# NEUROLOGICAL AND PSYCHIATRIC DISORDERS IN ENDOCRINE DISEASES

EDITED BY: Gianluca Tamagno and Jacques Epelbaum
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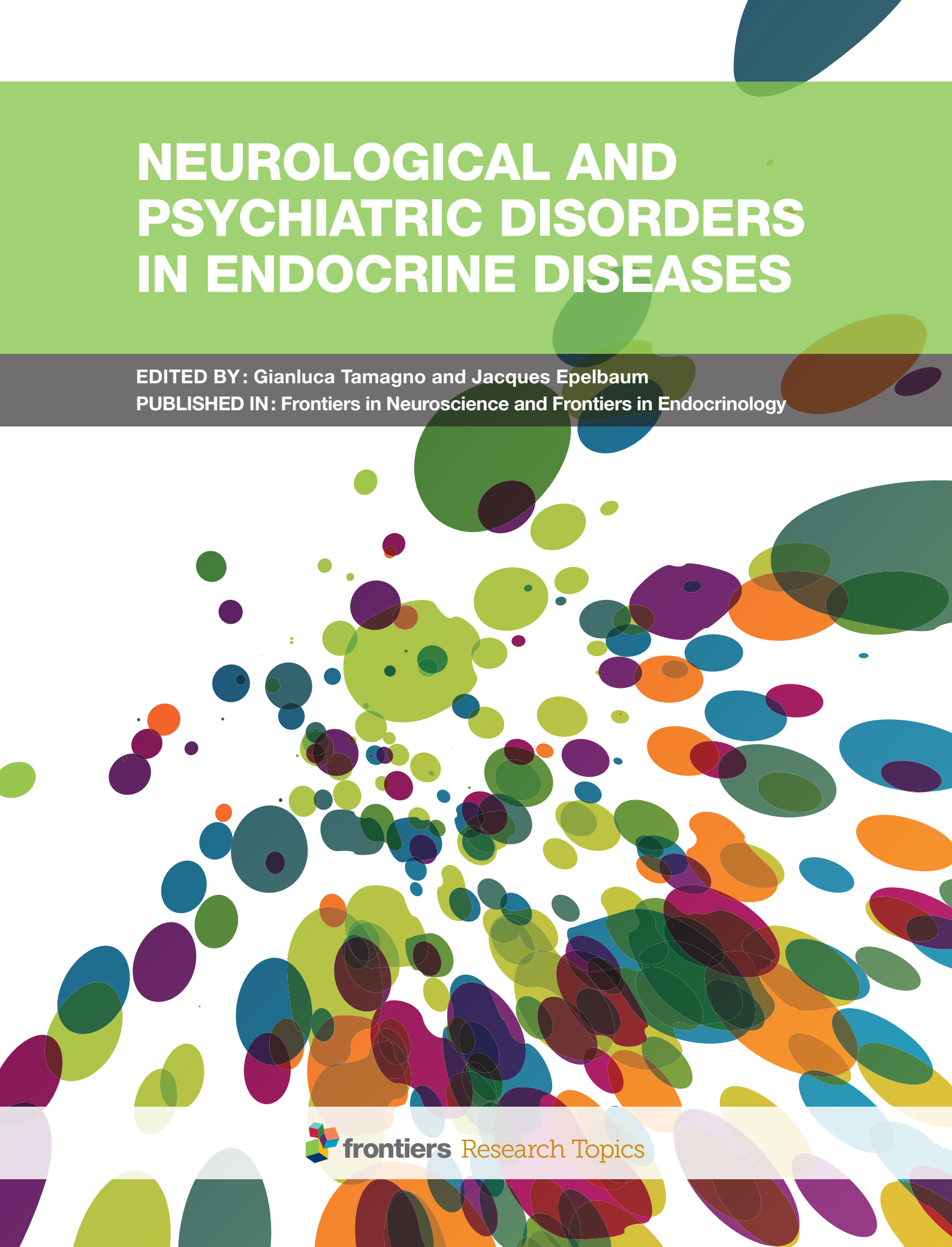

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# NEUROLOGICAL AND PSYCHIATRIC DISORDERS IN ENDOCRINE DISEASES

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Neurological and psychiatric disorders can occur in endocrine diseases either in the setting of the clinical manifestations of the same (i.e., hyper- or hyposecretion of hormones or peptides from the endocrine glands) or as events secondary to the pathogenetic mechanisms of the endocrinopathy (i.e., autommunity affecting endocrine glands and the brain). Also the medical or surgical treatment of the endocrine disease can sometimes determine the occurrence of neurological or psychiatric abnormalities. Moreover some genetic alterations can lead to syndromes affecting both the endocrine and the nervous system with a variety of possible manifestations. In the last couple of decades a number of associations between dysfunctions of the endocrine system and neurological or psychiatric manifestations have appeared and only in the minority of the cases this link has been fully elucidated. Often the neurological or psychiatric alterations still represent a relevant challenge for clinicians with regard to the management of the patients. The complexity of the topic and the limited availability of laboratory research models for the study of the endocrine system-nervous system cross-interaction are making the scientific progresses intricate and, sometimes, slow. A dedicated focus to such broad and often still obscure topic might help and clarify the current state-of-the-art in the field and direct the goals of future research.

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EDITORIAL
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# Editorial: Neurological and psychiatric disorders in endocrine diseases

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**Keywords: eating disorders, exercise, gender identity, acromegaly, Cushing syndrome, polycystic ovary syndrome, obesity, hypogonadism, thyroid surgery, quality of life**

In the last few decades, a number of reciprocal associations between endocrine system dysfunctions and neurological or psychiatric manifestations have appeared, and only in the minority of the cases this link has been fully elucidated (1). Unfortunately, the complexity, and sometimes also the severity, of the clinical issues and the limited availability of reliable experimental models for the study of the endocrine system–nervous system cross-interactions do not help the fast achievement of scientific progresses.

Isolated neurological or psychiatric symptoms as well as more complex disorders can occur in the setting of most endocrine diseases. Such manifestations can be the direct result of hyper- or hyposecretion of hormones from the endocrine glands or may occur as events secondary to the pathogenic mechanisms of the endocrinopathy, like in the case of autoimmunity affecting both endocrine glands and the brain (2). Moreover, the medical or surgical treatment of endocrine diseases can sometimes determine the occurrence of neurological or psychiatric syndromes. Some genetic aberrations can also lead to conditions affecting both the endocrine and the nervous system with a variety of possible manifestations. On the other side, many psychiatric or neurological diseases, including but not limiting to conditions, which affect the hypothalamus or the pituitary gland, may impair the physiological endocrine functions and demonstrate once again the intricate complexity of the psychoneuroendocrine connection.

Covering all basic, translational, and clinical aspects in a single Research Topic on the "Neurological and psychiatric disorders in endocrine diseases" cannot be realistically feasible. The scope is simply raising a number of interesting points from experts in the field to bring a contribution to the knowledge and maybe direct future research in this very intriguing area. Thus, we have collected reviews and original research articles, which may speak to a wide audience, including neurologists, psychiatrists, endocrinologists, and basic scientists in neuroscience, endocrinology, and metabolism.

In the clinical area, an interesting study assesses the plasma levels of the muscle-derived hormone irisin in anorexic women, without finding any significant correlation between irisin level and physical exercise (3). Another fascinating study analyzes the complexity of psychiatric and personality disorders in women with polycystic ovary syndrome, highlighting the issue of psychological distress in this patient group (4). A comprehensive picture of gender dysphoria, a psychiatric condition, which requires endocrine management, in Ireland is presented (5). In a short review, the most relevant reproductive, neurodevelopmental, and genetic aspects of hypogonadotropic hypogonadal syndromes are outlined (6). A German study indicates that psychopathology significantly predicts quality of life in patients with acromegaly and suggests that depressive symptoms and anxiety, being modifiable factors, may represent relevant targets for a broad treatment intervention in acromegalic patients (7). In a concise review, the psychiatric alterations, the neurocognitive impairment, and the altered quality of life affecting at some extent the majority of the patients with Cushing's syndrome are summarized, and the authors highlight that resolution of hypercortisolism, a challenging and



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non-granted achievement, does not always lead to the complete remission of the neuropsychiatric changes or restore the quality of life (8). With an incursion in the surgical field, the complications potentially affecting the laryngeal nerves during thyroid surgery are extensively reviewed, suggesting that still the incidence of laryngeal nerves damage secondary to thyroid surgery cannot be suppressed but may be maintained in a low range with thorough surgical techniques and the use of intraoperative neuromonitoring (9). Three articles address the complex interactions between metabolism and neuropsychiatric symptoms. The first one focuses on biological differences between restrictive anorexia nervosa and constitutional thinness, a controversial concept to describe young girls who follow a normal diet and differ from restrictive anorexia nervosa on a number of endocrine parameters (10). At the opposite of the spectrum, the second one reviews the role of inflammatory processes in the neuropsychiatric comorbidity associated with obesity (11). Finally, the last one summarizes the fascinating link through ghrelin peptides between appetite/reward/growth hormone axis and psychiatric disorders (12).

## References


1. Brambilla F. Psychoneurendocrinology: a science of the past or a new pathway for the future? *Eur J Pharmacol* (2000) **405**(1–3):341–9. doi:10.1016/S0014-2999(00)00564-1
2. Tamagno G, Federspil G, Murialdo G. Clinical and diagnostic aspects of encephalopathy associated with autoimmune thyroid disease (or Hashimoto's encephalopathy). *Intern Emerg Med* (2006) **1**(1):15–23. doi:10.1007/BF02934715
3. Hofmann T, Elbelt U, Ahnis A, Kobelt P, Rose M, Stengel A. Irisin levels are not affected by physical activity in patients with anorexia nervosa. *Front Endocrinol* (2014) **4**:202. doi:10.3389/fendo.2013.00202
4. Scaruffi E, Gambineri A, Cattaneo S, Turra J, Vettor R, Mioni R. Personality and psychiatric disorders in women affected by polycystic ovary syndrome. *Front Endocrinol* (2014) **5**:185. doi:10.3389/fendo.2014.00185
5. Judge C, O'Donovan C, Callaghan G, Gaoatswe G, O'Shea D. Gender dysphoria – prevalence and co-morbidities in an Irish adult population. *Front Endocrinol* (2014) **5**:87. doi:10.3389/fendo.2014.00087
6. Valdes-Socin H, Rubio Almanza M, Tomé Fernández-Ladreda M, Debray FG, Bours V, Beckers A. Reproduction, smell, and neurodevelopmental disorders: genetic defects in different hypogonadotropic hypogonadal syndromes. *Front Endocrinol* (2014) **5**:109. doi:10.3389/fendo.2014.00109
7. Geraedts VJ, Dimopoulou C, Auer MK, Schopohl J, Stalla GK, Sievers C. Health outcomes in acromegaly: depression and anxiety are promising targets for improving reduced quality of life. *Front Endocrinol* (2015) **5**:229. doi:10.3389/fendo.2014.00229
8. Pivonello R, Simeoli C, De Martino MC, Cozzolino A, De Leo M, Iacuaniello D, et al. Neuropsychiatric disorders in Cushing's syndrome. *Front Neurosci* (2015) **9**:129. doi:10.3389/fnins.2015.00129
9. Varaldo E, Ansaldo GL, Mascherini M, Cafiero F, Minuto MN. Neurological complications in thyroid surgery: a surgical point of view on laryngeal nerves. *Front Endocrinol* (2014) **5**:108. doi:10.3389/fendo.2014.00108
10. Estour B, Galusca B, Germain N. Constitutional thinness and anorexia nervosa: a possible misdiagnosis? *Front Endocrinol* (2014) **5**:175. doi:10.3389/fendo.2014.00175
11. Castanon N, Lasselin J, Capuron L. Neuropsychiatric comorbidity in obesity: role of inflammatory processes. *Front Endocrinol* (2014) **5**:74. doi:10.3389/fendo.2014.00074
12. Labarthe A, Fiquet O, Hassouna R, Zizzari P, Lanfumey L, Ramoz N, et al. Ghrelin-derived peptides: a link between appetite/reward, GH axis, and psychiatric disorders? *Front Endocrinol* (2014) **5**:163. doi:10.3389/fendo.2014.00163


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GENERAL COMMENTARY
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# Corrigendum: "Editorial: Neurological and psychiatric disorders in endocrine diseases"

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**Keywords: eating disorders, exercise, gender identity, acromegaly, Cushing syndrome, polycystic ovary syndrome, obesity, hypogonadism, thyroid surgery, quality of life**

**A corrigendum on**

**Editorial: Neurological and psychiatric disorders in endocrine diseases**
*by Tamagno G, Epelbaum J (2015). Front Endocrinol* ***6****:75. doi: 10.3389/fendo.2015.00075*

We have added the following correction to the Editorial article, now including a reference to Kern et al. "Apo-ghrelin receptor (apo-GHSR1a) regulates dopamine signaling in the brain."

Four articles address the complex interactions between metabolism and neuropsychiatric symptoms. The first one focuses on biological differences between restrictive anorexia nervosa and constitutional thinness, a controversial concept to describe young girls who follow a normal diet and differ from restrictive anorexia nervosa on a number of endocrine parameters (10). At the opposite of the spectrum, the second one reviews the role of inflammatory processes in the neuropsychiatric comorbidity associated with obesity (11). The third one summarizes the fascinating link through ghrelin peptides between appetite/reward/growth hormone axis and psychiatric disorders (12). Finally, the last one proposes a molecular mechanism through allosteric interactions between dopamine/DRD2 and GHSR1 receptors for controlling appetite and the uncontrollable hyperphagia associated with Prader–Willi syndrome (13).

Addition of reference:

13. Kern A, Grande C, Smith RG. Apo-ghrelin receptor (apo-GHSR1a) regulates dopamine signaling in the brain. *Front Endocrinol* (2014) **5**:129. doi:10.3389/fendo.2014.00129

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# Irisin levels are not affected by physical activity in patients with anorexia nervosa

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Irisin was recently identified as muscle-derived hormone that increases energy expenditure. Studies in normal weight and obese subjects reported an increased irisin expression following physical activity, although inconsistent results were observed. Increased physical activity in a subgroup of patients with anorexia nervosa (AN) complicates the course of the disease. Since irisin could account for differences in clinical outcomes, we investigated irisin levels in anorexic patients with high and moderate physical activity to evaluate whether irisin differs with increasing physical activity. Hospitalized female anorexic patients ($n = 39$) were included. Plasma irisin measured by enzyme-linked immunosorbent assay and locomotor activity were assessed at the same time. Patients were separated into two groups ($n = 19$/group; median excluded): moderate and high activity ($6331 \pm 423$ vs. $13743 \pm 1047$ steps/day, $p < 0.001$). The groups did not differ in body mass index ($14.2 \pm 0.4$ vs. $15.0 \pm 0.4$ kg/m$^2$), irisin levels ($558.2 \pm 26.1$ vs. $524.9 \pm 25.2$ ng/ml), and body weight-adjusted resting energy expenditure ($17.6 \pm 0.3$ vs. $18.0 \pm 0.3$ kcal/kg/day, $p > 0.05$), whereas body weight-adjusted total energy expenditure ($46.0 \pm 1.4$ vs. $41.1 \pm 1.1$ kcal/kg/day), metabolic equivalents (METs, $1.9 \pm 0.1$ vs. $1.7 \pm 0.1$ METs/day), body weight-adjusted exercise activity thermogenesis ($1.8 \pm 0.5$ vs. $0.6 \pm 0.3$ kcal/kg/day), duration of exercise ($18.6 \pm 4.7$ vs. $6.2 \pm 3.1$ min/day), and body weight-adjusted non-exercise activity thermogenesis ($21.6 \pm 1.0$ vs. $18.8 \pm 0.8$ kcal/kg/day) were higher in the high activity compared to the moderate activity group ($p < 0.05$). No correlations were observed between irisin and activity parameters in the whole sample ($p > 0.05$). In conclusion, the current data do not support the concept of irisin being induced by exercise, at least not under conditions of severely reduced body weight like AN.

**Keywords: brown adipose tissue, energy expenditure, exercise, FNDC5, myokine, SenseWear™ armband**

## INTRODUCTION

Irisin is a recently identified muscle-derived hormone produced by cleavage from fibronectin type III domain containing 5 (FNDC5) (1). The expression of FNDC5 is induced by the peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α (PGC-1α) that has been demonstrated to play an important role in the expression of uncoupling protein 1 (UCP1), a key factor of thermogenesis in brown adipose tissue (2). Irisin drives the browning of subcutaneous white adipose tissue by stimulating the expression of UCP1 in beige fat cells (1). These beige fat cells have been shown to be a cell type distinct from white or brown fat cells being located in white adipose tissue (3) and exhibiting properties of brown adipose tissue which – for long thought to exist only in newborns – was recently also detected in adult humans (4, 5) and demonstrated to be activated in response to cold exposure (4). Besides its occurrence in skeletal muscle, irisin was detected – although in much smaller quantities – in several human tissues containing smooth muscle as well as heart muscle, and organs including liver, kidney, and lung (6). Additionally, this peptide is also present in adipose tissue (7, 8) and rodent cerebellar Purkinje cells (9). Lastly, irisin was detected in the circulation (1, 6, 10, 11) with decreased levels in patients with type 2 diabetes mellitus (10).

In their initial study, Boström et al. described an increase of circulating irisin levels following physical activity in mice and in male subjects after a 10-week endurance exercise program (1). In a subsequent study, elevated circulating irisin levels were observed following acute exercise consisting of 80-m sprints, while this effect was weakened after a 8-week sprint training program completed by young healthy men (6). However, one study detected a modest 30% higher FNDC5 mRNA expression only in a subgroup of older male subjects after an endurance training program compared to sedentary controls, whereas no changes were observed in a large sample of younger adults (12). While data on an exercise-dependent production of irisin in muscle and its secretion into the circulation are inconsistent in humans, an association of plasma irisin levels with body mass index (BMI) seems to be a robust finding. Extending the results of a previous study that showed a trend toward a correlation of circulating irisin with BMI in subjects with a BMI range from 20 to 48 kg/m$^2$ (6), we previously reported a positive correlation of plasma irisin levels with BMI

over a very broad spectrum of body weights with BMIs ranging from 8 to 85 kg/m$^2$ as well as positive associations with fat mass (FM), fat-free mass (FFM), and body cell mass (BCM) (11). One previous study on the association of irisin and energy expenditure reported that irisin levels correlated with 24-h energy expenditure measured by indirect calorimetry in overweight and obese postmenopausal women only in a subgroup of subjects whose energy expenditure was higher than predicted by an FFM-based equation (13), whereas it did not hold true when the whole sample was analyzed.

In light of these data, more research is needed in order to characterize the regulation of irisin under conditions of different activity patterns and to establish its possible role as a novel muscle-derived and exercise-induced hormone. In contrast to its potentially beneficial effect as health-promoting hormone in overweight or obese patients, the role as an exercise-induced and energy-dissipating myokine might be an additional biological mechanism explaining worse clinical prognosis and higher relapse rates of hyperactive anorexic subjects. Furthermore, anorexia nervosa (AN) might be viewed as a mirror image of obesity and thus investigation of this disease likely contributes to the understanding of weight disorders in general. Therefore, in the present cross-sectional study we investigated the relationship of circulating irisin levels with measures of physical activity and different aspects of energy expenditure in anorexic patients, since subjects suffering from AN are known to display very different activity patterns encompassing a relevant subgroup of hyperactive in contrast to moderately active patients (14–16).

## SUBJECTS AND METHODS

### SUBJECTS

We recruited 39 anorexic female inpatients at admission to their treatment in the Division for General Internal and Psychosomatic Medicine at Charité – Universitätsmedizin Berlin. Inclusion criteria were a BMI of <18 kg/m$^2$ and fulfillment of ICD-10 (International Statistical Classification of Diseases and Related Health Problems of the World Health Organization, 10th revision) criteria (17) for typical or atypical AN. Atypical AN is defined as a disorder meeting some criteria of the typical form (BMI below 17.5 kg/m$^2$, self-induced weight loss, body image distortion, and endocrine disorder most often reflected by secondary amenorrhea) without fulfilling all key symptoms (17). The restricting subtype of AN according to the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) differs from the purging subtype by the absence of binge-eating or purging behavior such as self-induced vomiting or misuse of appetite suppressants, thyroid hormones or laxatives, diuretics, and enemas (18).

According to their locomotor activity, anorexic patients were separated into two groups by dividing the patients at the median step count resulting in a moderate activity and a high activity group ($n = 19$/group, one patient with the median value was excluded). Additionally, since the restricting subtype was shown to be associated with excessive exercise (19, 20), calculations were also performed for three subgroups of AN: typical restricting, typical purging, and atypical AN.

Patients with current pregnancy or psychotic disorders and an age of <18 years were excluded. The investigations were conducted according to the Declaration of Helsinki. All patients gave written informed consent and the study was approved by the institutional ethics committee of the Charité – Universitätsmedizin Berlin (protocol number: EA1/114/10).

### LABORATORY ANALYSIS OF IRISIN PLASMA LEVELS

All blood samples were collected from a forearm vein between 7 and 8 a.m. after an overnight fast during the period of the data collection on physical activity. Participants were allowed to drink small amounts of water but were advised not to smoke or exercise before blood withdrawal. The blood was collected in pre-cooled EDTA tubes prepared with aprotinin (1.2 Trypsin Inhibitory Unit per 1 ml blood; ICN Pharmaceuticals, Costa Mesa, CA, USA) for peptidase inhibition. The tubes were placed back on ice immediately after blood withdrawal and then centrifuged at 4°C for 10 min at 3000 × *g*. Plasma was separated and stored at −80°C until further processing. At the day of the measurement, samples were diluted 1:10 and irisin plasma levels were analyzed using a commercial enzyme-linked immunosorbent assay (ELISA, catalog # EK-067-16, Phoenix Pharmaceutical, Inc., Burlingame, CA, USA). All samples were processed in one batch (intra-assay variability <5%).

### MEASUREMENTS AND CALCULATION OF ACTIVITY AND ENERGY EXPENDITURE

Weight and height were assessed on the day of the blood collection. Weight was measured to the nearest 0.1 kg and height to the nearest 0.5 cm. The BMI was calculated as kilogram per square meter. Physical activity data were continuously measured for 3 days (Friday to Sunday) during inpatient treatment using a portable armband device (SWA; SenseWear™ PRO3 armband; BodyMedia, Inc., Pittsburgh, PA, USA). One day was included in the data analysis if the minimum duration of data acquisition was 20 h and 30 min/day. Patients were not activity restricted during the measurement. Activity displayed by individuals is suggested to be affected only in part deliberately with a high predictive value of the activity level under sedentary conditions for daily life activity (21). Although activity patterns under conditions of hospitalization might differ from those in daily life, activity habits are likely to persist during inpatient treatment if activity is not restricted due to a genetic influence on physical activity (22).

The SWA uses a multisensory array including sensors measuring heat flux, galvanic skin response, skin temperature, near-body ambient temperature, and a two-axis accelerometer. Step counts were measured by the accelerometer and directly taken for analyses. Obtained data were analyzed using a generalized proprietary algorithm developed by the manufacturer (SenseWear™ Software, Version 6.1, BodyMedia, Inc.) and prepared for statistical analysis.

Total energy expenditure (TEE) consists of resting energy expenditure (REE), thermic effect of food (TEF), and activity thermogenesis. Activity thermogenesis can be further separated into the two components exercise-related activity thermogenesis (EAT, deliberate physical exercise, sports) and non-exercise activity thermogenesis (NEAT, spontaneous daily physical activity) (23). Energy expenditure of more than five metabolic equivalents (METs) of a task (a physiological measure expressing the energy cost of physical activities) was classified as EAT, whereas

energy expenditure of up to five METs was classified as NEAT adapting the findings of Ainsworth et al. (24) and as described before by our group (25). While TEE, EAT, and duration of exercise were directly received from the proprietary algorithms of the SWA, NEAT was calculated according to the equation: NEAT = TEE − TEF − REE − EAT. TEF was estimated as 10% of TEE and calculated as TEE × 0.1 (26). REE, required for calculation of NEAT, was calculated using weight-/group-specific REE prediction equations provided by Müller et al. (27) since REE cannot be directly determined by the SWA.

### STATISTICAL ANALYSIS

Distribution of the data was determined by using the Kolmogorov–Smirnov test. Data are expressed as mean ± SEM. Differences between groups were calculated using the *t*-test. For the comparison of the subtypes of AN differences between groups were calculated using one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test. Correlations were determined by Pearson's or Spearman's analysis depending on the distribution of the data. Differences between groups were considered significant when $p < 0.05$. All statistical analyses were conducted using SigmaStat 3.1.

## RESULTS

### IRISIN PLASMA LEVELS DO NOT DEPEND ON ACTIVITY AND ENERGY EXPENDITURE

Per definition, the moderate activity and the high activity group differed significantly in the number of steps performed per day ($p < 0.001$; **Figure 1A**). However, irisin plasma levels were not different between these two groups ($p = 0.37$; **Figure 1B**). Associated with the higher activity, significant differences were observed for METs per day ($p = 0.03$; **Figure 1C**) and body weight-adjusted TEE ($p = 0.009$; **Figure 1D**) with higher values in the high compared to the moderate activity group. No differences were detected for body weight-adjusted REE ($p = 0.38$; **Figure 1E**), whereas duration of exercise ($p = 0.03$; **Figure 1F**) or body weight-adjusted EAT ($p = 0.03$; **Figure 1G**) and body weight-adjusted NEAT ($p = 0.03$; **Figure 1H**) were higher in the high activity compared to the moderate activity group.

In the whole sample no correlation was observed between plasma irisin levels and the number of steps as a measure of activity (**Figure 2A**), the amount of METs (**Figure 2B**) and other parameters of energy expenditure, namely body weight-adjusted TEE (**Figure 2C**), body weight-adjusted REE (**Figure 2D**), body weight-adjusted EAT (**Figure 2E**), and body weight-adjusted NEAT (**Figure 2F**).

### IRISIN PLASMA LEVELS SHOW A NEGATIVE CORRELATION WITH COMPONENTS OF ENERGY EXPENDITURE IN PATIENTS WITH RESTRICTING TYPE ANOREXIA NERVOSA ONLY

After division of the sample into subgroups according to the type of AN, namely purging type ($n = 10$), restricting type ($n = 20$), and atypical ($n = 9$) AN, the three groups did not differ in terms of age and BMI ($p > 0.05$; **Table 1**). As shown in **Table 1**, no differences among these three groups were observed for irisin, number of steps per day, METs per day, and the different components of energy expenditure ($p > 0.05$).

When investigating the three subgroups separately, irisin levels showed a negative correlation with the number of steps per day, duration of exercise, and body weight-adjusted EAT in patients with restricting type AN ($p < 0.05$; **Table 2**), whereas no correlations of plasma irisin and components of energy expenditure were observed for the subgroups of purging type and atypical AN ($p > 0.05$; **Table 2**).

## DISCUSSION

Irisin is a newly defined myokine that increases energy expenditure by stimulating the expression of UCP1 and thus the browning of white adipose tissue (1). In addition, it has been shown that irisin plasma levels are increased in response to different types of exercise (1, 28, 29). Consequently, irisin was proposed to mediate some of the health-promoting effects of physical activity. In turn, irisin could be an additional biological mechanism contributing to the course of the disease and therefore the prognosis in highly active patients suffering from AN (30, 31). However, in rejection of our initial hypothesis, we did not detect differences in irisin plasma levels between anorexic patients with high vs. moderate locomotor activity as determined by average daily step count. When investigating the whole cohort, we also did not observe any correlations of circulating irisin with physical activity, TEE or other parameters of energy expenditure, in particular EAT. Several points could contribute to this lack of correlation of irisin with measures of locomotor activity and energy expenditure in our study.

First, Timmons et al. reported that they failed to detect a strong increase of the irisin precursor, FNDC5 after exercise in a population of younger adults, whereas only in a subgroup of older subjects an increase of FNDC5 mRNA expression after exercise was observed (12). Since our data were collected in a population of young patients with a mean age of 27, the results could underline the hypothesis that exercise-related effects on irisin expression are associated with age and only (or predominantly) observed in elderly subjects.

Second, irisin is a cleavage product of FNDC5 expressed mainly in skeletal muscle. Irisin plasma levels have been shown to be associated with biceps circumference as a marker of muscle mass (6, 11). In addition, using bioelectrical impedance analysis (BIA) in a previous study, we showed a positive correlation of irisin with FFM and BCM (the measure best reflecting muscle mass using BIA) (11). Since patients suffering from AN have greatly diminished muscle mass as a consequence of malnutrition and weight loss, the total muscle mass might be too low in our patient population (BCM 15.54 ± 0.43 kg; FFM 37.02 ± 0.66 kg) to detect differences in the levels of the circulating myokine, irisin.

Third, in line with our finding of a lacking association between energy expenditure and circulating irisin, one recent study did not detect a correlation of plasma irisin levels with 24-h energy expenditure in post-menopausal women when assessed in a metabolic chamber (13). Subjects in this and our study are in a state of down-regulated sexual hormones that could contribute to the results. Another explanation could be that only exercise with respect to a defined acute or repetitive physical training program exerts significant effects on irisin muscle expression or plasma levels, whereas chronic changes in exercise do not. This might be due to counter-regulatory adaptive changes that have to be further investigated.

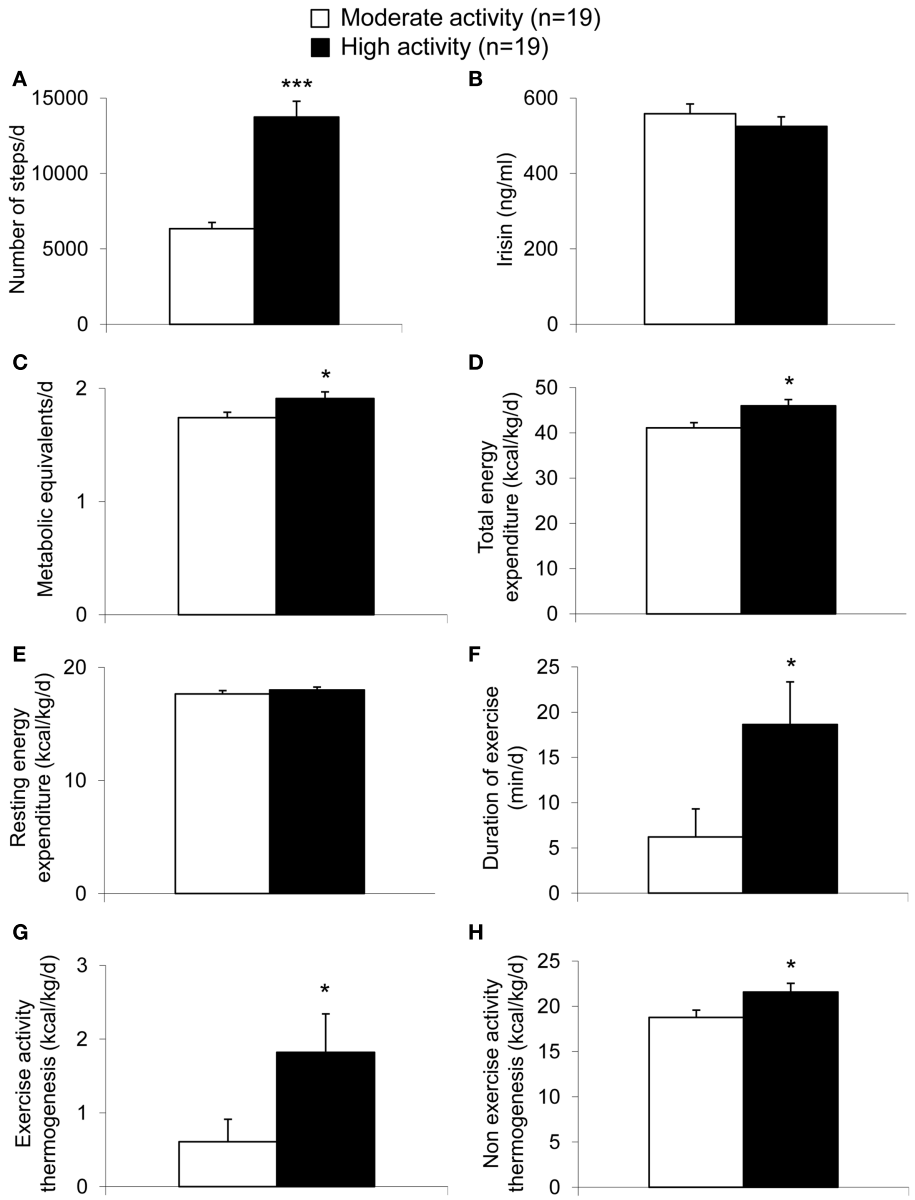


**FIGURE 1 | Parameters of energy expenditure in anorexic patients with moderate and high physical activity**. Per definition, the high activity group showed a higher number of steps compared to the moderate activity group **(A)**. Irisin levels did not differ between the two groups **(B)**. The metabolic equivalents per day **(C)** as well as body weight-adjusted total energy expenditure **(D)** were higher in the high compared to the moderate activity group, while no differences were observed for body weight-adjusted resting energy expenditure **(E)**. Duration of exercise **(F)**, body weight-adjusted exercise activity thermogenesis **(G)**, and body weight-adjusted non-exercise activity thermogenesis **(H)** were higher in the high compared to the moderate activity group. Data are expressed as mean ± SEM. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ in the high vs. moderate activity group ($n = 19$/group).

In addition, since irisin has been shown to initiate the browning of white adipose tissue and thereby eliciting an additional energy-dissipating effect (1, 3), the existence of a reasonable amount of beige adipocytes would be crucial to mediate these effects. However, little is known about the existence of brown and beige adipose tissue in fat-deprived anorexic subjects. The activity of brown fat has been reported to be inversely correlated with BMI with reduced activity in overweight or obese compared to lean young male subjects (4). However, a more recent study did not detect brown fat activity in anorexic patients, whereas it was detectable in constitutionally lean (average BMI 16.2 kg/m$^2$) subjects (32). The absence of brown (or beige) fat activity could therefore be another factor contributing to the lacking correlation of irisin with components of energy expenditure.

Fourth, parameters of physical activity were measured using the SenseWear™ armband device shown to be the best estimate of energy expenditure when comparing several monitors of physical activity in healthy volunteers under regular daily life

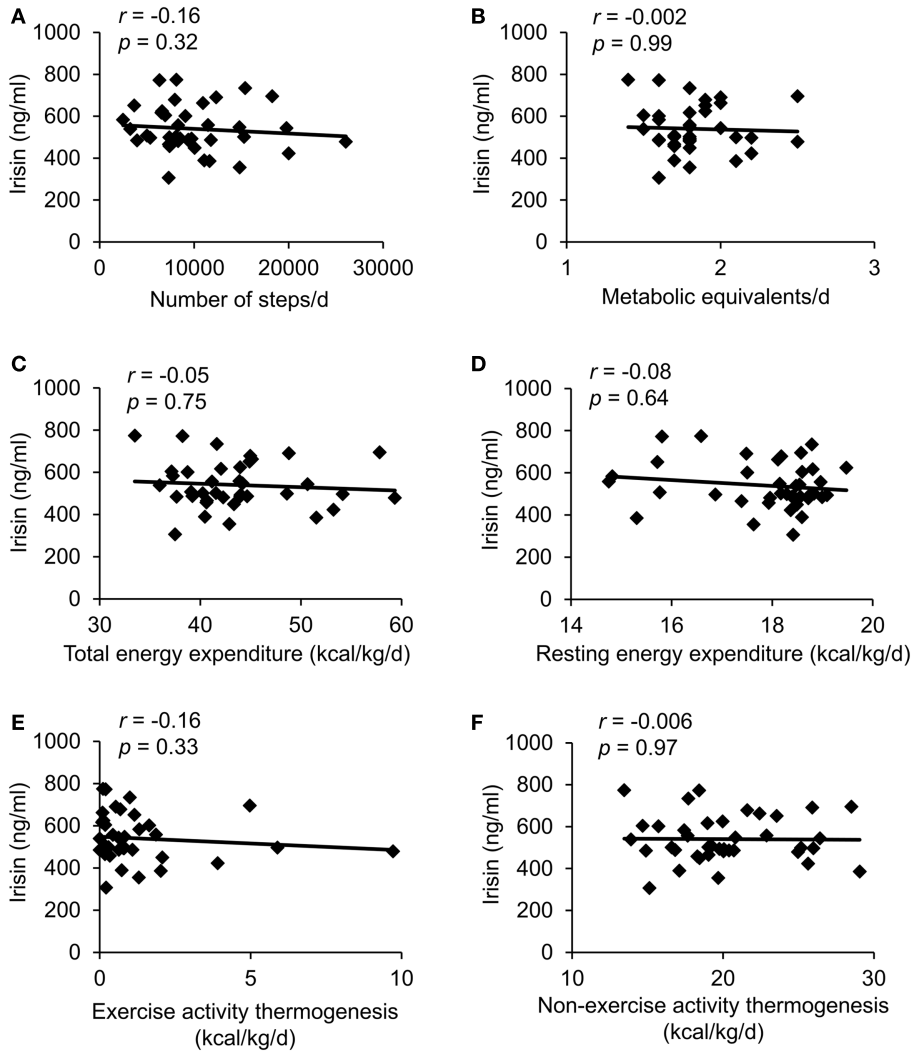


**FIGURE 2 | Correlations between circulating irisin concentrations and number of steps per day (A), metabolic equivalents per day (B), body weight-adjusted total energy expenditure (C), body weight-adjusted resting energy expenditure (D), body weight-adjusted exercise activity thermogenesis (E), and body weight-adjusted non-exercise activity thermogenesis (F)**. Values for *r* and *p* are indicated in each graph, $n = 39$ patients.

conditions (33). When compared with predictive equations (34), the SenseWear™ armband device and the doubly labeled water method have shown sufficient concordance for the assessment of resting and TEE in healthy volunteers and in overweight subjects with type 2 diabetes mellitus (35, 36). However, this comparison is still pending for subjects with reduced body weight. Therefore, the SenseWear™ armband device might have limitations for the use in anorexic patients. In addition, the measurement method of irisin could contribute to the lacking correlation of physical activity and circulating irisin. Erickson recently commented on the missing validity of the antibodies used to detect irisin in the existing studies since a quantitative western blot analysis has not been conducted yet and thus questioned the results on circulating irisin published so far (37). However, the antibody by Phoenix used in the present study seems to be the most suitable to date since it detects an amino acid sequence that is part of the cleaved irisin protein (full cross-reactivity with amino acids 42–112 of the 112-amino acid polypeptide irisin, while no cross-reactivity was observed with the C-terminal parts of the irisin precursor FNDC5 amino acids 149–196 and 162–209, manufacturer's information). This contrasts with other antibodies that were raised against the transmembrane sequence of FNDC5 (1, 8) unlikely to occur in the circulation.

