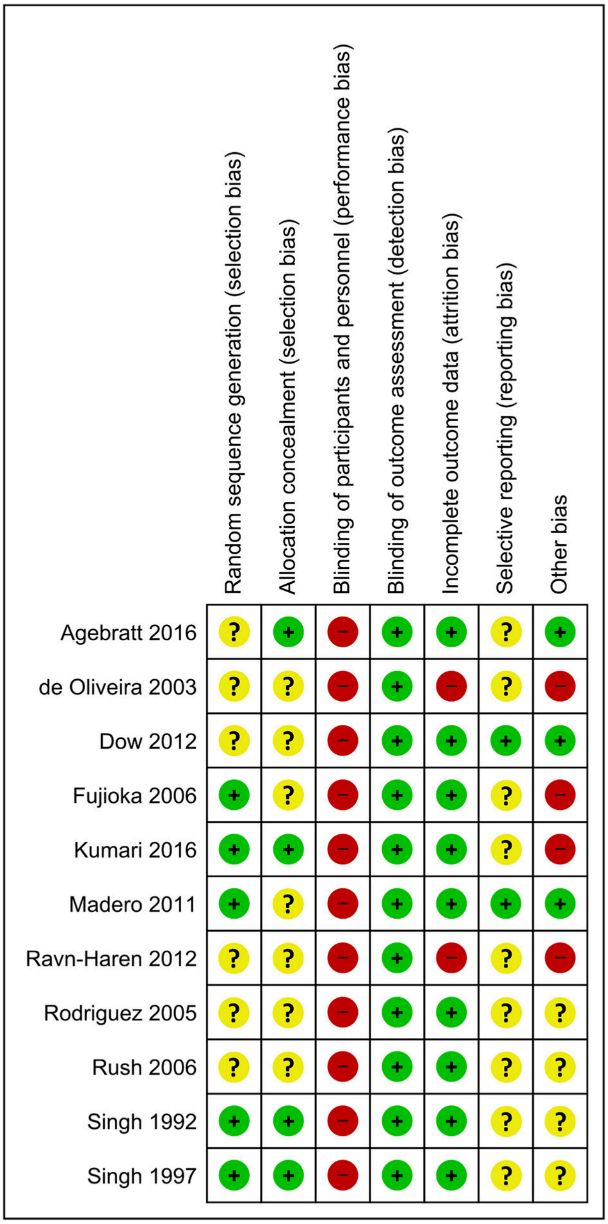

FIGURE 3 | Risk of bias summary for individual trials contributing to the primary outcome: the impact of whole, fresh fruit consumption on measures of body weight and adiposity in RCTs. Colored dots represent low risk (green), unclear risk (yellow), and high risk (red) in each risk of bias domain for each trial.

Of the five RCTs included, four reported numerical reductions of energy intake as a result of increased fruit consumption relative to a comparison group, while one reported a numerical increase of energy intake (**Table 2**). Among trials that reported numerical reductions of energy intake, two comparisons were statistically significant (35, 36), while the comparison suggesting numerically increased energy intake reported a small-magnitude effect (+47 kcal/d) that did not achieve statistical significance (27). This is consistent with the possibility that the finding of increased energy intake resulted from random chance. In support of this possibility, this study was likely statistically underpowered, with seven and eight subjects per group (**Table 1**). It did not report performing an *a priori* power calculation and was also at high risk of bias due to its use of a self-reported measure of energy intake, and not blinding subjects (unavoidable) or investigators (avoidable) (**Figure 5**).

Only one energy intake trial was preregistered, and its primary outcome was a change of hepatic fat content rather than energy intake (33). Among the three trials that reported performing a power calculation *a priori*, two reported statistically significant single-meal reductions of energy intake of 134–187 kcal from meals including a fruit preload relative to no preload or a confectionary snack (35, 36), and the third reported a non-significant 216-kcal reduction of daily energy intake from a high-fruit diet relative to a high-nut diet (33). The former two trials were the only two to employ direct measurement of energy intake by investigators rather than self-reported intake (**Table 2**). The three trials that performed power calculations received the most favorable risk of bias scores among the five trials identified, although none of the five trials received a low risk score in more than two of seven domains (**Figure 5**).

Consistent with the adiposity RCTs, it is evident that the trials reporting energy intake reductions, and particularly statistically significant ones, tend to have higher methodological quality than the trial reporting energy intake increase, suggesting that the former should be viewed as more informative. However, it is notable that the three trials reporting non-significant effects, while relying on self-reported data, were the only three with follow-up periods longer than a single meal (**Table 2**).

Flood-Obbagy and Rolls (35) and James et al. (36) appear to surpass the other trials in methodological quality due to direct measurement of energy intake, lower risk of bias score than the other three, and sufficient statistical power supported by *a priori* power calculations (35, 36). Flood-Obbagy and Rolls (35) also has the largest sample size of the five trials identified, and its effective sample size is amplified by its crossover design. These two trials will be discussed in detail and will contribute disproportionately to the overall conclusions of this review. Although these trials may provide a higher level of certainty than the others, they are less relevant to energy intake control in the context of obesity because their subjects were either lean (35) or mildly overweight (36) on average.

Flood-Obbagy and Rolls (35) received one of the lowest risk of bias scores among the five trials identified, although none of the trials were at a low risk of bias. It received a low risk of bias score in blinding of outcome assessment due to directly measured energy intake, and low risk of attrition bias due to low attrition (**Figure 5**). It received an unclear risk of bias score for random sequence generation, allocation concealment, selective reporting, and other bias due to insufficient information in the manuscript and the absence of preregistration. It received a high risk of bias score in participant blinding, in which high risk is unavoidable.

Fifty-nine men and women 18–45 years old, with body mass index of 23.7 kg/m^2 (M) and 24.3 kg/m^2 (W), received five food preloads in random order on different days, followed after 15 min by an *ad libitum* test meal of cheese tortellini, tomato

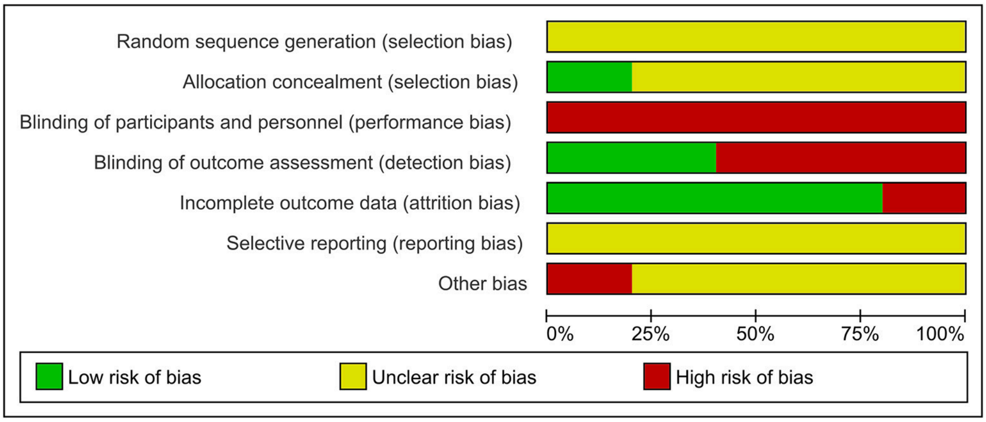

FIGURE 4 | Risk of bias graph for a secondary outcome: the impact of whole, fresh fruit consumption on energy intake in RCTs. Bars illustrate the proportion of trials that received a particular risk of bias score in each risk of bias domain.

sauce, and water (35). Preload conditions were whole apple, apple sauce, apple juice with added soluble fiber (pectin), apple juice, or no preload. All preloads were adjusted to contain equal energy (125 kcal) and weight (266 g) except the no-preload control. Total meal energy intake as directly measured by investigators, including preload, was lowest in the whole apple condition and highest in the no-preload condition, with a highly statistically significant difference of −187 kcal (**Table 2**). Energy intake in the whole apple condition was also significantly lower than all other conditions. Total meal energy intake increased with each processing step away from whole fresh apples, in the following order: whole apples < apple sauce < apple juice with fiber < apple juice.

James et al. (36) received a risk of bias score identical to Flood-Obbagy and Rolls (35) for similar reasons (**Figure 5**) (36). Twelve healthy pre-menopausal women, with body mass index of 26.6 kg/m^2, received two preloads in random order on different days, followed after 60 min by an *ad libitum* test meal of pasta with Bolognese sauce and olive oil. Preload conditions were fresh mixed berries vs. soft berry-flavored candies and were matched for energy content (65 kcal). The two preloads also contained a similar amount of sugar (12.1 vs. 15.5 g). Energy intake at the test meal, as directly measured by investigators, was 134 kcal lower in the mixed berry condition than in the candy condition ($p < 0.001$).

Informally weighting the strength of findings according to study quality and risk of bias, the overall RCT literature suggests that increasing whole, fresh fruit consumption reduces energy intake, particularly when consumed prior to a meal or instead of more energy-dense foods. However, these findings have uncertain relevance to energy intake control in obesity because the most informative trials were conducted in subjects who were lean or modesty overweight. In addition, the most informative trials used single-meal designs, limiting conclusions about the impact of fruit consumption on long-term energy intake. The overall quality of evidence according to the GRADE method is moderate, indicating that the true effect of pre-meal fruit consumption on short-term energy intake in people without obesity is probably close to the effect estimated in the RCTs considered here. Longer-term effects, and those in people with obesity, are less certain. Nevertheless, the energy intake RCT literature is broadly consistent with findings from the body weight RCT literature.

Prospective Observational Studies

A secondary outcome of this review is the association between whole, fresh fruit consumption and measures of adiposity including body weight, as measured by prospective observational studies. Of the 25 studies identified, 11 reported weight changes over time, and of those, ten reported that people who consumed larger amounts of fruit gained numerically less weight over time (or lost more weight) than people who consumed less fruit (**Table 3**). Seven of these associations were statistically significant, all suggesting that higher fruit intake is associated with superior weight control over time.

Eighteen studies reported changes in measures of adiposity other than weight over time, 12 of which reported statistically significant differences between higher and lower consumers of fruit (**Table 3**). Among these 12, all reported that markers of adiposity in people who consume larger amounts of fruit tend to increase less over time (or decline more rapidly) than in people who consume less fruit. Although the Cochrane risk of bias tool was not applied to these studies, as discussed previously they all appear to be at a high risk of bias due to a combination of unavoidable and potentially avoidable design features.

Nevertheless, some studies appear more informative than others. Bertoia et al. (55) [similar to Mozaffarian et al. (50)] and Vergnaud et al. (53) will be discussed further due to the fact that they have the largest sample sizes of the 11 studies identified, they rely on contextually-validated food frequency questionnaires, they have relatively long follow-up periods, and together they represent men and women of 11 nations (35, 36).

Bertoia et al. (55) compiled data from three cohorts representing 133,468 US male and female health professionals (55). Diet assessment was performed using food frequency questionnaires administered at 4-year intervals for a mean of

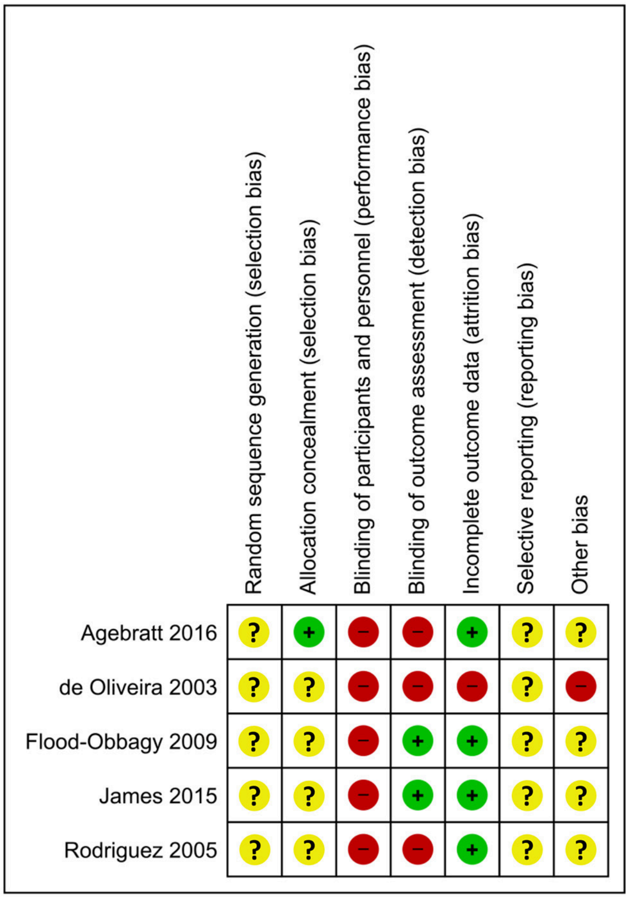

FIGURE 5 | Risk of bias summary for individual trials contributing to a secondary outcome: the impact of whole, fresh fruit consumption on energy intake in RCTs. Colored dots represent low risk (green), unclear risk (yellow), and high risk (red) in each risk of bias domain for each trial.

13–14 years. Analyses controlled for a wide variety of diet and lifestyle factors (**Table 3**), and although they did not control for demographic variables such as income and education, included cohorts were fairly homogeneous in these respects. Notably, analyses did not control for energy intake, which is preferable because energy intake is likely a mediating variable between whole, fresh fruit intake and adiposity.

In contrast to most other studies identified, Bertoia et al. (55) examined the association between *changes* in self-reported fruit intake and *changes* in measures of adiposity. In other words, if a person reported increasing fruit intake over the course of the follow-up period, were they also less likely to gain weight over time? Although this "change-on-change" method remains fundamentally observational, it may avoid some of the confounding potential of traditional nutritional epidemiology study designs (65). The study reported that a one-serving increase of daily fruit intake was associated with a highly statistically significant 0.24 kg reduction of body weight per 4-year period, and did not report associations with other measures of adiposity.

Vergnaud et al. (53) represents 373,803 Danish, French, German, Greek, Italian, Dutch, Norwegian, Spanish, Swedish, and UK men and women, making it the largest cohort of the 11 studies identified (53). Diet assessment was performed using food frequency questionnaires and the duration of follow-up was 5 years. In contrast to Bertoia et al. (55) but similar to most other nutritional epidemiology studies, Vergnaud et al. (53) reports the association between baseline self-reported fruit intake and weight change over a 5-year period. Analyses controlled for several basic diet, lifestyle, and demographic factors, including energy intake (**Table 3**). Systematic underestimation or overestimation of dietary intakes between study centers was addressed using a dietary calibration study. The study reported that 100 g higher daily fruit intake was associated with a non-significantly lower rate of weight gain of −0.001 kg per year in men and women, and did not report associations with other measures of adiposity.

Informally weighting the strength of findings according to study quality, the overall prospective observational literature suggests that habitually higher fruit intake is associated with no effect on weight, or modest protection against weight gain. Although these findings must be interpreted cautiously due to limitations of study design, they are broadly consistent with the findings of energy intake and body weight RCTs discussed previously and may suggest that the short- to medium-term effects observed in RCTs persist in the long term.

DISCUSSION

Summary of Main Findings

The primary outcome of this review is the impact of whole, fresh fruit consumption on measures of adiposity including body weight, as measured by RCTs. Overall, these RCTs suggest that increasing intake of whole, fresh fruit promotes weight maintenance or modest weight loss over periods of 3–24 weeks. High intakes of whole, fresh fruit in people with obesity may promote some degree of weight loss. RCTs provide little information about more direct measures of adiposity such as body fat percentage. The strength of evidence supporting this conclusion is moderate, indicating that the true effect of fruit consumption on measures of adiposity is probably close to the effect estimated here.

Secondary outcomes of RCTs reporting the impact of fruit consumption on energy intake, and prospective observational studies reporting associations between fruit intake and measures of adiposity, were broadly consistent with the primary outcome. The strength of evidence supporting conclusions regarding energy intake RCTs is moderate, while the prospective observational findings did not receive a GRADE assessment but are likely at high risk of bias. As with the primary outcome, RCTs and prospective observational studies of higher quality tend to support the hypothesis that higher intakes of whole, fresh fruit either do not impact weight or modestly attenuate weight gain over time.

Limitations

Limitations of this review relate both to the review itself, and to the studies that underlie it. Although quantitative pooling of RCT data appeared suboptimal in this context due to the limited number of studies and considerable heterogeneity in study methods and quality, narrative synthesis is inherently more subjective than quantitative meta-analysis. The author endeavored to limit the potential for bias by preregistering a detailed research plan and adhering to widely accepted defined methods for assessing and reporting evidence, such as the Cochrane risk of bias tool, the GRADE method, and PRISMA guidelines. The author attempted to be transparent in methods and reasoning so the reader may form his or her own views. In addition, the greater subjectivity of narrative reviews may be counterbalanced in some instances by a superior ability to focus on high-quality studies rather than diluting their evidence value by pooling them with lower-quality studies. Finally, the author was not funded for this work and has no connection with Big Fruit, eliminating this potential source of real or perceived bias.

An additional limitation of this review is that due to resource constraints, study selection, data extraction, risk of bias scoring, and GRADE evaluation were performed by one person. The Cochrane handbook for Systematic Reviews of Interventions recommends that systematic reviews be conducted by at least two people to reduce the risk of errors[4].

The conclusions of this review are also limited by the underlying evidence. Although 11 RCTs were available for the primary outcome of adiposity, most had serious limitations of sample size, lack of preregistration, and/or risk of bias. Conclusions of the primary outcome of this review rest disproportionately on two high-quality trials. Energy intake RCTs were fewer in number and tended to be lower-quality than adiposity RCTs. Prospective observational studies typically had serious limitations including lack of preregistration, lack of correction for multiple comparisons, and potential confounding and overcorrection, which together raise substantial concerns of bias. Some of these limitations are inherent to observational methods, while others are potentially avoidable. Nevertheless, the consistency of findings across the three primary and secondary outcomes is somewhat reassuring.

CONCLUSIONS

Consistent with earlier reviews on this topic (14, 17), available evidence suggests that increasing intake of whole, fresh fruit probably does not increase adiposity, but instead has either no impact on adiposity or constrains it modestly. Findings consistent with this hypothesis are observed in studies representing single meals, 3–24 week periods, and periods of five or more years. Although some uncertainty remains, these findings support increasing the consumption of whole, fresh fruit as part of a multi-factor approach to controlling excess energy intake and adiposity. These findings also suggest that if the sugar content of fruit favors increased energy intake and adiposity, this is outweighed by its other properties, such as lower calorie density, moderate palatability/reward value, higher fiber content, and micronutrient content, at least when consumed as part of typical diet patterns.

These findings support existing recommendations by organization such as the US Department of Agriculture and the World Health Organization to increase fruit consumption as a public health measure (66, 67). Although increasing consumption of whole, fresh fruit is unlikely to have a large impact on population obesity rates on its own, it may make a positive contribution as part of a broader public health strategy for obesity control. Similarly, healthcare providers should not expect large changes in adiposity as a result of increasing whole, fresh fruit consumption alone, but it is reasonable to include it as part of a broader package of slimming diet and lifestyle behaviors. Furthermore, it is unlikely to cause adiposity gain despite its sugar content, at least as part of a typical mixed diet. Increasing intake of whole, fresh fruit may be more effective as an adiposity control measure when presented as a replacement for calorie-dense dessert foods.

Several opportunities for reducing the uncertainty of conclusions on this topic are apparent. Additional high-quality RCTs with changes in adiposity as the preregistered primary endpoint would be useful, particularly if they report an accurate measure of total and regional adiposity such as dual-energy X-ray absorptiometry. High-quality energy intake RCTs, preregistered and with complete description of randomization processes, involving direct measurement of energy intake in people with obesity over longer periods of time would also contribute substantially. Additional energy intake RCTs could also compare the impact of fruit intake in different contexts, such as pre-meal vs. intra-meal vs. after-meal intake, to identify the most effective strategy for energy intake control. Finally, prospective observational studies that use accurate measurement instruments, are conducted according to a preregistered research plan, and adjust for multiple comparisons would reduce uncertainty.

AUTHOR CONTRIBUTIONS

SG is the sole contributor to this manuscript, aside from its search strategy, which was designed in collaboration with Ben Harnke.

ACKNOWLEDGMENTS

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[4]https://handbook-5-1.cochrane.org/chapter_2/2_3_4_1_the_importance_of_a_team.htm

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Conflict of Interest Statement: This research was not conducted in association with any public or private organization. SG is the author of a general-audience book on the neurobiology of overeating, The Hungry Brain, which does not currently yield royalties but may in the future. This book mentions fruit but does not place a special emphasis on it. SG is the co-creator of a weight management program, the Ideal Weight Program, from which he receives revenue. This program permits the consumption of fruit but does not place a special emphasis on it.

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An Intensive Lifestyle Intervention to Treat Type 2 Diabetes in the Republic of the Marshall Islands: Protocol for a Randomized Controlled Trial

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Background: The Republic of the Marshall Islands has the highest prevalence of type 2 diabetes (T2D) in the world, with the country's rapid rise of T2D attributed to its reliance on imported and refined foods laden with salt, sugar, and fat. As much as lifestyle factors can increase the risk of T2D, they can also reverse or treat the disease, with multiple studies demonstrating that plant-based diets and/or moderate exercise improve glycemic control and cardiovascular risk factors in T2D patients.

Objective: We therefore tested the hypothesis that a community-based, intensive, plant-rich lifestyle intervention with exercise is more effective for treating and managing T2D in the Republic of the Marshall Islands than the standard of diabetes care.

Methods: Building on a successful lifestyle program used at the Guam Seventh-day Adventist Clinic, we conducted a randomized controlled trial to test the effectiveness of an intensive lifestyle intervention involving a plant-rich diet and moderate exercise or the standard of care in T2D patients for 24 weeks. In this manuscript, we describe the clinical trial protocol, including the rationale, design, and methods of the clinical trial and the lifestyle program. The lifestyle intervention included a step-wise, intensive 12-week program of counseling and instruction on healthy eating, exercise, and stress management. The prescribed diet focused on high-fiber, whole plant foods, with foods grouped into a four-tiered system. The lifestyle intervention also involved hands-on cooking classes, meals prepared for participants, and group exercise classes—all tailored to be culturally appropriate. The study's main endpoints were glycemic control and cardiovascular disease risk factors.

Discussion: The present study is the first randomized clinical trial conducted in the Republic of the Marshall Islands and the first lifestyle intervention trial conducted in

Micronesia. The results of this study will help guide future medical care for indigenous populations in the Pacific Islands and will also shed light on how to effectively design and deliver intensive lifestyle interventions to treat and manage diabetes.

Clinical Trials Registration: www.ClinicalTrials.gov; identifier NCT03862963

Keywords: type 2 diabetes, lifestyle intervention, plant-based diet, exercise, Republic of the Marshall Islands

INTRODUCTION

Diabetes is a rising epidemic. More than 420 million people worldwide have the disease (1), and new estimates suggest that diabetes now afflicts about 9.4% of Americans (2). Yet, in some countries, such as within the Pacific Islands and Micronesia, the prevalence of diabetes among adults is as high as 25–31% (3).

The Republic of the Marshall Islands (RMI) has the highest prevalence of diabetes in the world, where the disease afflicts almost one-third (31.4%) of all adults aged 18–99 years (3). The explosion in diabetes rates in the RMI is a relatively recent phenomenon. In the 1950's, only three people in the RMI were known to have the disease, according to an oral report by an RMI health official in 2005. By the 1990's, the prevalence had reached 30% (4). Now, more than half of all hospital admissions in the capital city of Majuro are due to diabetes (5), and 60% of deaths in adults under the age of 60 years are attributed to the disease and its related comorbidities (5). The epidemic has been attributed to the growing population, which has strained supplies of healthier indigenous foods and increased the reliance on imported goods such as flour, rice, and foods high in salt, sugar, and fats (6).