Lastly, a recent study suggested that the human FNDC5 gene differs from other species by a mutation in the start codon sequence (38) which may result in a lower translation efficiency (39) and consequently could explain difficulties to translate animal data to humans.

After investigating the whole cohort, we separated the study population according to the type of anorexia (purging type, restricting type, and atypical) since there is evidence for differences in excessive exercise in these subtypes of AN (19, 20, 40,

**Table 1 | Parameters of energy expenditure according to the subtype of anorexia nervosa**.

| Parameter | Group | | |
|---|---|---|---|
| | **Purging type AN ($n = 10$)** | **Restricting type AN ($n = 20$)** | **Atypical AN ($n = 9$)** |
| Age (years) | 28.6 ± 3.1 | 27.8 ± 2.4 | 25.4 ± 1.7 |
| Body mass index (kg/m$^2$) | 14.1 ± 0.7 | 14.3 ± 0.4 | 15.7 ± 0.6 |
| Irisin (ng/ml) | 578.5 ± 41.5 | 522.8 ± 21.8 | 536.9 ± 38.6 |
| Number of steps per day | 11011 ± 2195 | 9804 ± 939 | 9307 ± 1567 |
| METs per day | 1.85 ± 0.12 | 1.83 ± 0.04 | 1.80 ± 0.08 |
| TEE (kcal/kg/day) | 43.7 ± 2.7 | 43.4 ± 0.9 | 43.9 ± 2.1 |
| REE (kcal/kg/day) | 17.7 ± 0.4 | 17.9 ± 0.3 | 18.0 ± 0.2 |
| Duration of exercise (min/day) | 17.5 ± 8.9 | 9.7 ± 2.4 | 12.4 ± 6.2 |
| EAT (kcal/kg/day) | 1.8 ± 1.0 | 0.9 ± 0.2 | 1.2 ± 0.6 |
| NEAT (kcal/kg/day) | 19.8 ± 1.5 | 20.3 ± 0.8 | 20.2 ± 1.7 |

*Data are expressed as mean ± SEM, $p > 0.05$. AN, anorexia nervosa; EAT, exercise activity thermogenesis; MET, metabolic equivalent; NEAT, non-exercise activity thermogenesis; REE, resting energy expenditure; TEE, total energy expenditure.*

**Table 2 | Correlation of irisin with parameters of energy expenditure according to the subtype of anorexia nervosa**.

| Parameter | Group | | |
|---|---|---|---|
| | **Purging type AN ($n = 10$)** | **Restricting type AN ($n = 20$)** | **Atypical AN ($n = 9$)** |
| Number of steps per day | $r = -0.055$; $p = 0.865$ | $r = -0.465$; $p = 0.039$* | $r = 0.283$; $p = 0.434$ |
| METs per day | $r = -0.043$; $p = 0.892$ | $r = -0.171$; $p = 0.464$ | $r = 0.332$; $p = 0.356$ |
| TEE (kcal/kg/day) | $r = -0.139$; $p = 0.681$ | $r = -0.251$; $p = 0.280$ | $r = 0.333$; $p = 0.356$ |
| REE (kcal/kg/day) | $r = -0.091$; $p = 0.785$ | $r = 0.000$; $p = 0.997$ | $r = -0.317$; $p = 0.381$ |
| Duration of exercise (min/day) | $r = -0.006$; $p = 0.973$ | $r = -0.524$; $p = 0.018$* | $r = 0.151$; $p = 0.676$ |
| EAT (kcal/kg/day) | $r = -0.018$; $p = 0.946$ | $r = -0.519$; $p = 0.019$* | $r = 0.150$; $p = 0.676$ |
| NEAT (kcal/kg/day) | $r = -0.127$; $p = 0.707$ | $r = -0.147$; $p = 0.529$ | $r = 0.367$; $p = 0.308$ |

*AN, anorexia nervosa; EAT, exercise activity thermogenesis; MET, metabolic equivalent; NEAT, non-exercise activity thermogenesis; REE, resting energy expenditure; TEE, total energy expenditure. Values for r and p are indicated in each row, *$p < 0.05$.*

41). However, irisin levels and all other parameters of energy expenditure assessed did not differ between these groups. Similarly to the whole sample, in the subgroups purging and atypical AN, no correlation was observed between irisin levels and any parameter of energy expenditure. However, when investigating the patients with restricting type AN separately, irisin levels negatively correlated with the number of steps per day and body weight-adjusted EAT which may indicate that this subgroup is a more homogeneous population of patients. This negative association is unexpected and in contrast to the reported positive association of exercise and circulating irisin for normal and overweight subjects (1, 6, 28). The present finding may point toward a differential regulation of irisin in patients with restricting type AN ($n = 20$) but should be confirmed in a larger cohort of patients.

In summary, irisin plasma levels do not differ in anorexic patients with moderate activity from those with high locomotor activity. Moreover, no association was observed for plasma irisin levels with various parameters of energy expenditure assessed under unrestricted conditions of physical activity in hospitalized patients. Therefore, the current data do not support the concept of irisin being induced by exercise, at least not under conditions of severely reduced body weight like AN. Thus, it is also unlikely that irisin contributes to exceeding energy expenditure in highly active anorexic patients. Further studies are needed to clarify whether the lacking correlation of irisin and physical activity described here is due to a missing association of irisin and exercise under conditions of reduced body weight *per se* or because of a unique characteristic of anorexic patients.

## AUTHOR CONTRIBUTIONS

Tobias Hofmann, Ulf Elbelt, and Andreas Stengel designed the study; Tobias Hofmann and Andreas Stengel coordinated and supervised the data collection, carried out the statistical analyses and drafted the manuscript. Ulf Elbelt, Anne Ahnis, Peter Kobelt, and Matthias Rose discussed the data and reviewed the article. All authors discussed the results, reviewed, and finalized the manuscript.

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## REFERENCES

1. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* (2012) **481**(7382):463–8. doi:10.1038/nature10777
2. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* (1998) **92**(6):829–39. doi:10.1016/S0092-8674(00)81410-5
3. Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* (2012) **150**(2):366–76. doi:10.1016/j.cell.2012.05.016
4. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, et al. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* (2009) **360**(15):1500–8. doi:10.1056/NEJMoa0808718
5. Yoneshiro T, Aita S, Matsushita M, Kameya T, Nakada K, Kawai Y, et al. Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obesity (Silver Spring)* (2011) **19**(1):13–6. doi:10.1038/oby.2010.105
6. Huh JY, Panagiotou G, Mougios V, Brinkoetter M, Vamvini MT, Schneider BE, et al. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. *Metabolism* (2012) **61**(12):1725–38. doi:10.1016/j.metabol.2012.09.002
7. Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F, et al. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. *J Clin Endocrinol Metab* (2013) **98**(4):E769–78. doi:10.1210/jc.2012-2749
8. Roca-Rivada A, Castelao C, Senin LL, Landrove MO, Baltar J, Crujeiras AB, et al. FNDC5/irisin is not only a myokine but also an adipokine. *PLoS One* (2013) **8**(4):e60563. doi:10.1371/journal.pone.0060563
9. Dun SL, Lyu RM, Chen YH, Chang JK, Luo JJ, Dun NJ. Irisin-immunoreactivity in neural and non-neural cells of the rodent. *Neuroscience* (2013) **240C**:155–62. doi:10.1016/j.neuroscience.2013.02.050
10. Choi YK, Kim MK, Bae KH, Seo HA, Jeong JY, Lee WK, et al. Serum irisin levels in new-onset type 2 diabetes. *Diabetes Res Clin Pract* (2013) **100**(1):96–101. doi:10.1016/j.diabres.2013.01.007
11. Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P, Klapp BF. Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity – correlation with body mass index. *Peptides* (2013) **39**:125–30. doi:10.1016/j.peptides.2012.11.014
12. Timmons JA, Baar K, Davidsen PK, Atherton PJ. Is irisin a human exercise gene? *Nature* (2012) **488**(7413):E9–10. doi:10.1038/nature11364 discussion E-1,
13. Swick AG, Orena S, O'Connor A. Irisin levels correlate with energy expenditure in a subgroup of humans with energy expenditure greater than predicted by fat free mass. *Metabolism* (2013) **62**(8):1070–3. doi:10.1016/j.metabol.2013.02.012
14. Davis C, Kennedy SH, Ravelski E, Dionne M. The role of physical activity in the development and maintenance of eating disorders. *Psychol Med* (1994) **24**(4):957–67. doi:10.1017/S0033291700029044
15. Klein DA, Mayer LE, Schebendach JE, Walsh BT. Physical activity and cortisol in anorexia nervosa. *Psychoneuroendocrinology* (2007) **32**(5):539–47. doi:10.1016/j.psyneuen.2007.03.007
16. Davis C, Katzman DK, Kaptein S, Kirsh C, Brewer H, Kalmbach K, et al. The prevalence of high-level exercise in the eating disorders: etiological implications. *Compr Psychiatry* (1997) **38**(6):321–6. doi:10.1016/S0010-440X(97)90927-5
17. World Health Organization. *International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10)*. New York, NY: World Health Organization (2008).
18. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Text Revision (DSM-IV-TR)*. 4th ed. Washington, DC: American Psychiatric Association (2000).
19. Bewell-Weiss CV, Carter JC. Predictors of excessive exercise in anorexia nervosa. *Compr Psychiatry* (2010) **51**(6):566–71. doi:10.1016/j.comppsych.2010.03.002
20. Dalle Grave R, Calugi S, Marchesini G. Compulsive exercise to control shape or weight in eating disorders: prevalence, associated features, and treatment outcome. *Compr Psychiatry* (2008) **49**(4):346–52. doi:10.1016/j.comppsych.2007.12.007
21. Westerterp KR, Kester AD. Physical activity in confined conditions as an indicator of free-living physical activity. *Obes Res* (2003) **11**(7):865–8. doi:10.1038/oby.2003.119
22. Joosen AM, Gielen M, Vlietinck R, Westerterp KR. Genetic analysis of physical activity in twins. *Am J Clin Nutr* (2005) **82**(6):1253–9.
23. Levine JA, Eberhardt NL, Jensen MD. Role of nonexercise activity thermogenesis in resistance to fat gain in humans. *Science* (1999) **283**(5399):212–4. doi:10.1126/science.283.5399.212
24. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc* (2000) **32**(9 Suppl):S498–504. doi:10.1097/00005768-200009001-00009
25. Elbelt U, Schütz T, Hoffmann I, Pirlich M, Strasburger CJ, Lochs H. Differences of energy expenditure and physical activity patterns in subjects with various degrees of obesity. *Clin Nutr* (2010) **29**(6):766–72. doi:10.1016/j.clnu.2010.05.003
26. Levine JA. Non-exercise activity thermogenesis (NEAT). *Best Pract Res Clin Endocrinol Metab* (2002) **16**(4):679–702. doi:10.1053/beem.2002.0227
27. Müller MJ, Bosy-Westphal A, Klaus S, Kreymann G, Lührmann PM, Neuhäuser-Berthold M, et al. World Health Organization equations have shortcomings for predicting resting energy expenditure in persons from a modern, affluent population: generation of a new reference standard from a retrospective analysis of a German database of resting energy expenditure. *Am J Clin Nutr* (2004) **80**(5):1379–90.
28. Kraemer RR, Shockett P, Webb ND, Shah U, Castracane VD. A transient elevated irisin blood concentration in response to prolonged, moderate aerobic exercise in young men and women. *Horm Metab Res* (2013). doi:10.1055/s-0033-1355381
29. Norheim F, Langleite TM, Hjorth M, Holen T, Kielland A, Stadheim HK, et al. The effects of acute and chronic exercise on PGC-1alpha, irisin and browning of subcutaneous adipose tissue in human. *FEBS J* (2013). doi:10.1111/febs.12619
30. Strober M, Freeman R, Morrell W. The long-term course of severe anorexia nervosa in adolescents: survival analysis of recovery, relapse, and outcome predictors over 10-15 years in a prospective study. *Int J Eat Disord* (1997) **22**(4):339–60. doi:10.1002/(SICI)1098-108X(199712)22:43.0.CO;2-N
31. Rigaud D, Pennacchio H, Bizeul C, Reveillard V, Verges B. Outcome in AN adult patients: a 13-year follow-up in 484 patients. *Diabetes Metab* (2011) **37**(4):305–11. doi:10.1016/j.diabet.2010.11.020
32. Pasanisi F, Pace L, Fonti R, Marra M, Sgambati D, De Caprio C, et al. Evidence of brown fat activity in constitutional leanness. *J Clin Endocrinol Metab* (2013) **98**(3):1214–8. doi:10.1210/jc.2012-2981
33. Welk GJ, McClain JJ, Eisenmann JC, Wickel EE. Field validation of the MTI Actigraph and BodyMedia armband monitor using the IDEEA monitor. *Obesity (Silver Spring)* (2007) **15**(4):918–28. doi:10.1038/oby.2007.624
34. El Ghoch M, Alberti M, Capelli C, Calugi S, Battistini NC, Pellegrini M, et al. Resting energy expenditure assessment in anorexia nervosa: comparison of indirect calorimetry, a multisensor monitor and the Muller equation. *Int J Food Sci Nutr* (2012) **63**(7):796–801. doi:10.3109/09637486.2012.658761
35. Mignault D, St-Onge M, Karelis AD, Allison DB, Rabasa-Lhoret R. Evaluation of the Portable HealthWear Armband: a device to measure total daily energy expenditure in free-living type 2 diabetic individuals. *Diabetes Care* (2005) **28**(1):225–7. doi:10.2337/diacare.28.1.225-a
36. St-Onge M, Mignault D, Allison DB, Rabasa-Lhoret R. Evaluation of a portable device to measure daily energy expenditure in free-living adults. *Am J Clin Nutr* (2007) **85**(3):742–9.
37. Erickson HP. Irisin and FNDC5 in retrospect: an exercise hormone or a transmembrane receptor? *Adipocyte* (2013) **2**(4):289–93. doi:10.4161/adip.26082
38. Raschke S, Elsen M, Gassenhuber H, Sommerfeld M, Schwahn U, Brockmann B, et al. Evidence against a beneficial effect of irisin in humans. *PLoS One* (2013) **8**(9):e73680. doi:10.1371/journal.pone.0073680

39. Kozak M. Context effects and inefficient initiation at non-AUG codons in eucaryotic cell-free translation systems. *Mol Cell Biol* (1989) **9**(11):5073–80.
40. Mond JM, Calogero RM. Excessive exercise in eating disorder patients and in healthy women. *Aust N Z J Psychiatry* (2009) **43**(3):227–34. doi:10.1080/00048670802653323
41. Shroff H, Reba L, Thornton LM, Tozzi F, Klump KL, Berrettini WH, et al. Features associated with excessive exercise in women with eating disorders. *Int J Eat Disord* (2006) **39**(6):454–61. doi:10.1002/eat.20247

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# Personality and psychiatric disorders in women affected by polycystic ovary syndrome

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**Background:** Polycystic ovary syndrome (PCOS) is the most prevalent endocrine disorder among fertile women. Studies show reduced quality of life, anxiety, depression, body dissatisfaction, eating disorder, and sexual dysfunction, but the etiology of these disturbs remains still debated. The aim of our study is to verify whether this hyperandrogenic syndrome characterizes a strong psycho(patho)logical personality.

**Method:** Sixty PCOS subjects (mean age 25.8 ± 4.7 years) were evaluated by anthropometric, metabolic, hormonal, clinical, and psychological parameters. After the certainty of the diagnosis of PCOS, the Rorschach test, according to Exner's comprehensive system (CS) and the Millon Clinical Multiaxial Inventory-III (MCMI-III) were administered to each patient. The control group, on which the comparison was carried out, was composed by 40 healthy and aged compared women who were exclusively administered the Rorschach test according to CS.

**Results:** MCMI-III evidenced axis II DSM-IV personality disorders [4.1% schizoid, depressive, sadistic, negativistic (passive–aggressive), and masochistic, 6.1% avoiding, 12.2% dependent, 20.4% histrionic, 16.3% narcissistic, 2.0% obsessive–compulsive], and axis I DSM-IV psychiatric disorders: 10.2% anxiety, 2.0% somatoform disorder and bipolar disorder, 16.3% major depressive disorder. Finally, we found 44.9% delusional disorder and 4.1% thought disorder. Rorschach test's results show 53.1% reduced coping abilities and social skills, 55.1% depression, 30.6% perceptual distortion and cognitive slippage, 24.5% constantly alert and worry, 8.1% at risk for suicide, and finally about 50% of our patients had chronic stress.

**Conclusion:** PCOS women have relevant personality and psychiatric disorders, when compared with normal subjects.

**Keywords: polycystic ovary syndrome, personality, Rorschach, MCMI-III, psychopathology, personality disorder, psychiatric disorder**

## INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous gynecological endocrine disorder, characterized by chronic anovulation, hyperandrogenism, and hyperinsulinism with insulin resistance, which affects about 6–10% of women in reproductive age (1–3) and profoundly affects the quality of life of these subjects (4, 5). In fact, this hyperandrogenic syndrome is frequently characterized by hirsutism, acne, alopecia, obesity, and irregular periods with infertility and it is often treated as cosmetic problem. The existence of a linkage between these latter clinical features and reduced quality of life was frequently suggested in several researches (6–8). Other studies evidenced how physical symptoms caused mental disorders, but depression and anxiety seem to be independent of obesity and infertility, the most frequent symptoms of PCOS (9, 10). Also, the relationship between androgen excess and mood remains still controversial because clinical and biochemical parameters of hyperandrogenism seem not directly cause of depression (11). Again, diagnosis of PCOS was related to negative feelings, such as frustration (67%), anxiety (16%), and sadness (10%), although the (self-reported) level of knowledge about the illness was high and the women were aware that PCOS can be treated and is not a deadly disease (12). Other authors reported reduced scores in the sub-categories of perception about health in general, physical performances, general behavior, and limitations in family activities due to the "girls' disease" (12). At the same time, higher scores were reported in the rating of health conditions, during the diagnostic pathway and above all the treatment period, further supporting a dependent effect between clinical features and psychological aspects. In different manner, several data obtained in PCOS subjects reported that differences for the QoL

are more related to the subjective perception of the illness's seriousness rather than to the clinical seriousness of the illness itself. These data, therefore, suggest that a subjective feeling of discomfort might be totally unrelated to the clinical picture, although objectively recorded. Again, other psychological aspects, such as depression (8, 13), and/or anxiety (14, 15), have been emphasized in PCOS, but only few articles analyzed women's personality structure (16). In women with PCOS, Sahingoz et al. (17) assessed a high prevalence of psychiatric and personality disorders, about 29.0 and 14.0%, respectively. They also reported 9.6% of mood and 26.0% of anxiety disorders, 13.7% of social phobia, 11.0% of generalized anxiety, and 12.3% of avoidant personality disorders, according to DSM-IV axis I and II Diagnostic Criteria, respectively. Again, Patients with borderline personality disorders, diagnosed by SCID for DSM-IV, about 30.0% was affected by PCOS (18). Personality disorders seem to be characterized by the tendency to exhibit a tenuous stability, or lack of resilience, under conditions of stress, by the inflexibility to adapt and by vicious cycles that repeats once again their pathology. Again, the interaction of psychosocial stressors and personality characteristics leads to the expression of psychological symptoms, that is, axis II and axis IV interact to produce axis I. Since no studies used Rorschach test or Millon Clinical Multiaxial Inventory to analyze the personality structure of subjects with PCOS, we believe it is useful to carefully study and develop the personality aspects related to this hyperandrogenic syndrome, as well as the psychopathological ones. Moreover, to minimize the influence of diagnostic and therapeutic pathways, we only studied young women, with comparable social and school conditions, undergoing for the first time to the endocrine evaluation for clinical features of hyperandrogenism and/or disorders of menstrual cycle.

This study was performed in collaboration with the Division of Endocrinology, Department of Internal Medicine of St. Orsola-Malpighi Hospital, University of Bologna, and with the Clinical Medical 3 of Health Centre/Hospital-University of Padua, on the white patients coming from the northern provinces of Italy.

## PATIENTS AND METHODS

### SUBJECTS RECRUITMENT

Sixty Caucasian women, all of Italian origin, affected by certain diagnosis of PCOS, were enrolled to participate in this research study: 30 subjects from the Endocrinology Unit of St. Orsola-Malpighi Hospital – University of Bologna and 30 subjects from the Clinical Medical 3 of Health Centre/Hospital-University of Padua. For all patients, it was the first time they turned to a Public Health Institution to resolve their hyperandrogenic symptoms.

All women were hyperandrogenic (clinical presence of hirsutism with Ferriman–Gallwey score >8, acne or alopecia and/or elevated androgen levels: testosterone ($T$) > 2.1 nmol/L and or androstenedione (A) > 10.4 nmol/L or Free Androgen Index > 5 [FAI = Testosterone(nmol/L)/SHBG(nmol/L) × 100] and met the diagnosis of PCOS, according to the Rotterdam criteria (19). Ovarian ultrasound findings were performed by transvaginal or transrectal pelvic ultrasound (US ESAOTE F.U.5, Probe of 6.5 MHz, Milan, Italy) and PCO diagnosis were considered according to previous criteria (20). All subjects were no-smokers, no dieting, had a normal glucose tolerance test and performed normal physical activities, none drank alcoholic beverages or took any continuous medications. According to PCOS diagnostic criteria, none had thyroid dysfunction, hyperprolactinemia, type 2 diabetes mellitus or concomitant cardiovascular, renal, and liver dysfunctions. Other causes of hyperandrogenism, such as Cushing syndrome/disease and congenital adrenal hyperplasia, were excluded, after nocturnal fasting, by basal and/or stimulated (0.25 mg Synacthen® iv, Biofutura Pharma S.p.A., Pomezia, Rome) plasma 17-OH-progesterone levels. At the beginning of the study, the insulin resistance and hyperinsulinism were, respectively, evaluated by using the homeostatic model assessment (HOMA-ir) [(fasting glucose (mmol/L) × fasting insulin (mIU/L)/22.5; n.v. <1.9 in our control group of at least 200 normal healthy subjects (data not shown)] and the 3-h oral glucose tolerance test (OGTT; 75 g of glucose) for plasma glucose, insulin, and C-peptide levels. Whole-body insulin sensitivity was calculated by using the insulin sensitivity index derived from the OGTT ($ISI_{COMPOSITE}$ n.v. >6.0) according to Matsuda and DeFronzo (21) considering normo-sensitive subjects with values higher than 6.0 and low-sensitive those with values lower than 4.0, respectively.

All women were studied in the follicular phase (days 1–7) of the cycle, or after 2 or more months of amenorrhea, after a negative pregnancy test. After this diagnostic pathway, but before of any pharmacological treatment, at each subject was administered the Millon Clinical Multiaxial Inventory-III (MCMI-III) (22). The MCMI-III has been adapted to the Italian population by a research group, which has involves the Universities of Milan-Bicocca, Rome-La Sapienza, and Padua, coordinated by Prof. Alessandro Zennaro, University of Turin, Italy (23, 24). We used Millon words to explain personality disorder and clinical syndrome.

In a subsequent day, for the first time in subject affected by PCOS, Rorschach, which is considered a scientific, valid, and reliable projective test to assess personality, was administered according to the Exner's comprehensive system (CS), once adapted to Italian population (24). The protocol was approved by the Local Ethics Committee of both Padua and Bologna Hospital, and all women gave their informed consent approval to collaborate with this research. Eleven women invalidated the Rorschach protocols because they have not given a sufficient number of answers in the first tables, so the original group of the patients was reduced to 49 patients. All subjects aged from 19 to a maximum of 39 years ($25.80 \pm 4.27$ years), with comparable social and school degree (from 13 to 18 years of school). Forty five women, with age (from 19 to 41 years; $25.2 \pm 6.0$), social, and school degree comparable, were selected from a total of 2.500 records present in our database, and considered as control group. Each control subject had a normal menstrual cycle, was not hirsute, not taking any drugs or abusing alcohol, did not smoke, and was matched to the subjects with PCOS for body weight and age. Anonymity was assured to each patient and names were substituted by an alphanumerical progression.

### TESTS AND SELECTION OF VARIABLES

The tests chosen for the personality analysis of the sample were (a) the *Rorschach Test* administered, scored, and interpreted according to the Exner's CS (23, 24); and (b) The MCMI-III (22) is

composed by 175 true/false items with 28 scales of which 14 refer to personality disorders and 10 refer to clinical syndromes and the remaining 4 refer to test validity indices. In MCMI-III scoring system vary from 1 to 115 with a median score of 60 base rate (BR), scores of 85 or above evidence a personality disorder, according to DSM-IV axis II classification and/or the prominence of a clinical syndrome, according to DSM-IV axis I diagnosis (24, 25). Italian version of MCMI-III shows good internal validity (Cronbach's alpha range for subscales = 0.66–0.90) and test-retest reliability ($r = 0.84$–$0.96$) (23). The Rorschach tables were administered, scored, and interpreted according to the Exner's CS (24, 26, 27). It was decided to select, besides the 27 variables proposed by Exner and Andronikof-Sanglade (28) and by Bihlar and Carlsson (29), further variables that we consider useful FD, SumT, SumC′, SumV, SumY, Sum Shading, Sum Color Shading Blends, Sum Shading Shading Blends, Wsum6, and An + Xy. Taking a cue from the work of Sultan et al. (30), conducted on patients with mellitus diabetes (IDDM), we wished to also consider part of their proposed indices.

### LABORATORY AND PLASMA ASSAYS

Glucose was measured by using the glucose oxidase method (Gluco-Quant®; Roche Diagnostics GmbH, Mannheim Germany), its detection limit was 0.11 mmol/L. Insulin by means of the two-site chemiluminescent immunometric immunoassay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA) and its analytic sensibility was 2.0 μIU/mL. The intra- and inter-assay coefficients of variation (CVs) were <5.5 and 7.3%, respectively. LH, FSH, and E2 were measured by a competitive immunoassay with the use of an electrochemiluminescence immunoassay (ECLIA; Roche Diagnostics GmbH, Mannheim, Germany). Analytic sensibility of LH and FSH was 0.2 and 0.34 UI/L, respectively, whereas for E2 was 0.05 pmol/L. The intra- and inter-assay CVs were 1.8 and 5.2% for LH, 2.0 and 5.3% for FSH, 5.7 and 6.2% for E2, respectively. Testosterone, DHEA-s, 17-OHP, and A, after celite column chromatographic separation, were assayed as previously described (31). Serum T and DHEA-s were further measured by competitive immunometric chemiluminescent enzyme immunoassay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA). The detection limit was 0.35 nmol/L for T and 0.08 μmol/L for DHEA-s, respectively, the inter- and intra-assay CVs were below 7.4% for T and below 6% for DHEA-s. A and OHP were measured by using an enzyme-linked immunosorbent assay, ELISA (DRG Instruments GmbH, Marburg, Germany) and their detection limit was 0.6 nmol/L for A and 0.18 nmol/L for OHP, respectively, with intra- and inter-assay CVs <9.1 and 12.1% for A and 7.8 and 9.4% for OHP, respectively. SHBG was measured by using two-site chemiluminescent immunometric assay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA), its analytic sensibility was 0.8 nmol/L with intra- and inter-assay CVs <5.3 and 6.6%, respectively.

### ANALYSIS OF THE DATA

The anthropometric and hormonal data were reported as mean ± SD. All continuous variables were compared using the Student's *t*-test for unpaired data. Glycometabolic and hormonal data from PCOS and control groups were compared and analyzed with repeated measures by ANOVA. Mann–Whitney *U* test and Wilcoxon paired rank test were also used for evaluating the distribution of non-parametric values. Values for $p < 0.05$ were considered significantly different.

For an in-depth approach to the psychological investigation of our samples, we carried out the following analyses – comparison of the BR points obtained from the administration of MCMI-III under the original norms proposed by Millon et al. (22); statistical comparison of the average values of the Rorschach variables selected with the most recent norms proposed by Exner and Erldberg (26) through the use of confidence intervals (CI 95%); statistical percentage comparison of the threshold values of the same variables with those proposed by Exner (25), ambitent subject, non-patient norms. The reference to the descriptive statistics of the ambitent subjects is justified by the confirmation that, on average, this coping style is representative of the M: WSumC relationship present in the PCOS sample ($M = 2.25$: SumC = 4.133). Comparison of Rorschach Indices [suicide constellation (S-Con), coping deficit index (CDI), depression index (DEP-I), perceptual-thinking index (PTI), schizophrenia index (SCZI), hypervigilance index (HVI)] obtained from subjects with PCOS were done with those obtained from control group recorded in our database.

## RESULTS AND DISCUSSION

Auxological and endocrine–metabolic parameters of our subjects are summarized in **Table 1**. As expected, hyperandrogenic parameters were elevated in women affected by PCOS and significantly different when compared with Control group. In particular, LH/FSH ratio and serum androgens, above all testosterone, were significantly higher in PCOS than in Controls. Also, metabolic parameters and insulin resistance were significantly elevated in patients with PCOS, whereas, as expected by the enrollment criteria, body weight (calculated as BMI) did not differ between the groups.

When we analyzed personality disorders scales, by MCMI-III, in PCOS group, we observed that several diseases, diagnosed in according to DSM-IV axis II, reached percentage significantly higher than in controls. In **Figure 1**, in fact, we summarized the

**Table 1 | Characteristics of women with PCOS and healthy controls**.

| | PCOS ($n = 49$) | Control ($n = 45$) | *p* |
|---|---|---|---|
| AGE (years) | 25.8 ± 4.7 | 25.2 ± 5.9 | n.s |
| BMI (kg/m$^2$) | 27.6 ± 4.8 | 24.9 ± 4.4 | n.s |
| Waist (cm) | 78.2 ± 6.8 | 69.5 ± 3.7 | <0.05 |
| PRL (μg/L) | 23.0 ± 8.6 | 11.9 ± 5.3 | <0.05 |
| LH/FSH (ratio) | 2.80 ± 0.4 | 1.09 ± 0.2 | <0.02 |
| DHEA-S (μmol/L) | 7.01 ± 2.5 | 5.50 ± 3.34 | n.s |
| 17-OH-P (nmol/L) | 2.68 ± 0.46 | 1.46 ± 0.38 | <0.05 |
| A (nmol/L) | 12.7 ± 1.2 | 6.08 ± 0.71 | <0.02 |
| T (nmol/L) | 2.96 ± 0.2 | 0.85 ± 0.3 | <0.005 |
| $E_2$ (pmol/L) | 123 ± 26 | 104 ± 34 | n.s. |
| SHBG (nmol/L) | 24.6 ± 7.9 | 54.7 ± 13.6 | <0.03 |
| FAI (free androgen index) | 12.0 ± 1.33 | 1.55 ± 0.6 | <0.005 |
| HOMA-ir | 2.46 ± 0.42 | 1.45 ± 0.36 | <0.05 |

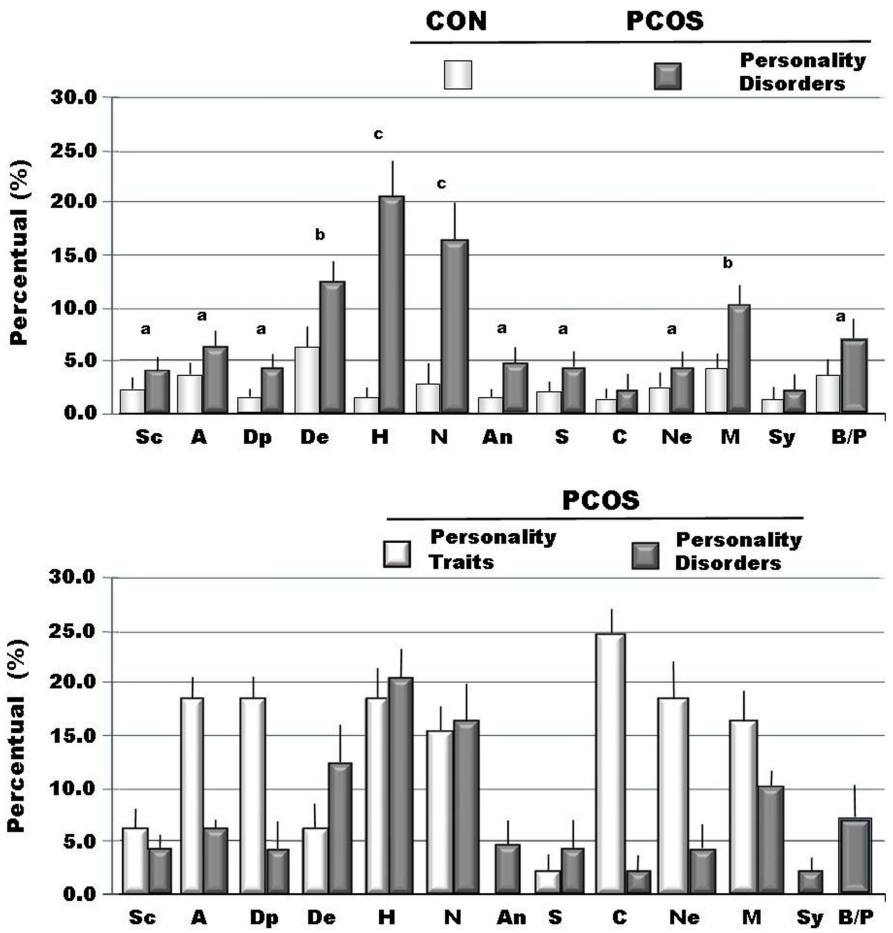


**FIGURE 1 | The incidence of traits and of personality disorders (DSM-IV axis II) recorded through MCMI-III in personality scales (BR ≥ 75 is personality trait; BR ≥ 85 is disorder) (22)**. Legend: Sc, schizoid; A, avoidant; Dp, depressive; De, dependent; H, histrionic; N, narcissistic; An, antisocial; S, sadistic; C, compulsive; Ne, negativistic; M, masochistic; Sy, schizotypal; B, borderline; P, paranoid; $^{a}p < 0.05$ vs. Cont; $^{b}p < 0.01$vs. Cont; $^{c}p < 0.005$ vs. Cont.

most important personality disorders in PCOS group compared with those observed in the control group. However, whether this increased incidence of personality disorders in patients affected by PCOS can be considered a link with clinical signs of hyperandrogenism remain at present unclear. In fact, the majority of studies showed a linkage between psychological disorders and major clinical PCOS symptoms, such as excessive body hairs, acne, infertility, or excessive body weight (4–6, 10, 13, 32, 33). Our results, in contrast, indicated that an intrinsic psychopathological characteristic of PCOS can be really consistent. According to our hypothesis, there are only three articles (17, 18, 34) that evidenced personality disorders in patients affected by PCOS, diagnosed by Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). However, these latter studies recorded lower percentage of psychological disorders, when compared with our data, so suggesting that a heterogeneous expression of the same psychopathological diseases can exist in PCOS or it was assessed a heterogeneous pool of subjects affected by PCOS, therefore, further studies that consider the same Diagnostic Criteria for PCOS and a more numerous group of person are needed. When we considered the results of clinical syndrome scales, diagnosed according to DSM-IV axis I Criteria, we also found several clinical psychopathological disorders, as summarized in **Figure 2**. The most representative disorders were anxiety (about 10.0%) and depression (about 16.0%). These analyses, in fact, seem to confirm recent data obtained in PCOS patients (17), which showed a frequent finding of psychiatric disorders. Interesting to note that in our sample we didn't observe neither alcohol addiction nor drug addiction or post-traumatic stress disorders. Because, we also observed different incidences (from 30.0% to about 70%) of psychopathological diseases, such as anxiety, mood, or thought disorders, using different psychometric test, it can be also suggested that different clinical expressions of psychopathological diseases can exist in PCOS. Also, other data, analyzed by different questionnaires, confirmed a prevalent expression of anxiety and depression, further suggesting that an elevated rate of mental illness in these hyperandrogenic patients (11, 14, 35). Again, Klipstein et al. (36) evidenced a rate of about 30.0% of bipolar disorder types I and II and Rassi (37) of about

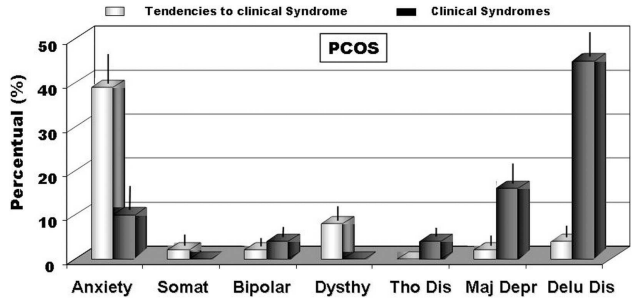


**FIGURE 2 | The incidence of clinical disorders scales (DSM-IV axis I) (BR > 75) and of full blown clinical syndromes (BR > 85) recorded through MCMI-III (22)**. Legend: anxiety; Somat, somatoform; bipolar; Dysthy, dysthymia; Tho Dis, thought disorder; Major Dep, major depression; Delu Dis, delusional disorder.

11.0% of patients, respectively. Our data are in agreement with these latter results, so further evidencing that women with PCOS had a rate of mental illness several times higher (three to five-fold), when compared with normal population. However, the absence of rigorous diagnostic criteria of PCOS can not exclude an enrollment of heterogeneous groups of patients, so reducing the diagnostic capacity and, of consequent, the percentage of psychopathological diseases in the PCOS *per se*. Results of Rorschach test, obtained from our patients, are summarized in **Table 2** and **Figure 3**. We used as control a group of 45 healthy Italian subjects already recorded in our database and gently provided by Zennaro (23). We used this trick because in Italy, at present, there are no any data about this pathology.

Using a statistical comparison (Student's *t*-test for independent groups) between the samples of patients and the control group, we have evidenced significant differences among several variables, as showed in **Table 2**. Calculating Cohen's *d*, the effect size (statistically significant $>0.6$) underlined a high effect for 10 of these variables: WSum6, AdjD, ego impairment index (EII), MOR, Popular, Shading Blends, SumT, SumV, Sum Shading e Sum6. Our data show an important deficit in cognitive functions (MEDIATION and IDEATION clusters). Cohen's *d* underlines as "high effects" ($\geq 0.6$) of the following variables: X + %, WSum6, AdjD, COP, GHR, Popular, SumV, SumY, Sum Shading, and Sum6. The following variables, WSum6, SumV, Sum Shading, Sum6, are significantly higher. The variables, X + %, COP, GHR, Popular, were significantly lower, while AdjD was actually negative. All these data evidenced that in subjects with PCOS exists a massive reality distortion (XA%, X − %, X + %, EII, PTI index, and SCZI index) with incoherent and disorganized thoughts (Sum6 and Wsum6), failures in the ability to judge, bizarre, or magical ideations and with interpersonal communication difficulties. PTI was positive for 30.6% of patients as well as SCZI for 20.4% of our sample, therefore, we can underline as "*daily life*" is really difficult for the majority of our patients affected by PCOS, because misperception leads to behavior often inappropriate in social life. Again, a large proportion (63.3%) of patients (X − % > 0.25) were displayed serious perceptual inaccuracies, stressing therefore how the reality was not adequately perceived because emotions distorted it (X − %, X + %). Again, 42.9% of subjects (Xu% = 0.2) were anti-conformism, displaying a tendency to originality, but 53.1% (Populars $\leq 4$) rejected conventional models, so adopting behaviors not socially adequate and/or accepted. Ninety-two percent of subjects with PCOS (X + % <0.70) showed a perception of the situations and words in an unusual manner. About four (4.1) % (Populars $\geq 8$) of our sample was exaggerated in conformism. These latter patients usually need a positive social judgment and they need the approval of other people. In our sample, 26.5% (lambda < 0.30) had an inadequate control over emotions that interfere with logical functions. EII showed serious impairment in 38.8% of the sample, moderate in 18.4%, average in 8.2%, and slight in 24.5% (X − %, Sum6, WSum6 and EII higher, XA%, WDA%, X + % lower). Only 10.0% of women analyzed, had a normal, non-pathological values. According all these results, we evidenced that our patients had really difficulties in organizing the stimulus when they face the reality, so forcing them to use more psychic energy than needed (WDA < XA%). However, in our study about 12.2% of patients (12 subjects) ignored the complexity of the reality and tended to simplistic situations and interpersonal relationships. Again, 40.8% were very self-involved (Egoindex $\geq 0.45$) and about 30% built up their own self-esteem, by considering themselves better than others (Fr + Rf = 1). This focus on self could be detrimental in the interpersonal relationship and could contribute to reality distortion. Our data seem to confirm Willmott's results (38) that evidenced as women with PCOS exhibit a lack of control over their own lives' events. Considering the CDI, we found it positive for 53.1% of the patients (vs. 29% of the controls), revealing a deficit in coping abilities, and consequently interpersonal relationships were superficial and rarely maintained. Also, social skills appeared to be reduced (CDI, EA) and interpersonal relationships were perceived as unsatisfying. About thirty (30.6) % of PCOS sample had a damaged and negative interpersonal relationship representation (PHR higher). Interactions of personal situations were perceived as not cooperative, but in a hostile manner. All of this creates, in our patients, a state of chronic stress, which was reflected in a cognitive slippage. More than 50% of our patients resulted depressed (DEP-I positive) and with a percentage significantly higher than observed in controls (55.1 vs. 25.0% controls). Unexpectedly, 8.1% of patients with PCOS (vs. 6% of the controls) were at risk for suicide (S-Con positive, MOR = 2, FD > 2, Shadings). These latter results were in agreement with previous studies that underlined how this psychopathological tendency was more frequently observed in women with PCOS (35). However, at present, whether suicide can be cause or effect of high level of discomfort induced by clinical or psychological signs, or both, remains still unknown. In expected manner, several authors (39, 40) reported a high prevalence of negative self image and poor body image in subjects with PCOS. Also, in our PCOS group, with respect of controls, we evidenced a significant higher, about 18.0%, a self image damaged (MOR = 2). Again, about 45% of patients had an exaggerated introspective behavior (FD $\geq 2$) that it could became a pathological self criticism and a ruminative thinking. This point of view is important, because women with PCOS had a negative and pessimistic view of themselves; therefore, they had constant need of reassurance about their own value

**Table 2 | Comparison between PCOS and control groups using *t*-test for independent samples**.

| Rorschach variables | PCOS group ($n = 49$) | Control group ($n = 45$) | *T* | *p* | Cohen's *d* |
|---|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | | | |
| Age | 25.80 ± 4.27 | 24.80 ± 6.77 | | | |
| R | 21.59 ± 6.68 | 23.35 ± 8.170 | | | |
| Lambda | 0.606 ± 0.5576 | 0.5728 ± 0.4800 | | | |
| FQM− | 0.88 ± 1.3 | 1.18 ± 1.279 | | | |
| FQS− | 1.12 ± 1.39 | 1.63 ± 1.628 | | | |
| FD | 0.92 ± 0.91 | 1.35 ± 1.562 | | | |
| XA% | 69.94 ± 12.27 | 71.35 ± 12.949 | | | |
| WDA% | 75.06 ± 12.62 | 73.45 ± 11.964 | | | |
| X + % | 42.45 ± 13.24 | 47.58 ± 13.769 | −1.777 | 0.079 | −0.37 |
| Xu% | 27.45 ± 1.49 | 23.78 ± 9.983 | | | |
| X − % | 28.49 ± 11.99 | 27.78 ± 3.318 | | | |
| S − % | 16.57 ± 21.51 | 22.98 ± 22.273 | | | |
| WSum6 | 23.55 ± 13.27 | 11.43 ± 10.77 | 4.759 | 0.000 | 1.003 |
| WSumC | 4.133 ± 2.345 | 3.338 ± 2.092 | | | |
| EA | 6.684 ± 3.556 | 7.888 ± 3.888 | | | |
| ES | 12.22 ± 6.28 | 11.68 ± 5.25 | | | |
| D | −1.73 ± 2.09 | −1.15 ± 1.46 | | | |
| AdjD | −0.96 ± 1.65 | −0.30 ± 1.159 | −2.212 | 0.030 | −0.464 |
| Afr | 0.445 ± 0.1605 | 0.488 ± 0.1281 | | | |
| EGO index | 0.42 ± 0.1685 | 0.4432 ± 0.1792 | | | |
| EII | 1.213 ± 1.0866 | 0.489 ± 1.1050 | 3.099 | 0.003 | 0.66 |
| AG | 0.20 ± 0.50 | 0.45 ± 0.815 | | | |
| MOR | 0.86 ± 1.17 | 2.05 ± 1.867 | −3.515 | 0.001 | −0.763 |
| COP | 0.51 ± 0.94 | 0.98 ± 1.121 | −2.092 | 0.040 | −0.454 |
| GHR | 2.12 ± 1.55 | 3.98 ± 1.790 | −5.156 | 0.000 | −1.110 |
| PHR | 3.47 ± 2.61 | 3.70 ± 2.44 | | | |
| POP | 4.20 ± 2.09 | 5.63 ± 1.917 | −3.338 | 0.001 | −0.712 |
| Blends | 5.67 ± 0.44 | 5.58 ± 3.727 | | | |
| Color Sh. blends | 1.41 ± 1.31 | 1.00 ± 1.320 | | | |
| Shading Sh. blends | 1.06 ± 1.34 | 0.33 ± 0.829 | 3.166 | 0.002 | 0.653 |
| SumC′ | 3.08 ± 2.26 | 2.53 ± 1.894 | | | |
| SumT | 1.43 ± 1.34 | 0.75 ± 0.776 | 2.986 | 0.004 | 0.621 |
| SumV | 1.65 ± 1.70 | 0.70 ± 0.966 | 3.319 | 0.001 | 0.686 |
| SumY | 2.53 ± 1.95 | 1.88 ± 2.174 | | | |
| Sum shading | 8.69 ± 4.85 | 5.85 ± 4.123 | 2.990 | 0.004 | 0.631 |
| Fr + Rf | 0.71 ± 0.87 | 0.83 ± 1.130 | | | |
| IsoIndex | 0.226 ± 0.1222 | 21.28 ± 12.649 | | | |
| Sum6 | 7.02 ± 3.53 | 3.68 ± 2.99 | 4.842 | 0.000 | 1.021 |
| Intell | 2.59 ± 2.04 | 2.85 ± 2.842 | | | |
| An + Xy | 1.96 ± 0.3 | 0.90 ± 1.215 | 2.783 | 0.007 | 0.576 |
| Schizo index | 2.88 ± 1.48 | 2.68 ± 1.70 | | | |
| PTI | 1.80 ± 1.41 | 1.63 ± 1.59 | | | |
| HVI 2–8 | 2.29 ± 1.53 | 3.23 ± 2.057 | −2.398 | 0.019 | −0.518 |
| DEP-I | 4.73 ± 1.22 | 4.25 ± 1.214 | 1.869 | 0.065 | 0.394 |
| CDI | 3.33 ± 1.3 | 2.90 ± 1.128 | | | |
| S-Con | 5.88 ± 1.18 | 5.40 ± 1.736 | | | |

and had serious problems of establishing intimate relationship (18.4% Fr + Rf ≥ 2). In our patients, we recorded about 25% of the samples (vs. 7% of healthy and normal subjects) constantly in alert (HVI) and consequently worried, therefore, this mood justifies how the majority part of the subjects examined, about 64% of PCOS, lived with a constant state of anxiety (SumY ≥ 2). It was interesting to underline that similar results were obtained both in depressed patients and in subjects with juvenile diabetes

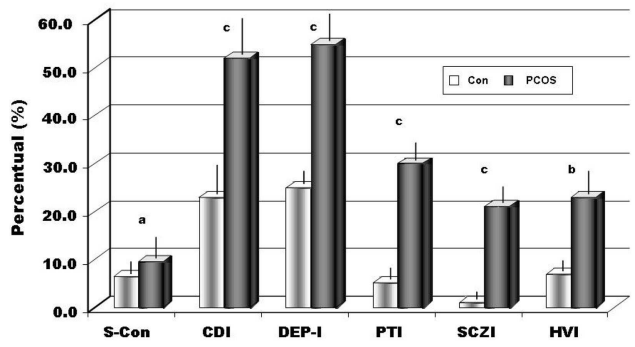


**FIGURE 3 | The percentage in the sample of positive results to Rorschach indices (S-Con ≥ 8, CDI ≥ 4, DEP-I ≥ 5, PTI ≥ 3, SCZI ≥ 4, HVI ≥ 4 if $T = 0$).** Legend: S-Con, suicide constellation; CDI, copy deficit index; DEP-I, depression index; PTI, perceptual-thinking index; SCZI, schizophrenia index; HVI, hypervigilance index; $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.002$.

mellitus type I (30). And again, it was suggested that the observation of Monzani et al. (41) who emphasized how in subjects affected by PCOS scores of somatization, anxiety, and depression variables reached higher values when compared with normal people. At the same time, these authors interestingly did not observe any relevant psychopathological disorders, but differently noticed that the subjects tend to hidden and/or clear behaviors typical of the opposite gender. Moreover, the same women tended to conform themselves to social standards, so probably reducing the perception of their "diversity." Our data, therefore, seem to confirm these results that report how ill women have altered body self-perception, sexual intimacy, sexual dissatisfaction, and the strong opinion that their partners' sex life is as unsatisfactory as their own, so indicating that PCOS is perceived as a real disease (42).

About 60% of PCOS women experienced painful emotions and high sense of insecurity, confusion, and ambivalence in relation to emotions (Color Shading Blends > 1 in 69.4% and Shading Blends > 1 in 61.2%), showed a low self-esteem (36.7% with Egoindex < 0.32), and feel anxious, tense, nervous, and irritable (more than 71.0% showed FQS-higher). All these data evidenced that these women did not tolerate frustration and they were impulsive (D < 0). About 70.0% was feared to affective stimuli and avoid them (Afr lower) and experienced discomfort with introspection, because feelings of inferiority and depressed feelings (SumV ≥ 1). We observed that many of the samples (69.4%) did not use intellectualization (Intell > 3) as a defense mechanism against affects, but tended to avoid (Afr) situations where they were generated. Since several patients affected by PCOS tended to isolate themselves, to refuse interpersonal relationships, to present social phobia, or tended to be worried and keep at a certain emotional distance from others, it can be concluded that these subject are not very interested in interpersonal relationship. However, our results, in different manner, evidenced that about 60% of patients tended to actively research contact and interpersonal relationships (Isoindex < 0.25). Because, we studied a group of young patients, with the majority of them in a period of university study, where the interpersonal contacts are very high, we cannot exclude that we have enrolled a group of patients with different social or cultural impact.

## CONCLUSION

Our results clearly highlight that in our patients, affected by PCOS, exist mood and severe thought disorders with perceptual distortion in about half of subjects. In this hyperandrogenic syndrome, women have elevated dysphoric feelings, chronic emotional stress, and several difficulties in social skills and daily life. In our group of patients, to attenuate an important painful emotional overload, many avoid interpersonal relationships and emotional inputs. Again, intimate relationships can often cause fear and frustration. All these conditions suggest that the characteristics of psychological suffering in PCOS can constitute a distinguishing element, which, for some aspects, appears to be so represented and shared by the sample as to be promoted to a proper diagnostic indicator. We also underline that the knowledge of these psychopathologic aspects may ameliorate the doctor–patient interaction and relationship with a desirable improvement on the medical examination, sensibility, and probably reaching greater adherence to diagnostic and medical therapy.

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## REFERENCES

1. Rosenfield RL, Ghai K, Ehrmann DA, Barnes RB. Diagnosis of the polycystic ovary syndrome in adolescence: comparison of adolescent and adult hyperandrogenism. *J Pediatr Endocrinol Metab* (2000) **13**(Suppl 5):1285–9.
2. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. Task force on the phenotype of the polycystic ovary syndrome of the androgen excess and PCOS society. The androgen excess and PCOS society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril* (2009) **91**:456–88. doi:10.1016/j.fertnstert.2008.06.035
3. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocr Rev* (2012) **33**(6):1–29. doi:10.1210/er.2011-1034
4. Barnard L, Ferriday D, Guenther N, Strauss B, Balen AH, Dye L. Quality of life and psychological well being in polycystic ovary syndrome. *Hum Reprod* (2007) **22**(8):2279–86. doi:10.1093/humrep/dem108
5. Coffey S, Mason H. The effect of polycystic ovary syndrome on health-related quality of life. *Gynecol Endocrinol* (2003) **17**(5):379–86. doi:10.1080/09513590312331290268
6. Li Y, Li Y, Yu Ng EH, Stener-Victorin E, Hou L, Wu T, et al. Polycystic ovary syndrome is associated with negatively variable impacts on domains of health-related quality of life: evidence from meta-analysis. *Fertil Steril* (2011) **96**(2):452–8. doi:10.1016/j.fertnstert.2011.05.072

7. Hahn S, Janssen OE, Tan S, Pleger K, Mann K, Schelowski M, et al. Clinical and psychological correlates of quality of life in polycystic ovary syndrome. *Eur J Endocrinol* (2005) **153**:853–60. doi:10.1530/eje.1.02024
8. Barry JA, Kuczmierczyk AR, Hardiman PJ. Anxiety and depression in polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod* (2011) **26**(9):2442–51. doi:10.1093/humrep/der197
9. Deeks AA, Gibson-Helm ME, Paul E, Teede HJ. Is having polycystic ovary syndrome a predictor of poor psychological function including anxiety and depression? *Hum Reprod* (2011) **26**(6):1399–407. doi:10.1093/humrep/der071
10. Annagur BB, Tazegul A, Uguz F, Kerimoglu OS, Tekinarslan E, Celik C. Biological correlates of major depression and generalized anxiety disorder in women with polycystic ovary syndrome. *J Psychosom Res* (2013) **74**:244–7. doi:10.1016/j.jpsychores.2013.01.002
11. Sills ES, Perloe M, Tucker MJ, Kaplan CR, Genton MG, Schattman GL. Diagnostic and treatment characteristics of polycystic ovary syndrome: descriptive measurements of patient perception and awareness from 657 confidential self-reports. *BMC Womens Health* (2001) **1**(1):3. doi:10.1186/1472-6874-1-1
12. Trent ME, Rich M, Austin F, Bryn S, Gordon CM. Quality of life in adolescent girls with polycystic ovary syndrome. *Arch Pediatr Adolesc Med* (2002) **156**(6):556–60. doi:10.1001/archpedi.156.6.556
13. Dokras A, Clifton S, Futterweit W, Wild R. Increased risk for abnormal depression scores in women with polycystic ovary syndrome. *Obstet Gynecol* (2011) **117**(1):145–52. doi:10.1097/AOG.0b013e318202b0a4
14. Jedel E, Waern M, Gustafson D, Landern M, Eriksson E, Holm G, et al. Anxiety and depression symptoms in women with polycystic ovary syndrome compared with controls matched for body mass index. *Hum Reprod* (2010) **25**(2):450–6. doi:10.1093/humrep/dep384
15. Dokras A, Clifton S, Futterweit W, Wild R. Increased prevalence of anxiety symptoms in women with polycystic ovary syndrome: systematic review and meta-analysis. *Fertil Steril* (2012) **97**(1):225–30. doi:10.1016/j.fertnstert.2011.10.022
16. Stovall DW, Scriver JL, Clayton AH, Williams CD, Pastore LM. Sexual function in women with polycystic ovary syndrome. *J Sex Med* (2012) **9**:224–30. doi:10.1111/j.1743-6109.2011.02539.x
17. Sahingöz M, Uguz F, Gezginc K, Korucu DG. Axis I and axis II diagnoses in women with PCOS. *Gen Hosp Psychiatry* (2013) **35**(5):508–11. doi:10.1016/j.genhosppsych.2013.04.003
18. Roepke S, Ziegenhorn A, Kronsbein J, Merkl A, Bahri S, Lange J. Incidence of polycystic ovaries and androgen serum levels in women with borderline personality disorder. *J Psychiatr Res* (2010) **44**:847–52. doi:10.1016/j.jpsychires.2010.01.007
19. The Rotterdam Consensus ESHRE/ASRM: ESHRE/ASRM Rotterdam Consensus Meeting. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* (2004) **19**:41–7. doi:10.1093/humrep/deh098
20. Fulghesu AM, Angioni S, Frau E, Belosi C, Apa R, Mioni R, et al. Ultrasound in polycystic ovary syndrome – the measuring of ovarian stroma and relationship with circulating androgens: results of a multicentric study. *Hum Reprod* (2007) **22**(9):2501–8. doi:10.1093/humrep/dem202
21. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* (1999) **22**:1462–70. doi:10.2337/diacare.22.9.1462
22. Millon T, Davis R, Millon C. *MCMI III, Manual.* 2nd ed. Minneapolis, MN: NCS Inc. (1997).
23. Zennaro A, Ferracuti S, Lang M, Sanavio E. *L'adattamento italiano del MCMI-III. Studi di validazione [MCMI-III Italian Adaptation].* Firenze: Organizzazioni Speciali (2008).
24. Exner J. *The Rorschach Comprehensive System: Volume 1: Basic Foundations.* 2th ed. New York, NY: Wiley (1986).
25. Exner J. *The Rorschach Comprehensive System: Volume 1: Basic Foundations.* 2th ed. New York, NY: Wiley (1995).
26. Exner JE, Eldberg P. *The Rorschach: A Comprehensive System: Volume 2: Advanced Interpretation.* 3rd ed. New York, NY: Wiley (2005).
27. Lis A, Zennaro A, Salcuni S, Parolin L, Mazzeschi C. *Il Rorschach secondo il Sistema Comprensivo di Exner.* Milano: Raffallo Cortina Editore (2007).
28. Exner JE, Andronikof-Sanglade A. Roschach changes following brief and short-term therapy. *J Pers Assess* (1992) **50**(1):59–71. doi:10.1207/s15327752jpa5901_6
29. Bihlar B, Carlsson AM. An exploratory study of agreement between therapists' goals and patients' problems revealed by the Rorschach. *Psychother Res* (2000) **10**(2):196–214. doi:10.1080/10503307.2000.9721973
30. Sultan S, Jebrane A, Heurtier-Hartemann A. Rorschach variables related to blood glucose control in insulin-dependent diabetes patients. *J Pers Assess* (2002) **79**(1):122–41. doi:10.1207/S15327752JPA7901_08
31. Mioni R, Chiarelli S, Xamin N, Zuliani L, Granzotto M, Mozzanega B, et al. Evidence for the presence of glucose transporter 4 in the endometrium and its regulation in polycystic ovary syndrome patients. *J Clin Endocrinol Metab* (2004) **89**(8):4089–96. doi:10.1210/jc.2003-032028
32. Sonino N, Fava GA, Mani E, Belludo P, Boscaro M. Quality of life of hirsute women. *Postgrad Med J* (1993) **69**:186–9. doi:10.1136/pgmj.69.809.186
33. Ekback M, Wijma K, Benzein E. "It's always on my mind": women experiences of their bodies when living with hirsutism. *Health Care Women Int* (2009) **30**:358–72. doi:10.1080/07399330902785133
34. Marsh CA, Berent-Spillson AB, Love T, Persad CC, Pop-Busui R, Zubieta JK, et al. Functional neuroimaging of emotional processing in women with polycystic ovary syndrome: a case-control pilot study. *Fertil Steril* (2013) **100**(1):200–7. doi:10.1016/j.fertnstert.2013.02.054
35. Mansson M, Holte J, Landin-Wilhelmsen K, Dahigren E, Johansson A, Landen M. Women with polycystic ovary syndrome are often depressed or anxious – a case control study. *Psychoneuroendocrinology* (2008) **33**:1132–8. doi:10.1016/j.psyneuen.2008.06.003
36. Klipstein KG, Goldberg JF. Screening for bipolar disorder in women with polycystic ovary syndrome. *J Affect Disord* (2006) **91**(2–3):205–9. doi:10.1016/j.jad.2006.01.011
37. Rassi A, Veras AB, Dos Reis M, Pastore DL, Bruno LM, Bruno RV, et al. Prevalence of psychiatric disorders in. *Compr Psychiatry* (2010) **51**:599–602. doi:10.1016/j.comppsych.2010.02.009
38. Willmott J. The experiences of women with polycystic ovary syndrome. *Fem Psychol* (1999) **10**(1):107–16. doi:10.1177/0959353500010001013
39. Mansson M, Norstrom K, Holte J, Landin-Wilhelmsen K, Dahlgren E, Landen M. Sexuality and psychological wellbeing in women with polycystic ovary syndrome compared with healthy controls. *Eur J Obstet Gynecol Reprod Biol* (2011) **155**:161–5. doi:10.1016/j.ejogrb.2010.12.012
40. Bazarganipour F, Ziaei S, Montazeri A, Foroozanfard F, Kazemnejad A, Faghihzadeh S. Psychological investigation in patients with polycystic ovary syndrome. *Health Qual Life Outcomes* (2013) **11**:141. doi:10.1186/1477-7525-11-141
41. Monzani F, Pucci E, Caraccio N, Bagnolesi A, Molli D, Fenu A, et al. Correlati psicologici and psicopatologici nella sindrome della micropolicistosi ovarica. *Med Psicosom* (1994) **39**:225–36.
42. Elsenbruch S, Hahn S, Kowalsky D, Offner A, Schedlowski M, Mann K, et al. Quality of life, psychosocial well-being, and sexual satisfaction in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* (2003) **88**(12):5801–7. doi:10.1210/jc.2003-030562


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# Gender dysphoria – prevalence and co-morbidities in an Irish adult population

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**Introduction:** Gender dysphoria (GD) is a condition in which there is a marked incongruence between an individual's psychological perception of his/her sex and their biological phenotype. Gender identity disorder was officially renamed "gender dysphoria" in the DSM-V in 2013. The prevalence and demographics of GD vary according to geographical location and has not been well-documented in Ireland.

**Methods:** We retrospectively reviewed medical records of 218 patients with suspected or confirmed GD referred to our endocrine service for consideration of hormonal therapy (HT) between 2005 and early 2014. We documented their demographics, clinical characteristics, and treatment during the study period.

**Results:** The prevalence of GD in the Irish population was 1:10,154 male-to-female (MTF) and 1:27,668 female-to-male (FTM), similar to reported figures in Western Europe. 159 of the patients were MTF and 59 were FTM, accounting for 72.9% and 27.1% of the cohort, respectively. The rate of referral has increased year-on-year, with 55 patients referred in 2013 versus 6 in 2005. Mean ages were 32.6 years (MTF) and 32.2 years (FTM). 22 of the patients were married and 41 had children, with 2 others having pregnant partners. 37.6% were referred by a psychologist, with the remainder evenly divided between GPs and psychiatric services. There were low rates of coexistent medical illness although psychiatric conditions were more prevalent, depression being a factor in 34.4% of patients. 5.9% of patients did not attend a mental health professional. 74.3% are currently on HT, and 9.17% have had gender reassignment surgery (GRS). Regret following hormonal or surgical treatment was in line with other Western European countries (1.83%).

**Conclusion:** The incidence of diagnosis and referral of GD in Ireland is increasing. This brings with it multiple social, health, and financial implications. Clear and accessible treatment pathways supported by mental health professionals is essential.

**Keywords: gender dysphoria, gender identity disorder, transgender, gender reassignment surgery, gonadectomy, hormone therapy**

## INTRODUCTION

Gender dysphoria (GD) is a recognized condition in which there is marked incongruence between an individual's psychological perception of his/her sex and their biological phenotype (1). GD has replaced the term gender identity disorder (GID) in the 2013 publication of the DSM-V, which itself replaced "transexualism" in 1980. The cause is not well understood and is believed to be due to a complex interaction of psychosocial and biological factors during development (2, 3).

Prevalence varies based on geographical location, with higher rates in Western Europe and America (0.001–0.002%) (4–6) compared to lower rates in Japan (0.0009%) (7). In most studies, male-to-female (MTF) GD cases tend to significantly outnumber female-to-male (FTM) cases (7).

The DSM-V has strict criteria for the diagnosis of GD that differ between children and adults. However, both include a persistent and strong conviction that they are the wrong sex, feel discomfort, distress, or anxiety with their gender-role and sexual characteristics, and possess a strong and persistent desire to be a member of the opposite sex (1). These criteria are based on the assumption that GD is a purely psychiatric condition which is a topic of controversy.

The complex nature of GD requires that psychological, social, and medical issues are addressed by a multidisciplinary team.

The Department of Endocrinology in St. Columcille's Hospital (SCH), Dublin, has been managing patients with GD since 2000. Patients are referred for consideration of hormonal therapy (HT) by a mental health professional or GP in the majority of cases. We have previously reported the characteristics of the first 52 patients referred for HT in SCH (8), however, GD in Ireland remains poorly investigated. This paper aims to update the characteristics of GD in Ireland, show the current prevalence of the condition, and discuss the need for developing accessible, well-defined pathways of care.

## MATERIALS AND METHODS

We retrospectively reviewed the medical records of 218 patients referred to the GD clinic between 2005 and early 2014. A diagnosis had been made by a MHP based on DSM-IV/V criteria in most instances, however, some patients required a referral from the clinic to a mental health professional if this had not occurred This initial consultation included a full medical and surgical history and examination as well as baseline laboratory investigations; full blood count, renal, liver, and bone profiles, fasting lipids, and glucose as well as hormonal profiling.

Data collection focused on documentation of past medical and surgical history (including hypertension, coronary artery disease, thromboembolic disease, dyslipidemia, diabetes mellitus, etc.), psychiatric background (including depression and autistic spectrum disorder etc.), and family history (including cardiovascular conditions etc.). Smoking and alcohol use were also recorded. GD-specific questioning aimed to establish age at self-diagnosis, time spent in desired role, and hormonal or surgical therapy prior to presentation, etc (**Table 1**).

Consideration for HT required that patients were diagnosed by a minimum of one, preferably two mental health professionals.

In our service, we aimed to initiate HT at the second visit, thus this necessitated that a detailed discussion with the patient regarding the risks and benefits of HT be undertaken at the initial consultation. Providing there were no contraindications and the patient received a definitive diagnosis of GD from an appropriate mental health specialist, HT could commence on the second consultation. We also analyzed the incidence of regret or adverse effects to treatment in those presenting for follow-up. Those wishing to proceed with gender-related surgery were referred to appropriate specialists in Ireland or abroad.

**Table 1 | Demographics and co-morbidities**.

| Variable | MTF | FTM |
|---|---|---|
| Number | 159 | 59 |
| Age (years) (SD) | 32.5(13.26) | 32.2(11.40) |
| Married (previous or current) | 41 | 5 |
| Number with children | 37 | 3 |
| Prior hormone therapy | 36 | 6 |
| Living in role | 66 | 44 |
| **MEDICAL HISTORY** | | |
| Hypertension | 17 | 3 |
| Dyslipidemia | 42 | 5 |
| Diabetes | 1 | 2 |
| Cigarettes | 40 | 17 |
| **PSYCHIATRIC CO-MORBIDITY** | | |
| Depression | 55 | 19 |
| Schizophrenia | 8 | – |
| Bipolar affective disorder | 2 | 2 |
| Deliberate self-harm/suicide attempt | 13 | 6 |

*SD, standard deviation; MTF, male-to-female; FTM, female-to-male.*

**Table 2 | Number of referrals per year**.

| Year of referral | MTF | FTM | Total |
|---|---|---|---|
| 2005 | 6 | – | 6 |
| 2006 | 9 | 2 | 11 |
| 2007 | 7 | 2 | 9 |
| 2008 | 10 | 2 | 12 |
| 2009 | 16 | 5 | 21 |
| 2010 | 15 | 6 | 21 |
| 2011 | 25 | 5 | 30 |
| 2012 | 30 | 17 | 47 |
| 2013 | 37 | 18 | 55 |
| 2014 (to March) | 3 | 1 | 4 |

*MTF, male-to-female; FTM, female-to-male.*

## RESULTS

### DEMOGRAPHICS

In the study, 218 patients with GD were referred to our clinic between 2005 and early 2014 (**Table 1**). 72.9% of these were MTF and 27.1% FTM, with a MTF:FTM ratio of 2.7:1. Based on the 2011 census reports (9), this indicates a prevalence of GD in 1:14,756 people (0.0067% of the population), categorized into 1:10,154 MTF (0.0098%) and 1:27,668 FTM (0.0036%). Seventy-one percent of the patients were Irish, with Leinster providing the most patients when subdividing the cohort based on region. The numbers of patients being referred increased steadily over the 9 years study period, from 6 referrals in 2005 to 55 in 2013 (**Table 2**; **Figure 1**).

The average age at referral was similar between both gender groups at 32.6 years for MTF and 32.2 years for FTM, ranging from 15 to 69 years. There was a clear trend in the study showing people are being referred at younger ages as time goes on. The average age at referral from 2005 to 2009 inclusive was 36.77 years, compared with the average age between 2010 and 2013, which was 30.14 years. Twenty-one percent of the patients were married at presentation or in the past, and 18.8% reported having children. Two patients reported having pregnant partners, one of whom conceived using banked sperm.

One hundred thirty-five patients (61.9%) declared their age at self-diagnosis of GD as being pre-pubertal, with 14.7% stating they realized their issues around gender as adolescents. 50.9% of patients were living in their desired gender-role on a full-time basis, with approximately one quarter of patients not living in role prior to initial presentation.

The majority of referrals were made by a psychologist (37.6%), with the remainder evenly divided between psychiatrists and GPs. Although most patients were seen by at least one MHP, 5.96% of patients did not attend either a psychiatrist or psychologist. Of these, five patients received HT without prior MHP consultation, however, one patient obtained the medications via the internet and three others were on HT and also had gender-related surgery prior to presentation. Forty-four patients (20.2%) were on HT prior to initial visit to the GD clinic, 9 of whom ordered it via the internet, 16 individuals sourced hormones from practitioners either in Ireland or the UK, 9 sourced it from overseas, and in the case of the remainder the source was unknown.

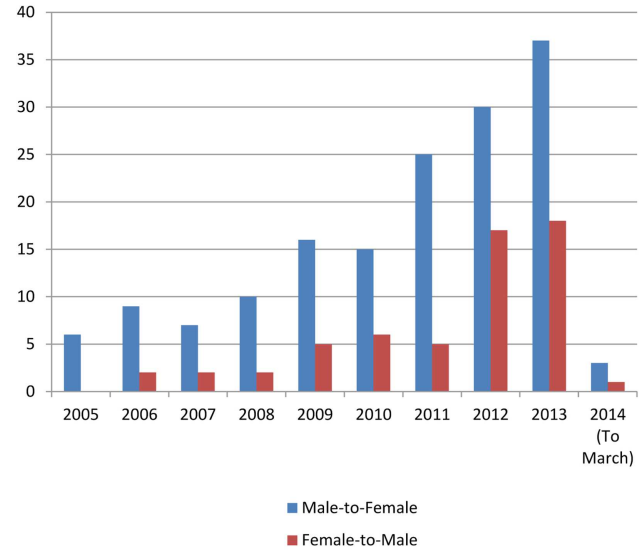


**FIGURE 1 | Number of referrals per year.**

### MEDICAL AND PSYCHIATRIC CO-MORBIDITIES

Several patients had co-existing medical co-morbidities, with dyslipidemia (21.6%), asthma (11.45%), and hypertension (9.17%) being the most prevalent. Twenty-six percent of the patients reported being smokers at the time of presentation, with 23.85% having smoked regularly in the past. Twelve patients consumed more than 15 units of alcohol per week, however, eight patients (3.67%) reported abusing alcohol to a damaging degree in the past. Seventy-five (34.4%) patients had a history of depression, 25.3% of those admitting to acts of deliberate self-harm. Other psychiatric conditions encountered included schizophrenia (3.67%), bipolar disorder (2.29%), and Asperger's syndrome (2.29%) (**Table 1**).