To combat the growing global diabetes epidemic, the American Diabetes Association has called for increased use of lifestyle interventions as part of primary care (7). Indeed, dietary interventions can lower hemoglobin A1c (HbA1c) by 0.5–2.0% (7), with several trials reporting values between 1.0 and 3.4% (8–20) as well as reductions in hyperglycemic medications and/or even T2D remission (8, 10–12, 15, 17, 19, 21–27). In particular, several trials suggest that vegetarian and vegan low-fat diets are superior to conventional diets for lowering glucose, HbA1c, and insulin levels; decreasing diabetes medication dosages; and reducing diabetes complications, such as neuropathy, in T2D patients (12, 19, 20, 22, 26–40). This is corroborated by prospective cohort studies, such as the Nurses' Health Study and Adventist Health Study-2, which report that plant-based diets reduce the risk of developing type 2 diabetes by about half (41–45). Moreover, among populations such as the Seventh-day Adventists, the prevalence of diabetes among vegans is as low as 0.22 times that among non-vegetarians (45, 46). Plant-based diets have also been found to improve lipids, renovascular health, and oxidative stress levels in adults with T2D more effectively than conventional diets (12, 19, 22, 26, 28, 31–35, 38, 47). Plant-based diets likely mediate these effects through multiple mechanisms (47–49), including through improvements in body weight and visceral fat [e.g., (32, 36, 50, 51)]; insulin sensitivity and beta-cell function (32, 39, 52, 53); incretins and gastrointestinal hormones (39, 52); oxidative stress (32); phytochemical intake [including polyphenols and plant sterols (49, 54)]; fiber and prebiotic intake, which positively modulate gut microbiota (55); lower oxidant intake (such as heme iron) (49); and increased well-being (37). Lifestyle interventions that combine plant-diets with regular exercise may be even more effective. Coupling a plant-based diet with exercise significantly reduces the incidence of diabetes in glucose-impaired individuals (56, 57), and clinical trials show that the combination is effective for treating diabetes, cardiovascular disease, obesity, and other chronic conditions (32, 58–64). To date, however, only one previous clinical trial has combined a plant-rich diet with exercise for treating T2D (65).

Despite the promise of such lifestyle interventions, implementing them in indigenous populations such as the RMI presents unique challenges. Research has shown that such interventions are more effective when they are culturally-sensitive and delivered to communities, rather than to individuals or families (66). In the late 1990's, Canvasback Missions, Inc., recognized the need for such interventions in the RMI and conducted two lifestyle intervention programs with excellent results, using a modified version of the NEWSTART® wellness program operated by the Guam Seventh-day Adventist Clinic (67). The program emphasizes a whole-food, plant-rich diet with moderate exercise and is used to treat T2D, obesity, and other chronic diseases. Although no studies have been published on the intervention, Guam physicians have found it effective and continue to refer their patients to wellness centers (68).

Building on the success of these lifestyle intervention programs in Guam and the RMI, we designed and conducted the first randomized controlled trial to rigorously evaluate whether a comprehensive, culturally-sensitive, lifestyle intervention program can treat T2D in the RMI. The goal of the study was to determine the effectiveness of an intensive lifestyle intervention consisting of a mostly plant-based diet and regular moderate exercise in Marshall Islanders with T2D. The present study was also the first randomized clinical trial ever conducted in the RMI and the first lifestyle intervention trial ever conducted in Micronesia. The primary aim was to determine whether the lifestyle intervention can improve glycemic control. The primary endpoints included fasting glucose, fasting insulin, HbA1c, insulin resistance as measured by the Homeostatic

Abbreviations: T2D, Type 2 diabetes; RMI, Republic of the Marshall Islands; BMI, body mass index; HbA1c, hemoglobin A1c; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; hs-CRP, high-sensitivity-C-reactive protein; MOH, Ministry of Health; DWC, Diabetes Wellness Clinic; NEWSTART, Nutrition, Exercise, Water, Sunshine, Temperance, Air, Rest, and Trust in divine power.

Model Assessment of Insulin Resistance (HOMA-IR), and usage of diabetes medications. The secondary aim was to determine whether the lifestyle intervention could improve cardiovascular risk factors, including lipids, blood pressure, heart rate, high-sensitivity C-reactive protein (hs-CRP), body weight, body mass index (BMI), and waist circumference. We hypothesized that study participants would be able to make sufficient lifestyle changes—including by modifying their diet and increasing their physical activity—to achieve clinically meaningful improvements in glycemic control and cardiovascular risk factors, relative to the control group.

MATERIALS AND METHODS

Study Design

Study Overview

This trial was conducted in partnership with the RMI's Ministry of Health (MOH). The study was designed as an open-label, parallel-arm, randomized controlled trial and was conducted at the MOH's Diabetes Wellness Center (DWC) in the city of Majuro in the RMI. Adults with T2D were randomized to receive either the standard of diabetes care (control group) or a lifestyle intervention comprising a whole-foods, largely plant-based diet combined with increased physical activity (intervention group). The intervention group received intensive counseling, support, and group sessions over a 12-week period and then continued the lifestyle intervention on their own for the remainder of the 24-week intervention. Health outcomes were assessed at weeks 0 (baseline), 2, 6, 12, and 24. At the end of the study intervention, those who were randomized to the control group were offered the option to crossover to the experimental arm and complete the lifestyle program. The study was approved by the Loma Linda University Institutional Review Board (IRB #: 59105) and an *ad-hoc* Institutional Review Board in the RMI that was set-up specifically for the trial. The study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to enrolling in the study and were given a stipend for their participation. Due to the nature of the intervention, the study was not blinded.

Recruitment

During year 1 of the study, participants were initially recruited from the general public. Two local leaders assisted in building interest among and recruiting Majuro residents. During year 2, participants were recruited both from the general public and from the MOH Diabetes Clinic through its diabetes registry. Of those screened, 18% or 31 out of 169 eligible participants dropped from the trial before beginning the intervention. The first cohort began the study intervention in June 2006 and the last cohort began in July 2008. Due to a lack of funding after the clinical trial ended, the study data was not analyzed and published until the present.

Eligibility Criteria

Recruited individuals were screened against a series of inclusion and exclusion criteria. The inclusion criteria were: (1) resident of RMI, (2) aged 18–75 years, (3) HbA1c $\geq$ 8.0% or diagnosed with T2D and taking diabetes medication, and (4) medical clearance to participate from DWC physicians. The exclusion criteria included (1) recent ($\leq$3 months) change in a diabetes-related medication dosage, (2) a physical or medical condition that would impede participation in the lifestyle intervention (e.g., wheel-chair bound, unstable angina), (3) evidence of significant coronary heart disease, and (4) previous participation in an intensive lifestyle intervention. There were no eligibility criteria related to BMI.

Screening

Prospective participants were screened during a single in-person visit, which included a review of their medical history, physical fitness testing, a dietary assessment based primarily on a food frequency questionnaire, and fasting blood draws to measure glucose and HbA1c. Screening was performed at the DWC. To ensure suitability for the exercise portion of the intervention, participants were queried about their cardiovascular fitness and history. Those with ischemic heart disease were required to have special clearance from their physician. A physician reviewed all dietary, medication, and medical history data for medical clearance. Individuals who passed the screening process later attended an oral presentation explaining the study. During these group sessions, the study was explained to the participants in detail. Translators were assigned by the MOH to explain the study to participants who did not understand English. Interested participants then read and signed the informed consent in English or their native language.

Randomization

Following enrollment, participants were randomized to the two groups. Spouses who enrolled in the trial were randomized in pairs to keep them together. Non-eligible spouses were encouraged to attend counseling sessions but were not enrolled in the study.

Cohorts

Participants were enrolled in five overlapping cohorts, with each cohort including both a control and intervention group (described below). Within each cohort, participants were randomized to one of the two groups in approximately equal numbers. The cohorts were spread over a two-year period, with Cohorts 1 and 2 finishing in year 1 and Cohorts 3–5 finishing in year 2. The cohorts 1–5 consisted of 27 (13 in the intervention group vs. 14 controls), 41 (21 vs. 20), 31 (9 vs. 22), 27 (14 vs. 13), and 43 (22 vs. 21) participants, respectively. The control arm received the usual standard of diabetes care, while the intervention arm received a lifestyle intervention. Unfortunately, not all participants in the control group in year 1 sought and received the usual standard of care for diabetes treatment as instructed, so starting in year 2, participants were primarily recruited from the MOH Diabetes Clinic, in order to ensure that the control group was compliant with the standard of care. Furthermore, to improve the delivery of the intervention, Cohorts 3–5 received an enhanced version of the lifestyle intervention. The enhanced version was identical to the standard version of the intervention, with the exception of more patient education and contact during weeks 4 and 6 of the study. The nature of the dietary and exercise intervention itself was not changed and did not vary across cohorts.

Study Intervention

Usual Diabetes Care

The control group received the standard of diabetes care in the RMI, which consisted of the standard treatment protocols used by the MOH Diabetes Clinic. These included placing T2D patients on anti-hyperglycemic agents appropriate to their HbA1c levels (including sulfonylureas, metformin, and insulin) and providing oral and written information about the importance of maintaining a healthy weight, eating a healthy diet, and getting regular exercise. Participants in the control group were instructed not to make changes in their diet and activity levels during the study.

Lifestyle Intervention

The lifestyle intervention consisted of a high-fiber, low-fat, mostly plant-based diet, and moderate exercise. Participants initially received 12 weeks of group educational classes and meals prepared by the DWC Wellness Kitchen and then followed the lifestyle intervention on their own for the remaining 12 weeks (**Table 1**). Group classes included informative sessions on healthy eating, exercise (both aerobic and strength training), and stress management, as well as hands-on cooking classes. Classes were delivered as a combination of PowerPoint presentations, practical workshops, dine-outs, shopping tours, and cooking classes with spouses. To foster access to affordable produce, participants were also taught by soil and gardening experts how to grow their own vegetables and were taken on agricultural field trips.

The initial 12-week program was structured as follows. During the first 2-week intensive phase, participants in all cohorts attended 5–6 h/day of group classes on Mondays–Thursdays (4 days/week) at the DWC, totaling 36 h of health education over the first two weeks. **Figure 1** displays the schedule for the intensive phase. On clinic days, group sessions included 1 h/day of exercise, and participants were fed 3 meals/day made by the DWC Kitchen, according to the menus shown in **Table 2**. Participants ate breakfast at the DWC and took lunch boxes with them to eat at work or at home. The participants then returned to the DWC for a 5-h session from 3:00 to 8:00 p.m., where they helped prepare the meals and then ate dinner. Participants prepared the main dish, and the kitchen staff made the side dishes (e.g., green salad). During weeks 3–6, participants who received the standard version of the intervention (Cohorts 1–2) spent 4–5 h twice per week (Tuesdays and Thursdays) in group sessions, including 60 min/day of structured exercise, a 10–20-min walk after each meal, and also consumed an evening meal provided by the DWC Wellness Kitchen (see menus in **Table 3**). For those receiving the enhanced version of the intervention (Cohorts 3–5), weeks 4 and 6 of the standard intervention were replaced with weeks 1 and 2 to increase the intensity of instruction, as shown in **Table 1**. During weeks 7–12 of the program, participants in all cohorts participated in a 4–5 h once per week session with structured exercise and group classes and were provided an evening meal. Finally, during the last 12 weeks of the trial (weeks 13–24), no instruction or counseling was provided, and participants came to the center weekly to turn in their pedometer readings.

Diet

The dietary intervention was adapted from the NEWSTART® program (67), a plant-based diet used by the Guam Seventh-day Adventist Clinic Wellness Center, and from concepts presented in the book *Defeating Diabetes* (69). (NEWSTART® stands for Nutrition, Exercise, Water, Sunshine, Temperance, Air, Rest, and Trust in divine power.) The diet used in this trial was mostly plant-based and emphasized whole plant foods, high fiber intake (roughly 35 g fiber per 1,000 kcal), low fat intake (20–25% of energy intake), moderate protein intake (10–15% of energy intake), and moderate sodium intake (<2,400 mg/day) while encouraging local cuisine (70). The diet was designed to be high in phytochemicals and antioxidants, low in dietary oxidants, and to have a low glycemic load. Concentrated fats and oils were minimized, and only fat from healthy sources was permitted. Saturated fat was limited to <7% of caloric intake, and no trans fatty acids were permitted. The diet included sufficient omega-3 fatty acids sourced from plants (primarily ground flaxseeds and walnuts), and fish, for those who chose to include it at home after the 2-week intensive phase of the intervention.

In the first 2 weeks of the dietary intervention (the intensive phase), participants consumed a 100% plant-based diet that minimized ground grains (e.g., flour, pasta, bread) and refined carbohydrates (e.g., refined sugar and starches). Also during the intensive phase, participants consumed 12 meals/week that were prepared for them by the DWC's Wellness Kitchen (see menus in **Table 2**), and they were instructed not to eat any outside food or snacks the remainder of those days. After the 2-week 100% plant-based phase, participants were allowed to consume boiled, steamed, or grilled fish or seafood if desired, and they continued to eat a smaller number of meals provided by the DWC (see **Tables 2**, **3**).

When not eating meals prepared by the DWC, participants were counseled to make healthy food choices on their own using a tiered food classification system, with foods classified into four groups: 1st, 2nd, 3rd, and 4th classes (**Table 4**). Participants were encouraged to eat freely from the 1st class, moderately from the 2nd class, and sparingly from the 3rd class, and to completely avoid items in the 4th class. The food classification system encouraged participants to eat mostly first-class foods (75–100% of daily calories), which were defined as unprocessed, whole plant foods. To maximize nutrient density and reduce glycemic load, the first class category emphasized non-starchy vegetables, legumes, and foods rich in viscous fiber (e.g., ground flaxseed, psyllium, oats, barley, beans, and some vegetables and fruits, such as sweet potatoes, local pumpkin, turnips, and citrus fruits). Second-class foods—defined as minimally processed products such as non-dairy milks, meat substitutes, canned vegetables, and whole grain flour products—were permitted but used less often. Third-class foods included moderately processed foods, such as processed whole grains (e.g., flaked and puffed cereals) and white flour products, and animal products with moderate fat content. Fish that was not fried was permitted after the intensive phase

TABLE 1 | Standard and enhanced versions of the intensive lifestyle intervention.

Standard version	Enhanced version	Frequency	Meals/day	Group classes	Exercise
Weeks 1–2	Weeks 1–2, 4, and 6	4 days/week	3	5–6 h	1 h
Weeks 3–6	Weeks 3 and 5	2 days/week	1	4–5 h	30–60 min
Weeks 7–12	Weeks 7–12	1 day/week	1	4–5 h	30–60 min
Weeks 13–24	Weeks 13–24	1 day/week	0	Turn in Pedometer Readings	

The standard and enhanced versions of the lifestyle intervention differ only during weeks 4 and 6.

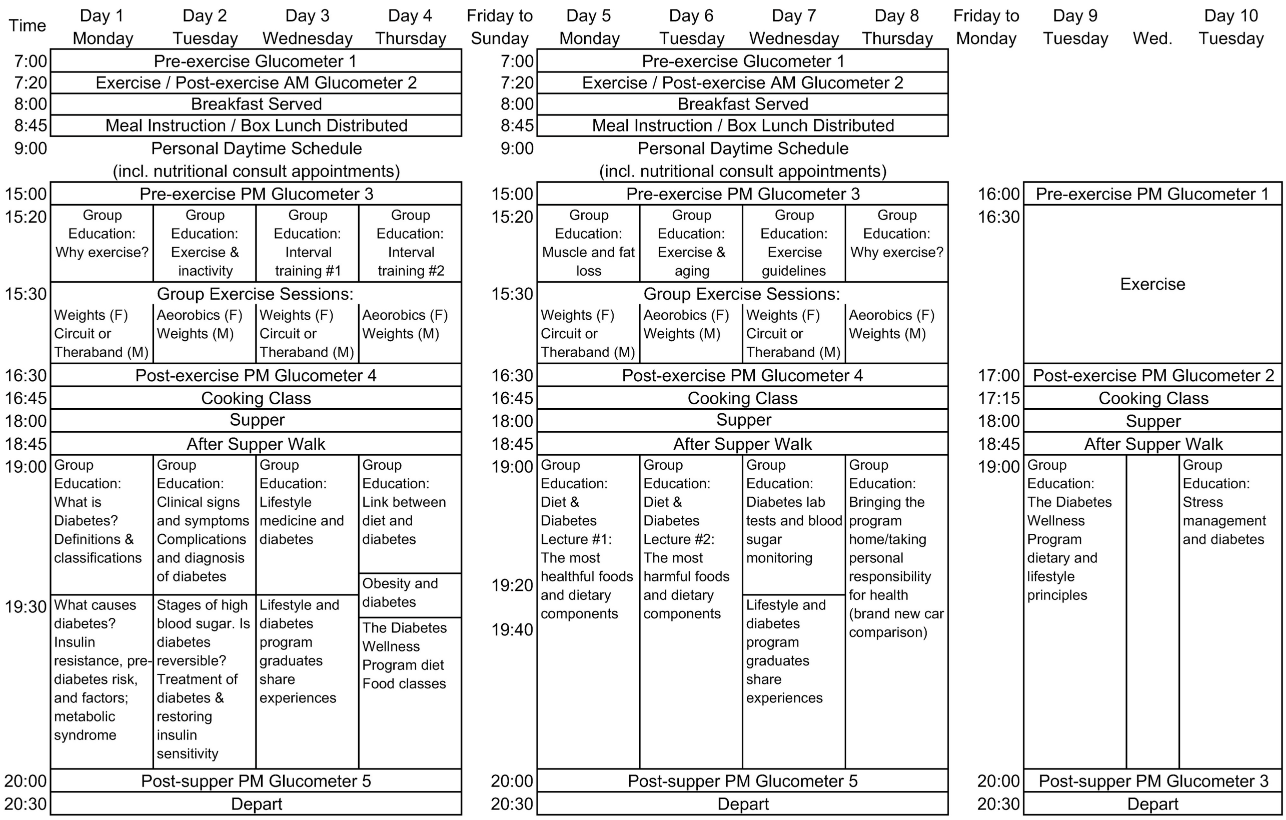

FIGURE 1 | Schedules for the first 3 weeks of the lifestyle intervention.

of the program, and non-fish animal products—such as poultry, eggs from local chickens, and lower-fat dairy—were permitted, but participants were encouraged to minimize consumption of these foods. Fourth-class foods—such as beverages with added sugar, processed meat, fried foods, fast foods, and confections—were prohibited. Within all food categories, lower glycemic index foods were selected. For example, barley was emphasized in the grain group, as it has the lowest glycemic index of all grains. Portions of concentrated carbohydrate foods such as whole grains and starchy vegetables were controlled, and portion sizes of very high-fat foods such as nuts, seeds, and coconut were small to help keep calories controlled.

Exercise

Participants were asked to exercise at least 30–60 min per day throughout the study, which included both solo exercise and structured group exercise classes that decreased in intensity as the study proceeded (**Table 1**). The structured group exercise included culturally acceptable physical activities as determined by focus groups. Group exercise included walking, calisthenics, elastic-band resistance training, Marshallese-style dance classes, and use of gym equipment, such as treadmills, cardio gliders, rowing machines, and free weights. During the 2-week intensive phase of the intervention, participants attended a 1-h group exercise class 4 days per week and completed 2 daily walks on their own, with one before breakfast and one after lunch. They also completed one daily walk as a group after dinner. Each after-meal walk was 10–20 min. On non-clinic days, participants exercised solo. They were encouraged to exercise at the clinic when possible, and when not possible, they were asked to use their therabands and to exercise for at least 30–60 min at home. After the

TABLE 2 | Study menus for weeks 1–2.

	Breakfast	Lunch	Dinner
WEEK 1			
Monday	Not provided	Salad bar Sesame tahini dressing Pumpkin and/or breadfruit and red bean salad Cream of broccoli soup	*Crispy tofu fingers *Spicy eggplant Fresh fruit or vegetables
Tuesday	*Pineapple and coconut muesli *Beans and greens Ground flax Soymilk Jelée parfait	Salad bar Italian dressing Five-bean salad Spiral noodle salad Pea soup	*Garbanzo à la king Fresh fruit or vegetables
Wednesday	*Kamut/barley cereal Beans and greens Walnuts Ground flax Soymilk *Jelée parfait	Gado gado Savory tofu Steamed breadfruit cubes Bean and barley soup	*Papaya or pumpkin stew Fresh fruit or vegetables
Thursday	*Oatmeal *Crunchy granola Ground flax Beans and greens Soymilk Jelée parfait	Salad bar Creamy dill dressing Sesame crackers "Eggless" egg salad Hummus Lentil soup	*Chili Fresh fruit or vegetables
Friday	*Creamy barley cereal *Easy beans/greens Walnuts Ground flax Soymilk Jelée parfait	Not provided	Not provided
WEEK 2			
Monday	Not provided	Salad bar Yam, black bean, and greens salad with balsamic vinaigrette Cabbage soup	*Garbanzo kelel *Cabbage salad Fresh fruit
Tuesday	*Apple raisin muesli Ground flax Easy beans/greens Soymilk Jelée parfait	Salad bar Thousand Island dressing Rice and lentil salad Black bean and corn soup	*Ma stew Fresh fruit or vegetables
Wednesday	*Creamy barley cereal Walnuts Ground flax Beans and greens Soymilk *Jelée parfait	Salad bar Sesame dressing Thai noodle salad Oriental cabbage salad Eggplant black-eyed pea soup	*Tofu or vegetable stir fry *Long beans in black bean sauce Fresh fruit or vegetables
Thursday	*Multigrain cereal Ground flax Beans and greens Soymilk Jelée parfait	Tropical haystacks (rice, pinto beans, lettuce/cabbage, peppers, peanuts, coconut, and pineapple) Golden sauce Pumpkin ginger soup	*Lentil loaf *Mushroom gravy +Roasted vegetables Fresh fruit or vegetables
Friday	*Scrambled tofu with peppers, onions, and greens Easy oat burgers Jelée parfait	Not provided	Not provided

**Recipes prepared by participants. +Recipes demonstrated to participants*

2-week intensive phase, participants attended 60-min exercise group classes on clinic days and were counseled to exercise on their own on non-clinic days. They were also advised to continue with their daily walks (including 10–20 min walks after each meal) and to attend group fitness classes (such as dance classes).

TABLE 3 | Study menus for weeks 3–12.

	Tuesday dinner	Thursday dinner
Week 3	Green salad +Balsamic vinaigrette *Creole +Barley	Promotion night Finger food provided by Diabetes Wellness Center, including breadfruit wedges, and taro squares
Week 4 *(standard version only)*	Green salad *Thousand Island dressing *Vegetable and bean stew	Green salad +Italian dressing *Pasta primavera +Spicy roasted chickpeas
Week 5	Potluck and/or games night (all groups)	*Red lentil soup *Mexi dip +Pita wedges Raw vegetables +Banana bread
Week 6 *(standard version only)*	Cucumber salad Wet pumpkin curry Brown rice +Banana ice cream	Green salad Balsamic vinaigrette *Bean burger +Whole-wheat bread or rolls
ONCE-A-WEEK DINNER		
Week 7	Green salad +Tahini dressing *African stew +Barley	
Week 8	Green salad Italian dressing *Polynesian beans and vegetables *Polynesian fruit bars	
Week 9	Asian salad Sesame or ginger dressing *Tofu triangles with mushrooms *Brown rice	
Week 10	Papaya salad +Coconut mayonnaise *Stewed beans *International greens *Cornbread	
Week 11	Green salad +Creamy dill dressing *Tamale pie +Brownie bars or banana carob cake	
Week 12	Green salad +Oil and vinegar dressing *Sweet and sour tofu Brown rice +Peru pudding	

*The menus for weeks 3–12 were the same for both the standard and enhanced versions of the intensive lifestyle intervention, with the exception that the weeks 1 and 2 menus were used for weeks 4 and 6, respectively, of the enhanced version of the intervention. *Recipes prepared by participants. +Recipes demonstrated to participants.*

Stress Management

Participants in the intervention group were also provided with stress counseling and management activities, such as deep breathing, stretching, and words of encouragement. They were provided a lecture on suggestions for handling stressful situations and getting support.

Cultural Adaptation

The intervention was delivered in a culturally-sensitive manner by trained indigenous Marshallese and Canvasback Missions staff. Prior to designing the intervention, Canvasback Missions had more than 20 years of experience working with the Marshallese people and government, including operating NEWSTART lifestyle programs. The intervention was designed in partnership with the RMI MOH. The study team met with the MOH and health care providers to determine the appropriate level of content and preferred methods of learning. Consequently, the program was structured to be interactive and rely on active learning. All Canvasback Missions staff were provided with significant cultural sensitivity training that included instruction in the cultural traditions and local customs of respect. Marshallese staff were hired, trained, and performed as many study functions as possible, including meal preparation and group activities. Training included a trip to Guam to learn the NEWSTART program before coming back to Majuro to set-up the study. We also engaged local physicians, nurses, dance instructors, and our staff cooks to be a part of education sessions, in an effort to make the program culturally relevant. This was supported by strong relationships that were forged with Marshallese and American dignitaries, the MOH personnel, community group leaders, and store managers. Study documents, lectures, and instructions were provided in the Marshallese language. To tailor the dietary intervention, recipes from Marshallese cuisine were incorporated and adapted while still ensuring that the diet was plant-based and met nutritional targets. Finally, group exercise activities were led by a Marshallese staff member who was known in the community for leading such activities, and local dance routines were encouraged as aerobic activity.