Blood samples were taken as they are important to both assess initial risk and evaluate possible future adverse events, including cardiovascular disease (10). Twenty-five percent were found to have an initial fasting cholesterol of >5.3 mmol/L and 31% had a fasting LDL of >2.6 mmol/L, both of these figures being the upper reference range for the laboratory in SCH. Of these, 47 were known to have dyslipidemia (42 MTF versus 5 FTM), 12 people went on to commence lipid-lowering therapy, with others initiating diet control or having a repeat sample within normal ranges. Five people were found to have a fasting blood glucose of >5.9 mmol/L, however, none were found to have diabetes mellitus on repeat testing (**Table 1**). Nearly 20% of patients had a family history of vascular disease including myocardial infarction (9%), deep venous thrombosis (2%), and cerebrovascular disease (9%).

### POST PRESENTATION

To date, 55 patients (22.3%) have undergone surgery, 36 (65.5%) of these have been MTF patients. Mammoplasty was the most common procedure, with 19 (52.8%) of those undergoing surgery electing to have breast augmentation. Twenty (36.4%) surgical patients opted for gender reassignment surgery (GRS), which constitutes 9.17% of the total cohort. A very small number also had facial or laryngeal surgery for voice alteration (**Table 3**). As there are currently no GRS services in Ireland, patients are referred to a variety of centers in the United Kingdom and elsewhere. Additionally, there are currently 11 patients referred to Charing Cross Hospital for consideration of GRS.

**Table 3 | Surgical procedures in gender dysphoria patients.**

| Variable | MTF | FTM |
|---|---|---|
| GRS | 15 | 9 |
| Breast surgery | 16 | 19 |
| Facial surgery | 6 | – |
| Laryngeal surgery | 2 | – |

*GRS, gender reassignment surgery.*

Dual energy X-ray absorptiometry (DEXA) scanning is now part of the standard screening protocol of patients presenting to our clinic. In our cohort, 39 patients (17.9%) underwent this investigation at time of last known follow-up, with 20.5% of those studied discovered to have osteopenia, and 7.7% diagnosed with osteoporosis.

Thirty-seven patients (17%) reported adverse effects following hormonal or surgical therapy. Eight patients reported decreased mood or libido, six patients were found to have developed dyslipidemia, five patients had hot flushes/headaches, and one patient developed a fistula post GRS. Four patients (1.8%) expressed regret following treatment. All of the incidences of regret involved MTF, three of who were post GRS with the other in the hormonal phase of treatment.

## DISCUSSION

In this retrospective study, we characterized GD patients attending an endocrine unit in the Republic of Ireland. From this study, the prevalence of GD is 1:10,154 MTF (0.0098%) and 1:27,668 FTM (0.0036%). This is comparable to the prevalence of GD reported in Western Europe, which ranges from 1:11,900 to 1:18,000 for MTF and 1:30,400 to 1:54,000 for FTM (4, 10–12). Our analysis echoed similar international studies in that we collated data from a single specialist GD clinic. De Cuypere et al. reviewed 10 such studies spanning 39 years with prevalence very much matching our own (10). The prevalence rate of GD in our cohort is likely to reflect that of the Republic of Ireland as a nation, as our unit has been the main specialist center in the country over the study period.

In the seventh version of the World Professional Association for Transgender Health (WPATH) Standards of Care for the Health of Transsexual, Transgender, and Gender-Non-conforming People (13), it is highlighted that in more recent analyses there appears to be a higher prevalence of GD, possibly reflecting an increase in the number of people seeking help from healthcare professionals (13–15). This hypothesis is supported by our analysis, which shows 6 referrals to our service in 2005 versus 55 in 2013.

Our MTF/FTM sex ratio of 2.7:1 is similar to the ratio of 3:1 reported in our previous study (8) and the sex ratio reported in Western Europe (10, 11). It is in line international data ranging from 3 to 5:1 (16).

The age of initial assessment was the same for both MTF and FTM (32 years). This is significantly different to our previous figures which indicated FTM patients presented, on average, 8 years younger than MTF (8). Age of presentation for MTF has decreased dramatically (39 versus 32.6) while that of FTM patients has remained approximately the same (31 versus 32.2) (8). The reason for this is unclear but may represent the condition now being more socially acceptable facilitating earlier presentation. This age is in line with a Belgian study, which showed a mean age of initial presentation of ~30 years (10). Over 60% of patients declared their age at self-diagnosis to be pre-pubertal which highlights the need for early recognition and diagnosis of the condition.

21% of the patients were married at presentation or in the past, and 18.8% reported having children. This is lower than the reported rates in similar European studies (10). Two of the patients had pregnant partners, one of whom conceived using banked sperm.

The rate of co-existing medical illness such as hypertension and diabetes were low in GD patients, which could perhaps be expected as the patients are generally young (average age 32 years). It is important to identify and optimize control of these medical co-morbidities prior to initiating the hormonal phase of treatment, as they can be exacerbated by hormones such as estrogen (17–19). Close attention should be paid to the possibility of cardiovascular events, as accruing evidence has suggested that MTF GD patients may be at an increased cardiovascular risk (20–22). Twenty-six percent of patients were smokers at initial presentation which is in line with the national average of 29% (23). Patients were informed that they would not be commenced on HT until they had ceased smoking due to the increased risk of venous thrombosis (24).

Depression was common to 34.4% of patients, while the incidence of other major psychiatric illnesses was relatively low. A survey of Dutch psychiatrists regarding psychiatric co-morbidity in those with GD found that 61% of their cohort had a co-existing psychiatric disorder (25). The presence of such a high rate of co-morbid psychiatric illness underlines the need for a well-defined and rigidly adhered-to mental health consultation service. While our rate of patients who did not attend a MHP (5.96%) is low, it outlines the challenges faced by specialist centers in ensuring that patients with GD receive the appropriate mental health support in the setting of increased referrals.

Currently, there are no centers in Ireland which provide GRS, which may have contributed to only 11% of our patients undergoing sex reassignment surgery. However, it is worth noting that there is an agreement between the Irish Health Service Executive and certain GRS centers in other countries to facilitate ease of access for Irish patients wishing to undergo GRS. Unfortunately, this system is somewhat convoluted and frequently causes delays in access to surgery. This is a frustration experienced by many of the patients examined in our study.

The regret rate following both HT and GRS was low with four (2%) patients expressing regret (all MTF). This is slightly higher than previously reported figures of 1–1.5% for MTF (26, 27). Three of these had gender-related surgery and one had received hormones as the only treatment. Although low, the regret rate of 2% illustrates the need for continued input from mental health professionals beyond GRS. Unfortunately, these services are not always readily available, and this is a matter for further discussion.

## CONCLUSION

The prevalence of GD in the Republic of Ireland is comparable to that reported in Western Europe. However, the true prevalence may be difficult to assess due to patient worries regarding stigma and societal acceptance of the condition. The number of patients treated by our unit is increasing each year, which will pose considerable challenges with already limited resources. The liaison with mental health services is of critical importance to patients and to the success of treatment. Difficulties in obtaining ongoing mental health care are all too common and lead to the burden of care resting on the endocrinology service. It is vital that a well-defined and accessible treatment pathway is continually developed in order to ensure a gold standard of care for patients with GD.

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## REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders.* 5th ed. Arlington, VA: American Psychiatric Publishing (2013).
2. Meyer JK. The theory of gender identity disorders. *J Am Psychoanal Assoc* (1982) **30**(2):381–418. doi:10.1177/000306518203000204
3. Zhou JN, Hofman MA, Gooren LJ, Swaab DF. A sex difference in the human brain and its relation to transsexuality. *Nature* (1995) **378**(6552):68–70. doi:10.1038/378068a0
4. van Kesteren PJ, Gooren LJ, Megens JA. An epidemiological and demographic study of transsexuals in The Netherlands. *Arch Sex Behav* (1996) **25**(6):589–600. doi:10.1007/BF02437841
5. Roberto LG. Issues in diagnosis and treatment of transsexualism. *Arch Sex Behav* (1983) **12**(5):445–73. doi:10.1007/BF01542888
6. Landen M, Walinder J, Lundstrom B. Prevalence, incidence and sex ratio of transsexualism. *Acta Psychiatr Scand* (1996) **93**(4):221–3. doi:10.1111/j.1600-0447.1996.tb10645.x
7. Okabe N, Sato T, Matsumoto Y, Ido Y, Terada S, Kuroda S. Clinical characteristics of patients with gender identity disorder at a Japanese gender identity disorder clinic. *Psychiatry Res* (2008) **157**(1–3):315–8. doi:10.1016/j.psychres.2007.07.022
8. De Gascun C, Kelly J, Salter N, Lucey J, O'Shea D. Gender identity disorder. *Ir Med J* (2006) **99**(5):146–8.
9. Irish Central Statistics Office Census. *This is Ireland (Part 1). Statistical Tables and Appendices (PDF 327KB).* (2011). Available from: http://www.cso.ie/en/media/csoie/census/documents/census2011pdr/Tables,and,Appendices.pdf
10. De Cuypere G, Van Hemelrijck M, Michel A, Carael B, Heylens G, Rubens R, et al. Prevalence and demography of transsexualism in Belgium. *Eur Psychiatry* (2007) **22**(3):137–41. doi:10.1016/j.eurpsy.2006.10.002
11. Bakker A, van Kesteren PJ, Gooren LJ, Bezemer PD. The prevalence of transsexualism in The Netherlands. *Acta Psychiatr Scand* (1993) **87**(4):237–8. doi:10.1111/j.1600-0447.1993.tb03364.x
12. Wilson P, Sharp C, Carr S. The prevalence of gender dysphoria in Scotland: a primary care study. *Br J Gen Pract* (1999) **49**(449):991–2.
13. Coleman E, Bockting W, Botzer M, Cohen-Kettenis P, DeCuypere G, Feldman J, et al. Standards of care for the health of transsexual, transgender, and gender-nonconforming people, version 7. *Int J Transgend* (2011) **13**:165–232. doi:10.1080/15532739.2011.700873
14. Reed B, Rhodes S, Schofield P, Wylie K. *Gender Variance in the UK: Prevalence, Incidence, Growth and Geographic Distribution.* (2009). Available from: http://www.gires.org.uk/assets/Medpro-Assets/GenderVarianceUK-report.pdf

15. Zucker KJ, Bradley SJ, Owen-Anderson A, Kibblewhite SJ, Cantor JM. Is gender identity disorder in adolescents coming out of the closet? *J Sex Marital Ther* (2008) **34**(4):287–90. doi:10.1080/00926230802096192
16. Sadock BJ, Kaplan HI, editors. *Kaplan and Sadock's Synopsis of Psychiatry: Behavioral Sciences, Clinical Psychiatry.* 10th ed. Philadelphia: Lippincott Williams and Wilkins (2007). p. 322–537.
17. Feldman J, Safer J. Hormone therapy in adults: suggested revisions to the sixth version of the standards of care. *Int J Transgend* (2009) **11**(3):146–82. doi:10.1080/15532730903383757
18. Hembree WC, Cohen-Kettenis P, Delemarre-Van de Waal HA, Gooren LJ, Meyer WJ III, Spack NP, et al. Endocrine treatment of transsexual persons: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* (2009) **94**(9):3132–54. doi:10.1210/jc.2009-0345
19. Dhejne C, Lichtenstein P, Boman M, Johansson ALV, Langstrom N, Landen M. Longterm follow-up of transsexual persons undergoing sex reassignment surgery: cohort study in Sweden. *PLoS One* (2011) **6**(2):1–8. doi:10.1371/journal.pone.0016885
20. Biller J, Saver JL. Ischemic cerebrovascular disease and hormone therapy for infertility and transsexualism. *Neurology* (1995) **45**(8):1611–3. doi:10.1212/WNL.45.8.1611
21. Asscheman H, Giltay EJ, Megens JA, de Ronde WP, van Trotsenburg MA, Gooren LJ. A long-term follow-up study of mortality in transsexuals receiving treatment with cross-sex hormones. *Eur J Endocrinol* (2011) **164**(4):635–42. doi:10.1530/EJE-10-1038
22. Lioudaki E, Ganotakis ES, Mikhailidis DP, Nair DR. The estrogenic burden on vascular risk in male-to-female transsexuals. *Curr Pharm Des* (2010) **16**(34):3815–22. doi:10.2174/138161210794455049
23. Brugha R, Tully N, Dicker P, Shelley E, Ward M, McGee H. *SLÁN 2007: Survey of Lifestyle, Attitudes and Nutrition in Ireland. Smoking Patterns in Ireland: Implications for Policy and Services.* Dublin: Department of Health and Children, Stationary Office (2009).
24. Enga KF, Braekkan SK, Hansen-Krone IJ, Le Cessie S, Rosendaal FR, Hansen JB. Cigarette smoking and the risk of venous thromboembolism: the Tromsø Study. *J Thromb Haemost* (2012) **10**(10):2068–74. doi:10.1111/j.1538-7836.2012.04880.x
25. Campo J, Nijman H, Merckelbach H, Evers C. Psychiatric comorbidity of gender identity disorders: a survey among Dutch psychiatrists. *Am J Psychiatry* (2003) **160**(7):1332–6. doi:10.1176/appi.ajp.160.7.1332
26. Pf'afflin F. Regrets after sex reassignment surgery. *J Psychol Human Sex* (1993) **5**(4):69–85. doi:10.1300/J056v05n04_05
27. Smith YL, Van Goozen SH, Kuiper AJ, Cohen-Kettenis PT. Sex reassignment: outcomes and predictors of treatment for adolescent and adult transsexuals. *Psychol Med* (2005) **35**(1):89–99. doi:10.1017/S0033291704002776

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# Reproduction, smell, and neurodevelopmental disorders: genetic defects in different hypogonadotropic hypogonadal syndromes

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The neuroendocrine control of reproduction in mammals is governed by a neural hypothalamic network of nearly 1500 gonadotropin-releasing hormone (GnRH) secreting neurons that modulate the activity of the reproductive axis across life. Congenital hypogonadotropic hypogonadism (HH) is a clinical syndrome that is characterized by partial or complete pubertal failure. HH may result from inadequate hypothalamic GnRH axis activation, or a failure of pituitary gonadotropin secretion/effects. In man, several genes that participate in olfactory and GnRH neuronal migration are thought to interact during the embryonic life. A growing number of mutations in different genes are responsible for congenital HH. Based on the presence or absence of olfaction dysfunction, HH is divided in two syndromes: HH with olfactory alterations [Kallmann syndrome (KS)] and idiopathic hypogonadotropic hypogonadism (IHH) with normal smell (normosmic IHH). KS is a heterogeneous disorder affecting 1 in 5000 males, with a three to fivefold of males over females. KS is associated with mutations in *KAL1*, *FGFR1/FGF8*, *FGF17*, *IL17RD*, *PROK2/PROKR2*, *NELF*, *CHD7*, *HS6ST1*, *FLRT3*, *SPRY4*, *DUSP6*, *SEMA3A*, *NELF*, and *WDR11* genes that are related to defects in neuronal migration. These reproductive and olfactory deficits include a variable non-reproductive phenotype, including sensorineural deafness, coloboma, bimanual synkinesis, craniofacial abnormalities, and/or renal agenesis. Interestingly, defects in *PROKR2*, *FGFR1*, *FGF8*, *CHD7*, *DUSP6*, and *WDR11* genes are also associated with normosmic IHH, whereas mutations in *KISS1/KISSR*, *TAC3/TACR3*, *GNRH1/GNRHR*, *LEP/LEPR*, *HESX1*, *FSHB*, and *LHB* are only present in patients with normosmic IHH. In this paper, we summarize the reproductive, neurodevelopmental, and genetic aspects of HH in human pathology.

**Keywords: reproduction, male, Kallman syndrome, hypogonadotropic hypogonadism, olfaction, kisspeptin, genetics**

## INTRODUCTION

Reproductive system development and control in mammals is dependent on specific neurons located in the hypothalamus that secrete gonadotropin-releasing hormone-1 (GnRH-1) and control the pituitary–gonadal axis (**Figure 1**). During embryogenesis, these neurons originate in the nasal placode and migrate into the forebrain along the olfactory-vomeronasal nerves (1–3). Alterations in this migratory process lead to defective GnRH-1 secretion, resulting in heterogeneous genetic disorders such as idiopathic hypogonadotropic hypogonadism (IHH), and other reproductive diseases characterized by the reduction in or failure of sexual maturation and competence. Another consequence of these migratory neuronal defects can be olfactory dysfunction. Depending of the affected genes, other neurological developmental disorders can also be encountered (1–4).

Thus, idiopathic hypogonadotropic hypogonadism (IHH) is a genetic disease that can occur with a normal sense of smell (normosmic IHH) or in association with anosmia (Kallmann syndrome; KS). To date, mutations in many genes have been described in relations to KS and/or normosmic IHH (nIHH) (**Tables 1** and **2**). Hypogonadotropic hypogonadism (HH) can also be found in association with other distinctive clinical syndromic conditions, such as Prader Willi syndrome, that are outside the scope of the current review.

In this review, we focus on genetic central hypogonadism, which is more frequently encountered in males than in females. Congenital IHH is a clinically and genetically heterogeneous disorder (3, 4). Although sporadic cases predominate, families with congenital IHH have been reported with X-linked, autosomal dominant (AD) or autosomal recessive (AR) inheritance patterns (1–4). In some families, high variability in reproductive and non-reproductive phenotypic features suggests the presence of complex inheritance. In particular, polygenic (digenic or oligogenic) forms and variable forms of transmission can be found in selected cases (6–11). Indeed, further complexity is added by the remarkable observation

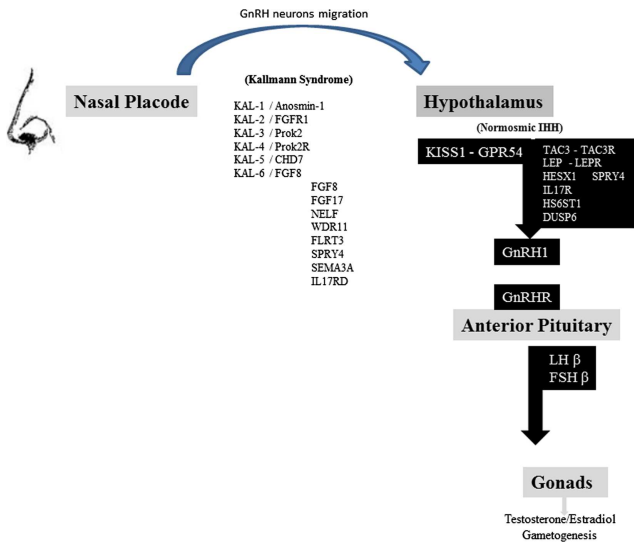


**FIGURE 1 | Schematic representation of the reproductive axis and the different genes invalidating mutations participating in Kallman syndrome and nIHH**. GnRH neurons migrate from nasal placode to hypothalamus in the first weeks of fetal life. Several genes are represented in the left column: their invalidating mutations are responsible for Kallmann syndrome and olfactory dysfunction. Kiss1 neurons integrate different hormonal, metabolic, circadian inputs from other hypothalamic and brain areas and thus stimulate GnRH neurons firing. Several genes are represented in the right black column, including KISS1-GPR54 system, which invalidating mutations lead to normosmic idiopathic hypogonadotropic hypogonadism. GnRH secretion leads to gonadotropins FSH and LH pituitary release. LH and FSH control sex steroids secretion and gametogenesis [adapted from Valdes-Socin et al. (1), with permission].

of reversibility of the phenotype in some cases of genetically determined hypogonadism (12–16).

## THE HUMAN REPRODUCTIVE AXIS

Normal human reproduction and sexual characteristics rely on an intact hypothalamic–pituitary–gonadal axis (HPG; **Figure 1**). Hypogonadism is defined as the insufficient production of sex hormones with or without disturbed gametogenesis. HH results from a dysfunction of the hypothalamic–pituitary axis interfering with control of gonadotropin secretion (1–3, 16).

During life, the activity of the HPG axis has a tri-phasic pattern of "on-off-on." A first phase of activity occurs from the 16th week of intrauterine life as well as in the period between the 4th and 10th weeks of postnatal life (or "mini-puberty"). Mini-puberty is characterized by an increase in gonadotropin and steroid hormone secretion. Gonadotropins and sex hormones levels rise to a lesser extent than in true puberty. After mini-puberty, the HPG axis is repressed ("off") until puberty, when the system is reactivated ("on"). HPG axis activity is maintained throughout adult life in men whereas in women, menopause intervenes, and low sex steroids and compensatory high gonadotropin levels are characteristic (1–3).

The immediate postnatal period can be a window of opportunity for pediatricians and neonatologists to diagnose certain forms of HH. The congenital gonadotropin deficiency phenotype is variable and depends on the gender, the magnitude of the deficit, and the specific genetic abnormalities (**Figure 1**). At the time of puberty, the diagnosis of HH may be suspected due to the absence in the onset of puberty and development of secondary sex characteristics in both sexes. In adulthood, gonadotropin deficiency can be suspected in a woman without breast development or who presents with primary amenorrhea. In adult men, gynecomastia, small testes (<14 mL), penile hypoplasia, and/or oligo-azoospermia raise the clinical suspicion of congenital hypogonadism (1–3).

**Table 1 | Genes and phenotype related only with normosmic IHH.**

| Genes | Locus | Inheritance | Phenotype | Comment |
|---|---|---|---|---|
| *GNRH1* | 8p21-11.2 | Autosomal | Normosmic | Cryptorchidism |
| *GNRH-R* | 4q13.2-3 | recessive | IHH | – |
| *KISS1* | 1q32 | Autosomal | Normosmic | – |
| *KISS1R* | 19p13.3 | recessive | IHH | – |
| *LEP* | 7q31.3 | Autosomal | Normosmic | Severe obesity |
| *LEPR* | 1p31 | recessive | IHH | |
| *TAC3* | 12q13.3 | Autosomal | Normosmic | – |
| *TACR3* | 4q25 | recessive | IHH | – |
| *DUSP6* | 12q21.33 | Complex trait | Normosmic IHH | – |
| *LHB* | 19q13.32 | Polymorphism and mutations (homozygous and heterozygous) | Normosmic IHH | – |
| *FSHB* | 11p13 | Polymorphism and mutations | Normosmic IHH | – |

### NORMOSMIC IDIOPATHIC HYPOGONADOTROPIC HYPOGONADISM

The genetic abnormalities described below are infrequent or rare (see **Table 1**). In contrast to KS, patients with nIHH have a normal sense of smell and tend not to have other clinical signs. From a biological point of view, sex steroids secretion and gametogenesis are compromised, but to varying degrees. As it would be expected, reproductive phenotypes are more pronounced in subjects in whom the receptor is inactivated as compared to those harboring hormone inactivating mutations.

#### *GNRH-1 and GNRHR mutations*

Gonadotropin-releasing hormone (GnRH) is encoded by the *GNRH1* gene, which is located on chromosome 8p21-11.2. GNRH-1 mutations are rare and have been described in only two families (17, 18). A single homozygous mutation (c.18-19insA) affecting the peptide precursor preproGnRH was described in a Romanian family (17). It encoded a truncated and biologically inactive peptide in a male patient and his sister, both of whom had delayed puberty and normal sense of smell. The phenotype was reversed by pulsatile GnRH administration. Another homozygous *GNRH1* mutation was identified in a prepubertal boy from Armenia, with cryptorchidism and microphallus (18).

**Table 2 | Genes, genes product, function, and phenotypes associated to congenital hypogonadism hipogonadotropic with anosmia/hyposmia (KS, Kallmann syndrome)**.

| Genes | Locus | Gene product | Function | Inheritance | Type of hypogonadism | Clinical phenotype |
|---|---|---|---|---|---|---|
| *KAL-1 (KS-1)* | Xp22.3 | Anosmin-1 | Migration of GnRH and olfactory neurons | X-linked | Kallmann syndrome or normosmic IHH | Unilateral renal agenesis, synkinesia |
| *FGF8 (KS-6)* | 10q24 | Fibroblast growth factor 8 | Migration of GnRH neurons | Autosomal dominant | Kallmann syndrome or normosmic IHH | Cleft lip/relatively common (mid-line defects) |
| *FGFR1 (KS-2)* | 8p11.22 | Fibroblast growth factor receptor | Migration of GnRH neurons | Autosomal dominant | Kallmann syndrome or normosmic IHH | |
| *FGF 17* | 8p2.3 | Fibroblast growth factor 17 | Migration of GnRH neurons | Autosomal recessive | Kallmann syndrome or normosmic IHH | |
| *FLRT3* | 20p12.1 | Fibronecting like domain containing leucine enrich transmembrane protein 3 | Interaction with FGFR | Complex trait | Kallmann syndrome | FGF network<br>KO mouse is embryonic lethal |
| *DUSP6* | 12q21.33 | Dual specific inhibitor phosphatases | Inhibitor of MAPK pathway | Autosomal recessive | Kallmann syndrome | FGF network |
| *IL17RD* | 3p14.3 | Interleukin-17 receptor | Early stage of GnRH specification | Autosomal recessive | Kallmann syndrome | FGF network |
| *SPRY4* | 5q31.3 | Sprouty homolog interactor with FGFR1 | Inhibitor of MAPK pathway | Autosomal recessive | Kallmann syndrome | FGF network |
| *CHD7 (KS-5)* | 8q12.1-q12.2 | Chromatin remodelating factor | | Autosomal dominant | Kallmann syndrome or normosmic IHH | CHARGE Syndrome |
| *SEMA3A* | 7q21.11 | Semaphorine 3A | Axonal path finding of GnRH neurons | Autosomal dominant | Kallmann syndrome | – |
| *PROK2 (KS-3)* | 3p21.1 | Prokineticin-2 | Migration of GnRH neurons | Autosomal dominant and recessive | Kallmann syndrome or normosmic IHH | Obesity, epilepsy, sleep disorders, fibrous dysplasia, and synkinesia |
| *PROKR2 (KS-4)* | 20p13 | Prok receptor | | Kallmann syndrome or normosmic IHH | | |
| *NELF* | 9q34.3 | Nasal embrionic LHRH factor | Migration of GnRH neurons | Digenic model (in association wth FGFR1 and HS6ST1) | Kallmann syndrome or normosmic IHH | – |
| *WDR11* | 10q | WD repeat containing protein family | Development of neurons | Autosomal dominant | Kallmann syndrome or normosmic IHH | – |
| *HS6ST1* | 2q21 | Heparan sulfate 6-O Sulfotransferase | HS modifier<br>Regulates neural branching | Complex trait | Kallmann syndrome or normosmic IHH | – |

The *GNRHR* gene (locus on chromosome 4q13.2-3) encodes for the GNRH receptor. There is some variability in clinical expression of *GnRHR* mutations that is due to a partial loss of function. *GNRHR* mutations have been described in about 40–50% of familial AR nIHH cases, and in around 17% of sporadic nIHH (1–3).

#### *KISS1 and GFPR54 mutations*

The gene *KISS1* was described originally as a metastasis suppressor gene but it is a key gene in reproduction. It is localized on chromosome 1q32, encoding a protein called kisspeptin, which is, in turn, processed in four peptides Kp10, Kp13, Kp14, and Kp54. Kisspeptins stimulate GnRH neuronal firing and GnRH secretion, which then triggers an increased release of LH and FSH (**Figure 1**). The *KISS1R* gene (locus 19p.13.3), a G-protein-coupled receptor, is also known as the *GPR54* gene, and it is the receptor for kisspeptins. *GPR54* mutations can be compound heterozygous or homozygous (19–22). Loss of function mutations in *KISS1* (20) and *GPR54* cause HH in mice and men (19, 21, 22). Moreover, higher serum kisspeptins levels are found in obese hypogonadal men and in central hypogonadism than in controls (23). In patients with *GPR54* mutations, GnRH deficiency can be partial or complete, permanent, or reversible, and can have a congenital or adult onset. Six homozygous inactivating mutations have been described in 19 individuals with nIHH: their LH and FSH secretion was blunted but normal secretion was restored after exogenous GnRH stimulation (19, 21, 22). Kisspeptins are highly expressed in placenta during pregnancy; different patterns of spatiotemporal expression of *KISS1* and *KISSR* were described in normal and pathological placentas (24).

#### *TAC3R and TAC3 mutations*

The *TACR3* gene (chromosome 4q25) encodes the neurokinin 3 receptor (NK3R) and the *TAC3* gene (chromosome 12q13.3) encodes neurokinin B (NKB), its endogenous ligand. nIHH caused by mutations in *TAC3* and *TAC3R* have an AR heritance (25). As well as the *GPR54/Kisspeptin* system, *TACR3/TAC3* pathway stimulates GnRH neurons. In initial studies, defects in either *TAC3* or *TACR3* were found in 11 patients from 5 of 10 families studied, but in none of 50 sporadic cases (25–27). Francou et al. studied the gonadotropin axis dysfunction associated with nCHH due to TAC3/TACR3 mutations: it was related to a low GnRH pulsatile frequency leading to a low frequency of alpha-subunit pulses and to an elevated FSH/LH ratio (27). They suggested that this ratio might be useful for pre-screening nCHH patients for TAC3/TACR3 mutations. In another broad cohort of normosmic IHH patients, 7 of the 16 males and 5 of the 7 females with *TACR3/TAC3* mutations were assessed after discontinuation of therapy: 6 of the 7 males and 4 of the 5 females demonstrated evidence for reversibility of their hypogonadotropism (14).

#### *Leptin (Ob) and leptin receptor mutations*

Leptin is an adipocyte secreted protein that ensures a link between body fat and the reproductive axis. HH and severe obesity are seen in humans and ob/ob mice with genetic leptin deficiency. There are at least 12 patients with leptin deficiency and homozygous mutations. In such cases, recombinant leptin administration restores gonadotropin secretion and dramatically reduces body mass index. Defects in the leptin receptor are more common, being identified in 3% of severe early onset obesity patients. Interestingly, the leptin receptor is expressed on kisspeptin neurons whereas leptin administration induces the expression of *Kiss-1* in *ob/ob* mice (3, 28).

#### *LHB mutations*

The *LHB* subunit gene is located at chromosome 19q13.32. Five mutations have been published up to now; clinical and molecular data are summarized in **Table 3**. The syndrome of preserved spermatogenesis with androgenic failure (now known to be due to LH deficiency) was described for the first time by Pasqualini and Bur in 1950 (29). The term "fertile eunuch" was then coined to describe these men.

In affected men, sexual differentiation was normal, but the absence of or significantly reduced LH secretion restrained the induction of puberty and altered Leydig cell proliferation and maturation (30–34). These males have impaired spermatogenesis, ranging from azoospermia to oligospermia, which has been linked to the lack of LH stimulation and low intratesticular testosterone action (5, 30–35). In 2004, we described a man with a homozygous missense mutation (G36D) in the *LHB* subunit gene that abrogated subunit dimerization and rendered LH biologically and immunologically inactive (31). Treatment with human chorionic gonadotropin (hCG) induced near normalization of testicular structure (5). The patient and his wife conceived a child by intracytoplasmic sperm injection from ejaculated sperm. The male heterozygous child had normal LH, FSH, and testosterone levels, at the age of 4 weeks (5, 35).

In women, *LHB* mutations lead to a normal pubertal development but they can have primary amenorrhea and micropolycystic ovaries (32–34).

#### *FSHB mutations*

The β subunit of FSH (*FSHB*) is located at chromosome 11p13. Three men and four women with inactivating FSH mutations have been reported. Men have normal pubertal development although they have azoospermia, whereas women have abnormal pubertal maturation; in these patients high level of LH are found whereas FSH is low/undetectable. Estrogen and progesterone concentrations are low (1, 2).

#### *Gonadotropins receptor (LHR and FSHR) mutations*

Inactivating mutations affecting the gonadotropin receptors contrast with those affecting their ligands in that they are invariably associated with hypergonadotropic hypogonadism; hence, they are not discussed here.

### KALLMANN SYNDROME

Kallmann syndrome, involving the characteristic features of HH and anosmia was noted in the historical literature long before being properly characterized as a genetic disorder. A man with delayed puberty and the lack of olfactory bulbs was reported over 150 years ago by the Spanish doctor Aureliano Maestre de San Juan (1828–1890). The German Franz Kallmann (1897–1965) completed in the 1940s a description of hypogonadism and anosmia in two families, establishing the genetic basis of transmission.

**Table 3 | Clinical, biological, pathological, and genetic studies in patients with LH deficiency**.

| | Weiss et al. (30) | Valdes-Socin et al. (31) | Lofrano-Porto et al. (32) | Achard et al. (33) | Basciani et al. (34) |
|---|---|---|---|---|---|
| Mutation LH beta | Glut54Arg<br>Homozygous | Glyc36Asp<br>Homozygous | IVS + 1G > C<br>Homozygous | Del10HisProlLeu<br>Homozygous | IVS + 1G > C<br>12-bp deletion in Exon 2<br>Heterozygous |
| Exon localization | Exon 2 | Exon 2 | Intron 2 | Exon 2 | Exon 2 |
| LH Functional Studies | Reduced LH bioactivity | Knot cysteine<br>No LH dimerization | Abnormal tertiary structure<br>No LH dimerization | Reduced LH bioactivity | No LH secretion |
| Plasma LH | LH = 64 | LH undetectable | LH undetectable | No detectable LH | LH undetectable |
| Women | No | No | 1, amenorrhea | 1, amenorrhea | 1, oligomenorrhea |
| Men | One man, impuberism | One man, impuberism | Two men, high FSH et SUα | One man, impuberism | One man |
| | FSH = 113 | Hypoandrogenism<br>FSH = 23<br>αSU = 0.8<br>inhB = N | | FSH = 20.7<br>SUα = 1.28<br>inhB = N<br>High AMH | FSH = 8.7<br>inhB = N |
| Testis biopsy | Leydig = 0<br>Arrested SPG | Leydig+<br>SPG diminished | Leydig = 0<br>Arrested SPG | Leydig±<br>SPG+ | (after hCG) Leydig+<br>SPG+ |
| Fertility | – | Azoospermia | Azoospermia | Normospermia but abnormal forms. | Oligospermia |
| Treatment | T2 then hCG | T2 then hCG | T2 | T2 then hCG | T2 then hCG |

*All but one patient (Basciani et al.) are homozygotes for an inactivating βLH mutation.*

*SU α, alpha subunit; inhB, inhibin B; AMH, antimullerian hormone; SPG, spermatogenesis; T2, testosterone; N, normal; Anorm, abnormal; Dim, dimerization. Normal values: FSH (2–14 UI/L), LH (2–10 UI/L), alpha subunit (< 1.2 mUI/L).*

The Swiss scientist, Georges de Morsier (1894–1982) provided the neuropathological description of the syndrome. KS has a prevalence of 1/5000, with a clear male predominance (1–3, 36, 37). Several mechanisms of inheritance and molecular mutations are described here after and summarized in **Table 2**.

### *Anosmin-1 (KAL-1) mutations*

The *KAL-1* gene is located on the X chromosome at Xp22.3. *KAL-1* encodes anosmin-1, a glycoprotein playing an in important role in kidney, respiratory tract, digestive system, and brain embryogenesis (1–3, 37, 38). Anosmin-1 is an adhesion molecule located on cell surface, consistent with the underlying defect of embryonic neuronal migration in KS. Anosmin-1 is mainly involved in growth and migration of GnRH, mitral olfactory cells, and Purkinje cerebellum neurons. Mutations in *KAL-1* gene cause 14% of familial cases of KS and 11% of cases of sporadic cases (1–3, 38). *KAL-1* mutations lead to HH with or without anosmia and may include synkinesis (mirror movements), unilateral renal aplasia, and mid-line abnormalities such as cleft lip/palate (37, 38).

### *FGF8 (KAL-6), FGF17, and FGFR1 (KAL-2) mutations*

The *FGF8* gene (also known as *KAL-6*) is located on chromosome 10q24. Fibroblast growth factors (FGF) interact with FGF tyrosine kinase receptors to mediate growth and development. *FGF8* participates in gastrulation, regionalization of the brain, and organogenesis of the limb and face as an embryonic epithelial factor. *FGF8* and its receptor *FGFR1* are involved in GnRH neuron migration. *FGF8* inactivating mutations can lead to both KS and nIHH with an AD inheritance. Triallelic inheritance has also been described. In addition, cleft lip or palate and other midline defects have been described in patients with *FGF8* and *FGFR1* mutations. Other features such as corpus callosum hypoplasia-agenesis or nose, ear, and finger abnormalities are more specific of *FGFR1* defects (37, 39, 40).