Measurements and Outcomes

Assessments

Study assessments included demographics, anthropometrics, vital signs, serum cardiometabolic risk factors, medication usage, and behavioral assessments. All study assessments were performed at weeks 0, 2, 6, 12, and 24 unless otherwise stated below.

Anthropometrics and Vital Signs

Height, weight, waist circumference, blood pressure, and resting heart rate were all measured in duplicate using standard procedures. Height was measured using a stadiometer and was used to tabulate BMI. Waist circumference was measured at the level of the umbilicus above the iliac crest.

Serum Chemistry

Serum analytes included HbA1c and fasting glucose, insulin, lipids, and hs-CRP. Lipids included total cholesterol, HDL cholesterol, and triglycerides, with LDL cholesterol calculated using the Friedewald equation. Analytes were measured using the CLIA-approved laboratory at the MOH's Hospital. HOMA-IR, which is a product of fasting glucose and fasting insulin, was used to estimate insulin resistance. LDL cholesterol values above 400 mg/dl will be considered unreliable and be replaced with

TABLE 4 | Tiered food classification system.

	1ST CLASS FOODS: Whole Plant Foods (75–100% of calories)	2ND CLASS FOODS: Lightly Processed Foods and Low-Fat Animal Products (≤25% of calories)	3RD CLASS FOODS: Moderately Processed Foods and Mid-Fat Animal Products (≤10% of calories)	4TH CLASS FOODS: Heavily Processed Foods and High-Fat Animal Products (≤5% of calories)
Vegetables	Green leafy vegetables Non-starchy vegetables: broccoli, carrots, cauliflower, onions, cucumbers, egg plant, peppers, mushrooms, tomatoes, sprouts Starchy vegetables: potatoes, sweet potatoes, corn, winter squash	Green leafy vegetables that are canned or cooked in oil	Battered vegetables Vegetables in high-fat sauces	Vegetable chips French fries Deep-fried potatoes or sweet potatoes
Protein foods	Legumes: beans, lentils, peas	Tofu Tempeh Fish (not fried)	Lean meat Poultry (no skin) Eggs Fish canned in oil	Processed or canned meat (e.g., bacon, sausage, cold cuts, frankfurters) High-fat meat Poultry Fried meat
Grains and cereal	Whole grains: barley, rye, kamut, spelt, brown rice, quinoa Cut and rolled grains	Slightly processed whole-grain breads and cereals (incl. whole wheat or other whole grain flour)	Moderately processed grains (e.g., granola bars, white flour, pasta, bagels, white rice)	Heavily processed, white flour products (e.g., donuts, cookies, and other sweet baked goods) Corn chips
Fruit	Fresh, dried, and frozen fruit	Lightly processed fruits (e.g., canned fruit in natural juice)	Fruit canned in syrup Fruit juice	Fruit jams and jellies Fruit-flavored punch and drinks
Dairy/dairy alternatives	Fresh pressed nut and seed milks	Soymilk (unsweetened or original) Other commercial non-dairy milk (almond, hemp, rice)	Non- or low-fat dairy products (incl. skim or low-fat milk and cheese)	High-fat dairy products (e.g., whole milk, cream, cheese, ice cream, sour cream, whipping cream)
Higher fat whole foods, fats, and oils	Nuts, seeds and their butters (natural) Avocados Olives	Nuts and seeds with small amounts of added oil or salt Nut butters with sugar or salt	Vegetable oils Zero trans-fat margarines Nut butters with hydrogenated fats Nuts with fat, sugar, and salt	Lard Shortening Butter Hydrogenated margarine
Other			Natural sweeteners (e.g., maple syrup, blackstrap molasses, honey, brown rice syrup)	Refined sugar (e.g., white sugar, syrup) Soda Sugar-sweetened beverages Candy

the maximum reliable value of 400 mg/dl. hs-CRP values above 10 mmol/l were taken as indicative of an active acute infection, rather than as measures of chronic low-grade inflammation, and were therefore treated as missing values.

Medications

To monitor changes in glucose levels in the intervention group and adjust medication dosages accordingly, a glucometer was used during the first 2 weeks and fasting labs at weeks 2, 6, and 12 were relied on thereafter. Medication dosages were adjusted by participants' primary care physicians and/or the DWC clinicians. Medications and their dosages were recorded at each assessment. Changes in the average number of medications and in the percentage of participants taking one or more medications will be compared across groups for both all medications and diabetes-specific medications.

Glucometers

Participants in the intervention group were given glucometers during the intensive 2-week phase of the intervention to measure their glucose levels, as needed. These assessments were used both for educational purposes and to make medication adjustments.

Dietary Assessment

Participants completed a food frequency questionnaire at baseline (week 0) and at week 24. The questionnaire was tailored to Majuro residents and asked participants about the frequency with which they consumed 110 food items, as well as the typical serving size and any seasonal differences in consumption (for fresh fruits and vegetables only). Participants also described what they usually ate for breakfast, lunch, dinner, and snacks. Participants also completed a one-page, self-reported diet quality assessment at weeks 2, 6, and 12 to report any changes in food intake, including the foods they now avoid, eat less of, and eat more of. Changes in dietary intake and other lifestyle factors were used to help gauge the program's effectiveness. At the end of the trial, participants also self-reported their adherence to the diet using a 1–5 Likert scale.

Physical Activity Assessment

Cardiopulmonary fitness was assessed at each time point using the Harvard step test in year 1 and both the Harvard step test and the timed 1-mile walk in year 2. For the Harvard step test, participants stepped up onto a gym bench ~20 inches in height once every 2 s for 5 min or until the participant could not maintain the pace. Thereafter, they were seated in a chair, and their pulse was assessed 1, 2, and 3 min after completing the step tests, and fitness level was graded as 50 × (duration in seconds)/(sum of the three post-exercise heart rates). For the timed 1-mile walk test, potential participants were given pedometers to wear and were asked to walk a one-mile roadway loop outside the hospital at a pace they could maintain without running or stopping. Participants were instructed to walk alone at their own pace. After the individuals returned, their walking time and steps were collected and recorded. Physical activity levels were assessed by self-report using a modified version of the International Physical Activity Questionnaire at each time point and through weekly pedometer readings**.** All participants—including those in the control group—were asked to wear pedometers throughout the study and report their step count on a weekly basis. Participants came to the DWC to report their readings or, if they were unable to come in, called study staff to report their readings.

Safety

Participants were instructed to report adverse events as soon as they occurred and were also queried about adverse events at each assessment. To minimize the risk of complications from exercises, warm-up exercises were used to gently increase the intensity of the physical activity.

DATA ANALYSES

Statistical Power Calculation

Since the trial proceeded in phases, the initial data collected in year 1 was used to determine the overall sample size needed for the trial. Based on the variances observed in the primary endpoints in the year 1 cohorts, a sample size of $N = 120$ in total was needed to provide at least 80% power (two-sided, $\alpha = 0.05$) to detect between-group differences of 20 mg/dl in fasting glucose and 1.0% in HbA1c.

Statistical Analyses

All data will be analyzed using two-sided statistical tests and a Type I error rate of $\alpha = 0.05$. The intention-to-treat method will be used for the primary analyses but will be compared to outcomes from actual-treatment-received analyses (i.e., per protocol analyses). Categorical data will be compared between the two groups using chi-square or Fisher's exact tests. Continuous data will be compared using linear mixed models or non-parametric tests (as appropriate), with potential adjustments for changes in medication dosages and potential covariates such as baseline values, age, biological sex, and cohort number. Likelihood functions will be examined to determine the appropriateness of covariates and to avoid overfitting the data. Multivariable linear and logistic regression will be used to identify predictors for improved health outcomes. Lastly, subgroup analyses may be performed by sex, age, co-morbidity, length of diagnosis, and severity to evaluate heterogeneity in treatment responses.

DISCUSSION

This study leverages interventions that are clinically effective in an already-established wellness program in Guam and tested them for the first time in a randomized controlled trial. The main goal of the study was to determine the effectiveness of an intensive lifestyle intervention consisting of a mostly plant-based diet and regular moderate exercise in Marshall Islanders with T2D. We expected that study participants would be able to adopt a plant-based diet and engage in moderate exercise sufficiently to achieve clinically meaningful improvements in glycemic control and that many participants would reduce their reliance on diabetes medications and/or even potentially go into remission from T2D. We also expected that participants would experience improved lipid profiles, reduced blood pressure, and lower levels of inflammation, relative to the standard of care. To support these objectives, we leveraged ongoing organizational networks within the RMI, including Canvasback Missions' more than 11 years of experience in providing medical services to T2D patients in the RMI. Moreover, we forged partnerships with the local government to deliver a culturally-adapted version of an intensive lifestyle intervention using trained indigenous staff.

Through implementing this clinical trial, we achieved several major community-wide successes, which serve as a model for future lifestyle intervention trials and community-based participatory research. One of the major achievements is that we assisted in designing an expanded and improved 6,700-square foot Diabetes Wellness Center, which has now been established and is operating in Majuro. The intervention itself was so successful that supermarket buying patterns in Majuro have changed. Reports from the local supermarket indicate that the demand for healthy foods has increased to such an extent that these items are "gone in 2–3 days." The DWC received many requests to speak and present intervention programs for churches, schools, and service groups. The DWC also held a seminar for the members of the Nitijela (Parliament) and the president's cabinet. As a result of the influence of these leaders, the community has begun to respond by changing their food choices and exercising more. When DWC staff first arrived in Majuro, walking was frowned upon, and people tended to take taxis, even for short distance outings. Now, walking for exercise has become sufficiently popular that there are concerns for public safety, as most walking takes place on the roads. As a result, the mayor and police commissioner have agreed to close down the main highway in the early morning to provide the community with a safe place to walk during the cooler dawn hours. More markets are setting aside sections for health foods, and the island now has a good selection of these foods. Produce is being flown in to meet the new public demand. DWC-approved items are appearing on local restaurant menus. The MOH has asked Canvasback Missions to take over food preparation for the hospital. The Department of Education approached Canvasback Missions to develop a nutrition curriculum for grades K-6 in public schools in Majuro and to train teachers. Canvasback Missions was awarded a grant from the World Diabetes Federation. Brenda Davis, RD and Margie Colclough, SLP, were recently brought into Majuro to complete this work. They developed a comprehensive 13-module curriculum called *Get Healthy at School*. Teachers in all nine public schools in Majuro were trained to use these materials in their classrooms. The MOH also held a 2-day conference with its senior administrative staff and leading doctors to find ways to focus on preventive health care. Word of the success of this program has reached many of the surrounding Pacific Island jurisdictions, and the DWC often hosts visitors from these countries.

In achieving these successes, we overcame several challenges. First, recruitment was slower than expected in year 1, so in year 2, we increased enrollment rates by recruiting subjects from the MOH Diabetes Clinic. Another benefit of this change in our recruitment strategy is that it improved compliance to the standard of care in the control group. During year 1, when participants were screened from the general public, they did not always adhere to the standard of care. Recruiting subjects who were already receiving the usual care and regularly met with their physicians resolved this problem. In year 2, as the word spread throughout the country that the DWC program was controlling and reversing T2D naturally, more and more people came forward and wanted to participate, and recruitment was no longer a challenge. In fact, by the end of the trial, more than 100 people were on the waiting list for the DWC program. Another challenge faced in year 1 is that about 25% of the adults screened did not comprehend English well enough to understand materials presented in English. This may have introduced selection bias by enrolling better-educated participants in year 1. Therefore, in year 2, all program materials were translated into Marshallese and presented in Marshallese, initially by translating English presentations, and then later by replacing English presentations with Marshallese presentations. Cohort 5 was the first all Marshallese-run program. After an initial hurdle of training local staff, who had little previous experience and limited education, to become precise and accurate recordkeepers and accurate presenters, we were able to deliver an all Marshallese-version of the intervention.

As for the intervention itself, we observed that the 4-times-per-week intensive phase appeared to be highly effective in promoting adherence to the lifestyle intervention and improving glycemic control. However, once the intervention intensity dwindled to once-every-other-week, a majority of participants did not maintain adherence as well, so adherence waned in the later stages of the trial. This may partially be due to the fact that intensive lifestyle changes can be socially and culturally isolating, particularly because the RMI is a highly social and culturally engaged society, where food is often a central part of cultural events. After some trial and error, we noticed that moving from a 4-times-per-week intensive phase to a twice-per-week phase (rather than every other week) is a more effective approach. It is important for future trials on intensive lifestyle interventions to continue testing the optimal intensity of lifestyle interventions. One of the main unanticipated effects of the intervention is that a significant fraction of the control group attempted the intensive lifestyle program on their own, despite being randomized to the standard of care. Such participants were spotted purchasing foods, such as oatmeal and flaxseeds, at the grocery store, despite not having used these foods before; some of these participants admitted that they were trying to do the lifestyle intervention on their own. In total, we estimate that about one-fourth of the controls adopted significant lifestyle changes during the 24-week intervention, despite instructions to the contrary and despite having the opportunity to crossover to the intervention group after completing the control arm. Contamination of the control group tends to bias outcomes toward the null hypothesis, so our anticipated future results may underestimate the actual treatment effects. Another intervention-related concern is the potential for nutritional shortfalls, especially regarding vitamin B12. Although we used some vitamin B12-fortified foods, such as nutritional yeast and fortified soymilk, such fortified foods are not as readily available in the RMI, and there was concern that over time, vitamin B12 intakes could be inadequate, especially for participants taking metformin. Also, healthy foods, such as fruits and vegetables, are expensive in the RMI, and residents tend to have few resources.

The management of adverse events and data collection in a country with no prior research infrastructure presented the greatest challenges to the trial. The main challenge for adverse event management was adjusting medication dosages downwards to avoid hypoglycemic reactions in the intervention group. When participants were not in the clinic, they often had to return to their family physician to adjust their medication dosages, which meant long wait times and a fee. This was particularly problematic for participants on insulin, who had to be very carefully monitored so that insulin dosages could be reduced or stopped accordingly. Further, because medication dosing tended to increase for control subjects and decrease for intervention subjects, future reported outcomes will likely be biased toward the null hypothesis. We also faced problems with missing data due to participants not showing up for appointments, procedural failures by the laboratory, and the hospital being new to the extensive documentation requirements for clinical trials, in a country where clinical trials have never been performed before. It took several trips and digging through boxes of duplicate lab slips to fill in missing logbook entries. The positive outcome is that new policies and reporting procedures were established for any future lab work that may be required, and all lab work that was done is now backed up. To tackle the challenge of participant attendance at clinic visits, research staff attempted to reach every participant by phone, and in some cases, went to the participant's home or work to remind them of the importance of the blood draws and measures to complete the study. We have discovered that within the RMI, more effort is needed than in the U.S. to ensure that participants fully participate in the intervention.

Overall, though, the clinical trial was a success. We delivered a novel lifestyle intervention, which constituted the first randomized clinical trial conducted in the Republic of the Marshall Islands; the first lifestyle intervention trial conducted in Micronesia; and the second clinical trial to investigate the effects of a plant-rich diet combined with moderate exercise in T2D patients. We intend to publish future manuscripts reporting the results of the clinical trial. We expect that the results of this study will help guide future medical care in the Pacific Islands and provide insight into how to best direct resources to treat indigenous populations with T2D. Outside of the Pacific Islands, it will shed light on how to effectively deliver comprehensive, intensive lifestyle interventions for the treatment and management of diabetes. Finally, it will provide critical insight into the degree to which lifestyle interventions can treat and reverse type 2 diabetes.

ETHICS STATEMENT

The study was approved by the Loma Linda University Institutional Review Board and an *ad-hoc* Institutional Review Board in the RMI that was set-up specifically for the trial. The raw data may be requested by contacting the corresponding author, Dr. John Kelly, at jhkelly@bhhec.org, after the study results are published. The data request will be reviewed by the RMI Ministry of Health.

AUTHOR CONTRIBUTIONS

JK designed the study, with input from JS. BD, RH, and JK conducted the study. HJ, CP, and RK performed the analyses and drafted the manuscript. All authors revised and approved the final version of the manuscript.

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CASE REPORT
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Heart Failure and a Plant-Based Diet. A Case-Report and Literature Review

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A 54-year-old female with grade 3 obesity body mass index (BMI 45.2 kg/m^2) and type II diabetes (hemoglobin A1c 8.1%) presented to her primary care physician in May 2017 with a chief complaint of left lower extremity edema. Work-up revealed heart failure with depressed left ventricular systolic function. Upon diagnosis, she substantially altered her lifestyle, changing her diet from a "healthy western" one to a whole food plant-based one. Guideline directed medical therapy for heart failure was also utilized. Over five and a half months, she lost 22.7 kg and reversed her diabetes without the use of diabetes medications. Her left ventricular systolic function normalized. Although causality cannot be determined, this case highlights the potential role of a plant-based diet in helping to reverse heart failure with reduced ejection fraction. This article will review how a minimally processed whole food plant-based dietary pattern and similar dietary patterns, such as the Dietary Approach to Stop Hypertension diet, may contribute to the reversal of left ventricular dysfunction.

Keywords: diet, heart failure, hypertension, plant-based, vegetarian

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CASE

A 54-year-old female with grade 3 obesity body mass index (BMI 45.2 kg/m^2) and type II diabetes (hemoglobin A1c 8.1%) presented to her primary care physician in May 2017 with a chief complaint of left lower extremity edema. Venous duplex revealed no deep venous thrombosis and an X-Ray revealed lower extremity atherosclerosis with no fracture. She was sent to a cardiologist. Electrocardiogram demonstrated normal sinus rhythm and a left bundle branch block. Echocardiography revealed a left ventricular ejection fraction of 25% without significant valvular pathology; heart failure was diagnosed. Renal, liver, and thyroid function, as well as ferritin and potassium levels were within normal limits. HIV was non-reactive. She was not anemic. She was started on a beta-blocker, an ACE inhibitor, and a statin. Cardiac MRI in June 2017, revealed a dilated cardiomyopathy and an ejection fraction of 21%. Coronary CT angiogram revealed an Agatston coronary artery calcium score of 458. Extensive calcification on the CT angiogram precluded assessment of coronary artery stenosis. Hence cardiac catheterization was performed and revealed a cardiomyopathy out of proportion to coronary artery disease with a 30% proximal left anterior descending artery stenosis, a 25% proximal and a 60% distal left circumflex artery stenosis, and a 65% first obtuse marginal artery lesion. The left main and right coronary arteries were without stenosis. She was shaken by her diagnosis and became determined to adopt a more healthful diet. She changed her diet from "healthy western" to whole food plant-based (**Table 1**). She also started supplemental vitamin B12. She lost 22.7 kg in <6 months, resulting in a BMI of 35.1 kg/m^2. Her diabetes resolved, with her hemoglobin A1c falling to 5.7% without the use of diabetes medications. Her baseline dyspnea on exertion improved considerably. Repeat echocardiography in November 2017 revealed a normal left ventricular ejection fraction of 55% (**Table 2**).

TABLE 1 | Patient's dietary pattern pre- and post-dietary change.

"Healthy western" diet	Plant-based diet (daily)
• Chicken (without the skin) • Fish • Turkey • Eggs • Lean cuts of red meat • Small amounts of processed red meat • Diet soft drinks • Processed foods (e.g., chips and cakes) • 1–2 servings of vegetables or fruits daily	• *No* animal products • At least 3 servings dark leafy greens • At least 3 servings of vegetables • At least 3 servings of fruit • 1–3 servings of beans/legumes • 1–3 servings of whole grains • 1 Tablespoon herb/spice • 1 serving of raw unsalted nuts or seeds • 2 Tablespoons of hemp seeds/chia seeds /ground flax meal • At least one cup of tea/day • Limit packaged/processed foods

Regarding the plant-based diet, patients are not given caloric or macronutrient goals and are invited to consume freely within these parameters.

TABLE 2 | Health parameters at baseline and after five and a half months on a plant-based diet.

Parameter	Baseline	After 5 ½ months
BMI	45.2 kg/m^2	35.1 kg/m^2
Hemoglobin A1c	8.1%	5.7%
Ejection fraction	25%	55%

Although causality cannot be determined, this case highlights the potential role of a plant-based diet in helping to reverse systolic dysfunction, or heart failure with reduced ejection fraction.

This article will review how a minimally processed whole food plant-based dietary pattern and similar dietary patterns, such as the Dietary Approach to Stop Hypertension (DASH) diet and vegetarian diet, may contribute to the reversal of left ventricular dysfunction. For the purposes of this case report and literature review, the term plant-based diet will include dietary patterns that are exclusively plant-based and dietary patterns that are predominantly plant-based, such as the DASH diet and vegetarian dietary patterns.

BACKGROUND

Heart failure (HF) is a condition in which the heart is unable to provide adequate blood flow to meet the normal metabolic needs of the body and can occur with either a reduced or a preserved left ventricular ejection fraction (1). HF is a leading cause of morbidity and mortality with a prevalence of more than 5.5 million in the US and 23 million globally (2). Each year in the US, over 550,000 individuals are newly diagnosed with HF (3)–about half die within 5 years (1).

Numerous risk factors for the development and progression of HF are influenced by diet, including inflammation, hypertension, dysbiotic microbiome, hyperlipidemia, obesity, and diabetes (4–6). However, the medical community has traditionally focused on pharmacotherapy and devices and not on nutrition in both the primary and secondary prevention of HF (7, 8).

This focus may occur because cardiologists receive little instruction on either nutrition or nutrition counseling (9, 10). In a recent survey of more than 900 cardiologists, although 95% believed that their role should include counseling patients about nutrition, 90% received minimal or no related training (10). This training deficit is not unique to cardiology and extends to most fields, including internal medicine and obstetrics/gynecology (9, 11, 12).

This deficit may represent a preventive opportunity lost throughout the lifecycle. The Barker Hypothesis suggests that the intrauterine environment influences cardiovascular health later in life (13, 14). In human and animal models, the presence of maternal obesity adversely impacted cardiac morphology and metabolism, predisposing offspring to cardiovascular disease (15, 16). Offspring of maternal pigs fed a high fat, high-calorie diet versus a standard diet have numerous structural and metabolic cardiac derangements that may put them at risk for HF (16). Human mothers consuming more meat and fish had offspring with elevated cortisol levels which may predispose to hypertension and the metabolic syndrome (17). Consequently, more healthful diets may provide both primordial prevention, and prevention throughout the lifecycle (18).

PROSPECTIVE COHORT STUDIES

Prospective cohort studies support the beneficial impact of plant-based dietary patterns on incident HF (19–23). In a study of 38,075 Finnish people over a median of 14.1 years, higher consumption of vegetables was associated with a lower incidence of HF in men, but not in women (21). Similarly, among 20,900 healthy male physicians in the Physicians' Health Study I, greater consumption of fruits and vegetables was associated with a decreased risk of HF (19). A subset of the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Cohort of 15,569 persons with no Coronary Artery Disease or HF diagnosis was divided into five dietary patterns: Alcohol/Salads, Convenience, Plant-based, Southern, and Sweets. After a median follow-up of more than 7 years, patients with closer adherence to the Plant-based dietary pattern had lower risk of incident HF (23). In a prospective cohort from Sweden of 34,319 women without cardiovascular disease and cancer at initial assessment, after 12.9 years, greater fruit and vegetable consumption was associated with a lower rate of HF (22).

THE INFLUENCE OF DIET ON HEART FAILURE RISK FACTORS

Inflammation

Elevated serum levels of inflammation increase risk factors for HF, as well as incident HF (24, 25). Plant-based diets (PBD) decrease serum levels of inflammation (26–29) and may be

Abbreviations: BMI, Body mass index; DASH, Dietary Approach to Stop Hypertension; HDL, High density lipoprotein; HF, Heart Failure; HFpEF, heart failure with preserved ejection fraction; HTN, Hypertension; LDL, Low-density lipoprotein; PBD, Plant-based Diet.

protective (5). This protective effect is in part mediated through Reactive Oxygen Species (ROS) or free radicals (30, 31).

ROS are unstable molecules that promote inflammation and may react with and damage myocardial cells leading to interstitial fibrosis, myocyte hypertrophy, and decreased myocardial contractility (32). PBDs with high levels of antioxidants and other bioactive compounds may be protective by reducing levels of ROS whereas animal-based diets with lower levels of antioxidants, may not (30, 33). In addition, the consumption of animal foods may facilitate ROS creation via heme iron (34) and other pro-inflammatory compounds such as N-Glycolylneuraminic acid (Neu5gc) (35), Advanced Glycation End-Products (AGEs) (36), trimethylamine amine (TMAO) (37), and nitrates utilized to preserve processed meats (38), thereby potentially further inducing myocardial dysfunction and HF (23, 24).

Moreover, vegetarian versus omnivorous diets are associated with lower plasma levels of the pro-oxidant compound, myeloperoxidase, which promotes atherosclerotic plaque formation, and progression. This promotion may lead to atherosclerotic plaque rupture, myocardial infarction, and HF (39).

Hypertension

Hypertension (HTN) is a common precursor to HF, increasing its risk by 2–3 fold (40, 41). Accordingly, in the Framingham cohort, a blood pressure (BP) of ≥160/100 vs. <140/90 doubled the lifetime risk of incident HF (40). PBDs and predominantly PBDs, such as the DASH or vegetarian diet, may lower both systolic and diastolic BP (42–44), via a number of mechanisms, including favorably modifying the renin-angiotensin (45) and sympathetic nervous systems (46), greater potassium and decreased sodium consumption (47), improved blood vessel dilation (44, 46, 48), and changes in baroreceptors (46).

In prospective studies, red meat and poultry increased incident HTN (49) whereas plant proteins lowered it (50). In the Chicago Western Electric Study, greater consumption of fruits or vegetables was associated with significantly lower BP whereas beef, lamb, poultry, and veal consumption was associated with higher BP after 7 years (51). Similarly, in a large cohort from China of over 450,000 subjects, greater consumption of fresh fruit was associated with decreased systolic BP by 4 mmHg (52). The Diet Approach to Stop Hypertension (DASH) diet, which is low in animal products, total, and saturated fat and high in fruits and vegetables, reduced both systolic and diastolic BP by 5.5 and 3.0 mmHg, respectively, in hypertensive patients (53). Correspondingly, large prospective studies reported that greater adherence to a DASH diet was associated with a 22 and 37% decreased HF risk in men and women, respectively (54, 55). A PBD, however, may even further lower BP. A vegan diet has been associated with achieving a lower BP than omnivorous, pescatarian, and vegetarian diets (56).

Microbiome

The gut microbiome consists of around one hundred trillion microorganisms and impacts our immunologic, cardiovascular, and overall health (57). For example, the pro-atherogenic compound, Trimethylamine N-oxide (TMAO) is formed, in part, via the interplay of the gut microbiome with the nutrients L-carnitine and choline (58). Increasing TMAO levels are associated with increasing severity of HF, rates of MI, and overall mortality (58–64). Accordingly, TMAO may induce vascular inflammation, increase platelet reactivity (58), and reduce reverse cholesterol transport (4). A PBD rapidly selects for a different microbiome than does an animal-based diet and hence produces less TMAO than does an animal-based diet (4) possibly accounting, in part, for the fewer cardiovascular events seen in those consuming a minimally processed plant-based diet (58, 65).

Furthermore, soluble dietary fiber, found exclusively in plant-based foods (66), nourishes healthful bacteria in the colon enabling them to produce short-chain fatty acids (67). Short-chain fatty acids then nourish the enterocyte (68) which may lead to reduced cholesterol biosynthesis by the enterocyte by down-regulating the enterocyte's expression of genes involved in cholesterol biosynthesis, and thereby lower serum cholesterol levels (69). As elevated serum cholesterol is a key cause of coronary artery disease and myocardial infarction (70–73), and hence HF, plant-based nutrition may further lower HF risk by impacting cholesterol biosynthesis in the enterocyte (4, 69).

Hyperlipidemia

Plant-based diets lower serum lipid levels in part due to their soluble fiber, phytosterols, low saturated fat content, absence of cholesterol, and potentially due to their ability to reduce enterocyte cholesterol biosynthesis (69, 74, 75). Moreover, a PBD improves lipids levels specifically in patients with decreased left ventricular ejection fraction (76, 77). Conversely, animal-based diets are associated with increased serum cholesterol levels (78).

Furthermore, a PBD may render low-density lipoprotein (LDL) cholesterol particles less prone to oxidation (74, 79–81). Oxidized LDL particles are particularly atherogenic, damaging to endothelial cells, and promoting of inflammation and oxidative stress (82, 83).

While high-density lipoprotein (HDL) levels may decrease on a PBD (43), recent studies have suggested that increasing HDL levels may not be beneficial and may be harmful (84). In addition, it appears that a healthful lifestyle may render an HDL particle protective, while an unhealthful one renders it atherogenic (85). Accordingly, HDL efflux capacity, or its ability to perform reverse cholesterol transport, irrespective of the absolute HDL serum level, may be a more important measure of its potential beneficial health impact (43, 84, 86). Thus, aspects of a PBD, such as pistachios and almonds, may improve HDL efflux capacity (86, 87).

Obesity

Obesity is a risk factor for HF and may account for approximately 11 and 14 percent of HF cases in men and women, respectively. Increases in BMI are linearly associated with an increase in HF risk, where those with obesity having twice the risk of HF compared to those with a healthy BMI (88). Similarly, in the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, greater self-reported weight at 20 and 40 years of age was associated with increased HF risk later in life (89).

This increased risk may occur as obesity is associated with altered left ventricular remodeling (90). Furthermore, obesity may promote risk factors for HF, such as inflammation, HTN, dyslipidemia, DM, and sleep apnea (88, 91–93).

PBDs have been associated with greater weight loss (94, 95), decreased weight gain (96), and lower BMIs (97, 98) when compared with other dietary patterns. Conversely, greater intake of animal-based foods is correlated with a higher BMI (97, 98). Accordingly, a randomized controlled trial found a vegan diet to be associated with significantly greater weight loss (−7.5%) than a pesco-vegetarian, semi-vegetarian, or omnivorous diet (−3.2, −3.2, and −3.1%, respectively) over the course of 6 months (94). A prospective cohort study found each 250 g/d increase in meat (red meat, processed meat, and poultry) consumption to be associated with an increase in weight of 2 kg over 5 years (99). In the Seventh-Day Adventist study, mean BMI increased as diet became more animal-centric: 23.6 kg/m^2 in vegans, 25.7 kg/m^2 in lacto-ovo-vegetarian, 26.3 kg/m^2 in pesco-vegetarians, 27.3 kg/m^2 in semi-vegetarians, and 28.8 kg/m^2 in non-vegetarians (98). PBDs likely contribute to the obtention and maintenance of a healthy weight through their high fiber content, low caloric density, and potentially, their ability to augment resting energy expenditure (100).

Diabetes

Diabetes Mellitus (DM) is associated with an approximate 400% increase in the incidence of HF (101) and poorly controlled diabetes is associated with worse HF outcomes. Each 1% increase in hemoglobin A1c (HbA1c) was associated with a 12% increase in HF hospitalizations after controlling for other HF risk factors (102). Similarly, insulin resistance, often a precursor for diabetes, is associated with an increased risk of incident HF (103).

A PBD results in a lower incidence of diabetes (104, 105) and hemoglobin A1c levels (106, 107) and may reduce insulin requirements despite a lack of weight loss (108). As such, a PBD may improve HF incidence and outcomes (101, 102). As aforementioned, PBDs are associated with decreased inflammation (26–29), which may ameliorate diabetes by improving both insulin sensitivity and beta cell function (109, 110). Moreover, PBDs are low in saturated fat, which upon accumulation in muscle and hepatic cells, contributes to insulin resistance (100, 111, 112).

Accordingly, in the Seventh-Day Adventist Study, the relationship between Type 2 diabetes and degree of dietary plant consumption appeared to lie on a continuum, with a prevalence of: 2.9% in vegans, 3.2% in lacto-ovo vegetarians, 4.8% in pesco-vegetarian, 6.1% in semi-vegetarians, and 7.6% in non-vegetarians (98).

HEART FAILURE WITH PRESERVED EJECTION FRACTION

Heart failure with preserved ejection fraction (HFpEF), or diastolic heart failure, is common and has similar symptoms and adverse outcomes as heart failure with reduced ejection fraction (113). It is hypothesized that HFpEF may be secondary, in part, to diffuse endothelial dysfunction, including that of the myocardial microvasculature, due partly, to increased inflammation (113). Many plant-based foods are anti-inflammatory (26), and increase Nitric Oxide bioavailability (114), thereby improving vascular health and potentially ameliorating this microvascular dysfunction (24, 113, 115). A PBD may also prevent or treat numerous comorbid conditions, such as obesity, HTN, kidney disease, and diabetes (44, 98, 104–107, 116–118), potentially providing additional preventive and therapeutic benefit. The Dietary Approach to Stop Hypertension in "Diastolic" Heart Failure (DASH—DHF) pilot study evaluated the impact of a DASH diet on 13 post-menopausal women with diastolic HF. After 3 weeks, consuming the DASH diet was associated with significant improvements in blood pressure, arterial elasticity, and oxidative stress (115).

CONCLUSION

Our patient's improvements were temporally associated with her adoption of a whole food plant-based diet. Plant-based diets are associated with improvements in risk factors for heart failure and with direct benefits on cardiac metabolism and function. Given the burden of heart failure, its adverse prognosis, and the overall evidence to date, a plant-based diet should be considered as part of a multifaceted approach to heart failure care.

ETHICS STATEMENT

Full name of the ethics committee: The Montefiore Einstein Institutional Review Board.

Consent Procedure: Written informed consent was obtained from the patient to publish her identifiable/case report data.

AUTHOR CONTRIBUTIONS

RO identified the case and had sole access to patient information. RO, KA, and DG all performed background research, and contributed both to the writing and editing of the paper.

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Relation of Vegetarian Dietary Patterns With Major Cardiovascular Outcomes: A Systematic Review and Meta-Analysis of Prospective Cohort Studies

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Background: Vegetarian dietary patterns are recommended for cardiovascular disease (CVD) prevention and management due to their favorable effects on cardiometabolic risk factors, however, the role of vegetarian dietary patterns in CVD incidence and mortality remains unclear.

Objective: To update the European Association for the Study of Diabetes (EASD) clinical practice guidelines for nutrition therapy, we undertook a systematic review and meta-analysis of the association of vegetarian dietary patterns with major cardiovascular outcomes in prospective cohort studies that included individuals with and without diabetes using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach.

Methods: MEDLINE, EMBASE, and Cochrane databases were searched through September 6th, 2018. We included prospective cohort studies ≥1 year of follow-up including individuals with or without diabetes reporting the relation of vegetarian and non-vegetarian dietary patterns with at least one cardiovascular outcome. Two independent reviewers extracted data and assessed study quality (Newcastle-Ottawa Scale). The pre-specified outcomes included CVD incidence and mortality (total CVD, coronary heart disease (CHD) and stroke). Risk ratios for associations were pooled using inverse variance random effects model and expressed as risk ratios (RRs) with 95% confidence intervals (CIs). Heterogeneity was assessed (Cochran Q-statistic) and quantified (I^2-statistic). The overall certainty of the evidence was assessed using GRADE.

Results: Seven prospective cohort studies (197,737 participants, 8,430 events) were included. A vegetarian dietary pattern was associated with reduced CHD mortality [RR, 0.78 (CI, 0.69, 0.88)] and incidence [0.72 (0.61, 0.85)] but were not associated with CVD mortality [0.92 (0.84, 1.02)] and stroke mortality [0.92 (0.77, 1.10)]. The overall certainty of the evidence was graded as "very low" for all outcomes, owing to downgrades for indirectness and imprecision.

Conclusions: Very low-quality evidence indicates that vegetarian dietary patterns are associated with reductions in CHD mortality and incidence but not with CVD and stroke mortality in individuals with and without diabetes. More research, particularly in different populations, is needed to improve the certainty in our estimates.

Clinical Trial Registration: Clinicaltrials.gov, identifier: NCT03610828.

Keywords: vegetarian dietary patterns, vegetarian diets, cardiovascular disease, prospective cohort studies, systematic review, meta-analysis, GRADE

INTRODUCTION

Vegetarian dietary patterns, or vegetarian diets, are defined as diets that exclude meat, poultry, or fish and may or may not include dairy and eggs. Vegetarian dietary patterns are recognized for their health promoting properties as these diets are typically higher in fiber, antioxidants, phytochemicals, and plant protein and lower in saturated fat compared to non-vegetarian dietary patterns (1). Recent systematic reviews and meta-analyses of vegetarian dietary patterns have found that following a vegetarian dietary pattern was associated with reduced risk of coronary heart disease (CHD) in prospective cohort studies, but not with cardiovascular disease (CVD), or stroke (2). Vegetarian dietary patterns also improved cardiometabolic risk factors in randomized controlled trials in individuals with and without diabetes when compared to non-vegetarian dietary patterns (3–5). Currently, the *2015–2020 Dietary Guidelines for Americans* recommend a vegetarian dietary pattern, along with the Mediterranean and healthy U.S. style dietary patterns, as 1 of 3 healthy dietary patterns (6). A number of clinical practice guidelines for diabetes and CVD also recommend vegetarian dietary patterns based on the evidence from systematic reviews and meta-analyses of both prospective cohort studies and randomized controlled trials (2–4). The Canadian Cardiovascular Society (7), Diabetes Canada (8), the American Diabetes Association (9), and Diabetes UK (10) include vegetarian dietary patterns in their clinical practice guidelines for the reduction of cardiovascular disease (CVD) risk factors [including low-density lipoprotein cholesterol (LDL-C), blood pressure (BP) and body weight risk], CVD outcomes, and improvement in glycemic control for individuals with type 2 diabetes.

Despite the evidence supporting the widespread inclusion of vegetarian dietary patterns in dietary guidelines and clinical practice guidelines for nutrition therapy, the European Association for the Study of Diabetes (EASD) (11) has not assessed the evidence for the role of vegetarian dietary patterns in the prevention and management of CVD, an important outcome as CVD, particularly CHD, is the leading cause of premature death in individuals with diabetes (12, 13). To update the clinical practice guidelines for nutrition therapy to include recommendations for the role of vegetarian dietary patterns in the prevention and management of cardiometabolic diseases, the Diabetes and Nutrition Study Group (DNSG) of the EASD commissioned a series of systematic reviews and meta-analyses using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach, a standard in guideline development to assess the certainty of evidence for important research questions in healthcare. Additional systematic reviews and meta-analyses on vegetarian dietary patterns, diabetes incidence and cardiometabolic risk factors were conducted to update the EASD clinical practice guidelines (5). These systematic reviews and meta-analyses were conducted as the GRADE approach was not previously assessed on this topic. The present systematic review and meta-analysis using GRADE was conducted to address the question of whether the available evidence from prospective cohort studies of vegetarian dietary patterns in comparison with non-vegetarian dietary patterns shows an association with reduced risk of major cardiovascular outcomes in individuals with and without diabetes.

METHODS

Study Design

We conducted a systematic review and meta-analysis following the methodology from the Cochrane Handbook for Systematic Reviews and Interventions (14). Reporting followed the Meta-analysis of Observational Studies in Epidemiology (MOOSE) (15) and PRISMA guidelines (www.prisma-statement.org). The study protocol was registered on ClinicalTrials.gov (identifier NCT03610828).

Data Sources and Searches

We searched MEDLINE, EMBASE, and the Cochrane Library (through September 6th, 2018) for relevant prospective cohort studies in humans with no language restrictions. The search strategy is presented in **Supplementary Table 1**. We supplemented the search with manual searches by identifying cohort studies in the reference lists of included studies.

Study Selection

We included studies based on the following PICOS (population, intervention, comparator, outcomes, and study design): Population included individuals of all ages with and without diabetes; intervention included vegetarian dietary patterns; comparator included non-vegetarian dietary patterns; outcomes included major cardiovascular outcomes (CVD, CHD, stroke mortality, and incidence) and study design included prospective cohort studies of ≥1-year duration. Vegetarian dietary patterns were considered either as lacto-ovo vegetarian diets, which includes dairy and eggs but excludes all other animal products, or vegan diets, which exclude all animal products including dairy and eggs.

Data Extraction

Two reviewers (AJG and EV or MS) independently reviewed the articles, extracted relevant data, and assessed risk of bias.

Risk of Bias

The Newcastle-Ottawa Scale (NOS), a scoring system developed to assess the quality of nonrandomized studies, was used to assess the risk of bias. The studies are judged on three broad perspectives and can receive up to a total of 9 points. The first section is cohort selection (max 4 points), which includes representativeness of the exposed cohorts, selection of the non-exposed cohort and ascertainment of exposure. The second section is the comparability of cohort (max 2 points), which refers to the appropriate inclusion of confounding variables in the analysis. The last section is adequacy of the outcome measures (max 3 points), which includes assessment of outcome and adequacy of follow-up (16). Studies achieving 6 points or more were considered high quality. Differences were reconciled by consensus.

Outcomes

There were 6 primary outcomes included in the analysis: CVD mortality, CHD mortality, stroke mortality, CVD incidence, CHD incidence, and stroke incidence. CVD incidence and mortality includes all forms of CVD, including both CHD, and stroke outcomes. CHD includes incidence or mortality from atherosclerosis and/or myocardial infarction. Stroke incidence and mortality includes all forms of stroke, including ischemic, hemorrhagic, and unspecified.

Statistical Analyses

Primary and sensitivity analyses were conducted using Review Manager (RevMan), version 5.3 (Copenhagen, Denmark) and subgroup and publication bias analyses was conducted using STATA software, version 13.0 (College Station, TX, USA). Individual cohort comparison relative risks (RRs) from the most adjusted models were obtained comparing vegetarian vs. non-vegetarian dietary patterns. Hazard Ratios (HRs) were treated as RRs. To obtain summary estimates, we natural log-transformed the RRs and pooled them using DerSimonian-Laird random effects models (17). Heterogeneity was assessed (Cochran Q statistic) and quantified (I^2 statistic). If I^2 was ≥50% and $p < 0.10$, then we interpreted this finding as evidence of substantial heterogeneity (18). Sources of heterogeneity were investigated through sensitivity and subgroup analyses. Sensitivity analyses were performed by systematically removing each study from the meta-analysis with recalculation of the summary estimates in order to assess whether any single study exerted an undue influence on the summary estimates (change of significance and/or direction of association or change of significance of heterogeneity estimate). If ≥10 cohort comparisons were available, a priori subgroup analyses by sex (female, male), type of vegetarian diet (vegan, lacto-ovo), underlying disease status (i.e., diabetes), follow-up (<10 vs. ≥10 years), validation of dietary assessment methods (yes vs. no), NOS (<5 vs. ≥6), and funding source (agency, industry) was conducted using meta-regression. If ≥10 cohort comparisons were available, publication bias by visual inspection of funnel plots and formal testing using the Begg and Egger tests was conducted (19, 20). When publication bias was suspected, adjustment for funnel plot asymmetry was done by imputing missing study data using the Duval and Tweedie trim and fill method (21).

Grading of the Evidence

The certainty and strength of the evidence was assessed using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system (18, 22–33). Included observational studies started at low-certainty of evidence by default and then were downgraded or upgraded based on pre-specified criteria. Criteria to downgrade certainty included study limitations (weight of studies showing risk of bias by NOS), inconsistency (substantial unexplained inter-study heterogeneity, $I^2 \geq 50\%$ and $P < 0.10$), indirectness (presence of factors relating to the population, exposures and outcomes that limit generalizability), imprecision [95% CIs were wide or crossed a minimally important difference of 5% (RR 0.95–1.05) for all CVD outcomes] and publication bias (significant evidence of small-study effects). Criteria to upgrade included a large effect size (RR > 2 or RR < 0.5 in the absence of plausible confounders), a dose–response gradient and attenuation by plausible confounding effects.

RESULTS

Figure 1 shows the flow of the literature search. Of the 233 reports found, 6 reports with 7 unique prospective cohort studies met eligibility criteria. The 7 unique cohorts include: EPIC-Oxford (34, 35), Heidelberg Study (36), Adventist Health Study-2 (AHS-2) (37), Adventist Mortality Study (AMS) (38), Adventist Health Study-1 (AHS-1) (38), Oxford Vegetarian Study (39), and Health Food Shoppers Study (39) (**Table 1**). We included

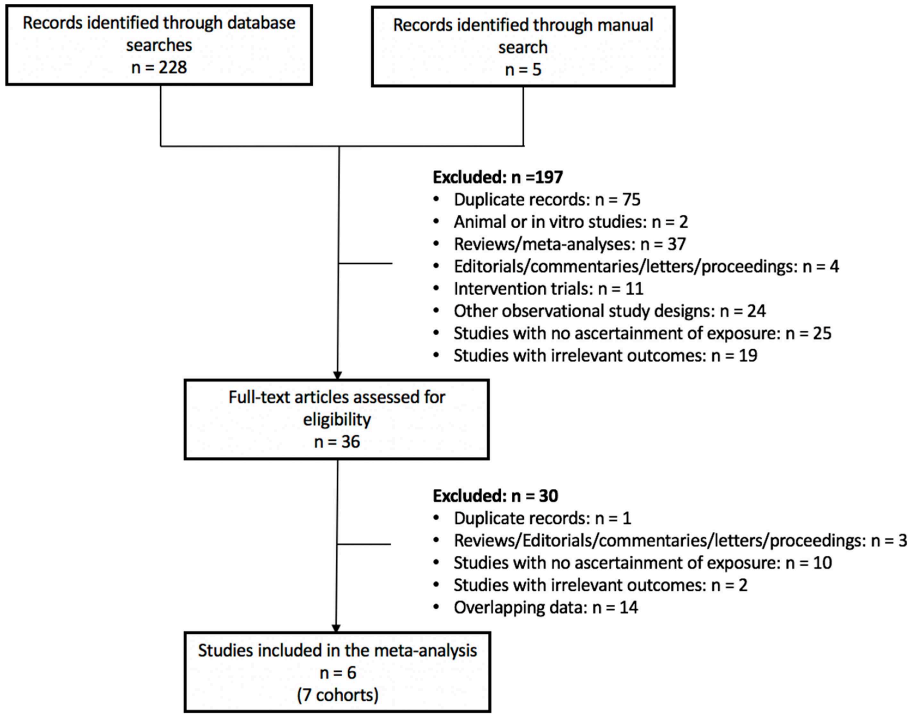

FIGURE 1 | Summary of evidence search and strategy.

sex-specific data from one cohort (37) as separate comparisons. Therefore, we included 6 cohort comparisons for CVD mortality (144,247 participants, 3,307 cases) (34, 36, 37, 39), 8 cohort comparisons for CHD mortality (197,737 participants, 2,988 cases) (34, 36–39), 5 cohort comparisons for stroke mortality (122,525 participants, 1,113 cases) (34, 38, 39), and 1 cohort comparison for CHD incidence (44,561 participants, 1,235 cases) (35) (**Figure 2**; **Table 1**). Data from two cohorts (AMS and AHS-1) were extracted from a previous pooled analysis (38). No studies were found that were exclusive to individuals with diabetes or that included subgroup analyses of individuals with diabetes. No studies were found reporting CVD and/or stroke incidence.

Study Characteristics

Table 1 shows the characteristics of the included prospective cohort studies. Participants were from the United States, the United Kingdom and Germany, mostly middle-aged (median age range: 33–58 years) and belonged to specific health-conscious groups (i.e., health food store shoppers, members of vegetarian societies, subscribers to vegetarian magazines, and members of the Seventh-Day Adventist church). Prevalence of individuals in the cohorts who followed a vegetarian dietary pattern ranged from 28 to 62%. While no studies excluded individuals with diabetes, only one cohort (EPIC-Oxford) reported prevalence of diabetes (1%) in their cohort (35) and no cohorts conducted subgroup analyses for individuals with diabetes. The mean follow-up durations ranged from 5.5 to 21 years. There were more female than male participants across all outcomes. Ascertainment of cases was done by medical record linkage for all cohorts except one (Heidelberg study), which ascertained mortality through death certificates (36). Vegetarian dietary patterns (combined lacto-ovo and/or vegan) were compared to non-vegetarian, or omnivorous, dietary patterns for all outcomes. This was because only one cohort (AHS-2) reported separate disease associations for different types of vegetarian dietary patterns: lacto-ovo and vegan diets (37). Although dietary intake was assessed by a food frequency questionnaire at baseline in most studies, the assignment of vegetarian status was often based on responses to global questions about the consumption of meat, poultry and/or fish. For example, one cohort (Health Food Shoppers) asked the question "Are you vegetarian?" and one cohort (Oxford Vegetarian Study) asked about never eating meat or fish (39). The EPIC-Oxford cohort assigned vegetarian diet status through four questions on diet groups (meat, fish, dairy, and eggs) or through a food frequency questionnaire (FFQ) (34, 35). All studies were funded by agency alone; except for one cohort (Heidelberg Study), which did not report funding sources (36).