*FGF17* is located at chromosome 8p2.3 and *FGF17* has a strong sequence identity with *FGF8*. *FGF17* might be implicated in GnRH neuron biology as an alternative to ligand *FGF8b*. Miraoui et al. have identified FGF17 heterozygous mutations in three patients with congenital HH and anosmia and in another individual. In a sporadic male patient with congenital, HH without anosmia (41).

*FGFR1* is located at 8p11.22-p11.23 and *FGFR1* mutations have been identified in 10% of KS. *FGFR1* related KS has an AD inheritance, associated with incomplete penetrance and interfamilial variability. FGFR1 encodes for type 1 FGF receptor, which is expressed in several embryonic tissues. The activation of the FGF–FGFR complex requires two FGF ligands. FLRT3 (Fibronecting like domain containing leucine enrich transmembrane protein 3) also interacts with FGFR (see **Table 2**). In addition, the binding of heparin or HS: heparan sulfate proteoglycan (see HS6ST1 gene

later) have been shown to be essential for FGF receptor dimerization and function (38). Mice with Fgfr1$^{-/-}$ mutations and patients with loss-of-function mutations in *FGFR1* have defective GnRH neuron migration (31). Thus, loss of function mutations in *FGFR1* which is involved in the development of the face, lead to the abnormal morphogenesis of the olfactory bulb, while specific gain-of function mutations in *FGFR1* cause craniosynostosis (37, 39–41).

#### *PROK2 and PROKR2 mutations*

*PROK2* (locus 3p21.1) and *PROKR2* (locus 20p13) genes encode for prokineticin-2 and its receptor (42). Prok2 and prokr2 gene knockout mice both have agenesis or hypoplasia of the olfactory bulbs, in association with HH. In this model, there is also abnormal GnRH neuron migration (43). Its heritance can be AD or AR. Mutations in these genes are described in up to 6% of KS and 3% of nIHH (1–3, 42). In pituitary deficits associated with septo-optical dysplasia, McCabe et al. (44), described a patient with a heterozygous L173R mutation in *PROKR2* gene, while its healthy mother is homozygous for this mutation. As some controversy exists on the pathogenic role of some PROKR2 mutations, the prevalence given should be interpreted cautiously. Indeed, digenic mutations are encountered (i.e., *PROKR2* and *KAL1*), while heterozygous patients (i.e., AD transmission) are present in families with healthy relatives presenting the same genotype. There are no reliable accessory pathognomonic features of *PROK2/PROKR2* function loss: patients have been described with obesity, sleep disorder, fibrous dysplasia, epilepsy, and synkinesia (2, 3, 43, 44).

#### *NELF mutations*

The *NELF* gene is located at chromosome 9q34.3. This gene encodes the nasal embryonic LHRH factor. The *NELF* gene is detected in olfactory sensory cells and GnRH cells during embryonic development. It constitutes a guidance molecule for the olfactory axon and GnRH neurons across the nasal region (45). *NELF* mutations have been described in patients with KS, in association with mutations in *FGFR1* or *HS6ST1*, indicating digenic inheritance. More studies are necessary to confirm a relationship between *NELF* and any reproductive and olfactory disorders (2).

#### *WDR11 mutations*

The *WDR11* locus is at chromosome 10q26.12 and its heritance is AD. It encodes murine Wdr11 that is expressed in the developing olfactory and GnRH migratory pathway and in the adult hypothalamus. *WDR11* biological function is not well understood: however, Kim et al. identified five different heterozygous mutations in nIHH and KS patients. WDR11 probably also plays an important role in puberty (46).

#### *CHD7 mutations*

The *CHD7* gene that encodes a chromatin-remodeling factor is located on chromosome 8q12.1. Mutations (AD inheritance) of this gene can cause CHARGE syndrome (Colobomata, Heart Anomalies, Choanal Atresia, Retardation, Genital, and Ear anomalies). *CHD7* was screened in nearly 200 patients: 7 KS and nIHH patients were found, 3 of them with olfactory abnormalities. CHD7 mutations were identified in 6% of KS and 6% of nIHH, respectively (1, 2, 47, 48). Laittinen et al. described in 2012, a KS patient with a truncating CHD7 mutation that underwent a reversal of central hypogonadism after therapy discontinuation (15).

#### *HS6ST1 mutations*

The *HS6ST1* gene (locus 2q.21) encodes a 6-*O*-sulfation enzyme, which is a member of the heparan sulfate enzyme family. The protein is involved in normal neuronal development and may play a role in limb development. In nematodes, HS 6-*O*-sulfate interacts with anosmin-1 and it is involved in function of FGFR1 and FGF8.

*HS6ST1* shows complex inheritance patterns, not following autosomal or recessive transmission. *HS6ST1* mutations were found in KS patients in combination with mutations affecting the FGFR1 gene. *HS6ST1* mutations were found in patients who had nIHH or variable degrees of olfactory dysfunction (KS) as well as with either normal or abnormal olfactory structures (49).

#### *IL17RD, DUSP6, and SPRY4 mutations*

The *IL17RD* gene (locus 3p14.3) encodes a membrane protein belonging to the interleukin-17 receptor (*IL-17R*) protein family. In a study with eight patients with congenital hypogonadism all had KS, 7/8 had absent puberty, 6/8 showed congenital hearing loss. One *IL17RD* allelic defect is likely to be insufficient, meaning that additional affected alleles in the same and/or other genes must be present to create the phenotype of KS with hearing loss (41).

*DUSP6* (locus 12q22-q23) encodes a member of the dual specificity protein phosphatase subfamily. They negatively regulate members of the mitogen-activated protein (MAP) kinase superfamily (25) Three patients were described with DUSP6 and *FGFR1* heterozygous mutation; they were hypogonadic, while one had hearing loss and the two others had abnormal speech. DUSP6$^{-/-}$ mice are however viable and fertile (41).

*SPRY4* (locus 5q.31.3) gene encodes a protein (sprouty homolog 4), which is an inhibitor of the receptor-transduced mitogen-activated protein kinase (MAPK) signaling pathway. It is positioned upstream of RAS gene activation and impairs the formation of active GTP-RAS. Diseases associated with SPRY4 include germ cell cancer, and testicular cancer. Miraoui et al. identified four anosmic patients with congenital HH (three females and one male) with heterozygosity for a c.530A-G transition in exon 3 of the SPRY4 gene. Another female patient had a heterozygosity for a c.910G-A transition in exon 3 of the SPRY4 gene. These mutations were not found in 155 controls. One of the patients also had hearing loss and another one had abnormal dentition (41).

#### *HESX1 mutations*

The *HESX1* gene (locus 3p14.3) encodes a protein that is a transcriptional repressor in the developing forebrain and pituitary gland (27). *HESX1* plays an important role in the temporal and sequential development of the forebrain, hypothalamus, optic nerve, and posterior pituitary (28). Mutations in *HESX1* have also been described in isolated growth hormone deficiency and combined pituitary deficiency (50, 51). *HESX1* mutations have been described in 1.4% of IHH/KS patients (50, 51), but as in *PROKR2* mutations this prevalence should be interpreted cautiously.

### *SEMA3A mutations*

The *SEMA3A* gene (7q21.11) encodes the semaphorin 3A protein, which regulates axonal path finding and participates in GnRH migration. Deletions and mutations of the *SEMA3A* gene validate a role for *SEMA3A* in KS. Moreover, SEMA3A knockout mice exhibit GnRH dependent hypogonadism and abnormal olfactory bulb innervation (52).

## CONCLUSION AND PERSPECTIVES

Kallmann syndrome and nIHH have the potential to unravel the processes behind normal embryonic development and reproductive neuroendocrine maturation (2). The complex biological path from childhood to the onset of human puberty is still incompletely understood (1–3). The molecular mechanism behind IHH remains unknown in a large number of cases. Over the past decade, the same genetic mutations have been described in associated with both KS and nIHH. Moreover, a significant clinical heterogeneity is seen in isolated GnRH deficiency, which might be explained to some extent by oligogenicity (6, 7, 9). In addition, over 60% of central hypogonadic patients have no identifiable mutations, suggesting that yet more disease loci remain to be discovered (1–3). These unidentified genes warrant an integrated research including clinicians, geneticists, and biological investigators to pursue further understanding of these fascinating cases.

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## REFERENCES

1. Valdes-Socin S, Debray FG, Parent AS, Lebrethon MC, Bourguignon JP, Bours V, et al. How to explore…congenital isolated hypogonadotropic hypogonadism? *Rev Med Liege* (2010) **65**(11):634–41.
2. Semple R, Topaloglu KA. The recent genetics of hypogonadotrophic hypogonadism – novel insights and new questions. *Clin Endocrinol (Oxf)* (2010) **72**(4):427–35. doi:10.1111/j.1365-2265.2009.03687.x
3. Nachtigall LB, Boepple PA, Pralong FP, Crowley WF Jr. Adult-onset idiopathic hypogonadotropic hypogonadism – a treatable form of male infertility. *N Engl J Med* (1997) **336**:410–5. doi:10.1056/NEJM199702063360604
4. Lippincott MF, True C, Seminara SB. Use of genetic models of idiopathic hypogonadotrophic hypogonadism in mice and men to understand the mechanisms of disease. *Exp Physiol* (2013) **98**:1522–7. doi:10.1113/expphysiol.2013.071910
5. Valdes-Socin H, Salvi R, Thiry A, Daly AF, Pralong FP, Gaillard R, et al. Testicular effects of isolated luteinizing hormone deficiency and reversal by long-term human chorionic gonadotropin treatment. *J Clin Endocrinol Metab* (2009) **94**:3–4. doi:10.1210/jc.2008-1584
6. Trarbach EB, Costa EM, Versiani B, de Castro M, Matias Baptista MT, Mendes Garmes H, et al. Novel fibroblast growth factor receptor 1 mutations in patients with congenital hypogonadotropic hypogonadism with and without anosmia. *J Clin Endocrinol Metab* (2006) **91**:4006–12. doi:10.1210/jc.2005-2793
7. Pitteloud N, Acierno JS Jr, Meysing A, Eliseenkova AV, Ma J, Ibrahimi OA, et al. Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci U S A* (2006) **103**:6281–6. doi:10.1073/pnas.0600962103
8. Pitteloud N, Hayes FJ, Boepple PA, DeCruz S, Seminara SB, MacLaughlin DT, et al. The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* (2002) **87**:152–60. doi:10.1210/jcem.87.1.8131
9. Sykiotis GP, Plummer L, Hughes VA, Au M, Durrani S, Nayak-Young S, et al. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci U S A* (2010) **107**:15140–4. doi:10.1073/pnas.1009622107
10. Fukami M, Maruyama T, Dateki S, Sato N, Yoshimura Y, Ogata T. Hypothalamic dysfunction in a female with isolated hypogonadotropic hypogonadism and compound heterozygous TACR3 mutations and clinical manifestation in her heterozygous mother. *Horm Res Paediatr* (2010) **73**:477–81. doi:10.1159/000313373
11. Lewkowitz-Shpuntoff HM, Hughes VA, Plummer L, Au MG, Doty RL, Seminara SB, et al. Olfactory phenotypic spectrum in idiopathic hypogonadotropic hypogonadism: pathophysiological and genetic implications. *J Clin Endocrinol Metab* (2012) **97**:136–44. doi:10.1210/jc.2011-2041
12. Raivio J, Falardeau A, Dwyer R, Quinton FJ, Hayes VA, Hughes LW, et al. Reversal of idiopathic hypogonadotropic hypogonadism. *N Engl J Med* (2007) **357**:863–73. doi:10.1056/NEJMoa066494
13. Pitteloud N, Acierno JS Jr, Meysing AU, Dwyer AA, Hayes FJ, Crowley WF Jr. Reversible Kallmann syndrome, delayed puberty, and isolated anosmia occurring in a single family with a mutation in the fibroblast growth factor receptor 1 gene. *J Clin Endocrinol Metab* (2005) **90**:1317–22. doi:10.1210/jc.2004-1361
14. Gianetti E, Tusset C, Noel SD, Au MG, Dwyer AA, Hughes VA, et al. TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. *J Clin Endocrinol Metab* (2010) **95**:2857–67. doi:10.1210/jc.2009-2320
15. Laitinen EM, Tommiska J, Sane T, Vaaralahti K, Toppari J, Raivio T. Reversible congenital hypogonadotropic hypogonadism in patients with CHD7, FGFR1 or GNRHR mutations. *PLoS One* (2012) **7**:e39450. doi:10.1371/journal.pone.0039450
16. Cariboni A, Maggi R. Kallmann's syndrome, a neuronal migration defect. *Cell Mol Life Sci* (2006) **63**(21):2512–26. doi:10.1007/s00018-005-5604-3
17. Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, et al. Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. *N Engl J Med* (2009) **360**:2742–8. doi:10.1056/NEJMoa0900136
18. Chan YM, de Guillebon A, Lang-Muritano M, Plummer L, Cerrato F, Tsiaras S, et al. GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci U S A* (2009) **106**:11703–8. doi:10.1073/pnas.0903449106
19. de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc Natl Acad Sci U S A* (2003) **100**:10972–6. doi:10.1073/pnas.1834399100
20. Topaloglu AK, Tello JA, Kotan LD, Ozbek MN, Yilmaz MB, Erdogan S, et al. Inactivating KISS1 mutation and hypogonadotropic hypogonadism. *N Engl J Med* (2012) **366**:629–35. doi:10.1056/NEJMoa1111184
21. Brioude F, Bouligand J, Francou B, Fagart J, Roussel R, Viengchareun S, et al. Two families with normosmic congenital hypogonadotropic hypogonadism and biallelic mutations in KISS1R (KISS1 receptor): clinical evaluation and molecular characterization of a novel mutation. *PLoS One* (2013) **8**(1):e53896. doi:10.1371/journal.pone.0053896
22. Tenenbaum-Rakover Y, Commenges-Ducos M, Iovane A, Aumas C, Admoni O, de Roux N. Neuroendocrine phenotype analysis in five patients with isolated hypogonadotropic hypogonadism due to a L102P inactivating mutation of GPR54. *J Clin Endocrinol Metab* (2007) **92**:1137–44. doi:10.1210/jc.2006-2147
23. Valdes-Socin H, Cavalier E, Beckaert A, Carlisi A, Chavez M, Beckers A. High plasma kisspeptins in obese men with acquired hypogonadism and in men with congenital hypogonadism: pilot study of plasma kisspeptins variations before and after hCG and testosterone administration. *Ann Endocrinol (Paris)* (2011).
24. Valdes-Socin H, Munaut C, Chavez M, Chantrain F, Delbecque K, Delvenne P, et al. Expression and spatio-temporal distribution of KISS1 and its receptor KISSR in human normal and pathological placentae. *Ann Endocrinol (Paris)* (2012) **4**:374. doi:10.1016/j.ando.2012.07.494
25. Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan D, Porter KM, et al. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nat Genet* (2009) **41**:354–8. doi:10.1038/ng.306
26. Guran T, Tolhurst G, Bereket A, Rocha N, Porter K, Turan S, et al. A novel missense mutation in the first extracellular loop of the neurokinin B receptor causes hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* (2009) **94**:3633–9. doi:10.1210/jc.2009-0551

27. Francou B, Bouligand J, Voican A, Amazit L, Trabado S, Fagart J, et al. Normosmic congenital hypogonadotropic hypogonadism due to TAC3/TACR3 mutations: characterization of neuroendocrine phenotypes and novel mutations. *PLoS One* (2011) **6**:e25614. doi:10.1371/journal.pone.0025614
28. Farooqi IS, Wangensteen T, Collins S, Kimber W, Matarese G, Keogh JM, et al. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *N Engl J Med* (2007) **356**:237–47. doi:10.1056/NEJMoa063988
29. Pasqualini RQ, Bur GE. Síndrome hipoandrogénico con gametogénesis conservada: clasificación de la insuficiencia testicular. *Rev Asoc Med Argent* (1950) **64**:15–30.
30. Weiss J, Axelrod L, Whitcomb RW, Harris PE, Crowley WF, Jameson JL. Hypogonadism caused by a single amino acid substitution in the subunit of luteinizing hormone. *N Engl J Med* (1992) **326**:179–83. doi:10.1056/NEJM199201163260306
31. Valdes-Socin H, Salvi R, Daly AF, Gaillard RC, Quatresooz P, Tebeu P-M, et al. Hypogonadism in a patient with a mutation in the luteinizing hormone-subunit gene. *N Engl J Med* (2004) **351**:2619–25. doi:10.1056/NEJMoa040326
32. Lofrano-Porto A, Barra GB, Giacomini LA, Nascimento PP, Latronico AC, Casulari LA, et al. Luteinizing hormone mutation and hypogonadism in men and women. *N Engl J Med* (2007) **357**:897–904. doi:10.1056/NEJMoa071999
33. Achard C, Courtillot C, Lahuna O, Me'duri G, Soufir JC, Lie're P, et al. Normal spermatogenesis in a man with mutant luteinizing hormone. *N Engl J Med* (2009) **361**:1856–63. doi:10.1056/NEJMoa0805792
34. Basciani S, Watanabe M, Mariani S, Passeri M, Persichetti A, Fiore D, et al. Hypogonadism in a patient with two novel mutations of the luteinizing hormone –subunit gene expressed in a compound heterozygous form. *J Clin Endocrinol Metab* (2012) **97**:3031–8. doi:10.1210/jc.2012-1986
35. Daly A, Valdes-Socin H, Beckers A. A tall man with hypogonadism. In: Wartofsky L, editor. *Diagnostic Dilemmas: Images in Endocrinology*. Washington DC: The Endocrine Society Press (2011). p. 204–7.
36. Legouis R, Hardelin JP, Levilliers J, Claverie JM, Compain S, Wunderle V, et al. The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. *Cell* (1991) **67**:423–35. doi:10.1016/0092-8674(91)90193-3
37. Sato N, Katsumata N, Kagami M, Hasegawa T, Hori N, Kawakita S, et al. Clinical assessment and mutation analysis of Kallmann syndrome 1 (KAL1) and fibroblast growth factor receptor 1 (FGR1, or KAL2) in five families and 18 sporadic patients. *J Clin Endocrinol Metab* (2004) **89**:1079–88. doi:10.1210/jc.2003-030476
38. Tsai PS, Gill JC. Mechanisms of disease: insights into X-linked and autosomal-dominant Kallmann syndrome. *Nat Clin Pract Endocrinol Metab* (2006) **2**:160–71. doi:10.1038/ncpendmet0119
39. Dodé C, Levilliers J, Dupont JM, De Paepe A, Le Dû N, Soussi-Yanicostas N, et al. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet* (2003) **33**:463–5. doi:10.1038/ng1122
40. Dodé C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, et al. Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet* (2006) **2**(10):e175. doi:10.1371/journal.pgen.0020175
41. Miraoui H, Dwyer AA, Sykiotis GP, Plummer L, Chung W, Feng B, et al. Mutations in FGF17, IL17RD, DUPS6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism. *Am J Hum Genet* (2013) **92**:725–43. doi:10.1016/j.ajhg.2013.04.008
42. Cole LW, Sidis Y, Zhang CK, Quinton R, Plummer L, Pignatelli D, et al. Mutations in prokineticin 2 and prokineticin receptor 2 genes in human gonadotrophin releasing hormone deficiency: molecular genetics and clinical spectrum. *J Clin Endocrinol Metab* (2008) **93**:3551–9. doi:10.1210/jc.2007-2654
43. Matsumoto S, Yamazaki C, Masumoto K, Nagano M, Naito M, Soga T, et al. Abnormal development of the olfactory bulb and reproductive system in mice lacking prokineticin receptor PKR2. *Proc Natl Acad Sci U S A* (2006) **103**:4140–5. doi:10.1073/pnas.0508881103
44. McCabe MJ, Gaston-Massuet C, Gregory LC, Alatzoglou KS, Tziaferi V, Sbai O, et al. Variations in PROKR2, but not PROK2, are associated with hypopituitarism and septo-optic dysplasia. *J Clin Endocrinol Metab* (2013) **98**:547–57. doi:10.1210/jc.2012-3067
45. Kramer PR, Wray S. Novel gene expressed in nasal region influences outgrowth of olfactory axons and migration of luteinizing hormone-releasing hormone (LHRH) neurons. *Genes Dev* (2000) **14**:1824–34.
46. Kim HG, Ahn JW, Kurth I, Ullmann R, Kim HT, Kulharya A, et al. WDR11, a WD protein that interacts with transcription factor EMX1, is mutated in idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. *Am J Hum Genet* (2010) **87**(4):465–79. doi:10.1016/j.ajhg.2010.08.018
47. Kim HG, Kurth I, Lan F, Meliciani I, Wenzel W, Eom SH, et al. Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. *Am J Hum Genet* (2008) **83**:511–9. doi:10.1016/j.ajhg.2008.09.005
48. Bergman JE, de Ronde W, Jongmans MC, Wolffenbuttel BH, Drop SL, Hermus A, et al. The results of CHD7 analysis in clinically well-characterized patients with Kallmann syndrome. *J Clin Endocrinol Metab* (2012) **97**(5):E858–62. doi:10.1210/jc.2011-2652
49. Tornberg J, Sykiotis GP, Keefe K, Plummer L, Hoang X, Hall JE, et al. Heparan sulfate 6-O-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotrophic hypogonadism. *Proc Natl Acad Sci U S A* (2011) **108**:11524–9. doi:10.1073/pnas.1102284108
50. McNay DE, Turton JP, Kelberman D, Woods KS, Brauner R, Papadimitriou A, et al. HESX1 mutations are an uncommon cause of septooptic dysplasia and hypopituitarism. *J Clin Endocrinol Metab* (2007) **92**:691–7. doi:10.1210/jc.2006-1609
51. Newbern K, Natrajan N, Kim H-G, Chorich LP, Halvorson LM, Cameron RS, et al. Identification of HESX1 mutations in Kallmann syndrome. *Fertil Steril* (2013) **99**:1831–7. doi:10.1016/j.fertnstert.2013.01.149
52. Young J, Metay C, Bouligand J, Tou B, Francou B, Maione L, et al. SEMA3A deletion in a family with Kallmann syndrome validates the role of semaphorin 3A in human puberty and olfactory system development. *Hum Reprod* (2012) **27**:1460–5. doi:10.1093/humrep/des022


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# Health outcomes in acromegaly: depression and anxiety are promising targets for improving reduced quality of life

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**Introduction:** Remission criteria of acromegaly are based on biochemical variables, i.e., normalization of increased hormone levels. However, the established reduction in Quality of Life (QoL) is suggested to be independent of biochemical control. The aim of this study was to test which aspects predict QoL best in acromegaly.

**Methods/design:** This is a prospective cohort study in 80 acromegalic patients, with a cross-sectional and longitudinal part. The main outcome measure was health-related QoL, measured by a generic and a disease-specific questionnaire (the SF-36 and AcroQoL). Main predictors were age, gender, biochemical control, disease characteristics, treatment modalities, and psychopathology.

**Results:** Our cohort of 80 acromegalics had a mean age $54.7 \pm 12.3$ years with an average disease duration of $10.8 \pm 10.0$ years. Ratio macro-/microadenoma was 54/26. In adjusted mixed method models, we found that psychopathology significantly predicts QoL in acromegaly (in models including the variables age, gender, disease duration, tumor size, basal hormone levels, relevant treatment modalities, and relevant comorbidities), with a higher degree of psychopathology indicating a lower QoL (depression vs. AcroQoL: $B = -1.175$, $p < 0.001$, depression vs. SF-36: $B = -1.648$, $p < 0.001$, anxiety vs. AcroQoL: $B = -0.399$, $p < 0.001$, anxiety vs. SF-36: $B = -0.661$, $p < 0.001$). The explained variances demonstrate superiority of psychopathology over biochemical control and other variables in predicting QoL in our models.

**Discussion:** Superiority of psychopathology over biochemical control calls for a more extensive approach regarding diagnosing depression and anxiety in pituitary adenomas to improve QoL. Depressive symptoms and anxiety are modifiable factors that might provide valuable targets for possible future treatment interventions.

**Keywords: acromegaly, anxiety, biochemical control, depression, pituitary adenomas, quality of life**

## INTRODUCTION

Objectives for health outcomes include reduction of mortality, morbidity, and the improvement of Quality of Life (QoL) (1).

However, in patients with pituitary adenomas, such as acromegaly, this third treatment goal remains often unfulfilled as they report to have a markedly reduced QoL, which often persists under biochemical control/remission (2–7).

Various factors have been suggested to be associated with reduced QoL in pituitary disease, particularly acromegaly; however, there is no clear consensus in this regard.

The current consensus criteria for cure and remission of acromegaly are based on biochemical variables, i.e., normalization of elevated hormonal levels of the biomarkers growth hormone (GH) and/or insulin like growth factor-1 (IGF-1) (8–10). However, the definition of such a "remission" remains insufficient with regard to absolute recovery: a drawback of a purely biochemical approach is that there are strong indications that biochemical control does not necessarily provide complete "cure" in patients' view, since their health-related QoL remains reduced in most patients with acromegaly.

However, there is no consensus on the value of these contributing factors. For instance, several articles reported biochemical control to have no significant association with reduced QoL (11–13), whereas other articles report exactly the opposite (14–16), indicating a clear need for elucidation.

Psychopathological variables are candidate modifiable factors to link pituitary disease, especially acromegaly, to a lower health-related QoL. On the one hand, acromegaly is reported to be associated with neuropsychiatric comorbidities such as depressive symptoms (17) and anxiety (5, 18, 19). On the other hand, there is a clear association between psychopathology and perceived QoL (20–22).

The aim of this study was therefore to test the predictive impact of psychopathology (depressive symptoms and anxiety) on health-related QoL in acromegalic patients.

For this purpose, a theoretical model (23) was applied on acromegaly and tested in two primary data sets: (a) a

cross-sectional cohort of 80 acromegalic patients, which was (b) subsequently validated in the longitudinal cohort of the same patients.

## MATERIALS AND METHODS

### STUDY DESIGN

This is a prospective cohort study including two analytic parts: a cross-sectional and prospective evaluation. For design and recruitment of the initial cross-sectional cohort, see previous publications from our group (18, 19). Six years after the cross-sectional evaluation (baseline), a longitudinal evaluation was performed in which the same patients were recontacted and asked to participate using the same diagnostic instruments.

### STUDY PARTICIPANTS

For the cross-sectional analyses, 80 acromegalic patients (response rate 56%) were included. Patients were recruited at the Endocrine Outpatient Clinic at the Max Planck Institute of Psychiatry and the Medizinische Klinik und Poliklinik IV, Ludwig-Maximilians-Universität Munich.

For the longitudinal analyses, patients were recontacted and recruited accordingly. At both timepoints, patients were contacted by a letter regarding the aim and design of the study. A further request for participation was issued via telephone for initial non-responders.

Thirty-six acromegalic patients (response rate 45%), were included at the follow-up timepoint.

The project was approved by the medical ethics committee of the Ludwig-Maximilians-Universität Munich; all patients gave their written informed consent.

### MEASUREMENT INSTRUMENTS

#### *Clinical characteristics*

Patients were given questionnaires with standardized psychometric instruments, which allowed assessment of disease-related variables, therapy history, symptoms, tumor characteristics, comorbidities and current complaints, and evaluation of psychopathological symptoms. Patients were seen either at the Max Planck Institute for Psychiatry or at the Ludwig-Maximilians-Universität Munich for a standardized clinical assessment, which included a physical examination and laboratory analyses. Additional information was retrieved from the patient files if necessary.

#### *Laboratory measurements*

Biochemical control (dichotomous classification) was based on a single serum sample of patients with confirmed disease. Biochemical control of acromegaly was defined as (1) GH levels $<1\,\mu g/l$ during a glucose-tolerance test over 2 h and (2) IGF-1 levels within 2 SD of an age- and gender-adjusted standardized sample (8).

Pituitary function was routinely assessed in all patients on a yearly basis, with basal fasting measurements of IGF-1, thyroid stimulating hormone (TSH), free thyroxine, total triiodothyronine, luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, and testosterone (men) or estradiol (female). Moreover, stimulation tests were administered including short ACTH test, GHRH/arginine test, and insulin hypoglycemia test, if indicated. All patients were studied under optimal replacement therapy (24).

#### *Neuropsychiatric assessment*

Quality of Life was measured using the specifically designed instrument AcroQoL (range 0–110) (25), and the general instrument SF-36 (range 0–100) (26, 27). All QoL instruments were arranged to have higher scores reflecting a better QoL.

The following neuropsychiatric variables were assessed: depressive symptoms [Becks Depression Inventory (BDI) (28), range 0–63] and anxiety [State-Trait Anxiety Inventory (STAI) (29), range 40–160]. Neuropsychiatric scoring-instruments were arranged to have higher scores reflect greater disability.

All questionnaires were self-completed.

### STATISTICAL ANALYSIS

All analyses were performed with the Statistical Package for the Social Sciences 20.0 Software (SPSS 20.0).

### DESCRIPTIVE ANALYSES

Differences between biochemically controlled and uncontrolled patients for demographic variables were analyzed using $\chi^2$-tests and independent samples *t*-tests. Significance was set at the 0.05 level.

### STATISTICAL MODELS

Six-Block linear regression analyses were carried out to assess the contribution of different variables to the outcome QoL. Block 1 contained age and gender, block 2 contained tumor

**Table 1 | Demographic variables according to disease control.**

| | Uncontrolled | Controlled | *P** |
|---|---|---|---|
| Subjects at baseline (*N*) | 80 | | |
| | 31 | 49 | |
| Subjects at follow-up | 36 | | |
| | 15 | 21 | |
| Follow-up time (years)[a] | 7.1 ± 0.7 | | |
| | 7.1 ± 0.5 | 7.1 ± 0.8 | 1.000 |
| Age (years)[a] | 54.7 ± 12.3 | | |
| | 51.9 ± 14.0 | 56.7 ± 10.8 | 0.088 |
| Disease duration (years)[a] | 10.8 ± 10.0 | | |
| | 10.6 ± 10.6 | 11.2 ± 9.6 | 0.822 |
| % Male[b] | 46.3 (37) | | |
| | 54.8 (17) | 40.8 (20) | 0.220 |
| % Macroadenoma[b] | 68.8 (55) | | |
| | 80.6 (25) | 61.2 (30) | 0.068 |
| Basal GH[c] | 2.8 ± 5.0 | | |
| | 5.3 ± 7.1 | 1.1 ± 0.9 | 0.003 |

**p-Values computed using independent t-tests, chi square tests for the variables % Male and % Macroadenoma.*

*[a] Mean ± SD,*

*[b] % (N).*

*[c] Basal hormonal levels: serum GH for acromegaly (μg/l).*

**Table 2 | Association of psychopathology/biochemical control and QoL at baseline**.

| Disease (scale) | Variable | *B* (SE) | *p** | $\Delta R^2$ after correction[a] | Model $R^2$ |
|---|---|---|---|---|---|
| Acromegaly (AcroQoL)[b] | **Depressive symptoms** | **−1.175 (0.170)** | **<0.001** | **0.256** | 0.688 |
| | Biochemical control | −1.157 (3.009) | 0.702 | 0.001 | |
| | **Anxiety** | **−0.399 (0.089)** | **<0.001** | **0.147** | 0.578 |
| | Biochemical control | −0.546 (3.497) | 0.876 | <0.001 | |
| Acromegaly (SF-36)[c] | **Depressive symptoms** | **−1.648 (0.256)** | **<0.001** | **0.279** | 0.618 |
| | Biochemical control | 2.724 (4.497) | 0.547 | 0.002 | |
| | **Anxiety** | **−0.661 (0.109)** | **<0.001** | **0.258** | 0.598 |
| | Biochemical control | 3.126 (4.614) | 0.501 | 0.003 | |

**p-Values were computed using linear regression.*

*[a]All models carried a correction for age, gender, disease duration, basal hormone levels and tumor size.*

*Additional correction for:*

*[b]radiation, pathological glucose intolerance and arthralgia,*

*[c]radiation, arthralgia.*

*Bold font marks those results with statistical significance.*

size (dichotomous: macro- vs. microadenoma), basal GH levels, and disease duration, block 3 contained treatment types [dichotomous: surgery, radiation therapy, octreotide, lanreotide, dopamine-agonists (bromocriptin, lisuride, cabergoline, quinagolide), pegvisomant], block 4 contained comorbidities (dichotomous: arrhythmia, cardiomyopathy, cerebrovascular diseases, arterial hypertension, coronary artery disease, history of myocardial infarction, arthralgia, arthropathy, carpal tunnel syndrome, diabetes mellitus type 2, pathological glucose-tolerance, pituitary insufficiency, sleep apnea, lung diseases, cancer), and block 5 contained the psychopathological variables (depressive symptoms, anxiety). Separate analyses were carried out for depressive symptoms and anxiety (block 5) due to large correlations (Pearson's $r = 0.713$, $p < 0.001$). Block 6 contained the variable biochemical control as a predictor. A forced entry method was used for block 1, 2, 5, and 6 as we deemed inclusion of these variables, a basic requirement for our model. A stepwise forward likelihood ratio method was used for blocks 3 and 4 to include only those predictors that carried significant predictive value. The likelihood ratio method is preferable over the other stepwise methods (30).

For the longitudinal analyses to determine predictors of long-term QoL, linear mixed-effect models with a first-order autoregressive covariance matrix for repeated effects were used. Linear mixed-effects models allow a flexible length of follow-up for separate patients and account for within-patient variations (31, 32). Separate regression coefficients and intercepts were created for each individual patient. To investigate the influence of psychopathology on progression of QoL, the interaction between time and depressive symptoms/anxiety was investigated. A similar approach was taken to investigate if biochemically controlled patients progress differently throughout time in terms of QoL than their uncontrolled counterparts by investigating an interaction between time and biochemical control. Variables that yielded a significant contribution in the baseline model were implemented as factors/covariates in all longitudinal models, as well as age, gender, disease duration, and basal hormonal levels, to account for confounding. Normality was confirmed by examining normal probability plots. Significance was set at the 0.05 level.

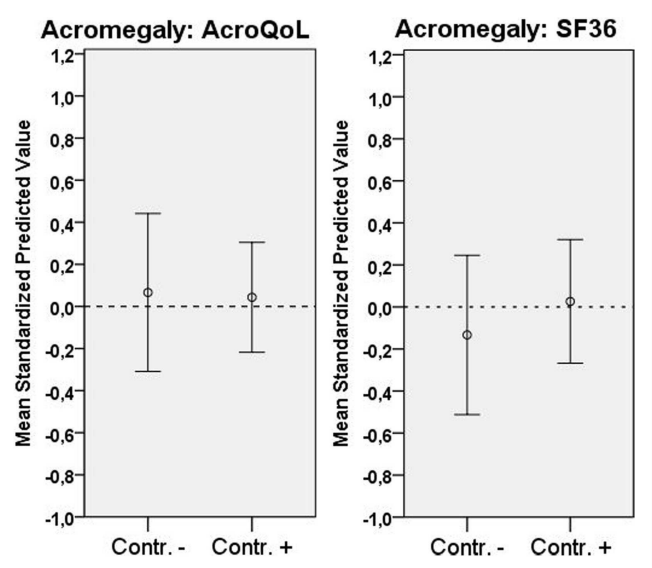


**FIGURE 1 | Distribution of QoL according to scale (mean standardized predicted values), and biochemical status**. Error bars represent 95% CI.

### MISSING VALUES

If 15% or more of the data from a questionnaire or scale was missing, data from that variable was excluded from analysis. Remaining missing values were filled in according to the corresponding scoring instructions or by using the median from the separate items if the scoring instructions lacked a suggestion for dealing with missing values. An exception was made for the covariate biochemical control; missing data in this domain resulted in exclusion from analysis for that patient.

## RESULTS

### DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Mean age for the total acromegalic cohort was $54.7 \pm 12.3$ years with an average disease duration of $10.8 \pm 10.0$ years. Fifty-four patients had a macroadenoma, 26 patients had a microadenoma.