Supplementary Table 2 show the statistical adjustments performed in the included studies. All studies adjusted for the pre-specified primary confounding variable (age). No studies adjusted for all 7 of 9 predefined secondary confounding variables for CVD outcomes (sex, family history of CVD, smoking, markers of overweight/obesity, diabetes, hypertension, dyslipidemia, energy intake, and physical activity) and only one cohort (Oxford Vegetarian Study) adjusted for diabetes status (39).

TABLE 1 | Summary of characteristics of prospective cohort studies assessing the association between vegetarian dietary patterns and major cardiovascular outcomes.

References	Cohort	Country	No. of participants	% vegetarians	Outcomes	No. of cases	Person-years	Age range, years	Mean follow-up, years (dates)	Diet assessment method to determine vegetarian status	Exposure	Methods of outcome assessment	NOS quality score	Funding source
Crowe et al. (35)	EPIC—Oxford	UK	10,602 (M) 33,959 (W)	34	Incident CHD	1,235	517,960	20–89	11.6 (1993–2009)	Validated 130-item FFQ and global questions about meat, fish, dairy, and eggs[f]	Vegetarian/vegan vs. non-vegetarian	Medical record linkage	7	Agency[a]
Chang-Claude et al. (36)	Heidelberg Study	Germany	858 (M) 1,046 (W)	61.2	CVD + CHD mortality	255 (CVD) 72 (CHD)	NR	10–85	21 (1976–1999)	FFQ included meat, fish, dairy, and eggs	Vegetarian/vegan vs. non-vegetarian	Death certificates	6	NR
Orlich et al. (37)	AHS-2	USA	25,105 (M) 48, 203 (W)	28.9	CVD + CHD mortality	987 (CVD) 372 (CHD)	NR	57[b]	5.79 (2002–2009)	Validated >200-item FFQ[g]	Vegetarian/vegan vs. non-vegetarian[d]	National Death Index	6	Agency[a]
Key et al. (38)	Adventist Mortality Study	USA	8,994 (M) 15,544 (W)	41.8	CHD + stroke mortality	598 (CHD) 182 (Stroke)	138, 304	35–80	5.5 (1960–1965)	21-item FFQ	Vegetarian vs. non-vegetarian	Medical record linkage	6	Agency[a]
Key et al. (38)	AHS-1	USA	12, 214 (M) 16, 738 (W)	27.6	CHD + stroke mortality	921 (CHD) 317 (Stroke)	320, 818	25–89	11.1 (1976–1988)	>60-item FFQ	Vegetarian vs. non-vegetarian	Medical record linkage	6	Agency[a]
Key et al. (34)	EPIC—Oxford	UK	11, 324 (M) 35, 930 (W)	34	CVD, CHD + stroke mortality	479 (CVD) 213 (CHD) 159 (Stroke)	506, 620	20–89	8–14[c] (1993–2007)	Validated 130-item FFQ and global questions about meat, fish, dairy and eggs[f]	Vegetarian/vegan vs. non-vegetarian[e]	Medical record linkage	7	Agency[a]
Appleby et al. (39)	Oxford Vegetarian Study	UK	4,174 (M) 6,871 (W)	42	CVD, CHD + stroke mortality	469 (CVD) 250 (CHD) 125 (Stroke)	NR	16–89	17.6 (1980–2000)	Never ate meat or fish statement	Vegetarian vs. non-vegetarian	Medical record linkage	5	Agency[a]
Appleby et al. (39)	Health Food Shoppers	UK	4,325 (M) 6,411 (W)	43	CVD, CHD + stroke mortality	1,117 (CVD) 562 (CHD) 330 (Stroke)	NR	16–89	18.7 (1973–1999)	"Are you vegetarian?" question	Vegetarian vs. non-vegetarian	Medical record linkage	6	Agency[a]

AHS-1, Adventist Health Study-1; AHS-2, Adventist Health Study-2; CHD, coronary heart disease; CVD, cardiovascular disease; EPIC, European Prospective Investigation Into Cancer; FFQ, food frequency questionnaire; M, men; NR, not reported; NOS, Newcastle-Ottawa Scale; USA, United States; UK, United Kingdom; W, women.

[a]*Agency funding is that from government, university or not-for-profit health agency sources.*

[b]*Mean age.*

[c]*Range of years of follow-up.*

[d]*Reported disease associations for other forms of vegetarianism in original publication (i.e., vegan, lacto-ovo, pescatarian, semi-vegetarian); only overall vegetarian diet included in current analysis.*

[e]*Reported disease associations for pescatarians in original publication; combined vegetarian and vegan diet data included in current analysis.*

[f]*Vegetarian dietary pattern determined by 4 global questions relating to never eating meat, fish, dairy or eggs or by intake of relevant food items reported in FFQ (first 1,300 participants); FFQ validated for nutrients only.*

[g]*Vegetarian dietary pattern determined by intake of food items of animal origin reported on FFQ; FFQ validated for animal foods/food groups.*

Risk of Bias Assessment

Supplementary Table 3 shows the NOS scores for the included prospective cohort studies. Although several studies lost points in several domains, only one cohort (Oxford Vegetarian Study) showed evidence of serious risk of bias (NOS <6) (39).

Vegetarian Dietary Patterns and CVD Mortality

Figure 2 and **Supplementary Figure 1** show the association between vegetarian dietary patterns and CVD mortality (6 cohort comparisons, 144,247 participants and 3,307 cases). We found no significant association (RR 0.92, 95% CI 0.84, 1.02, $p = 0.13$) with no evidence of heterogeneity ($I^2 = 34\%$, $P = 0.18$) when we compared vegetarian dietary patterns to non-vegetarian dietary patterns.

Vegetarian Dietary Patterns and CHD Mortality

Figure 2 and **Supplementary Figure 2** show the association between vegetarian dietary patterns with CHD mortality (8 cohort comparisons, 197 737 participants and 2,988 cases). We found a protective association (RR 0.78, 95% CI 0.69, 0.88, $p < 0.0001$) with no evidence of substantial heterogeneity ($I^2 = 46\%$, $P = 0.07$) when we compared vegetarian dietary patterns to non-vegetarian dietary patterns.

Vegetarian Dietary Patterns and Stroke Mortality

Figure 2 and **Supplementary Figure 3** show the association between vegetarian dietary patterns with stroke mortality (5 cohort comparisons, 122,525 unique participants and 1,113 cases). We found no significant association (RR 0.92, 95% CI 0.77, 1.10, $p = 0.36$) with no evidence of heterogeneity ($I^2 = 44\%$, $P = 0.13$) when we compared vegetarian dietary patterns to non-vegetarian dietary patterns.

Vegetarian Dietary Patterns and CHD Incidence

Figure 2 shows the association between vegetarian dietary patterns with CHD incidence (1 cohort comparison, 44,561 participants and 1,235 cases). We found a protective association (RR 0.72, 95% CI 0.61, 0.85) in this cohort between vegetarian dietary patterns and CHD incidence. As only one cohort comparison was found, a test for overall effect or heterogeneity was not possible.

Sensitivity Analyses

Supplementary Table 4 show the sensitivity analyses involving the systematic removal of each study for CVD, CHD and stroke mortality, respectively. For CVD mortality, removing each study did not change the direction or significance of the result, or result in significant heterogeneity. For CHD mortality, the direction or significance of the association did not change, however, individually removing the Adventist Mortality Study, the Heidelberg Study, the Adventist Health Study-2 (men), the Adventist Health Study-2 (women), and EPIC-Oxford introduced significant heterogeneity. Similarly, for stroke mortality, the direction or significance of the association did not change, however, individually removing the Adventist Health Study-1 and the Health Food Shoppers study introduced evidence of substantial heterogeneity.

Subgroup and Publication Bias Analyses

Subgroup and publication bias analyses were not undertaken as there were <10 cohort comparisons available for each of the CVD outcomes.

GRADE Assessment

Figure 2 and **Supplementary Table 5** show the GRADE assessments for the association between vegetarian dietary patterns and each CVD outcome. The evidence was rated as very low certainty for all outcomes. CVD and stroke mortality were rated as very low certainty owing to downgrades for serious imprecision and indirectness. CHD mortality was rated as very low-certainty owing to downgrades for serious indirectness. All mortality outcomes were downgraded for indirectness as the majority of the studies (comprising of 84–91% of weight in pooled analyses) were done in participants who belonged to specific health-conscious groups (e.g., vegetarian societies, health food store shoppers, subscribers to vegetarian magazines, or were members of the Seventh-day Adventist church). In addition, outcomes were downgraded for indirectness due to the lack of exclusive to or subgroups analyses in patients with diabetes. CHD incidence was rated as very low certainty due to serious indirectness (only one cohort was included in the analysis). Publication bias and a dose-response relationship were not assessed due to limited cohort comparisons.

DISCUSSION

Summary of Main Findings

The present systematic review and meta-analysis of prospective cohort studies including individuals with and without diabetes assessed the association between vegetarian dietary patterns (combined lacto-ovo and vegan) and major cardiovascular outcomes. A total of 8 cohort comparisons were included in the analysis: 6 for CVD mortality (144,247 participants and 3,307 cases), 8 for CHD mortality (197,737 participants and 2,988 cases), 5 for stroke mortality (122,525 participants and 1,113 cases), and 1 for CHD incidence (44,561 participants and 1,235 cases) (34–39). Pooled analyses showed that vegetarian dietary patterns were associated with a 22% decrease in CHD mortality and 28% decrease in CHD incidence. Vegetarian dietary patterns were not associated with reductions in CVD and stroke mortality. These findings will provide important evidence to update the clinical practice guidelines for nutrition therapy of the EASD on vegetarian dietary patterns.

Results in Relation to Other Studies

Our results are consistent with systematic-reviews and meta-analyses of prospective cohorts previously conducted in this area, where vegetarian dietary patterns were associated with a 25% reduced risk of CHD mortality (2). The same study also found that vegetarian dietary patterns were not significantly

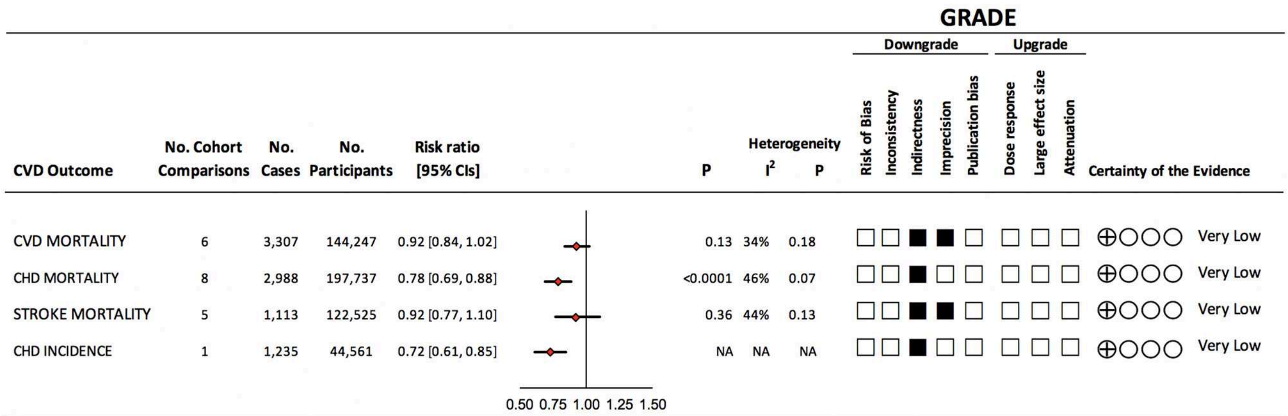

FIGURE 2 | Summary of the pooled effect estimates and certainty of evidence of prospective cohort studies assessing the association between vegetarian dietary patterns and major cardiovascular outcomes. Pooled risk estimate is represented by the diamond. Values of $I^2 \geq 50\%$ indicate substantial heterogeneity (18). Values > 1.0 indicate an adverse association. The Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) of prospective cohort studies are rated as "Low" certainty of evidence and can be downgraded by 5 domains and upgraded by 3 domains. The filled black squares indicate downgrade and/or upgrades for each outcome. CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; NA, not applicable.

associated with CVD and stroke mortality. While similar studies were included in both this study and the most recent past systematic reviews and meta-analyses (2), there were slight differences in methodology (i.e., we included sex-specific analysis as different comparisons when possible), and the study did not include a GRADE assessment of the certainty of the evidence, an important assessment for providing evidence-based recommendations for healthcare professionals and to identify knowledge gaps. Systematic reviews and meta-analyses of prospective cohort studies have also consistently shown that increased meat consumption has been associated with increased CVD outcomes, including CHD and stroke (40–43). Furthermore, the evidence from systematic reviews and meta-analyses of randomized controlled trials of intermediate CVD risk factors, including LDL-C, total cholesterol, BP and body weight, found that vegetarian dietary patterns and replacing animal protein with plant protein can significantly reduce these CVD risk factors compared to non-vegetarian dietary patterns or consuming animal protein (3–5, 44, 45).

We were unable to assess the quality of the vegetarian dietary patterns consumed in our pooled analysis due to the limited information on the exposure provided in the cohorts. This highlights an area to be considered in future studies as these aspects may be important for CVD prevention. Recently, an analysis of the Nurse's Health Study and Health Professionals Follow-up Study found that a healthy plant-based dietary pattern (which may or may not be vegetarian) was associated with reduced risk of CHD, whereas an unhealthy plant-based diet high in refined carbohydrates was associated with an increased risk of CHD (46). This finding suggests that the quality of a vegetarian dietary pattern may also be important and that healthy plant-based dietary patterns that include small amounts of meat, poultry or fish may also reduce risk of CHD. We were also unable to separate different types of vegetarian dietary patterns (e.g., vegan) in our pooled analysis due to limited sample size of vegans, highlighting another area to be considered in future studies. Vegan dietary patterns may impact health outcomes differently, as evidenced by an analysis of the Seventh-Day Adventist cohorts which showed that vegan dietary patterns may offer additional CVD protection, particularly in males (47).

Potential Mechanisms

Several potential mechanisms may explain the protective association found with CHD in our systematic review and meta-analysis and for the cardioprotective effects of vegetarian dietary patterns found in randomized controlled trials. Vegetarian dietary patterns are typically higher in whole grains, fruits, vegetables, nuts, pulses and soy compared to non-vegetarian dietary patterns (48). This difference in eating pattern results in a diet that is typically higher in fiber, phytochemicals (e.g., antioxidants and plant sterols), plant protein, plant-based unsaturated fatty acids, and lower in energy density and saturated fat, factors that have been shown to reduce CVD risk factors and impact overall CVD health through both intrinsic and food displacement mechanisms (1, 5, 45, 49–53). A lower intake of saturated fat may explain the association of reduced risk with CHD, as high saturated fat diets have been shown to increase LDL-C (54), and LDL-C is a known causal factor in the development of atherosclerosis and CHD (55). Other novel mechanisms include reduced intake of heme iron from animal products (56–59) and less trimethyl N-oxide (TMAO) production (60), as higher intake and levels have both been associated with increased CVD events (56–60). Similar mechanisms may also play a role in reduced CVD and stroke incidence or mortality, however, we did not find protective associations of vegetarian dietary patterns with CVD or stroke mortality.

Strengths and Limitations

The strengths of this study are that we identified all available prospective cohort studies through a systematic search strategy, performed quantitative syntheses and assessed the certainty of the evidence using the GRADE approach. The inability to rule out residual confounding is a limitation inherent in all observational research and explains why prospective cohort studies start at a GRADE of low certainty. Potential sources of residual confounding include reverse causality, the reliability of self-report dietary intake (61) and measurement of the exposure to vegetarian and non-vegetarian dietary patterns, measured and unmeasured confounders included in statistical models, and important collinearity effects from related dietary and lifestyle factors. In particular, as diet was only assessed at baseline in each cohort, we cannot determine if individuals changed their diet over time to include meat or fish, which could lead to misclassification of vegetarian diets during follow-up (62). Some self-reported vegetarians may also consume small amounts of meat and fish, which could also result in misclassification of their diet (63). Another important limitation is indirectness of the study populations. We downgraded the certainty of the evidence for all of the mortality outcomes for serious indirectness, as the majority of the studies (comprising of 84–91% of weight in pooled analyses) were conducted in participants who belonged to specific health-conscious groups (e.g., vegetarian societies, health food store shoppers, subscribers to vegetarian magazines, or were members of the Seventh-day Adventist church) and the populations did not provide subgroup analysis of individuals with diabetes, limiting generalizability. We also downgraded the certainty of the evidence for CHD incidence for indirectness as it was limited to one cohort from the UK. Furthermore, there were no available prospective cohort studies that assessed the relationship of vegetarian dietary patterns with CVD and/or stroke incidence. A final limitation was imprecision. We downgraded CVD and stroke mortality for serious imprecision as the 95% CIs were wide and could not rule out clinically important benefit and/or harm.

Weighing the strengths and limitations of the evidence, the certainty of evidence was considered to be very low certainty for each CVD outcome, owing to downgrades of indirectness due to the limitations of the populations included for all outcomes, and downgrades of imprecision for CVD and stroke mortality.

Implications

Although the evidence has been rated as very low certainty for all CVD outcomes, if we consider the current study results with findings from systematic reviews and meta-analyses of randomized controlled trials of vegetarian dietary patterns and findings from systematic reviews and meta-analyses of meat consumption in prospective cohort studies, we are provided with further and better quality evidence that vegetarian dietary patterns may be beneficial for CVD risk reduction. The evidence for vegetarian dietary patterns in CVD risk reduction is not only important for clinical practice and dietary guidelines to consider for individual health, but also for growing consumer concerns regarding climate change and animal welfare.

All prospective cohorts included in this analysis were conducted in North America or Europe, where following a vegetarian dietary pattern may be more beneficial for health than other areas in the world where small amounts of meat may improve nutritional status. Globally, in North America and Europe, the number of reported vegetarians is increasing but still remains low, with recent surveys in estimating 10% of the population follows a vegetarian or vegan diet (64–67). This highlights that there is room to shift more individuals toward this healthy dietary pattern as one strategy to improve cardiovascular health, including individuals who have diabetes. Interestingly, recent surveys have reported that 33% of Americans plan to buy more plant-based products in the next year (68), and in Europe recent surveys have indicated that 45% of consumers in Italy and France, 57% in Germany and 61% in Spain report that they regularly include meat-free days in their diet (69). Despite this increased interest in plant-based foods, there is some evidence from a small study in Canada that healthcare professionals are hesitant to recommend vegetarian dietary patterns as they are perceived as too difficult to follow (70). Moreover, the same study reported that 89% of patients living with diabetes did not know a vegetarian dietary pattern was an option to manage their disease and help prevent future CVD, however, 66% reported they would be willing to try the diet with the right support (70). Further, evidence supports that vegetarian dietary patterns have been shown to be as acceptable as other therapeutic diets, suggesting their suitability for long-term use (71–73).

The expanding plant-based food market and availability of vegetarian products in grocery stores and restaurants indicates that this dietary pattern may become easier to follow in the future. As mentioned previously, other implications of a vegetarian dietary pattern for ethical and environmental reasons may also be in line with the values and preferences of some individuals (74, 75). Many scientists have called for significant reductions in the consumption of animal products for sustainable planetary health, in which a vegetarian dietary pattern would fit, as recently described in the global scientific targets of the EAT-*Lancet* Commission (76). Given these consumer trends and concerns, healthcare professionals may have more clients interested in this dietary pattern. Therefore, there is an opportunity for healthcare professionals to consider a vegetarian dietary pattern as one dietary strategy, along with other dietary patterns such as the Mediterranean and Dietary Approaches to Stop Hypertension (DASH), to reduce CVD risk in their patients. Clinical practice guidelines, such as the EASD, should consider developing appropriate resources and tools for healthcare professionals to effectively counsel their patients and address barriers of those interested in following a vegetarian or more plant-based diet (77). Lastly, healthcare professionals can work with their clients to develop a vegetarian dietary pattern that is appropriately planned to ensure adequate nutrient intake, and to ensure that a reliable source of vitamin B12 is included in the diet of those following a vegan dietary pattern (65).

CONCLUSIONS

In conclusion, vegetarian dietary patterns were associated with a reduced risk of CHD mortality and incidence but were not associated with reductions in CVD and stroke mortality in predominantly middle-aged participants with and without diabetes. These findings and GRADE assessment provide a very low certainty of evidence for all CVD outcomes, which will be included in the EASD clinical practice guidelines for nutrition therapy. Sources of uncertainty include the observational study design from which one cannot infer causality, indirectness due to the specific groups studied, and imprecision in the pooled estimates for CVD and stroke mortality. Additional research will have an important influence on the certainty of our estimates. In the absence of randomized controlled trials, additional well-conducted prospective cohort studies in other populations assessing the relationship of vegetarian dietary patterns on CVD outcomes are needed. Future studies should also assess if there are differences between different forms of vegetarianism (e.g., vegan) and the nutritional quality of the vegetarian dietary patterns. There is also a need to assess the role of vegetarian dietary patterns in CVD prevention in patients with diabetes, as there were no studies exclusively in individuals with diabetes and no subgroups analyses conducted in those with diabetes. Lastly, and more importantly, there is a need for more high-quality evidence from large randomized trials assessing the effect of vegetarian dietary patterns on hard CVD outcomes in individuals with and without diabetes.

AUTHOR CONTRIBUTIONS

AG, HK, DR, JS-S, CK, and JS conceived and designed the study. All authors analyzed and/or interpreted the data. AG wrote the first draft of the manuscript. All authors revised the article critically for important intellectual content, gave final approval of the version to be published and agreed to be accountable for all aspects of the work**.**

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2019.00080/full#supplementary-material

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AG, DJ, and HK follow a vegetarian or vegan dietary pattern.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Beyond Meat: A Comparison of the Dietary Intakes of Vegetarian and Non-vegetarian Adolescents

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Dietary intake of adult vegetarians from large prospective studies has been well-characterized but is rarely reported in vegetarian adolescents. Our objective was to describe and compare the dietary intake of vegetarian adolescents with their non-vegetarian counterparts in a population known to espouse healthy living. Adolescents ($n = 534$) aged 12–18 years old from middle and high schools near major Adventist universities in Michigan and Southern California provided dietary, demographic, and anthropometric data. Dietary intake was measured with a validated 151-item self-administered web-based food frequency questionnaire; weight and height were measured during school visits. Vegetarian was defined as the combined intake of meat, meat derivatives, poultry, and fish of <1 serving per week. Descriptive statistics and ANCOVA were used to compare the intake of vegetarians and non-vegetarians. Vegetarians significantly ate more fruits, vegetables, and other plant-based foods, but significantly less foods of animal origin, sugar-sweetened beverages, and coffee/tea compared to non-vegetarians. Vegetarians had significantly higher intakes of carbohydrates and total protein but lower intakes of fats, animal protein, and zinc compared to their counterparts. A majority (75% or more) of both groups met the 2015 Dietary Guidelines' age-and-gender-specific recommendations for most nutrients but only 16–18% of vegetarians/non-vegetarians did not exceed the upper limit for sodium. More vegetarians (49%) than non-vegetarians (25%) had <10% of their caloric intake from SFA. More than 90% of both groups met dairy recommendations, but greater proportions of vegetarians met recommendations for vegetables, fruits, nuts/soy products, and legumes than non-vegetarians. Of the non-vegetarians, only 7% and 44% met the fish and meats/poultry/eggs recommendation, respectively, which none of the vegetarians met. Compared to the general US adolescent population, both diet groups ate more fruits, vegetables, dairy and protein foods, and also consumed more micronutrients but less macronutrients. Overall, vegetarian adolescents have a more favorable dietary intake profile than non-vegetarians, but both vegetarians and non-vegetarians in this study population have a more adequate diet than the general US adolescent population. The influence of the Adventist plant-based diet culture that is translated both at home and at school is evident in our findings.

Keywords: vegetarian, adolescence, dietary habits, nutritional status, Adventist Health Studies, plant-based diets, NHANES, Adventist

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INTRODUCTION

Dietary habits formed during childhood and adolescence have both short- and long-term impacts on health (1–3). Nutrient and energy demands increase to support the accelerated growth and development during adolescence, thus, it is crucial to establish or maintain healthy dietary habits during this period since this tends to persist through adulthood (4). A healthy diet during adolescence predisposes to better physical health since it is associated with lower weight gain (1) and lower risk of developing cardiovascular risk factors (5) during adulthood. High diet quality is also imperative to prevent depression (6), an increasing mental health problem among the youth and young adults (6–8).