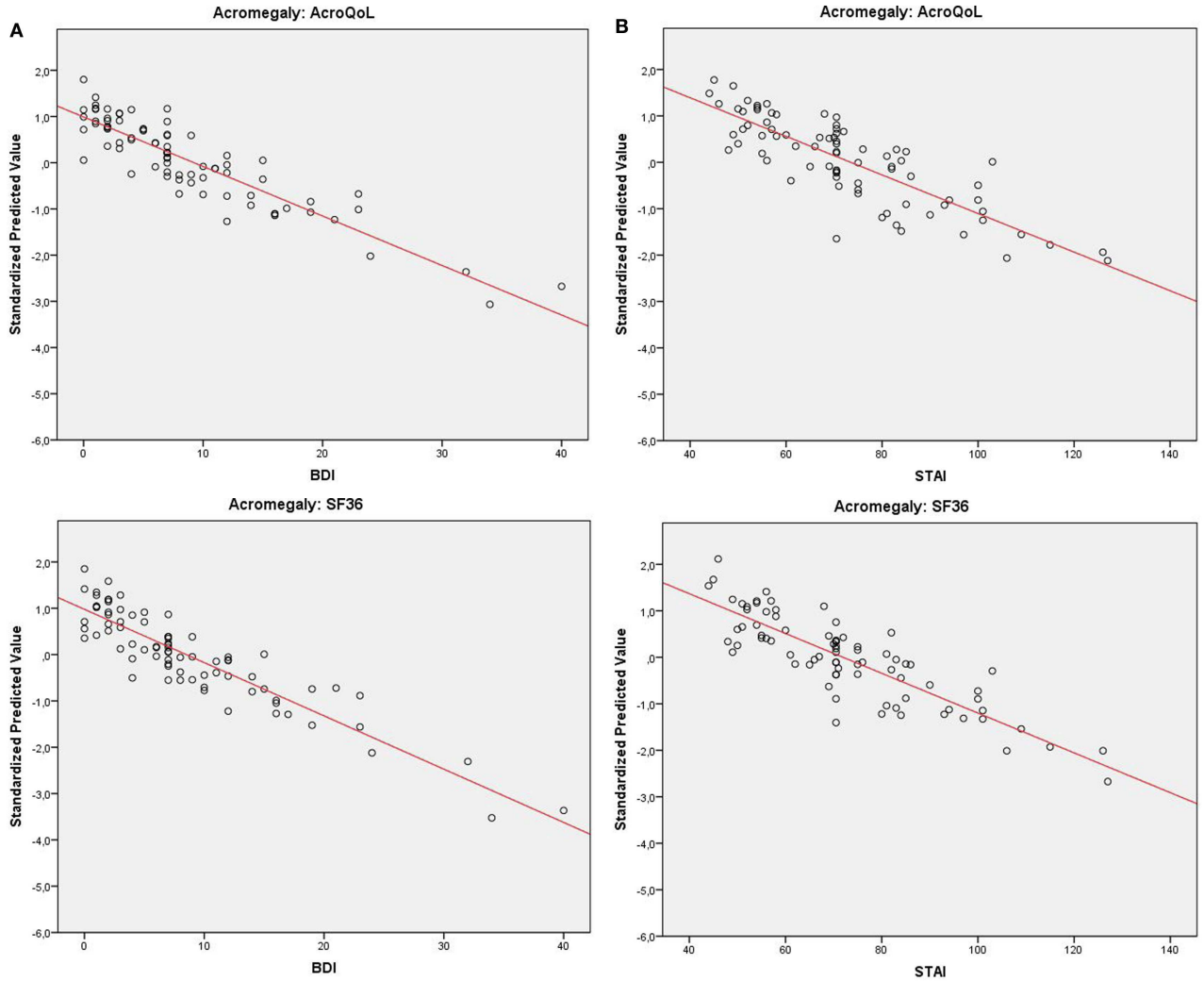


**FIGURE 2 | (A)** Distribution of QoL in acromegaly (standardized predicted values) relative to depressive symptoms with fitted trend-line. **(B)** Distribution of QoL in acromegaly (standardized predicted values) relative to anxiety with fitted trend-line.

At baseline, 31 patients were biochemically uncontrolled, and 49 patients were biochemically controlled. Mean basal hormonal level was $2.8 \pm 5.0\,\mu g/l$ for GH and $214.2 \pm 161.2$ nmol/l, for IGF-1.

For the follow-up analyses, 36 patients participated, mean follow-up time was $7.1 \pm 0.7$ years – 15 patients remained uncontrolled, whereas 21 patients were biochemically controlled at follow-up. Demographic and clinical characteristics (cross-sectional and longitudinal) are shown in **Table 1**.

### ASSOCIATION BETWEEN PSYCHOPATHOLOGY/BIOCHEMICAL CONTROL AND QoL AT BASELINE

At baseline, our analyses demonstrate that depressive symptoms significantly predicted QoL measured by AcroQoL ($p < 0.001$) and SF-36 ($p < 0.001$) in acromegaly in a model including age, gender, disease duration, basal GH levels, tumor size, and possible treatment modalities and comorbidities as covariates. Negative coefficients reflect a greater amount of depressive symptoms to be indicative of a greater impairment of QoL. The amount of variance explained ($\Delta R^2$) by depressive symptoms is 0.261 for the AcroQoL and 0.285 for the SF-36, which was the highest variance among all covariates in models explaining a variance ($R^2$) of 0.637 (AcroQoL) and 0.577 (SF-36).

Similarly, results demonstrate anxiety to be significantly predictive for the AcroQoL ($p < 0.001$) and the SF-36 ($p < 0.001$) in acromegaly. Negative coefficients reflect a greater amount of anxiety to be indicative of a greater impairment of QoL. $\Delta R^2$ is 0.149 for AcroQoL and 0.256 for SF-36 in models with an $R^2$ of 0.505 (AcroQoL) and 0.543 (SF-36).

Biochemical control was not significantly associated with QoL in acromegaly.

Coefficients, $p$-values, and explained variances ($R^2$) are shown in **Table 2**, **Figure 1** (biochemical control), and **Figure 2** (psychopathology).

### ASSOCIATION BETWEEN PSYCHOPATHOLOGY/BIOCHEMICAL CONTROL AND QoL AT FOLLOW-UP

At follow-up (longitudinal analyses), results demonstrate depressive symptoms to be significantly predictive of QoL measured by AcroQoL ($p < 0.001$) and SF-36 ($p = 0.001$) in acromegaly in a similar model with age, gender, disease duration, basal GH levels, tumor size, and possible treatment modalities and comorbidities as covariates. Similarly, results demonstrate anxiety to be predictive of QoL measured by AcroQoL ($p < 0.001$) and SF-36 ($p < 0.001$) in acromegaly. Negative estimates reflect a greater amount of depressive symptoms/anxiety to be indicative of impaired QoL.

Biochemical control was otherwise not significantly predictive of QoL in other analyses.

Estimates and *p*-values are shown in **Table 3**.

**Table 3 | Association of psychopathology/biochemical control and QoL at follow-up**.

| Disease (scale) | Variable | Estimate (SE)[a] | P* |
|---|---|---|---|
| Acromegaly (AcroQoL)[b] | **Depressive symptoms** | **−1.213 (0.154)** | **<0.001** |
| | Biochemical control | −1.973 (2.910) | 0.500 |
| | **Anxiety** | **−0.405 (0.074)** | **<0.001** |
| | Biochemical control | −1.281 (3.473) | 0.713 |
| Acromegaly (SF-36)[c] | **Depressive symptoms** | **−1.601 (0.236)** | **0.001** |
| | Biochemical control | 2.420 (4.072) | 0.555 |
| | **Anxiety** | **−0.666 (0.087)** | **<0.001** |
| | Biochemical control | 3.647 (4.471) | 0.418 |

**p-Values computed using linear mixed models.*

*[a]All models carried a correction for age, gender, disease duration, basal hormone levels, and tumor size.*

*Additional correction for:*

*[b]radiation, pathological glucose intolerance and arthralgia,*

*[c]radiation, arthralgia.*

*Bold font marks those results with statistical significance.*

**Table 4 | Association of psychopathology/biochemical control and progression of QoL**.

| Disease (scale) | Variable | Estimate (SE)[a] | P* |
|---|---|---|---|
| Acromegaly (AcroQoL)[b] | Depressive symptoms*time | −0.107 (0.222) | 0.632 |
| | Biochemical control*time | 0.272 (3.244) | 0.934 |
| | Anxiety*time | −0.005 (0.041) | 0.900 |
| | Biochemical control*time | 0.434 (3.743) | 0.908 |
| Acromegaly (SF-36)[c] | Depressive symptoms*time | −0.121 (0.335) | 0.922 |
| | Biochemical control*time | −1.512 (4.823) | 0.926 |
| | Anxiety*time | −0.052 (0.047) | 0.274 |
| | Biochemical control*time | −1.712 (4.271) | 0.691 |

**p-Values computed using linear mixed models.*

*[a]All models carried a correction for age, gender, disease duration, basal hormone levels and tumor size.*

*Additional correction for:*

*[b]radiation, pathological glucose intolerance, and arthralgia,*

*[c]radiation, arthralgia.*

### ASSOCIATION BETWEEN PSYCHOPATHOLOGY/BIOCHEMICAL CONTROL AND PROGRESSION OF QoL THROUGHOUT TIME

Interaction between time and depressive symptoms, time and anxiety, and time and biochemical control was not significantly associated with QoL in acromegaly, indicating no influence of depressive symptoms, anxiety, or biochemical control on the progression of QoL throughout time. Estimates and *p*-values are shown in **Table 4** and **Figure 3**.

## DISCUSSION

The aim of this study was to analyze the relative impact of previously described predictors in relation to psychopathology on the patient-related health outcome "QoL" in acromegaly.

Since most of the known variables associated with a poor health-related QoL in acromegaly are not modifiable (such as age, gender, disease duration and tumor size), we focused on psychopathology, which would reflect a treatable component that is commonly underdiagnosed.

Although the relationship between QoL and psychopathology has been described before (20–22) in parallel to a solid recognition of reduced QoL in pituitary adenomas and acromegaly (2,

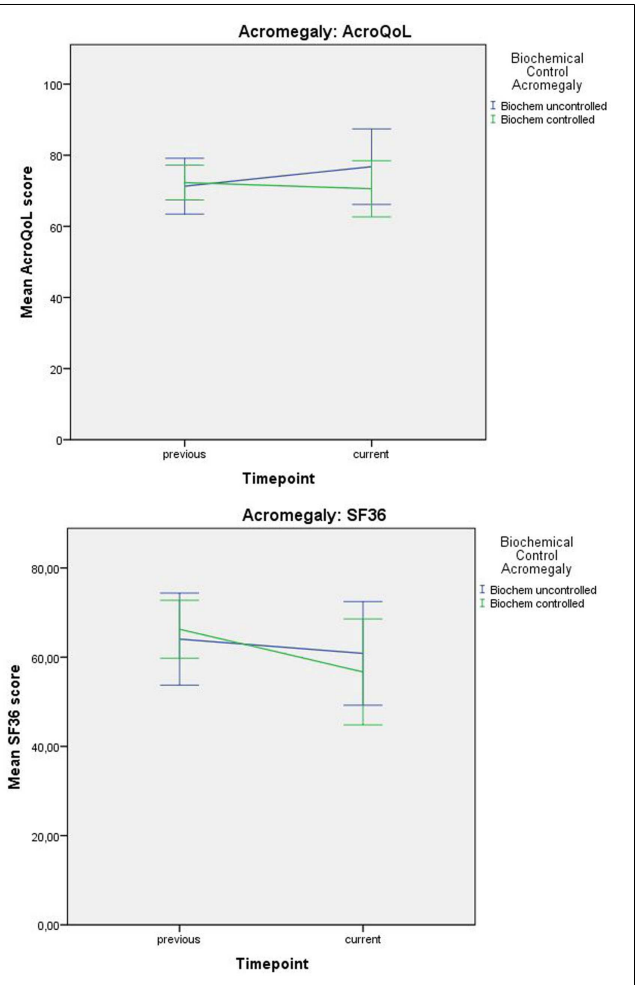


**FIGURE 3 | Progression of QoL (mean values) in acromegaly relative to biochemical status**. Error bars represent 95% CI.

3), we found that the marked reduction of QoL is driven dominantly by psychopathology rather than biochemical control or other factors, which is new information. A potential reason for this observation is that hormonal factors in acromegaly are not necessarily noticeable on a daily basis whereas psychopathological factors are much more dominantly present. The overbearing character of psychopathology may therefore exert a larger influence on a patient's QoL than the less obvious biochemical control.

It has been previously described that psychopathology is an independent predictor of QoL rather than a masked way of measuring QoL in pituitary patients (6). The scientific implications of our research are augmented by the World Health Organization, which has long recognized the crucial role of QoL in patient-oriented clinical approaches (33). Moreover, a clear association between high QoL scores and a longer survival duration in cancer patients has been described previously (34). These results are in agreement with an earlier study that argues the importance of adding a QoL component rather than sole biochemical considerations in order to improve patient management (35). The findings that psychopathology, rather than biochemical control, drives reduced QoL based on the demonstrated explained variances complements the well-recognized reduction of QoL in pituitary adenomas and leads to our recommendation to place greater emphasis on the role of psychopathology in acromegaly.

Aside from scientific implications, key in the clinical application of this research is the finding that important predictors of reduced QoL are depressive symptoms and anxiety, which are essentially modifiable predictors. A more complex treatment strategy including a more extensive psychopathological evaluation and therapy may be an attractive possibility to improve patient management in pituitary adenomas and especially acromegaly.

### STRENGTHS AND LIMITATIONS

Strengths of our study are the longitudinal design, the two-center approach, the large amount of potential confounders that are accounted for and the usage of validated and disease-specific questionnaires. Furthermore, our study yields new and additional information to expand on previous research and has obvious clinical relevance.

Limitations of our study as in each and every longitudinal study are the potential bias that is introduced due to a preferential "loss-to-follow-up." Reasons for this non-response were, e.g., disinterest to participate in a large questionnaire, feeling of being cured, and associated disinterest to participate in medical research, deterioration, and associated inability to fill out questionnaires or death. The reasons for non-compliance in our study were not systematically studied. Additionally, not all potential influential factors have been included in the study, e.g., no available data on antidepressant drug-usage, which may be influential on the severity of the depressive symptoms.

Future research should focus on improving the response rates and validate the observed findings in preferably larger cohorts. Systematic reviews of the literature should attest whether there are other (ideally modifiable) predictors of QoL in acromegaly aside from psychopathology to identify multiple targets for improving QoL (research in progress). Trials with modifying these variables could ultimately verify their clinical applicability (protocol submitted for ethical vote, phase 4 trial EudraCT 2014-000265-43).

## CONCLUSION

Results indicate biochemical control of acromegaly to be unassociated with both generic and disease-specific QoL. Psychopathology seems to predominantly drive reduced QoL in acromegaly. Hence, we recommend scrutinous systematic screening for psychopathology leading to subsequent specific therapy in acromegaly to test the effect on improving QoL.

## REFERENCES


1. Guilbert JJ. The world health report 2. *Educ Health (Abingdon)* (2003) **16**(2):230. doi:10.1080/1357628031000116808
2. Crespo I, Santos A, Resmini E, Valassi E, Martínez-Mombían MA, Webb SM. Improving quality of life in patients with pituitary tumours. *Eur Endocrinol* (2013) **9**(1):32–6.
3. Johnson MD, Woodburn CJ, Vance ML. Quality of life in patients with a pituitary adenoma. *Pituitary* (2003) **6**(2):81–7. doi:10.1023/B:PITU.0000004798.27230.ed
4. van der Klaauw AA, Biermasz NR, Hoftijzer HC, Pereira AM, Romijn JA. Previous radiotherapy negatively influences quality of life during 4 years of follow-up in patients cured from acromegaly. *Clin Endocrinol (Oxf)* (2008) **69**(1):123–8. doi:10.1111/j.1365-2265.2007.03169.x
5. van der Klaauw AA, Kars M, Biermasz NR, Roelfsema F, Dekkers OM, Corssmit EP, et al. Disease-specific impairments in quality of life during long-term follow-up of patients with different pituitary adenomas. *Clin Endocrinol (Oxf)* (2008) **69**(5):775–84. doi:10.1111/j.1365-2265.2008.03288.x
6. Biermasz NR, van Thiel SW, Pereira AM, Hoftijzer HC, van Hemert AM, Smit JW, et al. Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. *J Clin Endocrinol Metab* (2004) **89**(11):5369–76. doi:10.1210/jc.2004-0669
7. Psaras T, Milian M, Hattermann V, Will BE, Tatagiba M, Honegger J. Predictive factors for neurocognitive function and Quality of Life after surgical treatment for Cushing's disease and acromegaly. *J Endocrinol Invest* (2011) **34**(7):e168–77. doi:10.3275/7333
8. Giustina A, Barkan A, Casanueva FF, Cavagnini F, Frohman L, Ho K, et al. Criteria for cure of acromegaly: a consensus statement. *J Clin Endocrinol Metab* (2000) **85**(2):526–9. doi:10.1210/jcem.85.2.6363
9. Giustina A, Chanson P, Kleinberg D, Bronstein MD, Clemmons DR, Klibanski A, et al. Expert consensus document: a consensus on the medical treatment of acromegaly. *Nat Rev Endocrinol* (2014) **10**(4):243–8. doi:10.1038/nrendo.2014.21
10. Melmed S, Casanueva FF, Klibanski A, Bronstein MD, Chanson P, Lamberts SW, et al. A consensus on the diagnosis and treatment of acromegaly complications. *Pituitary* (2013) **16**(3):294–302. doi:10.1007/s11102-012-0420-x
11. Hua SC, Yan YH, Chang TC. Associations of remission status and lanreotide treatment with quality of life in patients with treated acromegaly. *Eur J Endocrinol* (2006) **155**(6):831–7. doi:10.1530/eje.1.02292
12. T'Sjoen G, Bex M, Maiter D, Velkeniers B, Abs R. Health-related quality of life in acromegalic subjects: data from AcroBel, the Belgian registry on acromegaly. *Eur J Endocrinol* (2007) **157**(4):411–7. doi:10.1530/EJE-07-0356
13. Matta MP, Couture E, Cazals L, Vezzosi D, Bennet A, Caron P. Impaired quality of life of patients with acromegaly: control of GH/IGF-I excess improves psychological subscale appearance. *Eur J Endocrinol* (2008) **158**(3):305–10. doi:10.1530/EJE-07-0697
14. Ben-Shlomo A, Sheppard MC, Stephens JM, Pulgar S, Melmed S. Clinical, quality of life, and economic value of acromegaly disease control. *Pituitary* (2011) **14**(3):284–94. doi:10.1007/s11102-011-0310-7
15. Trepp R, Everts R, Stettler C, Fischli S, Allemann S, Webb SM, et al. Assessment of quality of life in patients with uncontrolled vs. controlled acromegaly using the Acromegaly Quality of Life Questionnaire (AcroQoL). *Clin Endocrinol (Oxf)* (2005) **63**(1):103–10. doi:10.1111/j.1365-2265.2005.02307.x
16. Paisley AN, Rowles SV, Roberts ME, Webb SM, Badia X, Prieto L, et al. Treatment of acromegaly improves quality of life, measured by AcroQol. *Clin Endocrinol (Oxf)* (2007) **67**(3):358–62. doi:10.1111/j.1365-2265.2007.02891.x

17. Anagnostis P, Efstathiadou ZA, Charizopoulou M, Selalmatzidou D, Karathanasi E, Poulasouchidou M, et al. Psychological profile and quality of life in patients with acromegaly in Greece. Is there any difference with other chronic diseases? *Endocrine* (2014) **47**(2):564–71. doi:10.1007/s12020-014-0166-5
18. Sievers C, Ising M, Pfister H, Dimopoulou C, Schneider HJ, Roemmler J, et al. Personality in patients with pituitary adenomas is characterized by increased anxiety-related traits: comparison of 70 acromegalic patients with patients with non-functioning pituitary adenomas and age- and gender-matched controls. *Eur J Endocrinol* (2009) **160**(3):367–73. doi:10.1530/EJE-08-0896
19. Sievers C, Dimopoulou C, Pfister H, Lieb R, Steffin B, Roemmler J, et al. Prevalence of mental disorders in acromegaly: a cross-sectional study in 81 acromegalic patients. *Clin Endocrinol (Oxf)* (2009) **71**(5):691–701. doi:10.1111/j.1365-2265.2009.03555.x
20. Rapaport MH, Clary C, Fayyad R, Endicott J. Quality-of-life impairment in depressive and anxiety disorders. *Am J Psychiatry* (2005) **162**(6):1171–8. doi:10.1176/appi.ajp.162.6.1171
21. Angermeyer MC, Holzinger A, Matschinger H, Stengler-Wenzke K. Depression and quality of life: results of a follow-up study. *Int J Soc Psychiatry* (2002) **48**(3):189–99. doi:10.1177/002076402128783235
22. Mendlowicz MV, Stein MB. Quality of life in individuals with anxiety disorders. *Am J Psychiatry* (2000) **157**(5):669–82. doi:10.1176/appi.ajp.157.5.669
23. Wilson IB, Cleary PD. Linking clinical variables with health-related quality of life. A conceptual model of patient outcomes. *JAMA* (1995) **273**(1):59–65. doi:10.1001/jama.1995.03520250075037
24. Schneider HJ, Aimaretti G, Kreitschmann-Andermahr I, Stalla GK, Ghigo E. Hypopituitarism. *Lancet* (2007) **369**(9571):1461–70. doi:10.1016/S0140-6736(07)60673-4
25. Badia X, Webb SM, Prieto L, Lara N. Acromegaly quality of life questionnaire (AcroQoL). *Health Qual Life Outcomes* (2004) **2**:13. doi:10.1186/1477-7525-2-41
26. McHorney CA, Ware JE Jr., Lu JF, Sherbourne CD. The MOS 36-item Short-Form Health Survey (SF-36): III. Tests of data quality, scaling assumptions, and reliability across diverse patient groups. *Med Care* (1994) **32**(1):40–66. doi:10.1097/00005650-199401000-00004
27. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care* (1992) **30**(6):473–83. doi:10.1097/00005650-199206000-00002
28. Whisman MA, Perez JE, Ramel W. Factor structure of the Beck Depression Inventory-Second Edition (BDI-II) in a student sample. *J Clin Psychol* (2000) **56**(4):545–51. doi:10.1002/(SICI)1097-4679(200004)56:4<545::AID-JCLP7>3.0.CO;2-U
29. Spielberger C. Assessment of state and trait anxiety: conceptual and methodological issues. *South Psychol* (1985) **2**(4):6–16.
30. Field A. *Discovering Statistics using SPSS*. 3 ed. London: SAGE Publications Ltd (2009).
31. Morley JF, Xie SX, Hurtig HI, Stern MB, Colcher A, Horn S, et al. Genetic influences on cognitive decline in Parkinson's disease. *Mov Disord* (2012) **27**(4):512–8. doi:10.1002/mds.24946
32. Siderowf A, Xie SX, Hurtig H, Weintraub D, Duda J, Chen-Plotkin A, et al. CSF amyloid {beta} 1-42 predicts cognitive decline in Parkinson disease. *Neurology* (2010) **75**(12):1055–61. doi:10.1212/WNL.0b013e3181f39a78
33. Kuyken W, Sartorius N, Power M, Orley J. The World Health Organization Quality of Life assessment (WHOQOL): position paper from the World Health Organization. *Soc Sci Med* (1995) **41**(10):1403–9. doi:10.1016/0277-9536(95)00112-K
34. Montazeri A. Quality of life data as prognostic indicators of survival in cancer patients: an overview of the literature from 1982 to 2008. *Health Qual Life Outcomes* (2009) **7**:102. doi:10.1186/1477-7525-7-102
35. Haring R, Feng YS, Moock J, Volzke H, Dorr M, Nauck M, et al. Self-perceived quality of life predicts mortality risk better than a multi-biomarker panel, but the combination of both does best. *BMC Med Res Methodol* (2011) **11**:103. doi:10.1186/1471-2288-11-103

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# Neuropsychiatric disorders in Cushing's syndrome

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Endogenous Cushing's syndrome (CS), a rare endocrine disorder characterized by cortisol hypersecretion, is associated with psychiatric and neurocognitive disorders. Major depression, mania, anxiety, and neurocognitive impairment are the most important clinical abnormalities. Moreover, patients most often complain of impairment in quality of life, interference with family life, social, and work performance. Surprisingly, after hypercortisolism resolution, despite the improvement of the overall prevalence of psychiatric and neurocognitive disorders, the brain volume loss at least partially persists and it should be noted that some patients may still display depression, anxiety, panic disorders, and neurocognitive impairment. This brief review aimed at describing the prevalence of psychiatric and neurocognitive disorders and their characterization both during the active and remission phases of CS. The last section of this review is dedicated to quality of life, impaired during active CS and only partially resolved after resolution of hypercortisolism.

**Keywords: Cushing's syndrome, psychiatric disorders, neurological disorders, cognitive impairment, quality of life**

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## Introduction

Chronic glucocorticoid (GC) overproduction by the adrenal glands represents the cause of endogenous Cushing's syndrome (CS), a rare but serious endocrine disorder. In approximately 80% of cases, endogenous CS is consequence of an ACTH hypersecretion (ACTH-dependent CS), generally due to a corticotroph pituitary tumor (Pituitary-dependent CS, or Cushing's disease, CD), and rarely due to an ACTH-secreting or CRH-secreting extra-pituitary tumor (Ectopic Cushing Syndrome, ECS), whereas in the remaining 20% of cases CS is independent from ACTH hypersecretion (ACTH-independent CS) and GC excess is direct consequence of autonomous overproduction by the adrenal glands, because of unilateral adrenocortical tumors or bilateral adrenal hyperplasia, or dysplasia (Arnaldi et al., 2003; Newell-Price et al., 2006; Pivonello et al., 2008). Chronic endogenous GC exposure determines several clinical complications, including metabolic complications such as visceral obesity, insulin resistance with glucose intolerance and diabetes mellitus, and dyslipidemia, which configure a metabolic syndrome, cardiovascular complications such as systemic arterial hypertension, atherosclerosis, thromboembolism, bone complications such as osteoporosis and osteoarthritis, infective complications, ranging from an increase susceptibility to infections up to a fatal sepsis, as well as neuropsychiatric disorders (Pivonello et al., 2005, 2010; Tauchmanovà et al., 2006; De Leo et al., 2010; Pereira et al., 2010; van der Pas et al., 2013). These clinical complications negatively impact the quality of

life (QoL) of patients with CS and increase their morbidity and mortality, mainly due to cardiovascular and infective diseases (Gotch, 1994; Webb et al., 2008; Clayton, 2010). The current management of patients with CS cannot ignore psychiatric and neurocognitive disorders, their evolution during the active disease and after disease remission, and their impact on QoL. The first description of neuropsychiatric disturbances in CS occurred in 1932 when, in his original description of a series of 12 CS cases, Harvey Cushing highlighted the presence of "emotional disturbances" as a pathologic feature of CS (Cushing, 1932). During the following years, several studies were performed with the purpose to better characterize the spectrum and the frequency of neuropsychiatric disorders in patients with CS (Sonino and Fava, 2001). These disorders include psychiatric disorders such as major depression, mania and anxiety, and neurocognitive disorders, mainly characterized by impairment of memory and concentration (Dorn et al., 1997; Sonino and Fava, 2001). Neuropsychiatric disorders contribute to significantly impair health-related QoL (Gotch, 1994; Webb et al., 2008). The aim of this brief review was to describe the main psychiatric and neurocognitive disorders observed in patients with endogenous CS during active disease and their development after disease remission, also discussing their impact on QoL. Additionally, this brief review aimed at addressing a simple overview on this topic, highlighting the important role of long-term follow-up and careful periodical investigation of psychiatric and neurocognitive disorders in the management of CS both in the active phase and after disease remission.

## Neuropsychiatric Disorders during Active Disease

GCs have a crucial role in stress response and GC receptors have a pleiotropic distribution in central nervous system (CNS), mainly in the hippocampus; therefore, it is not surprising that chronic GC excess can lead to structural and functional changes in CNS, which are mainly based on brain atrophy (De Kloet et al., 1998; Sonino et al., 2010). The mechanisms by which GCs induce brain damage are largely unknown although four theories have been suggested. (1) The decrease of glucose uptake is responsible of brain atrophy. According with this theory, GCs induce brain damage by reducing glucose utilization in brain, as supported by the evidence of a generalized reduction in cerebral glucose metabolism in all areas of the brain in patients with CD. (2) The increase of excitatory aminoacids is responsible of toxic effects on nervous cells. GCs increase the release or enhance the effects of excitatory aminoacids, such as glutamate, which cause cell damage inducing the dendritic atrophy, particularly in the hippocampus. (3) The inhibition of "long-term potentiation" is responsible of cognitive deficits. GCs reduce the synthesis of neurotrophic factors, such as nerve growth factor-b and brain-derived neurotrophic factor, which through a presynaptic mechanism inhibit the long-term potentiation that is believed to be the mechanism behind learning process and memory formation. Additionally, this neurotrophic factors reduction can also be responsible of brain atrophy. (4) The suppression of neurogenesis in the dentate gyrus. GCs excess may suppress neurogenesis in the dentate gyrus, which determine hippocampal volume loss (De Kloet et al., 1998; Jacobs et al., 2000; Simmons et al., 2000; Bourdeau et al., 2002; Patil et al., 2007; Michaud et al., 2009). All these mechanisms seem to explain the GC-induced brain and, mainly, hippocampal damage responsible for neurocognitive disorders, although the atrophy of the prefrontal cortex (Patil et al., 2007) or the suppression of neurogenesis in the dentate gyrus (Jacobs et al., 2000) have been specifically identified as crucial pathophysiological events for the development of depression.

The most important psychiatric disorders observed in patients with active CS are: (1) major depression, which include alterations in mood, affective, vegetative, and cognitive functions; and (2) mania and anxiety. Major depression represents the most frequent and severe psychiatric disorder associated with chronic endogenous hypercortisolism. The prevalence of major depression in patients with CS is reported to be about 50–81%, as summarized in **Table 1** (Haskett, 1985; Hudson et al., 1987; Loosen et al., 1992; Sonino et al., 1993, 1998; Dorn et al., 1995; Kelly, 1996). Focusing on CD, Sonino and co-workers reported major depression to occur in 54% of 162 patients and observed that it was significantly associated with female gender, older ages, higher urinary cortisol levels, relatively more severe clinical conditions and undetectable pituitary tumor (Sonino et al., 1998). Different degrees of severity were reported, ranging from latent to very severe melanchonic forms, also associated with suicide thoughts and attempts (Starkman et al., 1981; Starkman, 2013). Therefore, a careful periodic investigation of psychiatric symptoms should be performed in all patients with active CS, also taking in consideration that depression in these patients is typically characterized by intermittent phases, with episodes of exacerbation recurring very frequently at irregular intervals (Starkman et al., 1981; Starkman, 2013). Additionally, all domains of the depressive syndrome including mood, affect, vegetative, and cognitive functions can be compromised (Starkman, 2013). As far as mood alterations are concerned, many patients with CS up to 86%, show irritability, which often has early onset, appearing even before than other clinical symptoms and signs (Starkman et al., 1981; Starkman, 2013). It has been reported that patients with CS described themselves as having an increased sensitivity and they perceived themselves unable to ignore minor irritations and feeling "impatient," with "over-reactivity," generalized hypersensitivity to stimuli and an easy development of anger (Starkman et al., 1981). Depressed mood has been reported in 74% of CS patients, without regular cyclicity, although a common depressive episode persists usually 1–2 days and rarely more than 3 days per week (Starkman, 2013). In some patients depressed mood could be present on awakening and remain throughout the day or the next day as well (Starkman et al., 1981); in other patients, the onset of depressed mood occurs suddenly during the day, determining "mood lability" (Starkman et al., 1981). Moreover, in patients with CS, depressed mood has been reported to be often characterized by hypersensitivity and oversentimentality, increased feeling of crying, short spells of sadness, and less frequently constant hopelessness, social withdrawal with feelings of discomfort in large groups, intermittent inability to experience pleasure, which rarely reaches the

**TABLE 1 | Prevalence of major depression in patients with active CS (adapted from Sonino and Fava, 2001).**

| References | Diagnosis | No. of PTS | Major depression: No. of PTS (%) |
|---|---|---|---|
| Sonino et al., 1998 | CD | 162 | 88 (54) |
| Haskett, 1985 | All forms | 30 | 24 (80) |
| Hudson et al., 1987 | CD | 16 | 9 (56) |
| Loosen et al., 1992 | CD | 20 | 13 (65) |
| Sonino et al., 1993 | CS excluding CD | 20 | 10 (50) |
| Kelly, 1996 | All forms | 209 | 120 (57) |
| Dorn et al., 1995 | All forms | 33 | 17 (51) |
| Total | | 490 | 281 (57) |

*CS, Cushing's syndrome; CD, Cushing's disease.*

levels of persistent anhedonia, and less often self-accusatory or irrational guilt (Starkman et al., 1981; Starkman, 2013). Conversely, a minority of patients, particularly in the first phase of active hypercortisolism, experiences elevated mood with episodes of hyperactivity, increased ambition and restlessness that generally disappears with the time over disease progression (Starkman et al., 1981; Starkman, 2013). Suicidal thoughts (17%) and suicide attempts (5%) have also been described (Starkman et al., 1981; Starkman, 2013) and frequently minimized by patients (Haskett, 1985; Sonino and Fava, 2001). In patients with active hypercortisolism symptoms and signs involving the vegetative functions are frequently reported. Indeed, fatigue has been described in almost all patients and a decreased libido in about two-thirds (Starkman et al., 1981). Disturbance of appetite and sleep have also been described; particularly, an increased or decreased appetite have been found in about 34% and 20% of patients, respectively (Starkman et al., 1981) and sleep disturbances such as middle insomnia (patients awakened at least one during the night) in 69% of CS patients, late insomnia (patients awakened earlier than desired in the morning) in 57% and early insomnia (inability to fall asleep at bedtime) in 29% (Starkman et al., 1981; Starkman, 2013). In about one third of patients, it has also been reported an alteration in the frequency and type of dreams which became more bizarre and vivid (Starkman et al., 1981; Starkman, 2013). Within the clinical frame of depressive syndrome, patients may also experience cognitive impairments that will be detailed below.

A significant percentage (66%) of CS patients reported generalized anxiety or panic disorders more frequently described in the chronic and advanced stage of active hypercortisolism (Starkman et al., 1981; Starkman, 2013). Also bipolar disorders including maniac and hypomaniac episodes have been observed in about 30% of CS patients and may represent an early manifestation of CS (Haskett, 1985; Hudson et al., 1987).

Neurocognitive impairment has been reported in about two thirds of CS patients with variable degrees from mild to severe (Whelan et al., 1980). An impairment of memory has been reported in 83% of CS patients, consisting of difficulty in processing new information and forgetfulness of information such as appointments, names of people, location of objects, important dates in their personal, and/or medical histories (Starkman et al., 1981; Starkman, 2013). Impaired concentration has been reported in 66% of CS patients that particularly complain of mind-wandering when reading, watching television and during the course of conversations, a decreased ability to "focus their minds" and more in general inattention, distractibility, shortened attention span, difficulties with reasoning ability, and comprehension (Starkman et al., 1981; Starkman, 2013). Moreover, disturbances in "thinking" may occur. Indeed, patients with CS can experience episodes of rapid and scattered thinking, or slow thinking with blocks, that are described as a mind, which suddenly "becomes blank" (Starkman, 2013). In addition, impairment in verbal control, non-verbal, visual, and spatial abilities has also been reported in CS patients (Starkman et al., 1981; Sonino and Fava, 2001; Starkman, 2013).

## Neuropsychiatric Disorders after Disease Remission

Several studies have reported that, after disease remission, the resolution of hypercortisolism, namely the normalization of cortisol secretion, is not constantly followed by the complete recovery of many clinical complications developed during the active disease; in particular, metabolic syndrome and the consequent cardiovascular risk have been found to partially persist after disease remission (Colao et al., 1999; Faggiano et al., 2003; Pivonello et al., 2005, 2007). Skeletal diseases, mainly osteoporosis, improve after disease remission, but the normalization of bone mass may require a long period of time or the use of specific treatments (Faggiano et al., 2001; Pivonello et al., 2007). Similarly, psychiatric and neurocognitive disorders generally improve after disease remission, but several studies reported that these disorders can persist, even long-term after the resolution of hypercortisolism and occasionally they can even exacerbate with the decrease and resolution of hypercortisolism. Consequently, this slow and partial recovery of different clinical alterations occurred during the active phase of the disease can contribute to the persistent impairment of QoL registered in CS patients after disease remission or cure (Sonino and Fava, 2001; Pivonello et al., 2007; Webb et al., 2008; Colao et al., 2012).

To date, the issue of whether remission of CS may completely resolve psychiatric and neurocognitive disorders remains controversial. Several studies demonstrated a significant improvement of psychiatric and neurocognitive disorders after resolution of hypercortisolism (Jeffcoate et al., 1979; Cohen, 1980; Kramlinger et al., 1985; Starkman et al., 1986; van der Lely et al., 1991; Verhelst et al., 1991; Kelly et al., 1996; Wolkowitz and Reus, 1999; Hirsch et al., 2000). In particular, Starkman and coworkers reported a significant improvement in depression scores of patients treated for CD, which were significantly correlated with the decrease in urinary cortisol excretion (Starkman et al., 1986). Different studies described significant improvements or even complete remission of depression, anxiety, irritability, psychosis, and neurocognitive impairment, after normalization of cortisol levels in CS patients treated with radiotherapy, surgery, or medical treatment (Jeffcoate et al., 1979; Cohen, 1980; Kramlinger et al., 1985; Starkman et al., 1986; van der Lely et al., 1991; Verhelst et al., 1991; Kelly et al., 1996; Dorn et al., 1997;

Wolkowitz and Reus, 1999; Hirsch et al., 2000). Particularly, some studies have reported the effects of successful reduction of GCs excess on psychopathology. These studies demonstrate that both reduction of GCs synthesis with ketoconazole or metyrapone and blockade of the GC receptor with mifepristone positively affect psychopathology. Interestingly, mifepristione has been demonstrated to have therapeutic effects in patients with psychotic depression. Additionally, this drug can mitigate the weight gain associated with the use of antipsychotic drugs and improve cognitive dysfunction in bipolar depression (Howland, 2013). However, as mentioned above, more recently, several studies showed that neuropsychiatric improvement, after a successful treatment of CS, might be delayed and incomplete (Dorn and Cerrone, 2000; Sonino and Fava, 2001; Forget et al., 2002; Heald et al., 2004; Merke et al., 2005; Maheu et al., 2008; Tiemensma et al., 2010; Ragnarsson et al., 2012; Resmini et al., 2012; Ragnarsson and Johannsson, 2013). Indeed, both psychiatric and neurocognitive alterations may require long time to recover after remission of hypercortisolism and they may sometimes persist even after CS cure. Long-lasting impairments were reported in many domains of cognitive function including attention, visuospatial orienting, alerting, reasoning, working and speed memory, verbal fluency, reading, and executive functions (Forget et al., 2002; Tiemensma et al., 2010; Ragnarsson et al., 2012; Resmini et al., 2012). Indeed, the brain volume loss, which is considered the main responsible for the neurocognitive decline of active CS patients, has been demonstrated to be partially persistent after remission (Bourdeau et al., 2002), but further studies are still required to determine whether these alterations can fully revert after long-term remission or better after a time long enough to consider the patients definitively cured. Additionally, in patients long-term remitted from CS, proton magnetic resonance spectroscopy, measuring brain metabolites in the hippocampus, suggested neuronal dysfunction or loss and consequently a repair mechanism of glial proliferation (Resmini et al., 2013). These alterations indicated the persistence of a neuronal damage associated with negative effects on hippocampal volume and memory (Resmini et al., 2013). To investigate whether neuropsychiatric disorders may persist in all patients treated for pituitary tumors, regardless the type of hormone secretion, Heald and co-workers compared patients treated for CD with patients treated for acromegaly, macroprolactinomas, or non-functioning pituitary tumors. In this study patients treated for CD appeared to perceive themselves as being more depressed, fatigued, anxious, having poorer physical health and environmental, and social adjustment, showing significantly impaired psychological well-being and psychosocial functioning compared with all other pituitary tumors patients (Heald et al., 2004). All these observations seem to validate that CS, even after resolution of hypercortisolism, has long-lasting adverse effects on CNS, suggesting that depression, anxiety, and neurocognitive impairment, may still dominate the clinical picture even after disease remission. Interestingly, Dorn and co-workers firstly reported that in CS patients some psychiatric symptoms might also exacerbate with cortisol decrease (Dorn et al., 1997). Particularly, it has been reported that the frequency of panic attacks and suicidal ideation increased after CS remission in a subgroup of patients. This phenomenon has been suggested to be due to the relative GC deficiency, which seems to allow unrestrained increase in catecholamines (Dorn et al., 1997). Moreover, in children and adolescents with CS, Merke and co-workers reported a significant decline of cognitive function 1 year after surgical correction of hypercortisolism, despite a rapid resolution of cerebral atrophy was observed (Merke et al., 2005).

Overall these data highlight the importance of a long-term follow-up and careful periodic investigation of neuropsychiatric symptoms in patients with CS also after hypercortisolism resolution, and the importance to consider the management of these disorders as one of the essential outcomes of CS patients.

## Effects of Neuropsychiatric Disorders on Quality of Life

Considering the systemic and neuropsychiatric complications associated with hypercortisolism it is expected that CS patients have impairment in QoL (Colao et al., 2012). Indeed, impairment in QoL has been demonstrated both by generic and disease-specific questionnaires, namely Cushing QoL, which has been reported to be the most appropriate to measure QoL in patients with CS (Webb et al., 2008). Multiple physical and psychological factors can interfere with QoL in CS patients, but the results of the ERCUSYN study, a recent published web-based, multicentre, observational study from 23 European countries, showed that only depression resulted an independent predictor of a lower CS QoL score, suggesting that psychiatric disorders, and mainly depression, may play a pivotal role in affecting QoL in patients with CS (Valassi et al., 2011). Indeed, patients suffering from CS most often complain of changes in physical appearance, depression, emotional instability, fatigue and/or weakness, sleeping difficulties, neurocognitive problems, interference with family life, relations with their partner, and with school/work performance (Gotch, 1994; Colao et al., 2012). As main psychiatric and neurocognitive disorders, the impairment in QoL of patients with CS is only partially resolved after treatment of hypercortisolism and a longer-term follow-up showed that a residual impairment of QoL may persist after long-term disease remission (Lindsay et al., 2006; Wagenmakers et al., 2012). Indeed, patients in remission upon proper treatment for CS displayed significantly higher scores in depression (feeling tired, guilty, hopeless, unworthy, poor appetite, loss of interest), anxiety (being nervous, tense, panicky, difficulties falling asleep, or early morning awakening), and psychotic symptoms (feelings of persecution, delusions, hallucinations), with a generalized compromised QoL compared with healthy subjects (Sonino et al., 2006). Particularly in patients with CD, despite long-term remission, QoL appeared reduced compared with reference values from the literature, especially in the presence of pituitary insufficiencies (van Aken et al., 2005). Interestingly, also in children and adolescents, CS is associated with impaired QoL, with residual impairment 1-year after treatment, suggesting that QoL is another important parameter of outcome also in these

patients and that the identification of factors that contribute to impair QoL may help to diminish the physical and psychological burden of disease in this population of patients (Keil et al., 2009).

## Conclusions

In conclusion, CS is frequently associated with an high prevalence of psychiatric and neurocognitive disorders and with a significant impairment in QoL. Major depression represents the most important and life-threatening psychiatric complication, occurring in approximately 50–60% of patients, but mania and anxiety are also reported in many cases. Additionally, neurocognitive disorders may occur both as a manifestation within the depressive syndrome clinical frame, and separately as isolated neurological disorder. It should be highlighted that psychiatric and mainly neurocognitive disorders, even long-term after CS remission, although generally improve, may persist, or even worsen, contributing to the persistent impairment of QoL registered also in patients treated for CS. Therefore, long-term follow-up and careful periodical investigation of psychiatric and neurocognitive symptoms should be always considered in the management of CS both in the active phase and after disease remission.

## References


Arnaldi, G., Angeli, A., Atkinson, A. B., Bertagna, X., Cavagnini, F., Chrousos, G. P., et al. (2003). Diagnosis and complications of Cushing's syndrome: a consensus statement. *J. Clin. Endocrinol. Metab.* 88, 5593–5602. doi: 10.1210/jc.2003-030871

Bourdeau, I., Bard, C., Noël, B., Leclerc, I., Cordeau, M. P., Bélair, M., et al. (2002). Loss of brain volume in endogenous Cushing's syndrome and its reversibility after correction of hypercortisolism. *J. Clin. Endocrinol. Metab.* 87, 1949–1954. doi: 10.1210/jc.87.5.1949

Clayton, R. N. (2010). Mortality and morbidity in Cushing's disease. *Neuroendocrinology* 92(Suppl. 1), 71–76. doi: 10.1159/000315813

Cohen, S. I. (1980). Cushing's syndrome: a psychiatric study of 29 patients. *Br. J. Psychiatry* 136, 120–124. doi: 10.1192/bjp.136.2.120

Colao, A., Cozzolino, A., and Pivonello, R. (2012). Quality of life in patients with Cushing's disease: a modern approach. *Clin. Endocrinol. (Oxf.)* 76, 776–777. doi: 10.1111/j.1365-2265.2012.04344.x

Colao, A., Pivonello, R., Spiezia, S., Faggiano, A., Ferone, D., Filippella, M., et al. (1999). Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. *J. Clin. Endocrinol. Metab.* 84, 2664–2672. doi: 10.1210/jc.84.8.2664

Cushing, H. (1932). The basophil adenomas of the pituitary body and their clinical manifestations (pituitary basophilism). *Bull. Johns Hopkins Hosp.* 50, 137–195.

De Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., and Joëls, M. (1998). Brain corticosteroid receptor balance in health and disease. *Endocr. Rev.* 19, 269–301.

De Leo, M., Pivonello, R., Auriemma, R. S., Cozzolino, A., Vitale, P., Simeoli, C., et al. (2010). Cardiovascular disease in Cushing's syndrome: heart versus vasculature. *Neuroendocrinology* 92(Suppl. 1), 50–54. doi: 10.1159/000318566

Dorn, L. D., Burgess, E. S., Dubbert, B., Simpson, S. E., Friedman, T., Kling, M., et al. (1995). Psychopathology in patients with endogenous Cushing's syndrome: 'atypical' or melancholic features. *Clin. Endocrinol. (Oxf.)* 43, 433–442. doi: 10.1111/j.1365-2265.1995.tb02614.x

Dorn, L. D., Burgess, E. S., Friedman, T. C., Dubbert, B., Gold, P. W., and Chrousos, G. P. (1997). The longitudinal course of psychopathology in Cushing's syndrome after correction of hypercortisolism. *J. Clin. Endocrinol. Metab.* 82, 912–919. doi: 10.1210/jc.82.3.912

Dorn, L. D., and Cerrone, P. (2000). Cognitive function in patients with Cushing syndrome: a longitudinal perspective. *Clin. Nurs. Res.* 9, 420–440. doi: 10.1177/10547730022158672

Faggiano, A., Pivonello, R., Filippella, M., Di Somma, C., Orio, F. Jr., Lombardi, G., et al. (2001). Spine abnormalities and damage in patients cured from Cushing's disease. *Pituitary* 4, 153–161. doi: 10.1023/A:1015362822901

Faggiano, A., Pivonello, R., Spiezia, S., De Martino, M. C., Filippella, M., Di Somma, C., et al. (2003). Cardiovascular risk factors and common carotid artery caliber and stiffness in patients with Cushing's disease during active disease and 1 year after disease remission. *J. Clin. Endocrinol. Metab.* 88, 2527–2533. doi: 10.1210/jc.2002-021558

Forget, H., Lacroix, A., and Cohen, H. (2002). Persistent cognitive impairment following surgical treatment of Cushing's syndrome. *Psychoneuroendocrinology* 27, 367–383. doi: 10.1016/S0306-4530(01)00059-2

Gotch, P. M. (1994). Cushing's syndrome from the patient's perspective. *Endocrinol. Metab. Clin. North Am.* 23, 607–617.

Haskett, R. F. (1985). Diagnostic categorization of psychiatric disturbance in Cushing's syndrome. *Am. J. Psychiatry* 142, 911–916. doi: 10.1176/ajp.142.8.911

Heald, A. H., Ghosh, S., Bray, S., Gibson, C., Anderson, S. G., Buckler, H., et al. (2004). Long-term negative impact on quality of life in patients with successfully treated Cushing's disease. *Clin. Endocrinol. (Oxf.)* 61, 458–465. doi: 10.1111/j.1365-2265.2004.02118.x

Hirsch, D., Orr, G., Kantarovich, V., Hermesh, H., Stern, E., and Blum, I. (2000). Cushing's syndrome presenting as a schizophrenia-like psychotic state. *Isr. J. Psychiatry Relat. Sci.* 37, 46–50.

Howland, R. H. (2013). Mifepristone as a therapeutic agent in psychiatry. *J. Psychosoc. Nurs. Ment. Health Serv.* 51, 11–14. doi: 10.3928/02793695-20130513-01

Hudson, J. I., Hudson, M. S., Griffing, G. T., Melby, J. C., and Pope, H. G. Jr. (1987). Phenomenology and family history of affective disorder in Cushing's disease. *Am. J. Psychiatry* 144, 951–953. doi: 10.1176/ajp.144.7.951

Jacobs, B. L., van Praag, H., and Gage, F. H. (2000). Adult brain neurogenesis and psychiatry: a novel theory of depression. *Mol. Psychiatry* 5, 262–269. doi: 10.1038/sj.mp.4000712

Jeffcoate, W. J., Silverstone, J. T., Edwards, C. R., and Besser, G. M. (1979). Psychiatric manifestations of Cushing's syndrome: response to lowering of plasma cortisol. *Q. J. Med.* 48, 465–472.

Keil, M. F., Merke, D. P., Gandhi, R., Wiggs, E. A., Obunse, K., and Stratakis, C. A. (2009). Quality of life in children and adolescents one-year after cure of Cushing syndrome: a prospective study. *Clin. Endocrinol.* 71, 326–333. doi: 10.1111/j.1365-2265.2008.03515.x

Kelly, W. F. (1996). Psychiatric aspects of Cushing's syndrome. *Q. J. Med.* 89, 543–551. doi: 10.1093/qjmed/89.7.543

Kelly, W. F., Kelly, M. J., and Faragher, B. (1996). A prospective study of psychiatric and psychological aspects of Cushing's syndrome. *Clin. Endocrinol.* 45, 715–720. doi: 10.1046/j.1365-2265.1996.8690878.x

Kramlinger, K. G., Peterson, G. C., Watson, P. K., and Leonard, L. L. (1985). Metyrapone for depression and delirium secondary to Cushing's syndrome. *Psychosomatics* 26, 67–71. doi: 10.1016/S0033-3182(85)72906-4

Lindsay, J. R., Nansel, T., Baid, S., Gumowski, J., and Nieman, L. K. (2006). Long term impaired quality of life in Cushing's syndrome despite initial improvement after surgical remission. *J. Clin. Endocrinol. Metab.* 91, 447–453. doi: 10.1210/jc.2005-1058

Loosen, P. T., Chambliss, B., DeBold, C. R., Shelton, R., and Orth, D. N. (1992). Psychiatric phenomenology in Cushing's disease. *Pharmacopsychiatry* 25, 192–198. doi: 10.1055/s-2007-1014405

Maheu, F. S., Mazzone, L., Merke, D. P., Keil, M. F., Stratakis, C. A., Pine, D. S., et al. (2008). Altered amygdala and hippocampus function in adolescents with hypercortisolemia: a functional magnetic resonance imaging study of Cushing's syndrome. *Dev. Psychopathol.* 20, 1177–1189. doi: 10.1017/S0954579408000564

Merke, D. P., Giedd, J. N., Keil, M. F., Mehlinger, S. L., Wiggs, E. A., Holzer, S., et al. (2005). Children experience cognitive decline despite reversal of brain atrophy one year after resolution of Cushing syndrome. *J. Clin. Endocrinol. Metab.* 90, 2531–2536. doi: 10.1210/jc.2004-2488

Michaud, K., Forget, H., and Cohen, H. (2009). Chronic glucocorticoid hypersecretion in Cushing's syndrome exacerbates cognitive aging. *Brain Cogn.* 71, 1–8. doi: 10.1016/j.bandc.2009.02.013

Newell-Price, J., Bertagna, X., Grossman, A. B., and Nieman, L. K. (2006). Cushing's syndrome. *Lancet* 367, 1605–1617. doi: 10.1016/S0140-6736(06)68699-6

Patil, C. G., Lad, S. P., Katznelson, L., and Laws, E. R. Jr. (2007). Brain atrophy and cognitive deficits in Cushing's disease. *Neurosurg. Focus* 23, E11. doi: 10.3171/foc.2007.23.3.13

Pereira, A. M., Tiemensma, J., and Romijn, J. A. (2010). Neuropsychiatric disorders in Cushing's syndrome. *Neuroendocrinology* 92(Suppl. 1), 65–70. doi: 10.1159/000314317

Pivonello, R., De Leo, M., Vitale, P., Cozzolino, A., Simeoli, C., De Martino, M. C., et al. (2010). Pathophysiology of diabetes mellitus in Cushing's syndrome. *Neuroendocrinology* 92(Suppl. 1), 77–81. doi: 10.1159/000314319

Pivonello, R., De Martino, M. C., De Leo, M., Lombardi, G., and Colao, A. (2008). Cushing's Syndrome. *Endocrinol. Metab. Clin. North Am.* 37, 135–149, ix. doi: 10.1016/j.ecl.2007.10.010

Pivonello, R., De Martino, M. C., De Leo, M., Tauchmanovà, L., Faggiano, A., Lombardi, G., et al. (2007). Cushing's syndrome: aftermath of the cure. *Arq. Bras. Endocrinol. Metabol.* 51, 1381–1391. doi: 10.1590/S0004-27302007000800025

Pivonello, R., Faggiano, A., Lombardi, G., and Colao, A. (2005). The metabolic syndrome and cardiovascular risk in Cushing's syndrome. *Endocrinol. Metab. Clin. North Am.* 34, 327–339. doi: 10.1016/j.ecl.2005.01.010

Ragnarsson, O., Berglund, P., Eder, D. N., and Johannsson, G. (2012). Long-term cognitive impairments and attentional deficits in patients with Cushing's disease and cortisol-producing adrenal adenoma in remission. *J. Clin. Endocrinol. Metab.* 97, E1640–E1648. doi: 10.1210/jc.2012-1945

Ragnarsson, O., and Johannsson, G. (2013). Cushing's syndrome: a structured short- and long-term management plan for patients in remission. *Eur. J. Endocrinol.* 169, R139–R152. doi: 10.1530/EJE-13-0534

Resmini, E., Santos, A., Gómez-Anson, B., López-Mourelo, O., Pires, P., Vives-Gilabert, Y., et al. (2013). Hippocampal dysfunction in cured Cushing's syndrome patients, detected by (1) H-MR-spectroscopy. *Clin. Endocrinol.* 79, 700–707. doi: 10.1111/cen.12224

Resmini, E., Santos, A., Gómez-Anson, B., Vives, Y., Pires, P., Crespo, I., et al. (2012). Verbal and visual memory performance and hippocampal volumes, measured by 3-Tesla magnetic resonance imaging, in patients with Cushing's syndrome. *J. Clin. Endocrinol. Metab.* 97, 663–671. doi: 10.1210/jc.2011-2231

Simmons, N. E., Do, H. M., Lipper, M. H., and Laws, E. R. Jr. (2000). Cerebral atrophy in Cushing's disease. *Surg. Neurol.* 53, 72–76. doi: 10.1016/S0090-3019(99)00197-4

Sonino, N., Bonnini, S., Fallo, F., Boscaro, M., and Fava, G. A. (2006). Personality characteristics and quality of life in patients treated for Cushing's syndrome. *Clin. Endocrinol.* 64, 314–318. doi: 10.1111/j.1365-2265.2006.02462.x

Sonino, N., Fallo, F., and Fava, G. A. (2010). Psychosomatic aspects of Cushing's syndrome. *Rev. Endocr. Metab. Disord.* 11, 95–104. doi: 10.1007/s11154-009-9123-7

Sonino, N., and Fava, G. A. (2001). Psychiatric disorders associated with Cushing's syndrome. Epidemiology, pathophysiology and treatment. *CNS Drugs* 15, 361–373. doi: 10.2165/00023210-200115050-00003

Sonino, N., Fava, G. A., Belluardo, P., Girelli, M. E., and Boscaro, M. (1993). Course of depression in Cushing's syndrome: response to treatment and comparison with Graves' disease. *Horm. Res.* 39, 202–206. doi: 10.1159/000182736

Sonino, N., Fava, G. A., Raffi, A. R., Boscaro, M., and Fallo, F. (1998). Clinical correlates of major depression in Cushing's disease. *Psychopathology* 31, 302–306. doi: 10.1159/000029054

Starkman, M. N. (2013). Neuropsychiatric findings in Cushing's syndrome and exogenous glucocorticoid administration. *Endocrinol. Metab. Clin. North Am.* 42, 477–488. doi: 10.1016/j.ecl.2013.05.010

Starkman, M. N., Schteingart, D. E., and Schork, M. A. (1981). Depressed mood and other psychiatric manifestations of Cushing's syndrome: relationship to hormone levels. *Psychosom. Med.* 43, 3–18. doi: 10.1097/00006842-198102000-00002

Starkman, M. N., Schteingart, D. E., and Schork, M. A. (1986). Cushing's syndrome after treatment: changes in cortisol and ACTH levels, and amelioration of the depressive syndrome. *Psychiatry Res.* 19, 177–178. doi: 10.1016/0165-1781(86)90096-X

Tauchmanovà, L., Pivonello, R., Di Somma, C., Rossi, R., De Martino, M. C., Camera, L., et al. (2006). Bone demineralization and vertebral fractures in endogenous cortisol excess: role of disease etiology and gonadal status. *J. Clin. Endocrinol. Metab.* 91, 1779–184. doi: 10.1210/jc.2005-0582

Tiemensma, J., Kokshoorn, N. E., Biermasz, N. R., Keijser, B. J., Wassenaar, M. J., Middelkoop, H. A., et al. (2010). Subtle cognitive impairments in patients with long-term cure of Cushing's disease. *J. Clin. Endocrinol. Metab.* 95, 2699–2714. doi: 10.1210/jc.2009-2032

Valassi, E., Santos, A., Yaneva, M., Tóth, M., Strasburger, C. J., Chanson, P., et al. (2011). The European registry on Cushing's syndrome: 2-year experience. Baseline demographic and clinical characteristics. *Eur. J. Endocrinol.* 165, 383–392. doi: 10.1530/EJE-11-0272

van Aken, M. O., Pereira, A. M., Biermasz, N. R., van Thiel, S. W., Hoftijzer, H. C., Smit, J. W., et al. (2005). Quality of life in patients after long-term biochemical cure of Cushing's disease. *J. Clin. Endocrinol. Metab.* 90, 3279–3286. doi: 10.1210/jc.2004-1375

van der Lely, A. J., Foeken, K., van der Mast, R. C., and Lamberts, S. W. (1991). Rapid reversal of acute psychosis in the Cushing's syndrome with the cortisol-receptor antagonist mifepristone (RU 486). *Ann. Intern. Med.* 114, 143–144. doi: 10.7326/0003-4819-114-2-143

van der Pas, R., Leebeek, F. W., Hofland, L. J., de Herder, W. W., and Feelders, R. A. (2013). Hypercoagulability in Cushing's syndrome: prevalence, pathogenesis and treatment. *Clin. Endocrinol. (Oxf.)* 78, 481–488. doi: 10.1111/cen.12094

Verhelst, J. A., Trainer, P. J., Howlett, T. A., Perry, L., Rees, L. H., Grossman, A. B., et al. (1991). Short and long-term responses to metyrapone in the medical management of 91 patients with Cushing's syndrome. *Clin. Endocrinol. (Oxf.)* 35, 169–178. doi: 10.1111/j.1365-2265.1991.tb03517.x

Wagenmakers, M. A., Netea-Maier, R. T., Prins, J. B., Dekkers, T., den Heijer, M., and Hermus, A. R. (2012). Impaired quality of life in patients in long-term remission of Cushing's syndrome of both adrenal and pituitary origin: a remaining effect of long-standing hypercortisolism? *Eur. J. Endocrinol.* 167, 687–695. doi: 10.1530/EJE-12-0308

Webb, S. M., Badia, X., Barahona, M. J., Colao, A., Strasburger, C. J., Tabarin, A., et al. (2008). Evaluation of health-related quality of life in patients with Cushing's syndrome with a new questionnaire. *Eur. J. Endocrinol.* 158, 623–630. doi: 10.1530/EJE-07-0762

Whelan, T. B., Schteingart, D. E., Starkman, M. N., and Smith, A. (1980). Neuropsychological deficits in Cushing's Syndrome. *J. Nerv. Ment. Dis.* 168, 753–757.

Wolkowitz, O. M., and Reus, V. I. (1999). Treatment of depression with antiglucocorticoid drugs. *Psychosom. Med.* 61, 698–711. doi: 10.1097/00006842-199909000-00011

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# Neurological complications in thyroid surgery: a surgical point of view on laryngeal nerves

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The cervical branches of the vagus nerve that are pertinent to endocrine surgery are the superior and the inferior laryngeal nerves: their anatomical course in the neck places them at risk during thyroid surgery. The external branch of the superior laryngeal nerve (EB) is at risk during thyroid surgery because of its close anatomical relationship with the superior thyroid vessels and the superior thyroid pole region. The rate of EB injury (which leads to the paralysis of the cricothyroid muscle) varies from 0 to 58%. The identification of the EB during surgery helps avoiding both an accidental transection and an excessive stretching. When the nerve is not identified, the ligation of superior thyroid artery branches close to the thyroid gland is suggested, as well as the abstention from an indiscriminate use of energy-based devices that might damage it. The inferior laryngeal nerve (RLN) runs in the tracheoesophageal groove toward the larynx, close to the posterior aspect of the thyroid. It is the main motor nerve of the intrinsic laryngeal muscles, and also provides sensory innervation to the larynx. Its injury finally causes the paralysis of the omolateral vocal cord and various sensory alterations: the symptoms range from mild to severe hoarseness, to acute airway obstruction, and swallowing impairment. Permanent lesions of the RNL occur from 0.3 to 7% of cases, according to different factors. The surgeon must be aware of the possible anatomical variations of the nerve, which should be actively searched for and identified. Visual control and gentle dissection of RLN are imperative. The use of intraoperative nerve monitoring has been safely applied but, at the moment, its impact in the incidence of RLN injuries has not been clarified. In conclusion, despite a thorough surgical technique and the use of intraoperative neuromonitoring, the incidence of neurological complications after thyroid surgery cannot be suppressed, but should be maintained in a low range.

**Keywords: superior laryngeal nerve, inferior laryngeal nerve, thyroid surgery, morbidity, dysphonia, dysphagia, neuromonitoring**

## BACKGROUND

The neurological issues that might appear after thyroid surgery are those related to lesions of motor or sensory nerves whose anatomical course is in the neck. The main structures that are jeopardized during thyroid surgery are those arising from the vagus nerve at various heights: the external branch of the superior laryngeal nerve (EB-SLN) and the inferior laryngeal nerve.

These nerves and their branches have mainly motor activity on the laryngeal muscles, being responsible for both the motility of the vocal cords and of all the distinctive features of one's voice.

The lesion for the main trunk of these nerves, or of their smaller motor branches, is responsible for the paralysis of different laryngeal muscles that might be clinically evident as a significant impairment of the voice either in its quality and intensity.

In a tertiary care referral center, this morbidity should be maintained in a due range, according to different factors that can generally be preoperatively identified: the incidence of a permanent lesion of the inferior laryngeal nerve in a surgery performed for a benign disease should be maintained below 1%. On the opposite side, a higher incidence of permanent lesions can be expected when the indication for surgery is a malignant disease or a recurrent benign or malignant disease (up to 6%). Another factor affecting the complication rate is the surgeon's experience with the low volume surgeons (those who perform <30 cases per year) being those more prone to have higher rates of permanent nerve damages (1).

Actually, a deep knowledge of the cervical anatomy, coming from a vast experience of thyroid surgery, is necessary to tailor the extent of every single operation according to the preoperative diagnosis, thus allowing to keep undesired and permanent post-operative consequences to a minimum.

## THE SUPERIOR LARYNGEAL NERVE

The EB-SLN, previously called the "Galli-Curci Nerve," has been named after the famous Italian opera singer who saw her career declining after a total thyroidectomy performed for a huge goiter although in the presence of a remarkably normal motility of the vocal cords. This event has been considered related to the lesion of this tiny motor branch (that for this reason was named after the singer) until recently, when it was clarified that Galli-Curci suffered from a physiological decline of her performances due to the normal aging process. The nerve has nevertheless maintained

its name in several textbooks and papers, although the true story should be remarked, to preserve the integrity of the surgeon who performed the thyroidectomy (2, 3).

The superior laryngeal nerve originates just below the inferior ganglion (nodose ganglion) of the vagus lining below the jugular foramen. About 1.5 cm inferiorly, the nerve divides into two branches: the internal one, which pierces the thyrohyoid membrane in association with the superior thyroid artery and supplies the sensory innervation to the mucosa of the larynx, and the external one (4, 5).

The EB-SLN is about 0.8 mm wide and 8–9 cm long, it courses anteriorly and inferiorly along the inferior pharyngeal constrictor muscles and the branches of the superior thyroid artery. It then curves anteriorly and medially close to the lower edge of the thyroid cartilage and then approaches the larynx within the sterno-thyro-laryngeal triangle, in the region known to surgeons as the "space of Reeve," whose limits are: the sternothyroid muscle superiorly, the inferior constrictor and cricothyroid muscles medially and the superior pole of the thyroid inferiorly (4, 6–8).

The surgical importance of the EB-SLN is due to the close relationship between the nerve and the superior thyroid vessels. At the level of the cricoid, it divides into two branches, entering separately at the pars recta and pars obliqua of the cricothyroid heads, respectively. In most circumstances, the EB-SLN passes well above the superior thyroid pole, but variations in its caudal extent in relation to the superior pole region have been well-described. For this reason, many anatomic classifications have been proposed (7–9). The most popular classification is the Cernea's one, based on the distance between the intersection of the EB-SLN and the superior thyroid artery, and the superior pole of the thyroid (10, 11).

Type 1 (about 60%): the EB-SLN crosses the superior thyroid vessels at least 1 cm above the superior thyroid pole.

Type 2a (17%): the EB-SLN crosses at a distance shorter than 1 cm.

Type 2b (20%): the EB-SLN crosses below the upper limit of the thyroid.

Type Ni (3%): EB-SLN not identified (subfascial/intramuscular course).

The surgical implication of this classification system is that both 2a and 2b types are considered at risk during thyroidectomy.

Variations in the incidence of Cernea's type were described in relation to: physical height (increased number of type 1), ethnicity (increased number in type 2 in Mexican, Chinese, and Indian ethnicities) and thyroid volume (increased number in type 2 in higher volumes) (5, 7, 8).

More recently, Friedman et al. proposed a classification based on the course of the EB-SLN with respect to the inferior constrictor muscle (8, 12). In type 1 variants, the EB-SLN descends with the superior thyroid vessels, either superficial or lateral to the inferior constrictor, until it terminates in the cricothyroid. In type 2 variants, the EB-SLN penetrates the inferior constrictor, 1 cm above the inferior and lateral edge of the thyroid cartilage. In type 3 variants, the EB-SLN penetrates the superior aspect of the inferior constrictor and then continues deep to the muscle before piercing the cricothyroid.

The EB-SLN carries motor fibers to the cricothyroid, the muscle that tilts the thyroid cartilage and tenses the vocal cord by modifying the distance between the cricoid and the thyroid cartilages. Vocal fold tension and thickness influence the frequency of the vibration, thus affecting the characteristic timbre of one's voice.

Injury of the EB-SLN causes paralysis and/or weakness of the cricothyroid, resulting in changes in voice quality, voice projection, and production of high-pitched sounds (6–8, 13–16).

The EB-SLN is jeopardized when the surgeon is dissecting around the superior pole of the thyroid gland to ligate the superior thyroid artery. The laryngeal head of the sternothyroid muscle should be considered as a landmark for the course of the nerve on the inferior constrictor (13). In cases of large goiters or in patients with a short neck, the complete or partial division of the sternothyroid muscle is recommended because it may improve access to the superior thyroid pedicle.

The anatomical variations of this nerve demand careful blunt dissection of the superior thyroid pole, which should start from the avascular space located between the medial aspect of the superior pole and the cricothyroid, allowing a good exposure of the region where the EB-SLN might be found.

A gentle traction of the thyroid in a lateral and caudal direction may be helpful to obtain a good exposure of the superior vascular pedicle; moreover, the selective ligation of the vessels of the upper pole as close to the thyroid pole as possible is recommended. Separation of the polar vessels should occur in the inferior to superior direction, to avoid the entrapment of the EB-SLN between the medial aspect of the superior pole and the cricothyroid.

Care must be taken to avoid both the stretching of the nerve and an indiscriminate use of monopolar diathermy or energy-based devices for sealing vessels, in order to prevent iatrogenic heat injuries (7, 8, 13).

Intraoperatively, due to its wide anatomic variability, the EB-SLN can be visually recognized in some cases only (15, 17–20), and its rate of visualization during thyroid surgery is summarized in **Table 1**. Recently, the diffusion of minimally invasive techniques relying on the use of the endoscope has led to a higher rate of EB-SLN visualization, basically due to the magnification of the anatomy of the neck (21) (**Figure 1**). Finally, the use of intraoperative neuromonitoring can be useful in both the identification and preservation of the EB-SLN, as described in several reports (7, 8, 13, 15–18, 21–23).

Post-operatively, patients with a lesion of the EB-SLN typically complain of voice fatigue, problems reaching high-pitch sounds that they were used to reach, and the need of an extra effort to speak; they can also complain of various rates of dysphagia.

The awareness to these symptoms, that may be subliminal to common people, might be more evident to the so-called "professional speakers," such as singers, teachers, and lawyers.

Several studies have investigated post-operative rates of EB-SLN injury varying from 0 to as much as 58% (the most relevant experiences reported are summarized in **Table 2**) (24–26), a result that reflects the need for standardized protocols looking forward a more accurate evaluation of this complication, even if the impairments coming from such lesions are the less prone to be improved with specific post-operative treatments.

Currently, videostroboscopy (which evaluates the regularity and symmetry of the mucosal traveling wave and the degree of

**Table 1 | Incidence of visualization of the external branch of the superior laryngeal nerve during thyroid surgery**.

| Reference | Patients | Study | Visualization of EB | Comment |
|---|---|---|---|---|
| Berti et al. (21) | 300 | Retrospective | 65% | "Incidental" visualization during MIVAT |
| Friedman et al. (12) | 884 | Retrospective | 85% | Identification followed transection of the sternothyroid muscle and NM |
| Dionigi et al. (23) | 72 | Prospective, randomized | 84% (NM) vs. 42% (visualization) | MIVAT |
| Pagedar and Freeman (18) | 112 | Prospective | 98% | Identification followed blunt dissection of the space of Reeve |
| Lifante et al. (22) | 47 | Prospective, randomized | 66% (NM) vs. 21% (no NM) | Improvement in patient-assessed voice quality after surgery but does not impact swallowing |

*EB-SLN, external branch of the superior laryngeal nerve; MIVAT, minimally invasive video-assisted thyroidectomy; NM, intraoperative neuromonitoring.*

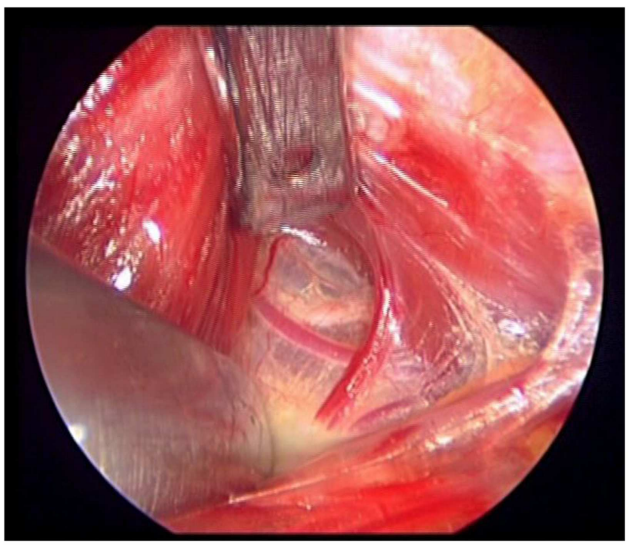

**FIGURE 1 | The loop of the external branch of the superior laryngeal nerve lies very cephalic, in the "space of Reeve," between minor vessels of the upper pedicle**. Notice that this endoscopic image is magnified 20×.

glottis closure) and/or electromyography of the cricothyroid (that documents a decreased recruitment, and polyphasic action potentials that might reach the electrical silence in extreme cases) are the only instrumental tools that might allow to achieve good diagnostic standards (5, 7, 8), although both are invasive methods, responsible for some discomfort to the patients.

## THE INFERIOR LARYNGEAL NERVE

The inferior laryngeal nerve (recurrent nerve, RLN) arises as the vagus courses anteriorly to the aortic arch. As the heart and the great vessels descend during the embryonic development, the RLNs are dragged down by the aortic arches. On the right side, the nerve recurs around the fourth arch (the right subclavian artery), whereas on the left, the nerve recurs around the sixth arch (the ligamentum arteriosum). The right RLN curves around the right subclavian artery and enters the base of the neck from a more lateral position, with respect to the nerve on the opposite side, a consideration that is extremely important from a strictly surgical point of view, since the anatomy is significantly different on the two different sides. The approximate length of the right RLN from the subclavian artery to the cricothyroid joint, is about 5–6 cm, whereas on the left side is about 12 cm long (from the aorta to the cricothyroid joint) (4). After leaving the superior mediastinum, the RLN runs toward the larynx in the trachea–esophageal groove, in a close anatomical relationship with the thyroid gland and the parathyroids. Immediately before or after crossing the Berry ligament, the RLN divides in smaller branches entering the esophagus (mainly sensory), and the main ones that enters the larynx at the inferior constrictor muscle, posterior to the cricothyroid joint (the motor ones).