Increased independence during adolescence and other factors, such as the family's socio-economic condition and education of parents (9), may influence dietary habits. Increased portion sizes of marketplace foods (10) have contributed to increased caloric consumption (11, 12) and low diet quality among US adolescents (13). Although there has been a decrease in sugar- sweetened beverages intake since 1999 (14), high-calorie-low nutrient-density foods have remained major sources of calories among the youth (12–14). On the contrary, intake of nutrient-dense foods (i.e., fruits and vegetables), remains below recommendations (15–18).

With a growing trend toward plant-based diets, there is an increasing movement toward vegetarian eating in different population groups, including adolescents. A vegetarian diet excludes meat, seafood, and products containing both, but may include eggs and dairy. Vegan diets eliminate all animal products. A national online survey conducted by Harris Poll for the Vegetarian Resource Group in 2014 indicated that 4% of youth ages 8–18 years are vegetarians while a total of 32% eat at least one vegetarian meal per week (19). In a 2016 national poll conducted in the United States among a representative sample of adults ages 18 years and above, 3.3% were self-reported vegetarians, half of whom claimed to be vegans (20).

Vegetarian and vegan diets are associated with a lower risk of chronic diseases among adults (21, 22). However, while plant-based or vegetarian diets and their health effects are well studied in adults, reports on vegetarian children and adolescents are scarce. We have access to the Adventist population, a group of people known to espouse a healthy lifestyle that includes a plant-based diet. A sizable proportion of this population are vegetarians, 4.2% being vegan and ∼32% being lacto-ovo vegetarian (23). Although several studies have been published on Adventist adults, only very few have been reported on young people and none of these investigated their dietary intakes. Thus, our objectives were to describe and compare the dietary intake of Adventist vegetarian and non-vegetarian adolescents, determine if their reported intakes meet the recommend intakes for their age group, and compare their dietary profile with those of the general US adolescent population.

METHODS

Study Design and Participants

In previous reports, we have described a cross-sectional study—the Teen Food and Development Study—that we conducted among adolescents to examine associations between dietary intake and certain health outcomes, particularly physical growth and pubertal development (24–26). We used the dietary data from this study in this report. Briefly, we collected data on 601 adolescents (262 males and 339 females) aged 12–18 years old from selected Adventist and public middle and high schools near major Adventist universities in Michigan and Southern California. A website was especially created for the study that served as the interface in the enrolment of potential participants and their parents, completing informed consent, and providing information on demographics, lifestyle habits, physical development, and dietary intake using the web-survey. Anthropometrics were measured during school visits.

Parents of potential participants 17 years old and younger provided their consent for their children's participation in the study by checking a box and providing their name and at least one telephone number at which they could be reached. In the same manner, the children indicated their assent to be part of the study by checking the assent box and providing their name on the same consent form. To ascertain that a parent actually provided the consent, a call was made at the telephone number provided. The informed consent was made official and printed for record-keeping only after the parent had confirmed his/her identity and consent, and the child's assent to participate. All study protocols, including data collection and informed consent, were approved by the Institutional Review Boards of Andrews University in Michigan (IRB#12-113) and Loma Linda University in Southern California (IRB#5120014).

Assessment of Dietary Intake

Dietary intake was measured using a validated 151-item self-administered semi-quantitative web-based food frequency questionnaire designed for the study (27). Briefly, the questionnaire included 8 food groups: convenience foods (32 items), protein-rich foods (29 items), starches/cereals (17 items), vegetables/fruits (21 items), dairy products (10 items), beverages (24 items), snacks/sweets (11 items), and soups/legumes (7 items). Participants reported frequency of their intake of a food item using a drop-down list: never/rarely, 1–3 times per month, once per week, 2–4 times per week, 5–6 times per week, once per day, 2–3 times per day, and 4 or more times per day.

Assessment of Vegetarian Status

Vegetarian status was determined based on self-reported intake of foods that differentiate vegetarians from non-vegetarians. Vegetarian was defined as intake of less than one combined portion (<3 ounces) of meat, meat derivatives, poultry, and fish per week.

Assessment of Other Measures

During school visits, trained personnel measured weight and height of the participants. In the web-based survey, participants also self-reported their weight and height after measuring themselves. Clinical data for the anthropometrics were used for the statistical analysis; however, for participants who were absent during the school visits (about 4%), self-reported measures were used in the analysis since self-reported weight and height were found to be highly correlated to the clinic-measured values (28).

A section of the self-administered web survey included items on demographics and lifestyle (time spent on vigorous physical activity and sleep). The parents' educational levels were used as surrogate for socio-economic status. Some of these variables were controlled for when dietary intake comparisons were made between vegetarians and non-vegetarians.

Data Management and Analysis

Of the 601 original participants, 13 were excluded due to completely missing diet or demographic data and 54 were excluded due to implausible energy intake (<500 calories and >3,500 calories for girls, <800 calories and >4,500 calories for boys). The final analytical dataset included 534 participants.

Descriptive and univariate analyses for all participants and by dietary preference (vegetarian vs. non-vegetarian) were conducted using either independent *t*-test or Chi-square test to check differences between vegetarians and non-vegetarians. ANCOVA was used to compare the food and nutrient intake of vegetarians and non-vegetarians while controlling for age, sex, ethnicity, education of mother, education of father, total energy intake, BMI z-score, and physical activity level. Before analysis, the food and nutrient variables were log-transformed due to non-normal distributions.

Food Groupings and Nutrient Calculations

Foods were grouped according to the following categories: (1) breads, grains and pastas; (2) cereals; (3) fresh fruits; (4) 100% fruit juices, canned and dried fruits; (5) non-starchy vegetables; (6) starchy vegetables; (7) legumes; (8) meats (red meats, processed meats, and poultry); (9) fish; (10) meat alternatives; (11) nuts and nut butters; (12) eggs; (13) dairy cheese; (14) dairy milk; (15) dairy substitutes; (16) dairy desserts; (17) water; (18) sugar-sweetened beverages; (19) coffee and tea; and, (20) pastries and chips. The first 16 food groups were further re-categorized into breads/grains/pastas/cereals, total fruits, total vegetables, total protein foods, total dairy. **Supplementary Table 1** shows the list of foods under each of the food groups. Given that our webFFQ was semi-quantitative, i.e., has fixed serving sizes, intake of these food groups was determined based on the sum of the intake frequency per day of the foods under each group, where frequency per day is equivalent to serving per day.

The nutrient profile of each food in the FFQ was determined using the Nutrition Data System for Research (29) database. Nutrient intake per day was computed using the sum-product method, i.e.,

$$A = \sum_{i=1}^{n} F_i a_i \qquad (1)$$

where A = total intake of nutrient A per day; F_i = frequency of food i intake per day; and a_i = amount of nutrient A in food i. The nutrients assessed in the study were: energy, total carbohydrates, added sugar, total fat, saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), linoleic acid (LA), alpha linolenic acid (ALA), total protein, animal protein, vegetable protein, total dietary fiber, insoluble fiber, soluble fiber, vitamin B12, vitamin C, thiamin, riboflavin, vitamin D, vitamin E, folate, calcium, iron, potassium, magnesium, sodium, and zinc.

Intake Comparisons With the US Adolescent Population

Food intakes of the study population were compared with their counterparts in the general US population using the values for those aged 14–18 years old in the 2007–2010 published estimates for the US population in the National Cancer Institute's Epidemiology and Genomics Research Program website (30). Weighted mean intake values for selected foods were computed since mean intakes were reported separately for males ($n = 808$) and females ($n = 727$). Percent differences between the mean intakes of the vegetarians and non-vegetarians in the study population were then calculated against the weighted means of the US adolescent population.

Nutrient intakes of the study population were also compared with the general US population ages 12–19 years using What We Eat in America, NHANES 2011–2012 (31) values from the Nutrient Intakes from Food and Beverages table. Since mean intake values were separated for male and female adolescents, weighted means were computed to represent the whole group. In the same manner as for foods, percent differences between the mean intakes of the vegetarians and non-vegetarians were then calculated against the general US population/NHANES values.

Intake Comparisons With Dietary Guidelines and Dietary Reference Intakes

The US Dietary Guidelines Recommendations and Nutritional Goals for Age-Sex Groups Based on Dietary Reference Intakes and Dietary Guidelines Recommendations found in Appendix 7 of the 2015–2020 Dietary Guidelines for Americans (2015DGA) (32) was used to determine what proportions of the study population met the recommended intake amounts for selected nutrients. To determine if the participants met the recommended food groups intake, Healthy U.S.-Style Eating Pattern: Recommended Amounts of Food from Each Food Group at 12 Calorie Levels found in Appendix 3 of the 2015DGA was used. Comparisons were made according to age and gender group recommendations for nutrients and calorie levels of 1,600 kcal (for female participants ages 12–13 years old), 1,800 kcal (for male participants ages 12–13 years old and female participants ages 14–18 years old), and 2,200 kcal (for male participants ages 14–18 years old).

RESULTS

The demographic profile of the study population is presented in **Table 1** for both vegetarians and non-vegetarians. Girls were more likely to be vegetarians compared to boys and more than half (52%) of the vegetarians were non-Hispanic whites. Although both groups had parents with college education or higher, the vegetarians had a significantly greater proportion of parents with higher education, especially at the graduate level.

TABLE 1 | Demographic characteristics according to vegetarian status.

Variable	Non-vegetarians, *n* = 397		Vegetarians, *n* = 137		*p*-value	
	n (%)	Mean (*SD*)	*n* (%)	Mean (*SD*)		
Sex						0.012
Girls	212 (53.4)		90 (65.7)			
Boys	185 (46.6)		47 (34.3)			
Age, years		15.0 (1.8)		15.0 (1.7)	0.986	0.458
12	28 (7.1)		10 (7.3)			
13	75 (18.9)		18 (13.1)			
14	64 (16.1)		25 (18.2)			
15	66 (16.6)		32 (23.4)			
16	70 (17.6)		25 (18.2)			
17	63 (15.9)		16 (11.7)			
18	31 (7.9)		11 (8.0)			
Mother's educational level						0.008
High school and below	66 (7.6)		8 (6.1)			
Vocational	18 (4.8)		5 (3.8)			
College	161 (42.9)		57 (43.2)			
Master's	101 (26.9)		42 (31.8)			
Doctoral	29 (7.7)		20 (15.2)			
Father's educational level						<0.001
High school and below	84 (22.4)		10 (7.6)			
Vocational	20 (5.3)		4 (3.0)			
College	128 (34.1)		34 (25.8)			
Master's	86 (22.9)		48 (36.4)			
Doctoral	57 (15.2)		36 (27.3)			
Ethnicity						0.001
Black	34 (9.1)		13 (9.8)			
Non-Hispanic White	123 (32.8)		69 (52.3)			
Hispanic	60 (16.0)		13 (9.8)			
Asian	49 (13.1)		13 (9.8)			
Other ethnicities	109 (29.1)		24 (18.2)			
Site						0.325
California	225 (56.7)		71 (51.8)			
Michigan	172 (43.3)		66 (48.2)			
Physical activity, min/day		33.3 (25.7)		27.5 (23.1)	0.022	
Duration of sleep, hours/day		7.8 (2.5)		7.9 (1.1)	0.746	
BMI Z-score		0.42 (0.97)	0.14 (0.89)		0.005	

Vegetarians were also leaner compared to non-vegetarians. Non-vegetarians, however, spent significantly more time in vigorous physical activity compared to their vegetarian counterparts.

As shown in **Table 2**, there were significant differences in the food intakes of vegetarians and non-vegetarians. **Table 2** displays the estimated marginal means with their 95% confidence intervals after controlling for relevant covariates. Vegetarians ate about one-half serving more of fruits, nearly ¾ serving more of vegetables, and over one serving more of nut/nut butters and meat alternatives than their non-vegetarian peers. Not surprisingly, non-vegetarians ate slightly over one serving more of animal protein foods than the non-vegetarians, but their egg intake amounted to approximately one-third of a serving compared to about one-fourth of a serving for their vegetarian counterparts. Intake of dairy products by non-vegetarians was higher by just a small amount (between 0.07 to 0.30 of a serving) compared to vegetarians. Although intakes of sugar-sweetened beverages and coffee/tea were small for both groups (much less than a serving), the non-vegetarians significantly consumed more of these beverages than the vegetarians.

Table 3 shows a comparison of the nutrient intakes between the two diet groups. Vegetarians had significantly higher intakes of carbohydrates (∼27 g more), polyunsaturated fatty acids (∼3 g more PUFA and LA, and ∼2 g more ALA), LA:ALA ratio, vegetable protein (∼21 g more), dietary fiber (∼8 g more), vitamin C (∼20 mg more), thiamin (∼1.7 mg more), vitamin E (∼1.4 mg more), folate (∼135 mcg more), calcium (∼130 mg more), iron (3.6 mg more), potassium (∼296 mg

TABLE 2 | Comparison of the intake of selected foods by diet groups.

Foods, servings per day	Means (95% CI)[a]		*p*-value
	Non-vegetarians	Vegetarians	
Breads/Grains/Pastas/Cereals, total	5.42 (5.25, 5.59)	**5.82 (5.53, 6.13)**	0.022
Breads, Grains, Pastas	4.75 (4.60, 4.91)	4.97 (4.69, 5.26)	0.207
Cereals	0.58 (0.53, 0.63)	**0.75 (0.65, 0.86)**	0.003
Fruits, total	2.17 (2.03, 2.31)	**2.71 (2.44, 3.00)**	0.001
Fresh fruits	1.53 (1.42, 1.65)	**1.93 (1.70, 2.17)**	0.002
100% FJ; canned and dried fruits	0.60 (0.54, 0.66)	0.68 (0.58, 0.79)	0.177
Vegetables, total	3.54 (3.37, 3.71)	**4.32 (3.99, 4.67)**	<0.0001
Non-starchy vegetables	2.66 (2.52, 2.81)	**3.11 (2.85, 3.40)**	0.004
Starchy vegetables	0.30 (0.27, 0.32)	0.30 (0.26, 0.34)	0.958
Legumes	0.47 (0.43, 0.52)	**0.76 (0.68, 0.85)**	<0.0001
Protein foods, total	2.44 (2.35, 2.54)	2.30 (2.15, 2.46)	0.144
Meat, poultry, eggs	**1.37 (1.30, 1.44)**	0.29 (0.22, 0.36)	<0.0001
Meats (red meats, poultry)	**0.97 (0.92, 1.03)**	0.06 (0.01, 0.11)	<0.0001
Eggs	**0.37 (0.34, 0.40)**	0.23 (0.18, 0.28)	<0.0001
Fish	**0.10 (0.09, 0.12)**	0.01 (-0.01, 0.03)	<0.0001
Nuts, nut butters, meat alternatives	0.86 (0.79, 0.93)	**1.97 (1.77, 2.17)**	<0.0001
Meat alternatives	0.55 (0.49, 0.61)	**1.34 (1.19, 1.50)**	<0.0001
Nuts and nut butters	0.29 (0.26, 0.33)	**0.53 (0.45, 0.61)**	<0.0001
Dairy, total	3.25 (3.16, 3.34)	3.08 (2.94, 3.22)	0.058
Cheese, dairy	**2.03 (2.01, 2.06)**	1.96 (1.92, 2.00)	0.003
Milk, dairy	**0.53 (0.47, 0.59)**	0.23 (0.15, 0.31)	<0.0001
Dairy desserts	**0.30 (0.27, 033)**	0.22 (018, 0.27)	0.007
Dairy substitutes	0.24 (0.19, 0.28)	**0.51 (0.42, 0.61)**	<0.0001
Water	2.75 (2.57, 2.95)	3.08 (2.74, 3.45)	0.118
Sugar-sweetened beverages	**0.62 (0.57, 0.69)**	0.41 (0.32, 0.50)	<0.0001
Coffee and tea	**0.17 (0.14, 0.20)**	0.09 (0.04, 0.13)	0.002
Pastries and chips	1.10 (1.02, 1.17)	1.10 (0.97, 1.24)	0.938

[a]*Estimated marginal means; controlled for age, gender, ethnicity, education of mother, education of father, total energy intake, BMI z-scores, and physical activity.*
Values in bold are significantly greater than the other diet group.

more), magnesium (∼72 mg more), as well as sodium (∼150 mg more) and phosphorus (57 mg more). On the other hand, non-vegetarians significantly consumed more total (∼6 g more), saturated (∼5 g more) and monounsaturated (∼3 g more) fats, animal protein (∼23 g more), and zinc (1 mg more) compared to vegetarians.

As seen in **Table 4**, at least 75% of both vegetarians and non-vegetarians in the study population met the recommended intake amounts for carbohydrates, protein, vitamin B12, riboflavin, vitamin C, thiamin, folate, and iron, and had adequate intakes of the omega-3 fatty acid, alpha linolenic acid (ALA). About 77% of vegetarians reported adequate intake of linoleic acid compared to about 72% of non-vegetarians. Very small proportions (≤10%) of both vegetarians and non-vegetarians met the dietary recommendations for vitamins D and E and potassium, and <20% consumed sodium below the upper limit. Almost 50% of the vegetarians consumed <10% of their total caloric intake from saturated fatty acids (SFA) whereas only half of that proportion (∼25%) met the SFA intake recommendation among non-vegetarians. Compared to non-vegetarians, a larger proportion of vegetarians met the dietary fiber (53 vs. 36%), calcium (44 vs. 36%), and magnesium (56 vs. 48%) recommendations. On the other hand, a larger proportion of non-vegetarians met the zinc recommendations compared to their vegetarian counterparts (77 vs. 64%).

Table 4 also shows the percentage of study participants who met the recommendations for vegetables, fruits, grains, dairy, and protein foods. Compared to their non-vegetarian counterparts, a larger percentage of vegetarians ate more vegetables (80 vs. 68%), non-starchy vegetables (85 vs. 77%), legumes (88 vs. 74%), fruits (72 vs. 62%), and nuts/soy products (98 vs. 70%). Only a small proportion of both groups met the recommendations for starchy vegetables, while more non-vegetarians met the recommendations for grains (41 vs. 38%) and protein foods (61 vs. 39%) than vegetarians. No vegetarian participants met the meat/poultry/eggs and fish intake recommendations.

Figures 1, **2** show comparisons of percent intake differences for food groups (**Figure 1**) and nutrients (**Figure 2**) between the two diet groups and the general US adolescent population. Intakes of dairy, fruits, total vegetables, non-starchy vegetables, and protein foods were relatively greater (ranging from about 25% to 300% more), whereas intakes of breads/grains/pastas, starchy vegetables, and fish were relatively lower (ranging from about 20 to 90%) for both diet groups than average US adolescents. However, only vegetarians reported lower intake of fish while non-vegetarians had a fish intake similar to that of US adolescents. Intakes of ALA, dietary fiber, vitamin B12, vitamin C, thiamin, vitamin E, folate, iron, potassium, and magnesium were higher for both groups (ranging from ∼10 to ∼290%) except for zinc, which was only higher for the non-vegetarian group compared to the US adolescent population. Only vegetarians had higher intakes of polyunsaturated fatty acids (PUFA), linoleic acid (LA), and calcium. Intakes of total fat, SFA, monounsaturated fatty acids (MUFA), LA:ALA ratio, total protein, vitamin D, and sodium were lower for both groups compared to the US adolescents, but zinc intake was only lower for vegetarians.

DISCUSSION

In this study, we described and compared the dietary intake of vegetarian and non-vegetarian adolescents in a population largely composed of Adventists. We further compared these intakes with the DRI and 2015 DGA to determine dietary intake adequacy, and with those of the general US adolescent population. As expected, vegetarians significantly consumed more plant-based foods (i.e., fruits, vegetables, legumes, cereals), meat alternatives, and dairy substitutes compared to their non-vegetarian counterparts while the non-vegetarians consumed more dairy and protein foods from animal sources (i.e., meats, eggs, and fish). Vegetarians also consumed higher amounts of most nutrients except those that are associated with animal

TABLE 3 | Comparison of the intake of selected nutrients by vegetarians and non-vegetarians.

Nutrients (servings per day)	Means (95% CI)[a]		*p*-value
	Non-vegetarians	Vegetarians	
Energy, kcal	1990.22 (1972.39, 2008.21)	2010.22 (1980.29, 2040.60)	0.277
Total carbohydrates, g	248.64 (244.69, 252.90)	**275.06 (267.20, 283.16)**	<0.0001
Added sugars	40.73 (38.74, 42.78)	37.15 (34.09, 40.49)	0.077
Fat	**77.09 (75.57, 78.65)**	70.95 (68.58, 73.48)	<0.0001
SFA, g	**26.68 (25.89, 27.47)**	21.41 (20.35, 22.53)	<0.0001
MUFA, g	**25.97 (25.41, 26.52)**	22.71 (21.89, 23.59)	<0.0001
PUFA, g	17.41 (16.98, 17.85)	**20.78 (19.91, 21.69)**	<0.0001
LA	15.27 (14.89, 15.67)	**18.38 (17.58, 19.20)**	<0.0001
ALA	1.70 (1.66, 1.74)	**1.86 (1.78, 1.93)**	<0.0001
LA:ALA ratio	8.98 (8.83, 9.14)	**9.90 (9.60, 10.22)**	<0.0001
Total protein, g	79.84 (78.34, 81.37)	77.48 (74.89, 80.08)	0.124
Animal protein	**38.21 (36.60, 39.92)**	15.72 (14.57, 16.96)	<0.0001
Vegetable protein, g	37.68 (36.45, 38.98)	**58.62 (55.37, 62.12**)	<0.0001
Total dietary fiber, g	21.87 (21.24, 22.49)	**29.84 (28.42, 31.37)**	<0.0001
Insoluble fiber, g	15.35 (14.88, 15.82)	**21.31 (20.19, 22.47)**	<0.0001
Soluble fiber, g	6.45 (6.28, 6.62)	**8.46 (8.08, 8.86)**	<0.0001
Vitamin B12. ug	5.69 (5.44, 5.95)	5.85 (5.42, 6.32)	0.539
Vitamin C, mg	142.74 (134.96, 150.96)	**162.39 (147.23, 178.93)**	0.029
Thiamin, mg	2.32 (2.20, 2.44)	**4.03 (3.69, 4.42)**	<0.0001
Riboflavin, mg	2.03 (1.98, 2.08)	2.10 (2.01, 2.19)	0.174
Vitamin D, mcg	4.69 (4.42, 4.98)	4.18 (3.77, 4.64)	0.067
Vitamin E, mg α-tocopherol	8.46 (8.20, 8.73)	**9.84 (9.31, 10.39)**	<0.0001
Folate, ug	540.77 (524.79, 557.80)	**675.19 (640.34, 711.94)**	<0.0001
Calcium, mg	1091.16 (1059.97, 1122.15)	**1221.70 (1162.12, 1284.34)**	<0.0001
Iron, mg	16.58 (16.15, 17.03)	**20.19 (19.30, 21.14)**	<0.0001
Potassium, mg	2702.68 (2643.87, 2765.56)	**2998.90 (2884.19, 3118.17)**	<0.0001
Magnesium, mg	314.51 (308.28, 320.86)	**386.45 (373.16, 399.81)**	<0.0001
Sodium, mg	3248.67 (3190.71, 3310.98)	**3398.20 (3291.18, 3508.70)**	0.022
Zinc, mg	**11.93 (11.66, 12.19)**	10.92 (10.52, 11.36)	<0.0001

[a]Estimated marginal means; controlled for age, gender, ethnicity, education of mother, education of father, total energy intake (except for energy), BMI z-scores, and physical activity level. Values in bold are significantly greater than the other diet group.

foods (total fat, SFA, animal proteins, and zinc) and MUFA compared to non-vegetarians. However, in comparison to the reported intake of US adolescents, both diet groups in our study population ate more plant-based foods (fruits, vegetables), dairy, and protein foods but relatively less breads/grains/pastas, starchy vegetables, and fish. Regarding nutrients, both groups had similar intakes of total energy, added sugars, total protein, vitamin B12, riboflavin, and vitamin D. Compared to US adolescents, both diet groups have lower intakes of total energy and macronutrients except for PUFA, ALA and dietary fiber; however, intake of LA was only higher for vegetarians but similar for non-vegetarians. Intakes of nutrients associated with fruits and vegetables (e.g., folate and vitamin C) and nuts/nut butters (e.g., vitamin E and ALA) as well as most of the selected micronutrients, except vitamin D and sodium, were higher for both groups compared to their US adolescent counterparts. However, zinc intake was higher for non-vegetarians yet lower for vegetarians compared to US adolescents. Most of the nutrient recommendations were met by more than three-fourths of both vegetarians and non-vegetarians except for linoleic acid and zinc. About half of vegetarians but just 25% of non-vegetarians met the recommendation for SFA intake to be <10% of total energy intake, while over half of vegetarians and about one-third of non-vegetarians met the dietary fiber recommendation. Lower proportions of both groups met the recommendations for intake of the nutrients important for bone health: calcium (44 vs. 36%, vegetarians vs., non-vegetarians, respectively); magnesium (56 vs. 48%); and, vitamin D (3.6 vs. 3.3%).

Our findings are consistent with previous findings of Perry et al. (33) regarding intakes of fruits, vegetables, total and saturated fats, and iron among vegetarian and non-vegetarian adolescents. However, unlike their findings, vegetarians in our study had similar vitamin B12 intake as non-vegetarians, probably due to most of them being lacto-ovo vegetarians. Vitamin D intake in both vegetarians and non-vegetarians is lower in this adolescent group compared to the mean intake

TABLE 4 | Percentage who met age-and-gender specific recommendations for intake of selected nutrients and food groups.

	Met Recommendations, %		
	All subjects	**Non-vegetarians**	**Vegetarians**
NUTRIENTS[a]			
Carbohydrates	95.1	94.7	96.4
Protein	92.5	93.2	90.5
SFA < 10% of energy intake	31.1	24.9	48.9
Linoleic acid (LA)[c]	73.0	71.5	77.4
Linolenic acid (ALA)[c]	77.7	77.6	78.1
Dietary fiber	40.6	36.3	53.3
Vitamin B12	92.7	94.5	87.6
Riboflavin	93.8	94.2	92.7
Vitamin C	88.8	88.4	89.8
Thiamin	94.6	92.9	99.3
Folate	82.0	80.1	87.6
Vitamin D	3.4	3.3	3.6
Vitamin E	15.4	15.1	16.1
Calcium	37.6	35.5	43.8
Iron	78.5	76.8	83.2
Potassium[c]	9.0	8.6	10.2
Magnesium	50.0	48.1	55.5
Sodium[d]	17.6	18.1	16.1
Zinc	73.6	76.8	64.2
Phosphorus	57.7	58.2	56.2
FOOD GROUPS[b]			
Vegetables[e]	71.2	68.0	80.3
Starchy vegetables[f]	7.9	8.3	6.6
Non-starchy vegetables[f]	78.7	76.6	84.7
Legumes[f]	77.3	73.6	88.3
Fruits[e]	64.8	62.5	71.5
Grains, oz-eq/day	40.4	41.3	38.0
Dairy[e]	95.1	95.5	93.8
Protein foods[g]	55.4	61.0	39.4
Meats/poultry/eggs[h]	33.0	44.3	0.0
Fish[h]	5.2	7.1	0.0
Nuts and soy products[h]	77.3	70.3	97.8

[a] *Intake of study participants were compared with Appendix 7 Nutritional Goals for Age-Sex Groups Based on Dietary Reference Intake and Dietary Guidelines Recommendations (27).*

[b] *Intake of study participants were compared with Table A3-1 Healthy U.S.-Style Eating Pattern: Recommended Amounts of Food from Each Food Group at 12 Calorie Levels (27).*

[c] *Based on adequate intake (AI) goal.*

[d] *Based on upper limit (UL) goal.*

[e] *Based on cup-equivalents per day.*

[f] *Measured in cup-equivalents per week.*

[g] *Measured in oz-equivalents per day.*

[h] *Measured in oz-equivalents per week.*

(5.6 mcg) of the NHANES 2011–2012 adolescent population (31). In the NHANES 2007–2010, it was reported that 88% of US youth ages 2–18 did not meet the estimated average requirement (EAR) for vitamin D (34). When compared with other populations, the mean intake of vitamin D is also lower in our population than that of adolescent Finnish vegans (5 mcg) and omnivores (14 mcg) (35). As a vitamin involved in bone metabolism, this is a concern; however, a better measure of the vitamin D status is serum 25(OH)D, which should be measured in this population to assess vitamin D adequacy.

The LA:ALA ratios for our study population-—8.98 for non-vegetarians and 9.90 for vegetarians—are lower compared to the US adolescent population (weighted mean = 11.1). A lower LA:ALA ratio is considered favorable since the dietary ratio of LA (the parent n-6) to ALA (the parent n-3) had been shown to affect ALA conversion to the physiologically important very long-chain n-3 fatty acids, EPA and DHA, in the red blood cells (36, 37). A lower LA intake and a LA:ALA ratio < 10 may improve this conversion and also reduce arachidonic acid (37), the precursor of pro-inflammatory eicosanoids.

The majority of both diet groups (82% of non-vegetarians and 84% of vegetarians) have exceeded the recommended upper limit (2,200 mg for ages 9–13 and 2,300 mg for ages 14–18) of sodium; these proportions are slightly lower than the reported excessive sodium intake prevalence in the US (89% among adults and >90% among children) (38). This is alarming given that the average intakes for both groups exceeded sodium intake limits by about 1,000 mg. Excessive sodium intake had been consistently linked to negative health effects, specifically elevated blood pressure and risk of kidney disease (39). Upon further analysis using stepwise multiple regression, we found that bread/pasta/grains explained 59.4% of the total variance in sodium intake. This was followed by legumes, which contributed 14.1% to the variance, succeeded by dairy cheese and non-starchy vegetables (each contributed 6.5% of total variance), meat alternatives (3.8%), meats/poultry (2.0%), starchy chips/pastry (1.4%), and others that contributed <1% to the total sodium intake variance of 96.7%. Foods under these groupings are similar to what were identified as major sources of sodium intake among US children ages 6–18 years as reported in NHANES 2011–2012 (40) and NHANES 2003–2008 (41), most of which are either manufactured/processed or from fast foods. Also consistent with the NHANES 2005–2008 report (42), we found sugar-sweetened beverages to be a significant predictor of sodium intake, but it only explained 0.1% of the total variance.

Using the same stepwise algorithm, we explored further the main sources of variation in SFA intake in our study population and found dairy cheese to be the main contributor (48.3% of the 72.5% total variance). Other foods that are significant contributors were meats and chicken (9.3%), dairy desserts (6.3%), chips and pastries (4.4%), milk/yogurt (2.1%), non-starchy vegetables (1.0%), and <1% each from eggs, nuts/nut butters, and starchy vegetables. The top five sources of SFA intake among US adolescents based on the NHANES 2011–2014 data were pizza, sweet bakery products, milk, Mexican dishes, and cheese (43). Cheese intake in our population is correlated with intake of pizza aside from other foods, such as pasta and sandwiches. Meats, poultry, cured meats/poultry and mixed dishes containing meat/fish/poultry were ranked 9th, 10th, 12th, and 15th, respectively, as sources of SFA among US adolescents (43), whereas meats and chicken were ranked second as a source of SFA in our population. Again, it should be noted that most foods identified to be determinants of saturated fat intakes are

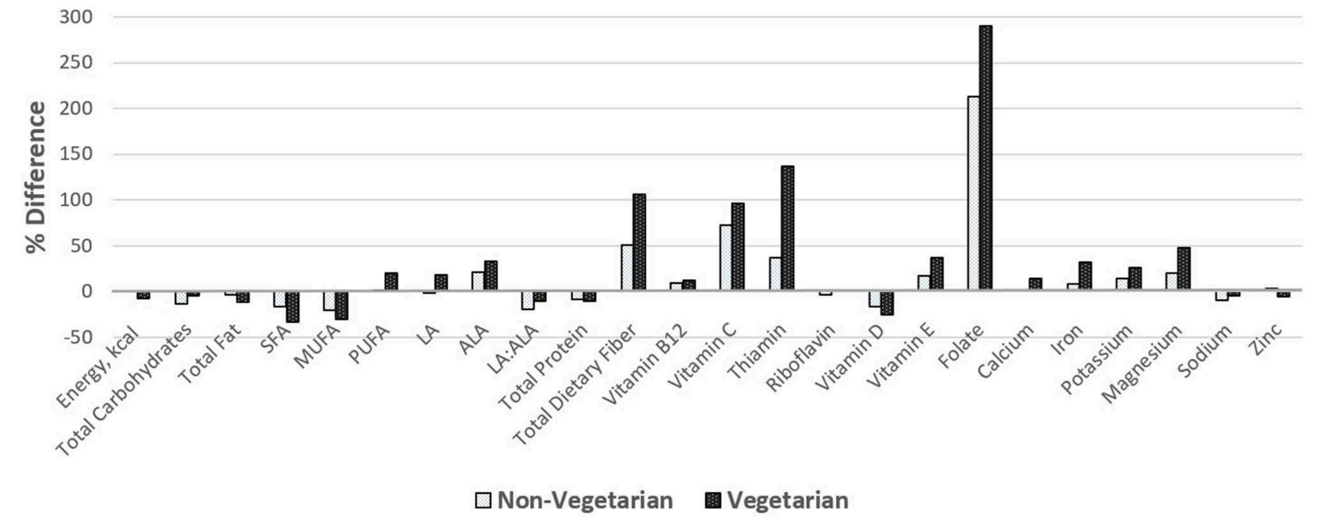

FIGURE 1 | Percent difference in food group intake of vegetarian and non-vegetarian participants compared to the average intake of the US adolescent population. Comparisons were made using data from *Usual Dietary Intakes: Food Intakes, US Population, 2007–2010* (30). Bars above the reference "0" line indicate higher intake while bars below the line indicate lower intake compared to the average intake of US adolescents.

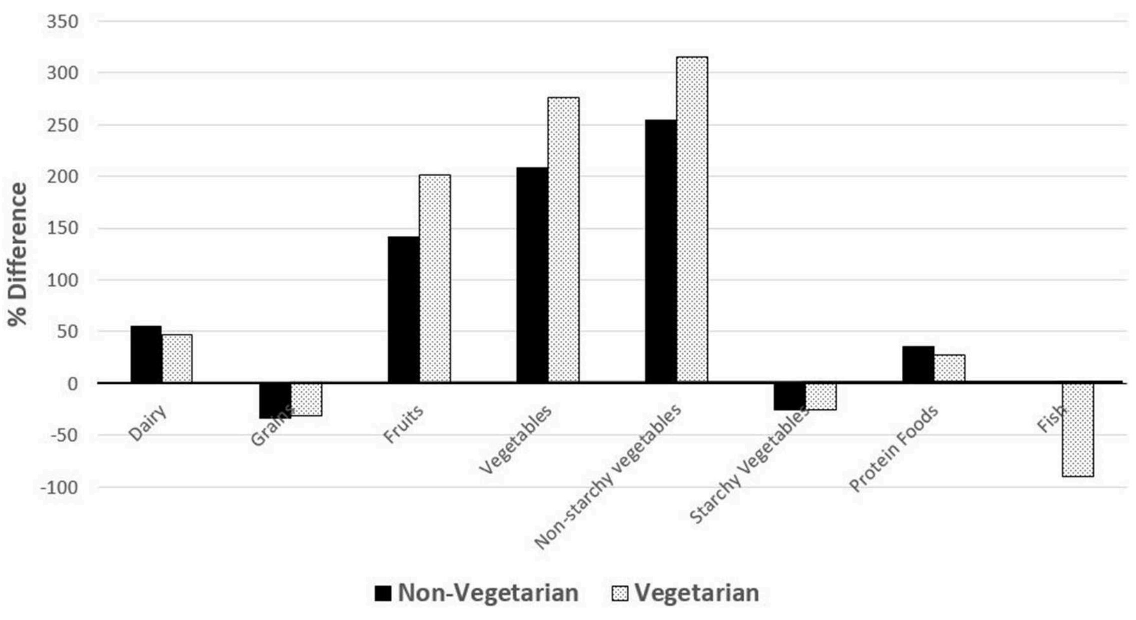

FIGURE 2 | Percent difference in nutrient intake of vegetarian and non-vegetarian participants compared to the average US adolescent population intake. Comparisons were made with nutrient intakes of adolescents from *What We Eat in America*, NHANES 2011–2012 for US population ages 12–19 years (31). Bars above the 0 horizontal line means greater intake while bars below the 0 horizontal line means lesser intake for participants compared to the reported intakes of the reference US adolescent population.

also manufactured/processed or from fast foods. In light of these findings, studies are needed to determine the impact of excessive salt intake during adolescence, both among vegetarians and non-vegetarians, as well as saturated fat intake >10% of total caloric intake, on health during adulthood.

Many other studies on vegetarian adolescents compared dietary intakes for only a few foods or nutrients (44), or were conducted on younger non-US populations (45–47). Our study is one of the very few that investigated the dietary intake of vegetarian adolescents. One strength of our study is the multi-ethnicity and the large percentage of vegetarians (25%) that provided the power necessary to determine significant intake differences between the diet groups. However, since our study population may not be representative of the US adolescent population, this limits the generalizability of our findings. On the other hand, our findings were also consistent with those of the previous study (33), despite methodological differences, and thus add additional evidence that vegetarian adolescents tend to have a more favorable dietary intake profile. One limitation of our study is the use of a dietary assessment method that has inherent biases, including the possibility of over- or under-estimation of reported intakes. However, food frequency questionnaires continue to be the mainstay in assessing habitual diet because they are low-cost, time-efficient, and easy to administer especially in the context of large epidemiological studies. The lack of biomarkers used to verify nutrient status based on reported intake is another limitation. Currently, however, only a few valid and reliable nutritional biomarkers have been identified that can be feasibly used in population studies (48). Considering that a substantial number of 9–13 (30%) and 14–18 (26.3%) years old US children use dietary supplements (49), we may have underestimated the nutrient intake of our participants by not accounting for their supplement use. However, our intent was to a focus on the nutrient amounts that our adolescent population derive from their food intake. The comparison values from the reference US adolescent population were also only based on nutrient intake from foods and beverages (31).

CONCLUSIONS

Overall, the influence of the SDA plant-based diet culture that is translated both at home and at school is evident in our findings. We found vegetarian SDA adolescents to have a more favorable dietary intake profile than non-vegetarians as indicated by the higher intake of plant-based foods and lower intake of nutrients and foods associated with detrimental health effects, such as saturated fats and animal-based products. It is notable that both vegetarians and non-vegetarians in this study population ate healthier than the general US adolescent population. This is indicated by vegetarians' greater intakes of foods considered to be beneficial to health and their overall better nutrient intake profile, including lower intakes of energy and SFA, and higher intakes of dietary fiber and most nutrients of concern—folate, iron, and the bone minerals calcium, potassium, and magnesium. However, a large majority of both the vegetarians and non-vegetarians exceeded the upper intake limits for sodium just like the general US adolescents. Our study does not allow us to draw conclusions about the potential beneficial effects of a long-term vegetarian diet in adult life endpoints. Thus, we recommend further investigations of the dietary tracking from adolescence to adulthood and the impact of dietary intake during adolescence to future health.

DATA AVAILABILITY

The datasets for this manuscript are not publicly available because we are still using the data for writing other manuscripts/reports. Requests to access the datasets should be directed to Joan Sabate, jsabate@llu.edu.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Institutional Review Board of Loma Linda University with written informed consent from parents and assent of all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Institutional Review Boards of Loma Linda University and Andrews University.

AUTHOR CONTRIBUTIONS

JS and GS-S designed the study. GS-S supervised the data collection, analyzed the data, and prepared the manuscript. JS, NB-C, SH, and GS-S interpreted the data and critically reviewed, edited, and finalized the 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/fnut.2019.00086/full#supplementary-material

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REVIEW
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Nutrition Interventions in Rheumatoid Arthritis: The Potential Use of Plant-Based Diets. A Review

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Rheumatoid arthritis (RA), a chronic inflammatory autoimmune disease, affects roughly 1% of the world's population. RA pathogenesis remains unclear, but genetic factors account for 50–60% of the risk while the remainder might be linked to modifiable factors, such as infectious diseases, tobacco smoking, gut bacteria, and nutrition. Dietary triggers may play an inciting role in the autoimmune process, and a compromised intestinal barrier may allow food components or microorganisms to enter the blood stream, triggering inflammation. In addition, excessive body weight may affect pharmacotherapy response and the likelihood of disease remission, as well as the risk of disease mortality. Evidence suggests that changes in diet might play an important role in RA management and remission. Several studies have shown improvements in RA symptoms with diets excluding animal products. Studies have also shown that dietary fiber found in these plant-based foods can improve gut bacteria composition and increase bacterial diversity in RA patients, thus reducing their inflammation and joint pain. Although some of the trigger foods in RA patients are individualized, a vegan diet helps improve symptoms by eliminating many of these foods. This review examines the potential role of a plant-based diet in mediating RA symptoms. Further research is needed to test the effectiveness of plant-based diets on joint pain, inflammation, and quality of life in patients with RA.

Keywords: autoimmune, diet, inflammation, plant-based, vegan, vegetarian, rheumatoid arthritis

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INTRODUCTION

Rheumatoid arthritis (RA), a chronic inflammatory autoimmune disease, affects roughly 1% of the world's population (1). Hands, wrists, and knees are most commonly bilaterally affected causing inflammation, pain, and eventually permanent joint damage (2). Genetic factors may account for a portion of risk (3–5), while the rest might be linked to environmental factors or a combination of genetic and environmental factors. Infectious diseases, tobacco smoking, and gut bacteria have all been considered to play a role in the development or progression of RA (6). Medications are a mainstay of treatment, but have unwanted side effects or are often expensive (7). Thus, changes in diet might be an easy and economical intervention in the management of RA.

Several studies have shown a correlation between modifiable risk factors and improvement of symptoms and outcomes in RA patients. Excessive body weight and diets that include animal products (e.g., dairy and red meat) tend to impair RA management efforts and exacerbate symptoms, presumably due to their pro-inflammatory effects (8). In contrast, diets rich in vegetables, fruits, and fiber are associated with lower BMI (9–11), have anti-inflammatory properties and help reduce pain and inflammation in these patients (12). Specifically, a 4-weeks low-fat vegan diet has been shown to significantly improve RA symptoms such as joint pain, stiffness, swelling and limitation in function (13). Likewise, a 1-year intervention tested the effects of a 7–10 day fast, followed by 3.5 months of a gluten-free vegan diet and gradual adoption of a vegetarian diet for the remainder of the study period. Significant improvements in several RA disease activity variables were observed after 1 month, including: number of tender joints, Ritchie's articular index, number of swollen joints, pain score, duration of morning stiffness, grip strength, erythrocyte sedimentation rate, C-reactive protein, white blood cell count, and a health assessment questionnaire score. These improvements were maintained after 1 year (14).

Several studies have reported lower risk of autoimmune diseases with a vegan diet. A 2013 study, using data from the Adventist Health Study-2 (AHS-2) cohort ($n = 65{,}981$), described a lower incidence and prevalence of hypothyroidism in people following vegan diets, compared to omnivorous, lacto-ovovegetarian, semi-vegetarian, and pesco-vegetarian diets even after controlling for BMI and demographic variables. The researchers speculated that the inflammatory properties of animal products could explain the lower risk in vegans (15).

Tonstad et al. also examined the correlation between dietary patterns and hyperthyroidism in the AHS-2 study population. Noting that the most common cause of hyperthyroidism is Graves' Disease, an autoimmune disorder, the researchers observed a 52% lower risk of hyperthyroidism with those consuming a vegan diet when compared to omnivores (16). Compared to non-vegetarians and lacto-ovovegetarians, vegans reported the lowest intake of saturated and *trans* fats, the highest intake of fiber, and displayed the lowest mean BMI (16), all of which could be relevant for the risk of hyperthyroidism. Potential down-regulation of insulin-like growth factor (IGF-1) (17) and higher consumption of polyphenols (18) in vegans are other possible protective mechanisms against hyperthyroidism.

Lauer et al. examined risk factors for multiple sclerosis, an autoimmune disease of the central nervous system, in male World War II veterans using the 1993 nationwide case-control study ($n = 10{,}610$) (19, 20). In the U.S, meat and dairy sales were significantly correlated with multiple sclerosis risk, while inverse associations were found with fruit and vegetable sales. Affluence was also positively associated with multiple sclerosis risk, corresponding with increased meat and dairy consumption with higher socioeconomic status.

These results suggest that a vegan diet, with a high intake of fruits and vegetables and the elimination of animal products, could protect against the development of autoimmune conditions. In contrast, diets high in animal products and low in fiber might increase the risk of developing these autoimmune conditions.

Intestinal gut health might play a role in the observed anti-inflammatory effects of dietary fiber. Studies have shown that dietary fiber can alter the composition of gut bacteria and increase the bacterial diversity, which is oftentimes lacking in RA patients, thus preventing intestinal damage (21).

Accumulating scientific evidence supports the health advantages of vegetarian diets (22). Vegetarian diets are characterized by reduced or eliminated consumption of animal products but may include dairy products and/or eggs, while vegan diets contain only plant foods. Both vegetarian and vegan diets typically emphasize vegetables, fruits, grains, legumes, and nuts. This paper summarizes the associations between diet and RA and makes a case for the potential benefits of a vegan diet in RA management.

PATHOGENESIS OF RHEUMATOID ARTHRITIS

Rheumatoid arthritis is an autoimmune disorder characterized by inflammation of the synovial lining. Inflammation results in an increase in the number of synoviocytes and immune cells. As a result, the synovial membrane becomes hyperplastic, resulting in eventual cartilage and bone erosion (23). The pathogenesis of rheumatoid arthritis is illustrated in **Figure 1**.

Studies have suggested that RA risk is influenced by a genetic predisposition, environmental factors, or a combination of both. It is clear that immune cells, such as lymphocytes, neutrophils, and macrophages, play an important role in the pathophysiology of RA (24). Within the synovium of RA patients are macrophages and T cells that produce cytokines which promote inflammation and cell migration. Cytokines tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), produced by macrophages, and cytokine interleukin-17 (IL-17), produced by CD4+ T cells, are commonly involved in the inflammatory response and subsequent cartilage destruction.

These cytokines activate synoviocytes and cause them to proliferate, creating proteases in the synovial fluid, which lead to the breakdown of cartilage and hypertrophied synovial tissue, known as pannus (25). Pannus can be further exacerbated by angiogenesis. The additional blood supply to invaded cartilage and bone allows immune cells to infiltrate the joints, worsening the synovial hyperplasia (24). Cytokines also combine with receptor activator of nuclear factor kappa-β ligand (RANKL) to stimulate osteoclast activity, which leads to bone erosion. The expression of RANKL is also affected by T cells (26).

Synovial dendritic cells stimulate immune response by attracting T lymphocytes and activating antigen-specific T cells and, in turn, B cells. In this positive feedback loop, activated B cells stimulate CD4+ T cells, producing more cytokines (27, 28). B cell proliferation can also lead to the creation of plasma cells, which produce autoantibodies, including rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) (29). These autoantibodies infiltrate the joint through newly developed blood vessels and are currently used in the diagnosis and prognosis of RA (30).

WEIGHT CONTROL AND RA

Studies show that excessive body weight increases the risk for developing RA (31). A 2011 report defined obesity as having higher than normal levels of all triglycerides found in adipose

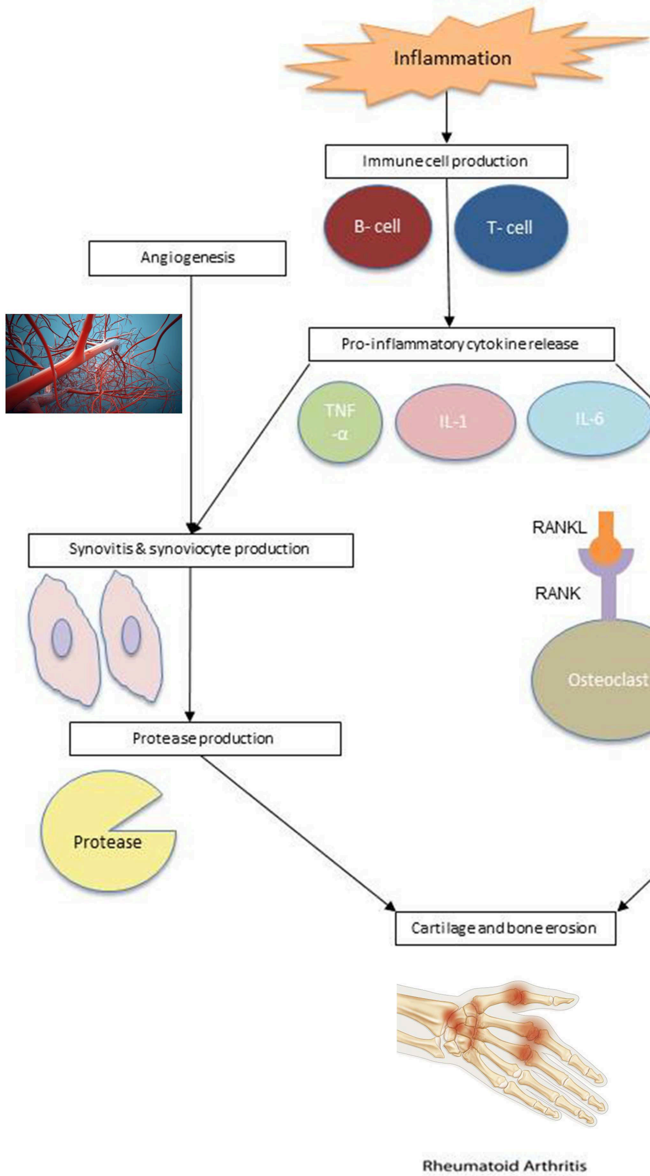

FIGURE 1 | RA pathogenesis. Angiogenesis: Reproduced from Sitox / E+ via Getty Images (https://www.gettyimages.com/detail/photo/vascular-system-veins-royalty-free-image/155351346). RA Hand: Reproduced from BSIP / Universal Images Group via Getty Images (https://www.gettyimages.com/detail/news-photo/illustration-of-a-hand-suffering-from-rheumatoid-arthritis-news-photo/586117004?adppopup=true).

tissue, which can contribute to negative health outcomes such as increased inflammation, type 2 diabetes, insulin resistance, and cardiovascular disease (32). Excessive adipose tissue secretes pro-inflammatory cytokines (adipokines) into circulation, which can increase adipose tissue growth, leading to a positive feedback cycle of adipokine secretion and tissue inflammation (33).

Adipose tissue stores energy in the form of fat, which helps regulate several physiological processes such as insulin sensitivity, metabolism, and inflammation. However, having excess fat in adipose tissue and non-adipose tissue cells can hinder these physiological processes. Recent studies have found that increased fat inside cells is related to increased inflammation (32). In addition, the extra stress placed on weight-bearing joints by excess body weight further exacerbates inflammation in these patients; therefore, weight loss could be an effective therapy for individuals diagnosed with RA.

Multiple studies have concluded that RA patients who are overweight have worse outcomes than those with a normal body mass index (BMI≤24.9) (34, 35). The Canadian Early Arthritis Cohort (CATCH) study ($n = 982$) showed that being overweight or obese were independently associated with a decreased chance for achieving sustained RA remission. Overweight patients were 25 and 47% less likely to achieve sustained remission within 3 years, respectively (36). Similarly, the Nurses' Health Study I and II, two prospective cohort studies including a total of 239,131 U.S. female nurses, reported that being overweight at 18 years of age was associated with a 35% increased risk of developing RA and a 50% risk of developing seropositive RA in adulthood (33).

Weight loss could be useful for alleviating the effects of inflammation and obesity in RA patients. A 2018 retrospective analysis ($n = 174$) evaluated the association between weight loss and RA disease activity, and found that overweight individuals who lost ≥5 kg had a three-fold increased odds of disease activity improvement compared to those who lost <5 kg. The clinical disease activity scale quantifies RA disease activity from 0 to 76 (higher scores indicating higher disease activity) by measuring the number of tender and swollen joints as well as a physician and patient global assessment on a 0–10 scale. The study concluded that each kilogram of weight lost was associated with a clinical disease activity index improvement of 1.15 ($p = 0.0026$) (37). Sparks et al. investigated the effect of weight loss in RA patients after bariatric surgery ($n = 53$) and had similar findings. At a 12-month, post-surgery follow-up, 6% of patients had moderate/high disease activity compared to 57% at baseline. At the most recent follow-up (mean 5.8 years after surgery) 74% of patients were in remission compared to 26% at baseline. Furthermore, inflammatory markers were significantly lower at the 6-, 12-month, and most recent follow up visits of 5.8 ± 3.2 years following surgery ($p < 0.05$, $p < 0.001$, and $p < 0.001$, respectively) (38). Weight loss may be a key non-pharmacologic approach in reducing inflammation and RA disease activity.

In addition to the importance of weight management, a 2015 nested case-control study ($n = 33,456$) found an association between elevated serum cholesterol and the subsequent development of RA. Women diagnosed with RA at follow-up had higher total cholesterol levels at baseline compared with healthy controls (OR 1.42; 95% CI 1.08–1.87). However, this relationship was not observed in men (39). The positive association between total cholesterol levels and RA development in women suggests a possible mechanism related to female sex hormones. A low-fat, vegan diet, which reduces plasma cholesterol and has a hormone-stabilizing effect (40–42), may therefore help protect against the development of RA in women (39).

Vegetarian and vegan diets have been consistently shown to be effective weight loss and cholesterol-lowering strategies compared with other conventional calorie-restricted diets. Two meta-analyses of randomized clinical trials showed a benefit of vegetarian, especially vegan, diets on body weight, compared with other diets (43, 44). The strong evidence is supported by observational studies (9–11). Likewise, the evidence for the effectiveness of vegetarian, particularly vegan, diets in lowering total and LDL-cholesterol in clinical trials is consistent (45, 46) and is further supported by observational studies (47, 48). Elkan et al. ($n = 66$) observed reductions in BMI, LDL, and total cholesterol after both 3 and 12 months of a gluten-free vegan diet ($p < 0.01$). These results correspond with the improvements in Disease Activity Score of 28 joints, Health Assessment Questionnaires, and CRP levels ($p \leq 0.008$) after 12 months (49). These findings suggest that by improving weight loss and lowering serum cholesterol levels, plant-based diets might improve RA symptoms and decrease the risk of developing the disease.

ASSOCIATIONS BETWEEN DIET AND RA

Diet and Inflammation

A 2015 study ($n = 50$) observed reductions in inflammatory scores in overweight or obese, otherwise healthy, participants randomized to a 2-month vegan, vegetarian, or pesco-vegetarian dietary intervention ($p < 0.05$) compared to those placed on a semi-vegetarian or omnivorous diet. Subjects in all five groups were counseled to choose low-fat foods, but only the vegan participants met the mean percentage energy from fat and saturated fat (≤30% energy from fat and ≤7% energy from saturated fat) recommendations. The researchers attributed this observation to the elimination of the leading sources of fat in the western diet (beef, cheese, milk, and poultry; 8). Diets high in fat and processed meat have been positive associated with inflammatory markers C-reactive protein (CRP), interleukin-6 (IL-6), and homocysteine, while diets high in whole grains and fruit have been inversely associated with these biomarkers (50). Likewise, vegetarian diets are negatively associated with CRP levels ($p < 0.000$) (51). Furthermore, a 3-week vegan lifestyle intervention resulted in a 33% reduction in CRP levels ($p < 0.001$), which was attributed to the anti-inflammatory components of the vegan diet, such as high fiber intake (>49 g/day) (12).

Research has found that a low-fat vegan diet improves RA symptoms, such as the degree of pain, joint tenderness, and joint swelling (13). A randomized clinical trial found that a gluten-free vegan diet decreases immunoglobulin G (IgG) in RA patients, an oftentimes elevated pro-inflammatory antibody (52). A Cretan Mediterranean Diet, rich in olive oil, cereals, vegetables, fruits, and legumes, also resulted in significant improvements in Disease Activity Score (DAS28), Health Assessment Questionnaire (HAQ), C-Reactive Protein (CRP), and swollen joint counts in patients with RA (53). However, further investigation is needed as previous research has also shown that a high-fat diet may change the composition of the gut bacteria and be linked to inflammation (54–56).

The naturally low-fat, fiber-rich components of a vegan diet might mediate the pathways that alleviate joint inflammation and pain, as observed through reduced CRP levels and improved inflammatory scores. These findings highlight the need for a randomized study that objectively measures biomarkers of inflammation related to plant-based dietary changes.

Relationship Between Inflammation and Protein Quantity and Source

Rheumatoid arthritis is a type of inflammatory polyarthritis, characterized by inflammation in more than four joints (57). Higher red meat intake has been positively associated with inflammatory polyarthritis ($p = 0.04$). Participants consuming the highest levels of red meat (OR 1.9, 95% CI 0.9–4.0), total meat (OR 2.3, 95% CI 1.1–4.9), and total protein (OR 2.9, 95% CI 1.1–7.5), displayed a higher risk for inflammatory polyarthritis when compared to participants with lower meat and protein intakes (58). These findings suggest that meat intake increases the risk of inflammatory arthritis.

Gögebakan et al. ($n = 932$) examined the effects of weight loss from varying dietary compositions on high sensitivity C-reactive protein (hsCRP) using data from the Diet, Obesity, and Genes study (DiOgenes) (59). After an initial weight loss of at least 8%, participants were randomized to 1 of 5 dietary interventions for 26 weeks. These interventions included: (1) low protein, low glycemic index; (2) low protein, high glycemic index; (3) high protein, low glycemic index; (4) high protein, high glycemic index; and (5) control diet based on national dietary guidelines. The initial calorie-restricted period resulted in a significant improvement of hsCRP, which is likely due to calorie restriction-stimulated activation of protective metabolic pathways, thus reducing inflammatory markers. Low-glycemic index diets resulted in a further decrease of hsCRP of −0.46 mg/L greater than in the high-glycemic-index groups. Similarly, hsCRP values decreased −0.25 mg/L more in the low-protein groups than in the high-protein groups. Gögebakan et al. postulate that the lower post-prandial glucose levels on low-glycemic index diets decrease inflammatory gene expression, resulting in reduced hsCRP levels (59).

A high-protein diet (28% protein, 43% carbohydrate, 13 g fiber) reduced insulin sensitivity by 12% while a high cereal fiber diet (17% protein, 52% carbohydrate, 43 g fiber) improved insulin sensitivity by 13% in 111 overweight and obese participants (60). Participants assigned to the high fiber diet displayed 25% higher insulin sensitivity than those on the high protein diet after the 6-week intervention ($p = 0.008$). These results indicate that high dietary protein (≥25–30% of energy) induces insulin resistance. Interestingly, insulin sensitivity was not significantly altered after 6 weeks of a mixed diet (23% protein, 44% carbohydrate, 26 g fiber). While dietary proteins are normally degraded by enzymes in the upper gut, these results indicate that cereal fibers may impede protein absorption in the small intestine. Thus, a low-glycemic, high-fiber and low-protein diet could mediate inflammation by decreasing pro-inflammatory gene expression and improving insulin sensitivity, even after significant reductions in inflammatory markers due to weight loss (59, 60). Ley et al. ($n = 3,690$) sought to examine the association between red meat intake and inflammatory biomarkers. Cross-sectional data from the Nurses' Health Study was analyzed and the association between total, unprocessed, and processed meat intake with CRP and adiponectin were measured. Greater total, unprocessed, and processed red meat intakes were associated with significantly higher plasma CRP concentrations and lower adiponectin levels for participants in the highest quintiles of these groups. Similarly, lower CRP values were associated with substituting a serving of total red meat with a combination of alternative protein sources (including poultry, fish, legumes, or nuts) ($\beta = -0.106$, $p \leq 0.02$). However, these associations were no longer significant after adjustment for BMI, as well as medical and lifestyle variables (61).

BMI accounted for a statistically significant proportion of associations with these biomarkers. The results of this study indicate that while greater red meat intake is associated with higher plasma concentrations of inflammatory biomarkers in diabetes-free women, adiposity accounted for a statistically significant proportion of these associations. Excluding animal products has been shown to reduce adiposity and improve CRP and adiponectin levels (61).

These findings highlight some of the potential mechanisms by which vegan diets could improve inflammation in RA patients. Apart from eliminating leading triggers, reducing animal protein has been linked to lower inflammatory markers and increased insulin sensitivity. Research also suggests that the low-glycemic index and high fiber content of the diet could reduce inflammatory gene expression. Thus, a naturally anti-inflammatory vegan diet could improve RA symptoms.

Fat Intake and Inflammation

A 3-month Mediterranean dietary intervention which significantly increased the ratio of monounsaturated fats:saturated fats improved rheumatoid arthritis symptoms as measured by pain score and physical function (62). These findings suggest that saturated fat might be linked to poorer rheumatoid arthritis symptoms, while monounsaturated fats are associated with improved outcomes (63).

A 2013 study ($n = 15$) examined the acute antioxidant and inflammatory response following a high-fat meal in overweight participants over an 8 h period. Pro-inflammatory TNF-α and IL-6 concentrations increased significantly in response to the meal. TNF-α levels increased by 12 pg/mL ($p < 0.001$) in the first hour and remained significantly above fasting values throughout the 8 h. IL-6 levels constantly rose after the meal, doubling its basal value after 2 h (+0.3 pg/mL, $p < 0.05$) and reached a maximum concentration after 8 h. Likewise, total cholesterol levels increased throughout the post-meal study period and peaked at the 8 h point (+7 mg/dl; $p < 0.01$) (64).

High-fat meal ingestion also increased endogenous antioxidants, uric acid and thiols, indicating the presence of oxidative stress. The area under the curve of both uric acid and thiols was significantly correlated with the triglycerides area under the curve (Pearson coefficient 0.923). This concomitant antioxidant response to high-fat meal ingestion highlights the pronounced impact of dietary-induced inflammation, due to a single meal (64).

A moderate (3.68–13.67 g) or high (>13.67 g) reduction in saturated fatty acid consumption has been shown to reduce leptin, a pro-inflammatory adipokine, and increase adiponectin, an anti-inflammatory adipokine, in obese adolescents. Likewise a decrease in the pro-inflammatory leptin/adiponectin (L/A) and an increase in the anti-inflammatory adiponectin/leptin (A/L) ratio was observed after the reduction in saturated fatty acid consumption. A negative correlation between the change in SFA and adiponectin as well as A/L ratio ($p < 0.05$ for both) was observed. Participants with the greatest reduction of SFA increased their adiponectin levels by 50%, followed by 20% and 23% in the moderate and low SFA reduction groups, respectively ($p \leq 0.05$) (65). This study demonstrates that a moderate change in SFA intake can yield significant changes in inflammatory measures.

The primary source of saturated fat in the U.S. is dairy products, followed by meat, eggs, and various processed foods (66). A low-fat, vegan diet is naturally free of animal products and low in SFA, potentially improving RA symptoms by up-regulating anti-inflammatory markers and down-regulating pro-inflammatory adipokines.

Complex Carbohydrates, Fiber Intake, and Inflammation

A 2009 systematic review ($n = 554$) sought to determine the influence of dietary fiber on CRP values in clinical trials (67). Increased fiber consumption, with corresponding altered fat intake and weight loss, was associated with lower CRP concentrations of 25–54% ($p = 0.05$) in six of the seven reviewed studies. The use of psyllium fiber supplementation in the seventh study did not result in lower CRP values, indicating that the effects of psyllium do not replicate those of a high-fiber diet (68). A significant decrease in inflammatory marker levels follows decreased fat intake (69), increased fiber consumption (70–72), and weight loss (69). Thus, a vegan diet, structured around low-glycemic foods and naturally high in fiber, has potential to lower inflammatory markers.

Dietary fiber is fermented by gut microbiota to produce short chain fatty acids (SCFAs), which have a beneficial effect on colonocytes (21, 73). Damage to colonocytes can result in intestinal permeability, endotoxemia, and inflammation. Thus, a diet rich in fiber provides an abundant energy source to colonocyte, reducing the risk of pathogens entering the bloodstream and inducing an inflammatory response.

These findings suggest that dietary fiber can reduce local and systemic inflammation, and that modulating effects on the gut bacteria composition, SCFA production, and intestinal barrier integrity could be involved.

Microbiome and Inflammation

The gut may play a key role in the pathophysiology of RA. Permeability of the intestinal barrier allows for food components or bacterial endotoxins to enter the bloodstream. Absorption of endotoxins into circulation can trigger a systemic inflammatory response (74). This process may help explain the oftentimes elevated self-reactive antibodies and pro-inflammatory T lymphocytes in RA patients (13, 21, 75). The maintenance of the intestinal barrier is largely dependent on the composition of the gut microbiome. As discussed in the previous section, particular microbes ferment dietary fiber into the short chain fatty acids (SCFAs) that serve as the primary energy source for colonocytes. Certain SCFAs, particularly butyrate, have also been shown to ameliorate colonocyte DNA damage (21, 73). A microbiome lacking in diversity can result in lower concentrations of these SCFAs, and therefore, impair the intestinal barrier, allowing pathogens to enter the bloodstream, and inducing an inflammatory response (74). Previous research has shown that the microbiomes of RA patients not only lack microbial diversity but are dominated by *Prevotella copri* (75, 76). This bacterial species appears to lower the abundance of other beneficial species and thrive in untreated RA patients (75). Thus, the gut microbiome and dietary fiber intake might have a significant impact on RA disease activity.

The gut microbiome could mediate the connection between diet, inflammation, and RA, although these relationships remain speculative (77). Some studies suggest a connection between intestinal inflammation and joint inflammation (78). Kim et al. observed that a vegan diet lowers the relative abundance of *Enterobacteriaceae* in the gut, which in turn reduces fecal lipocalin-2 (Lcn-2), a sensitive biomarker of intestinal inflammation, within 28 days (54). **Table 1** lists the type of correlation between bacterial species, dietary factors, and inflammation.

Elimination Diets in the Treatment of RA

As explored throughout this review, a growing body of research suggests that RA may have a gastrointestinal component and may even originate in the gut, at least for some individuals. In addition to other dietary considerations, antigenic load and sensitivities to specific foods may contribute to both the onset and severity of RA (81).

An early review by van de Laar et al. revealed that arthritic symptoms are associated with multiple gut-related conditions, including celiac disease, intestinal bypass, and inflammatory bowel disease. Moreover, mast cells, which are activated in response to foreign antigens, often in a process mediated by immunoglobulin E (IgE), are present in elevated numbers in the synovial tissues of patients with RA (82, 83). Even more telling, cross-reactive antibodies to various foods are found in the small intestine of those with RA at markedly higher levels than in healthy individuals (84).

Multiple studies have found improvements in RA patients placed on an elemental diet in which all antigenic proteins are eliminated from the patient's diet. The individual is given, as a sole source of nutrition, a complete formula in which all proteins have been broken down into free amino acids. A study conducted by Podas et al. found that placing patients with RA on an elemental diet was as effective as 15 mg/day of oral prednisolone over a 2-week period (81). However, symptoms returned upon cessation of the elemental diet, just as for cessation of prednisolone. Similar benefits were not seen for patients on a peptide diet containing protein fragments 3–6 amino acids in length (85).

Elimination diets, which remove one or more foods likely to trigger symptoms, have also been shown to induce clinical improvement in RA patients in clinical trials (52, 86). These improvements disappear when patients resume their normal diet (86).

However, response to elimination diets is highly individualized. Darlington et al. reported that some RA patients were "good responders." These individuals, who reported feeling "better" or "much better" after an elimination diet (75% of respondents), showed profound improvements in all or nearly all measures of disease activity. Interestingly, after analyzing a number of personal attributes, the only factor significantly associated with a good response to the elimination diet was a family history of atopy (86).

One challenge in studying elimination diets for RA is that, as for food allergies, trigger foods are often unique to each individual (87). Multiple methods have been tried to identify individual food sensitivities, with varying degrees of success. Skin prick testing (SPT) is a tool used to identify IgE antibody response

TABLE 1 | Microbiota and inflammatory associations.

Genus	Dietary association	Association with inflammation/RA	References
Ruminococcus	↑Fruit and vegetables	↓Endotoxemia	(79)
	↑Non-digestible carbohydrates	↓Colorectal adenomas	(80)
Roseburia	↓High protein/low carbohydrate diet ↑Mediterranean diet ↓Animal protein	↓Inflammatory Bowel Disease ↓Colorectal adenomas	(80)
Bifidobacterium	↑Non-digestible carbohydrates ↑Plant polyphenols ↑Low fat diet ↑Unsaturated fat ↑Date fruits ↑Mediterranean diet ↓Western diet ↓Beef	↓hs-CRP ↑Immune-modulation ↑Gut mucosal barrier	(80)
Lactobacillus	↑Non-digestible carbohydrates ↑Plant polyphenols ↑Unsaturated fat ↑Mediterranean diet ↓High fat diet ↓Western diet	↑Anti-inflammatory activities ↓hs-CRP ↓Intestinal dysbiosis ↓Inflammatory Bowel Disease	(80)

↑*Increase.*
↓*Decrease.*

to a stimulus, but does not consistently correspond with reactions to offensive foods (88).

However, SPT and oral food challenges have both identified foods capable of worsening RA symptoms in some individuals. For example, SPT was used to identify corn, wheat, coffee, soybeans, and other foods as possible triggers in 20 RA patients who demonstrated reactivity in SPT (Note: the researchers did not test dairy products or red meat due to bovine spongiform encephalopathy concerns). Of the 18 patients who subsequently underwent an elimination period wherein they omitted common food allergens, followed by a challenge with foods they reacted to in SPT, 13 (72%) experienced worsening symptoms after reintroduction of SPT-positive foods (89).

Darlington et al. used elimination and oral food challenge to identify foods capable of inducing symptoms in RA patients. Forty-eight patients undertook a 6-week elimination diet; forty-one were found to have foods that triggered symptoms. Foods triggering symptoms for reactive patients are described in **Table 2** (90).

Serum levels of food-specific antibodies and even rectal food protein challenge have also been tried; however, these methods have largely failed to identify a reliable link between specific foods and clinical symptoms (87). Additional foods have been implicated in some individuals using these and other methodologies. For example, the level of antibodies specific to *Saccharomyces cerevisiae* (baker's or brewer's yeast) in the blood of RA patients strongly correlates with C-reactive protein levels and erythrocyte sedimentation rate, both markers of inflammation (91).

Animal foods, including milk, eggs, and dairy, have also been found to be particularly problematic for RA patients, as evidenced by studies showing symptomatic improvement with a vegan diet (13, 52). Certain cereals may also pose problems in addition to animal products. Confirming findings from food challenge studies, one trial found strikingly higher levels of antibodies to milk, egg, pork, and codfish antigens, along with wheat, oat, and soy antigens, in the jejunal fluid of RA patients when compared to controls. Given evidence suggesting these results were not due to increased intestinal permeability from NSAID exposure, study authors concluded that mucosal immune activation in the intestine could play a role in the pathogenesis of RA (84).

TABLE 2 | Foods inducing symptoms in food-reactive RA patients ($n = 41$).

Food	Percent of patients affected	Food	Percent of patients affected
Corn	57	Malt	27
Wheat	54	Cheese	24
Bacon/pork	39	Grapefruit	24
Oranges	39	Tomato	22
Milk	37	Peanuts	20
Oats	37	Sugar (cane)	20
Rye	34	Butter	17
Eggs	32	Lamb	17
Beef	32	Lemons	17
Coffee	32	Soy	17

From Darlington and Ramsey (90).

However, a 2009 Cochrane review found "uncertain" effects of elimination and vegan diets as a result of inadequate data reporting (92); little if any research has since been published on the effects of elimination diets on RA. The lack of recent research on this topic is concerning, since emerging research has shown that diets eliminating specific foods can be effective for other inflammatory and autoimmune conditions, such as Crohn's disease (93, 94) and eosinophilic esophagitis (95–98). Eliminating gluten has also been found to not only ameliorate intestinal signs and symptoms in celiac disease (CD) but also to improve arthritis/arthralgia in some CD patients (99, 100).

The relationship between diet and RA is complex, and foods that trigger reactions in patients are individualized and therefore can be challenging to detect. However, a vegan diet comprised of fruits, grains, legumes, and vegetables can be a beneficial start for RA patients. In addition to being associated with lower BMI and greater fiber intake, this diet may help improve symptoms by eliminating many common trigger foods. Further elimination may be beneficial depending on the individual.

CONCLUSION

Several studies have shown that joint pain and other RA symptoms may be modified by dietary factors. Excessive body weight and diets that include animal products (e.g., dairy, red meat) exacerbate the RA symptoms likely due to their pro-inflammatory effects. In contrast, diets rich in vegetables, fruits, and fiber are associated with lower BMI, have anti-inflammatory properties and help reduce pain and inflammation in these patients. Studies have shown that dietary fiber found in these plant-based foods can improve the gut bacteria composition and increase the bacterial diversity in RA patients, potentially reducing inflammation and joint pain. Moreover, although some of the trigger foods in RA patients are individualized, a vegan diet comprised of fruits, whole grains, legumes, and vegetables, can improve symptoms by eliminating many common trigger foods. Elimination of additional food triggers may be necessary depending on the individual food sensitivities. Further research is needed to test the effectiveness of plant-based diets on joint pain, inflammation, and quality of life in patients with RA.

AUTHOR CONTRIBUTIONS

JA and HK contributed organization of the manuscript. MC drafted the manuscript and composed the outline. JA, HK, ER, WY, SD, MC, NBu, and LC wrote sections of the manuscript. NBa reviewed and approved the submitted version. All authors had full access to data and revised and approved the manuscript for publication.

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