The RLN in the neck is supplied by both its own vessels ("vasa nervorum") and by branches of the inferior thyroid artery: this vascularization is particularly important since an excessive dissection of the nerve might theoretically represent a cause of undesired post-operative issues (4).

The course of the RLN with respect to inferior thyroid artery is quite variable: it more commonly courses deep to the inferior thyroid artery, but can also travel anterior to or between its branches (11, 27) (**Figure 2**).

Several anatomical variations of the RLN may be found: in some cases, these variations might represent an unfavorable situation and thus an additional cause of morbidity. The more common anatomical variants are: the variable relationship with the branches of the inferior thyroid artery and the ligament of Berry, the various patterns of extralaryngeal branching, and the non-recurrent variant (27).

Various anatomical relationships between the RLN and the ligament of Berry are described: although the nerve is generally located posterior and lateral to this ligament, it can also have a medial course with respect to it, and, in some cases, an anterior (motor) branch can penetrate this ligament (11, 28). This latter situation is considered the one associated with the highest surgical morbidity, since the terminal branches might be completely hidden inside the ligament, and therefore sectioned during the final part of the thyroidectomy.

Variations in the pattern of distal bifurcation of the RLN have been widely reported, since, within the larynx, the nerve divides into an external branch providing motor function to the four intrinsic laryngeal muscles, and an internal branch with only

**Table 2 | Incidence of lesions of the external branch of the superior laryngeal nerve during thyroid surgery: the sequence is progressive, starting from the series reporting the lowest incidence**.

| Reference | Patients | Study | Visualization of EB | Rate of injury |
|---|---|---|---|---|
| Jonas et al. (17) | 108 | Prospective | 37.5% | 0 (nerve monitoring) |
| Bellantone et al. (19) | 289 | Prospective, randomized | 0 (not searched) vs. 89% (searched) | 0 vs. 0 |
| Inabnet et al. (20) | 10 | Prospective | 53% (out of 15 nerves at risk) | 0 (nerve monitoring) |
| Teitelbaum et al. (24) | 20 | Retrospective | Not described | 5% |
| Aluffi et al. (25) | 45 | Retrospective | Not described | 14% |
| Hurtado-Lopez et al. (26) | 100 | Prospective, randomized | 0 (not searched) vs. 78% (searched) | 20% (non-visualized) vs. 8% (visualized) |

*EB-SLN, external branch of the superior laryngeal nerve.*

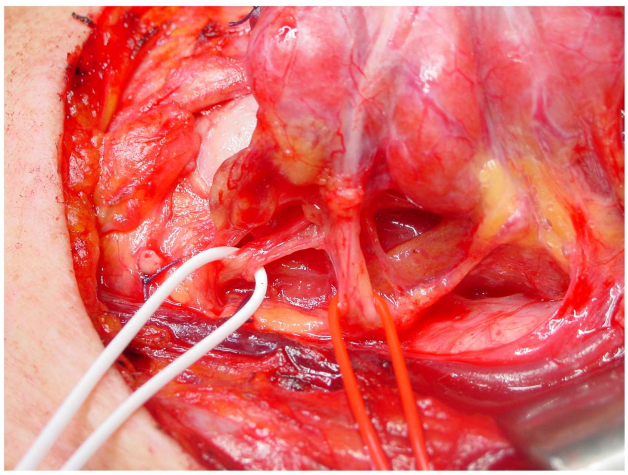

**FIGURE 2 | The relationship between the inferior laryngeal nerve and the inferior thyroid artery, on the left side**. In this case, the nerve (white loop) runs posterior to the artery (red loop).

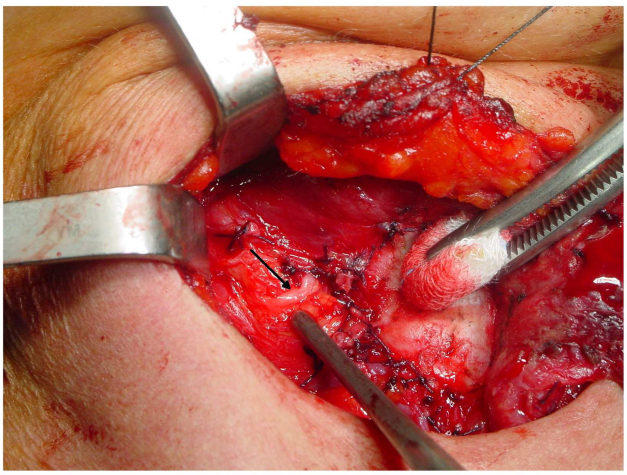

**FIGURE 3 | A relatively high variant of a non-recurrent inferior laryngeal nerve on the right side: in this case, the nerve (arrow) runs almost horizontally from the vagus toward the larynx**.

sensory activity for the glottis. This division may occur before the RLN enters the larynx near the Berry ligament or the inferior thyroid artery giving origin to up to several extralaryngeal branches extremely varying in sizes. Functional studies of the extralaryngeal nerve branches have well-demonstrated that motor branches to both the abductor and adductor muscles of the larynx are generally located in the anterior division (4, 27, 29), this adding further potential morbidity, since the motor branches are those located closest to the thyroid lobe.

The non-recurrent laryngeal nerve is associated to a major vascular variant characterized by the absence of the brachiocephalic artery and the formation of a right aberrant subclavian artery that originates from the last branch of the aortic arch and extends rightwards, running behind the esophagus (the "lusorian artery"). The nerve then originates directly from the cervical part of the vagus, at various heights, since it cannot be pulled down by the right subclavian artery during its embryological descent (**Figure 3**). For this reason, this variant is present almost exclusively on the right side (incidence: 0.3–1.6%), although a few cases have been described on the left side (incidence: 0.04%), mainly in patients with situs viscerum inversus (4, 29–34), since the corresponding anatomical anomaly on the left side (the absence of the ductus arteriosus) is generally incompatible with survival of the fetus. Soustelle has classified the non-recurrent laryngeal nerve into two types according to their courses. TYPE 1 arises directly from the cervical vagus and enters the larynx at the upper pole of the thyroid gland, running together with the vessels of the superior thyroid pedicle. TYPE 2 arises from the vagus nerve near the origin of the inferior thyroid artery and enters the larynx above the inferior thyroid artery (TYPE 2A), or under the inferior thyroid artery (TYPE 2B) (32, 34).

From a surgical point of view, TYPE 1 anomaly implies that the nerve could be injured during superior thyroid pedicle ligation, while TYPE 2 variant implies that, over its transverse course, the non-recurrent laryngeal nerve can mimic the course of the inferior thyroid artery, and thus be misinterpreted by the surgeon, and finally interrupted. Preoperative computed tomography showing the presence of an arteria lusoria or the presence of a "horizontal Y" at US scan (due to the bifurcation of the brachiocephalic trunk into the right common carotid artery and the right subclavian artery) could predict the presence of a non-recurrent laryngeal nerve and therefore reduce the risk of injuring it (32–34).

Transient partial RLN dysfunctions are related to segmental demyelination or focal conduction block, and transient or

permanent total nerve dysfunctions result from severe myelin sheath or axonal damage and neural degeneration. Transient palsy can be regarded either as a consequence of neuroapraxia or axonotmesis, depending on whether local myelin damage, usually secondary to compression (edema, blood clot, and suffusion) or a loss of continuity of axons. An excessive manipulation during surgery might lead to a significant edema or to diffuse microhemorrhage by injury of fragile capillaries, possibly resulting in neuroapraxia or axonotmesis (29).

The final outcome of the damage to a RLN is represented by the paralysis of the sole abducting muscle (posterior cricoarytenoid) of the vocal cords, which might result in a clinical situation ranging from an impaired motility of the cord, up to its atrophy.

**Table 3 | The incidence of morbidity on the inferior laryngeal nerve according to different experiences reported in literature: the sequence is, again, progressive, starting from the series reporting the lowest incidence**.

| Reference | Patients/nerves at risk | Nerve injuries (%) (transient/permanent) |
|---|---|---|
| Efremidou et al. (39) | 932/1864 | 1.3/0.2° |
| Bergamaschi et al. (40) | 1163/2010 | 2.9/0.3 |
| Chiang et al. (41) | 521/704 | 5.1/0.9 |
| Lo et al. (42) | 500/787 | 5.2/0.9 |
| Thomusch et al. (43) | 7266/13436 | 2.1/1.1° |
| Rosato et al. (38) | 14934/n.a. | 3.4/1.4 |
| Lefevre et al. (44) | 685/- (reoperations) | ?/1.5 |
| Toniato et al. (45) | 504/1008 | 2.2*§ |
| Echternach et al. (46) | 1001/1365 | 6.6 |

*°Only thyroidectomies for benign disease were considered; *only thyroidectomies for malignant disease were considered. § Post-operative laryngoscopy not routinely performed.*

Consequent symptoms range from hoarseness in unilateral lesions, to stridor and acute airway obstruction in bilateral damage. Temporary or permanent post-operative vocal changes can have a serious impact on the patient's quality of life, especially in professional voice users.

The posterior branches of the RLN often provide innervation to both the cricopharyngeus muscle and the esophagus, thus justifying the impairment in swallowing that is frequently complained by patients after thyroidectomy (31, 35–37). These branches are extremely short and thin and might be difficult to visualize and, therefore, spared by the surgeon performing the thyroidectomy. Nerve monitoring cannot obviously be used to identify such branches since they are almost exclusively sensorial.

Post-operative RLN lesion is a relatively rare complication of thyroid surgery in expert hands, the rate of permanent damages occurring approximately between 0.3 and 3% of the cases, and transient palsies in up to 8% of the cases. This wide range of results is described in **Table 3** (38–46), where the most numerous studies recording the morbidity of thyroid surgery are summarized. This rate is mainly related to the diagnosis of the patient, with patients undergoing reoperative thyroid surgery and those undergoing extensive surgery for cancer being at greater risk of morbidity, and to the surgeon's experience (1). The risk of RLN injury in reoperative surgery is described between 2 and 30%, with rate that is also significantly different when the surgery is performed for recurrent benign or recurrent malignant disease: the injury to the nerve in such cases can be due to the difficulty in identifying and preserving the nerve encased in the scar tissue of the previous surgery (27, 29, 31, 38). These results are summarized and shortly commented in **Table 4** (42, 44, 47–49).

A cornerstone of the recommended surgical technique of thyroidectomy is the active search for and identification of the RLN. It is classically identified in the Simon triangle (4), formed by the esophagus medially, carotid artery laterally and inferior thyroid artery superiorly. The tubercle of Zuckerkandl is another landmark that can be useful in identifying the RLN, since it

**Table 4 | Risk factors affecting the final rate of inferior laryngeal nerve injuries according to different authors**.

| Reference | Patients | Study | RNL (%) | Comment |
|---|---|---|---|---|
| Dralle et al. (47) | 16448 | Retrospective, multicentric | – | Risk factors for permanent RNI: recurrent benign (4.7×) and malignant (6.7×) disease; thyroid malignancy (2×); lobectomy (1.8×) |
| Erbil et al. (48) | 3250 | Retrospective | 1.8 | Extended surgery (12×) and reoperations (3×) had a significant effect on the incidence of complications |
| Lefevre et al. (44) | 685 (reoperations) | Retrospective | 1.5 | "Permanent complication rates were higher than those for primary thyroid resection" |
| Lo et al. (42) | 500 | Prospective | 1.4 | "Thyroid surgery for malignant neoplasm and recurrent substernal goiter was associated with an increased risk of permanent nerve palsy" |
| Shindo and Stern (49) | 122 (TT + CC) Vs. 134 (TT) | Retrospective | 5 vs. 10 (temp.); 0 vs. 1 (perm.) | More extended surgery on the lymph nodes is burdened by a higher rate of temporary injuries |

*The cited papers are those with the highest statistical power (a high statistical power is necessary to evaluate a rare event), and this is reflected by the sequence of the citations, from the most to the less powerful ones.*

*RNL, inferior laryngeal nerve lesions; TT + CC, total thyroidectomy and central neck dissection; TT, total thyroidectomy.*

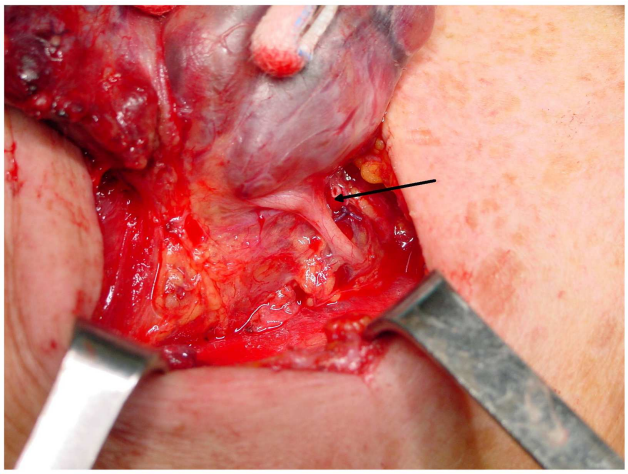

**FIGURE 4 | The strict anatomical relationship between the inferior laryngeal nerve and the tubercle of Zuckerkandl**. The nerve (arrow) almost adheres to the inferior side of the tubercle, immediately before entering the laryngeal muscles.

generally lies anterior to the nerve (**Figure 4**) (50). Obviously, the thyroid surgeon must be aware of the possible anatomical variations of the nerve, and always search for its visual control and gentle dissection of RLN, which are imperative since possible mechanisms of injury include transection, clamping, ligation, traction, thermal injury, and ischemia. A vast majority of injuries occur because of an excessive traction on this extremely delicate structure (these lesions are those more prone to be temporary ones), and in the setting of anatomical variants, especially when an extralaryngeal bifurcation of RLN is present (4, 27, 31).

The use of intraoperative nerve monitoring (IONM) in association to visual identification of the nerve has been safely applied, even if there is lack of high-level evidence that might elucidate the true RLN sparing effect of IONM: this tool should therefore not be used as the sole mechanism for identifying and preserving the nerve, although it can be used to aid in the identification and dissection of the nerve.

In conclusion, a "safe" technique for thyroidectomy cannot be described, since every expert thyroid surgeon is burdened by a morbidity rate that cannot ever reach the 0%. Nevertheless, this rate can be "kept to a minimum" when a thorough surgical technique is followed, and the surgeon should always keep in mind that different preoperative indications correspond to a different incidence of morbidity, accordingly informing the patients about it.

## AUTHOR CONTRIBUTIONS

All Authors (Emanuela Varaldo, Gian Luca Ansaldo, Matteo Mascherini, Ferdinando Cafiero, and Michele N. Minuto) made substantial contributions to conception and design, acquisition of data, and analysis and interpretation of data, and all participated in drafting the article and gave final approval of the version to be submitted and published.

## REFERENCES


1. Sosa JA, Bowman HM, Tielsch JM, Powe NR, Gordon TA, Udelsman R. The importance of surgeon experience for clinical and economic outcomes from thyroidectomy. *Ann Surg* (1998) **228**(3):320–30. doi:10.1097/00000658-199809000-00005
2. Crookes PF, Recabaren JA. Injury to the superior laryngeal branch of the vagus during thyroidectomy: lesson or myth? *Ann Surg* (2001) **233**(4):588–93. doi:10.1097/00000658-200104000-00016
3. Marchese Ragona R, Restivo DA, Mylonakis I, Ottaviano G, Martini A, Sataloff RT, et al. The superior laryngeal nerve injury of a famous soprano, Amelita Galli-Curci. *Acta Otorhinolaryngol Ital* (2013) **33**:67–71. doi:10.1002/lary.23708
4. MohEB-SLNati A, Shaha AR. Anatomy of thyroid and parathyroid glands and neurovascular relations. *Clin Anat* (2012) **25**(1):19–31. doi:10.1002/ca.21220
5. Hwang SB, Lee HY, Kim WY, Woo SU, Lee JB, Bae JW, et al. The anatomy of the external branch of the superior laryngeal nerve in Koreans. *Asian J Surg* (2013) **36**:13–9. doi:10.1016/j.asjsur.2012.08.003
6. Kochilas X, Bibas A, Xenellis J, Anagnostopoulou S. Surgical anatomy of the external branch of the superior laryngeal nerve and its clinical significance in head and neck surgery. *Clin Anat* (2008) **21**:99–105. doi:10.1002/ca.20604
7. Barczynski M, Randolph W, Cernea CR, Dralle H, Dionigi G, Alesina PF, et al. External branch of the superior laryngeal nerve monitoring during thyroid and parathyroid surgery: international neural monitoring study group standards guideline statement. *Laryngoscope* (2013) **123**:S1–14. doi:10.1002/lary.24301
8. Morton RP, Whitfield P, Al-Ali S. Anatomical and surgical considerations of the external branch of the superior laryngeal nerve: a systematic review. *Clin Otolaryngol* (2006) **31**:368–74. doi:10.1111/j.1749-4486.2006.01266.x
9. Kierner AC, Aigner M, Burian M. The external branch of the superior laryngeal nerve. *Arch Otolaryngol Head Neck Surg* (1998) **124**:301–3. doi:10.1001/archotol.124.3.301
10. Cernea CR, Ferraz AR, Nishio S, Dutra A, Hojaij FC, Dod Santos LR. Surgical anatomy of the external branch of the superior laryngeal nerve. *Head Neck* (1992) **14**:380–3. doi:10.1002/hed.2880140507
11. Cernea CR, Ferraz AR, Furlani J, Monteiro S, Nishio S, Hojaij FC, et al. Identification of the external branch of the superior laryngeal nerve during thyroidectomy. *Am J Surg* (1992) **164**:634–9. doi:10.1016/S0002-9610(05)80723-8
12. Friedman M, LoSavio P, Ibrahim H. Superior laryngeal nerve identification and preservation in thyroidectomy. *Arch Otolaryngol Head Neck Surg* (2002) **128**(3):296–303. doi:10.1001/archotol.128.3.296
13. Potenza A, Phelan EA, Cernea CR, Slough CM, Kamani DV, Darr A, et al. Normative intra-operative electrophysiologic waveform analysis of superior laryngeal nerve external branch and recurrent laryngeal nerve in patients undergoing thyroid. *World J Surg* (2013) **37**:2336–42. doi:10.1007/s00268-013-2148-9
14. Henry JF, Audiffret J, Denizot A, Plan M. The nonrecurrent inferior laryngeal nerve: review of 33 cases, including two on the left side. *Surgery* (1988) **104**(6):977–84.
15. Fellmer PT, Böhner H, Wolf A, Röher HD, Goretzki PE. A left nonrecurrent inferior laryngeal nerve in a patient with right-sided aorta, truncus arteriosus communis, and an aberrant left innominate artery. *Thyroid* (2008) **18**(6):647–9. doi:10.1089/thy.2007.0284
16. Yalcin B, Develi S, Tubbs RS, Poyrazoglu Y. A detailed study of the relationship between the external laryngeal nerve and the superior thyroid artery, including its glandular branches. *Clin Anat* (2013) **26**:814–22. doi:10.1002/ca.22198
17. Jonas J, Bahr R. Neuromonitoring of the external branch of the superior laryngeal nerve during thyroid surgery. *Am J Surg* (2000) **179**:234–6. doi:10.1016/S0002-9610(00)00308-1
18. Pagedar N, Freeman JL. Identification of the external branch of the superior laryngeal nerve during thyroidectomy. *Arch Otolaryngol Head Neck Surg* (2009) **135**(4):360–2. doi:10.1001/archoto.2009.9
19. Bellantone R, Boscherini M, Lombardi CP, Bossola M, Rubino F, De Crea C, et al. Is the identification of the external branch of the superior laryngeal nerve mandatory in thyroid operation? Results of a prospective randomized study. *Surgery* (2001) **130**(6):1055–9. doi:10.1067/msy.2001.118375
20. Inabnet WB, Murry T, Dhiman S, Aviv J, Lifante JC. Neuromonitoring of the external branch of the superior laryngeal nerve during minimally invasive thyroid surgery under local anesthesia: a prospective study of 10 patients. *Laryngoscope* (2009) **119**(3):597–601. doi:10.1002/lary.20071
21. Berti P, Materazzi G, Conte M, Galler D, Miccoli P. Visualization of the external branch of the superior laryngeal nerve during video assisted thyroidectomy. *J Am Coll Surg* (2002) **195**(4):573–4. doi:10.1016/S1072-7515(02)01338-8

22. Lifante JF, McGill J, Murry T, Aviv JE, Inabnet WB III. A prospective, randomized trial of nerve monitoring of the external branch of the superior laryngeal nerve during thyroidectomy under local/regional anesthesia and IV sedation. *Surgery* (2009) **146**(6):1167–73. doi:10.1016/j.surg.2009.09.023
23. Dionigi G, Boni L, Rovera F, Bacuzzi A, Dionigi R. Neuromonitoring and video-assisted thyroidectomy: a prospective, randomized case-control evaluation. *Surg Endosc* (2009) **23**(5):996–1003. doi:10.1007/s00464-008-0098-3
24. Teitelbaum BJ, Wenig BL. Superior laryngeal nerve injury from thyroid surgery. *Head Neck* (1995) **17**(1):36–40. doi:10.1002/hed.2880170108
25. Aluffi P, Policarpo M, Cherovac C, Olina M, Dosdegani R, Pia F. Post-thyroidectomy superior laryngeal nerve injury. *Eur Arch Otorhinolaryngol* (2001) **258**(9):451–4. doi:10.1007/s004050100382
26. Hurtado-Lopez LM, Pacheco-Alvarez MI, Montes-Castillo Mde L, Zaldivar-Ramirez FR. Importance of the intraoperative identification of the external branch of the superior laryngeal nerve during thyroidectomy: electromyographic evaluation. *Thyroid* (2005) **15**(5):449–54. doi:10.1089/thy.2005.15.449
27. Cernea CR, Hojaij FC, De Carlucci D, Gotoda R, Plopper C, Vanderlei F, et al. Recurrent laryngeal nerve. A plexus rather than a nerve? *Arch Otolaryngol Head Neck Surg* (2009) **135**:1098–102. doi:10.1001/archoto.2009.151
28. Yalcin B, Ozan H. Detailed investigation of the relationship between the inferior laryngeal nerve including laryngeal branches and ligament of Berry. *J Am Coll Surg* (2006) **202**:291–6. doi:10.1016/j.jamcollsurg.2005.09.025
29. Altorjay A, Rull M, Paal B, Csati G, Szilagyi A. Mystic transient recurrent nerve palsy after thyroid surgery. *Head Neck* (2013) **35**(7):934–41. doi:10.1002/hed.23048
30. Pelizzo MR. Thyroid resurgery. *G Chir* (2011) **32**:453–9.
31. Hayward NJ, Grodski S, Yeung M, Johnson WR, Serpell J. Recurrent laryngeal nerve injury in thyroid surgery: a review. *ANZ J Surg* (2013) **83**:15–21. doi:10.1111/j.1445-2197.2012.06247.x
32. Lee YS, Son EJ, Chang HS, Chung WY, Nam KH, Park CS. Computed tomography is useful for preoperative identification of nonrecurrent laryngeal nerve in thyroid cancer patients. *Otolaryngol Head Neck Surg* (2011) **145**(2):204–7. doi:10.1177/0194599811406670
33. Wang Z, Zhang H, Zhang P, He L, Dong W. Preoperative diagnosis and intraoperative protection of nonrecurrent laryngeal nerve: a review of 5 cases. *Med Sci Monit* (2014) **20**:233–7. doi:10.12659/MSM.889942
34. Gong R, Luo S, Gong Y, Wei T, Li Z, Huang J, et al. Prediction of nonrecurrent laryngeal nerve before thyroid surgery-experience with 1825 cases. *J Surg Res* (2014) **189**:75–80. doi:10.1016/j.jss.2014.02.010
35. Maeda T, Saito M, Otsuki N, Morimoto K, Takahashi M, Iwaki S, et al. Voice quality after surgical treatment for thyroid cancer. *Thyroid* (2013) **23**:847–53. doi:10.1089/thy.2012.0060
36. Medeiros Silva IC, De Pedro Netto I, Vartanian JG, Kowalski LP, Carrara de Angelis E. Prevalence of upper aerodigestive symptoms in patients who underwent thyroidectomy with and without the use of intraoperative laryngeal nerve monitoring. *Thyroid* (2012) **22**:1–6. doi:10.1089/thy.2011.0118
37. Van Lierde K, D'Haeseleer E, Wuyts FL, Baudonck N, Bernaert L, Vermeersch H. Impact of thyroidectomy without laryngeal nerve injury on vocal quality characteristics: an objective multiparameter approach. *Laryngoscope* (2010) **120**:338–45. doi:10.1002/lary.20762
38. Rosato L, Avenia N, Bernante P, De Palma M, Gulino G, Nasi PG, et al. Complications of thyroid surgery: analysis of a multicentric study on 14,934 patients operated on in Italy over 5 years. *World J Surg* (2004) **28**(3):271–6. doi:10.1007/s00268-003-6903-1
39. Efremidou EI, Papageorgiou MS, Liratzopoulos N, Manolas KJ. The efficacy and safety of total thyroidectomy in the management of benign thyroid disease: a review of 932 cases. *Can J Surg* (2009) **52**(1):39–44.
40. Bergamaschi R, Becouarn G, Ronceray J, Arnaud JP. Morbidity of thyroid surgery. *Am J Surg* (1998) **176**(1):71–5. doi:10.1016/S0002-9610(98)00099-3
41. Chiang FY, Wang LF, Huang YF, Lee KW, Kuo WR. Recurrent laryngeal nerve palsy after thyroidectomy with routine identification of the recurrent laryngeal nerve. *Surgery* (2005) **137**(3):342–7. doi:10.1016/j.surg.2004.09.008
42. Lo CY, Kwok KF, Yuen PW. A prospective evaluation of recurrent laryngeal nerve paralysis during thyroidectomy. *Arch Surg* (2000) **135**(2):204–7. doi:10.1001/archsurg.135.2.204
43. Thomusch O, Machens A, Sekulla C, Ukkat J, Lippert H, Gastinger I, et al. Multivariate analysis of risk factors for postoperative complications in benign goiter surgery: prospective multicenter study in Germany. *World J Surg* (2000) **24**(11):1335–41. doi:10.1007/s002680010221
44. Lefevre JH, Tresallet C, Leenhardt L, Jublanc C, Chigot JP, Menegaux F. Reoperative surgery for thyroid disease. *Langenbecks Arch Surg* (2007) **392**(6):685–91. doi:10.1007/s00423-007-0201-6
45. Toniato A, Boschin IM, Piotto A, Pelizzo MR, Guolo A, Foletto M, et al. Complications in thyroid surgery for carcinoma: one institution's surgical experience. *World J Surg* (2008) **32**(4):572–5. doi:10.1007/s00268-007-9362-2
46. Echternach M, Maurer CA, Mencke T, Schilling M, Verse T, Richter B. Laryngeal complications after thyroidectomy: is it always the surgeon? *Arch Surg* (2009) **144**(2):149–53. doi:10.1001/archsurg.2008.530
47. Dralle H, Sekulla C, Haerting J, Timmermann W, Neumann HJ, Kruse E, et al. Risk factors of paralysis and functional outcome after recurrent laryngeal nerve monitoring in thyroid surgery. *Surgery* (2004) **136**(6):1310–22. doi:10.1016/j.surg.2004.07.018
48. Erbil Y, Barbaros U, Issever H, Borucu I, Salmaslioglu A, Mete O, et al. Predictive factors for recurrent laryngeal nerve palsy and hypoparathyroidism after thyroid surgery. *Clin Otolaryngol* (2007) **32**(1):32–7. doi:10.1111/j.1365-2273.2007.01383.x
49. Shindo M, Stern A. Total thyroidectomy with and without selective central compartment dissection: a comparison of complication rates. *Arch Otolaryngol Head Neck Surg* (2010) **136**(6):584–7. doi:10.1001/archoto.2010.79
50. Pelizzo MR, Toniato A, Gemo G. Zuckerkandl's tuberculum: an arrow pointing to the recurrent laryngeal nerve (constant anatomical landmark). *J Am Coll Surg* (1998) **187**(3):333–6. doi:10.1016/S1072-7515(98)00160-4


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# Constitutional thinness and anorexia nervosa: a possible misdiagnosis?

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Clinical and biological aspects of restrictive anorexia nervosa (R-AN) are well documented. More than 10,000 articles since 1911 and more than 600 in 2013 have addressed R-AN psychiatric, somatic, and biological aspects. Genetic background, ineffectiveness of appetite regulating hormones on refeeding process, bone loss, and place of amenorrhea in the definition are widely discussed and reviewed. Oppositely, constitutional thinness (CT) is an almost unknown entity. Only 32 articles have been published on this topic since 1953. Similar symptoms associating low body mass index, low fat, and bone mass are reported in both CT and R-AN subjects. Conversely, menses are preserved in CT women and almost the entire hormonal profile is normal, except for leptin and PYY. The aim of the present review is to alert the clinician on the confusing clinical presentation of these two situations, a potential source of misdiagnosis, especially since R-AN definition has changed in DSM5.

**Keywords: anorexia nervosa, constitutional thinness, phenotyping, DSM IV, DSM5**

## INTRODUCTION

In a young population of women, between 15 and 30 years old, a low body mass index (BMI) associated with apparent healthy state suggests that the diagnosis of anorexia nervosa (AN). For the same age range, other etiologies of starvation are associated with specific symptoms leading to obvious diagnosis of blood or oncologic pathologies, digestive absorption disorders.

Although restrictive anorexia nervosa (R-AN) definition reported in the "diagnostic and statistical manual of mental disorders" (DSM) is in perpetual evolution, diagnosis remains easy for an experienced clinician. The last issue, DSM5, was published in 2013 (1). Previous DSM IV definition (2) included some psychological elements in the diagnosis such as "refusal to maintain a normal BMI for their age and height, weight loss leading to maintenance of body weight <85% of that expected, fearful of even the slightest weight gain and takes all precautionary measures to avoid weight gain and becoming overweight and disturbance in the way one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of the seriousness of the current low body weight." Amenorrhea, i.e., the absence of at least three consecutive menstrual cycles, was the only somatic symptom included in the DSM IV definition. In R-AN women, amenorrhea is related to a blunted/hibernating hypothalamic–pituitary–gonadal (HPG) axis activity. This criterion cannot be applied in pre-menarchal females, females taking oral contraceptives, post-menopausal females, or in case of delayed menarche. Moreover, male gonadal axis status was not mentioned in DSM IV definition. Giving these limitations, a psychiatric taskforce developed a new wide definition meant to be relevant for both genders. Thus, amenorrhea symptom was removed from the current DSM5 definition, entirely based on psychiatric symptoms (1). Major consequences of this truncated definition are further discussed as it can lead to misdiagnosis in front of a thin woman.

## DIFFERENCES BETWEEN ANOREXIA NERVOSA AND CONSTITUTIONAL THINNESS

In a similar population of women, a low BMI associated with apparent healthy state could also suggests that the diagnosis of a not yet well-known entity called constitutional thinness (CT). This natural and physiological low BMI ($<18.5\,kg/m^2$) state is associated with preserved menses (without contraceptive treatment) and reproductive function leading to constitutional thin families. CT prevalence is unknown in the developed world. These subjects want to gain weight and often consult in this perspective. We recently showed that they failed to gain weight in a controlled overfeeding study (3). Opposite to the presence of amenorrhea that advocates for a diagnosis of R-AN, the absence of amenorrhea advocates for a diagnosis of CT.

Thinness is defined by the World Health Organization which specified three levels of underweight for BMI $<18.5\,kg/m^2$ (4). Thinness is the only resemblance between R-AN and CT. Indeed, despite the same BMI as AN, CT subjects do not exhibit any typical clinical features such as amenorrhea, fear of weight gain, or hormonal abnormalities commonly seen in R-AN patients.

This review summarizes the main features opposing R-AN and CT including nutritional markers, bone loss physiology, appetite regulating hormones, and how the DSM5 definition can lead to misdiagnoses between those two etiologies of thinness. The results published by our group come from a large cohort of more than 600 R-AN and 100 CT.

Hormonal abnormalities data about R-AN reported so far in literature are commonly accepted and have been showed and published many times by different teams. Most of them are undernutrition markers such as low free-T3 (5, 6), low IGF-1, and elevated GH displaying a GH resistance (7), elevated SHBG (8), and blunted leptin reflecting a decreased fat mass (9). These biological features are adaptive to food restriction and hormonal levels return within normal range after refeeding (10, 11). In 2010, we published biological data on a large R-AN cohort of 200 patients. Despite a correlation between BMI and five specific nutritional or hormonal parameters including leptin, GH, cortisol, free-T3, and IGF-1, we noticed a large inter-individual heterogeneity of these parameters (12). In CT matched for BMI and gender, free-T3, IGF-1, mean 24-h GH levels (sampled six times over the day) are in the normal range (**Figure 1**). This absence of undernutrition markers is in line with the low levels of leptin, still higher than in AN, and with preserved circadian cycle (13). A girl with low BMI and normal free-T3 or IGF-1 is not a R-AN.

Anorexia nervosa is associated with well-known hypothalamus–pituitary–peripheral axis changes/disturbances such as "pseudo-Cushing syndrome" and hypogonadism. Indeed, the excess of the hypothalamic–pituitary–adrenal (HPA) axis activity includes high CRF (14), increased circadian cortisol levels (15), and rapid escape of cortisol from suppression in response to i.v. dexamethasone (16). Nevertheless, R-AN patients display no clinical Cushing-like features and is considered as a "pseudo-Cushing syndrome." These abnormalities regress after refeeding (17).

Restrictive anorexia nervosa amenorrhea, the only somatic trait in the DSM IV classification, is the consequence of blunted HPG axis associating low estradiol (18), low free testosterone levels (8), and absence of LH pulsatility (19). This low GnRH activity seems to be dependent on multiple neuropeptides/amino acids influences including opioid (20), gabaergic (21), or serotoninergic activity (22) changes. Refeeding restores menses only in 55–100% of R-AN women (23–25). GnRH pump is the current validated treatment to restore LH pulsatility and to stimulate ovulation (26). This treatment leads to ovulation in 100% of cases, most often between day 10 and 14, and to pregnancy in 55% of cases after 6 stimulations (unpublished data).

Oppositely, normal HPA axis activity suggested by normal six-point (8, 12, 16, 20, 24, 04 h) circadian levels of cortisol was found in CT subjects matched for BMI and gender. Functional HPG axis was also attested by normal LH, FSH, estradiol, and free testosterone plasma level without oral contraception, and preserved fertility (13). A young adult girl with low BMI and normal menses without pills, and normal hormonal profile is not a R-AN.

The alteration of bone quality in AN, a consequence to undernutrition and hormonal abnormalities, is widely accepted. Loss of bone density, correlated with the duration of low BMI (27) and explained by a bone turnover uncoupling (28) leads to increased fractures risk (29), also related with some genetic disturbances (30). An important issue comes from the lack of treatment to increase bone mineral density, except refeeding. Hormonal deficiency supplementation with IGF-1 (31), classical treatment with bisphosphonates (32), or PTH administration (33) are not approved. The role of estradiol in osteoporosis treatment is worldwide accepted, but in R-AN the supplementation is non-effective (34). The association of two hormonal supplementations without side effects such as SDHA and estradiol seems to be effective (35, 36). However, all authors agree on the interest to treat earlier in order to preserve and not to restore bone mineral density.

Bone loss with 44% of osteopenia is also found in 20 years old CT subjects (37). Oppositely to the bone uncoupling seen in R-AN, bone turnover is balanced in CT, with normal bone formation (normal circadian osteocalcin profile) and bone resorption (normal plasma circadian CTX profile). Furthermore, bone microarchitecture measured by pQCT is different (37). Combined normal bone marker and specific microarchitecture profile are other arguments to differentiate CT from R-AN and to avoid a misdiagnosis.

While low food intake characterizes R-AN subjects, equilibrated energy balance was noticed in CT (38). Food restriction behavior of R-AN patients was found to be in opposition with the theoretical action of most of the appetite regulating hormones. Orexigenic gastric hormone ghrelin and the related gene derived peptide obestatin are elevated in all studies without orexigenic effect (13, 39). Ghrelin gene variants (40) or circulating antibodies (41) are proposed to explain the "ghrelin-resistance." Interestingly, a normal ghrelin level is found in lean anorectic patients (AN) with bingeing and in normal weight bulimic patients. High ghrelin seems to be a mark of restrictive subtype of AN (restrictive only and binging associated) (42). A recently discovered hypothalamic orexigenic peptide called 26 RFa is also increased in R-AN, and oppositely normal in CT (43). These hormones act through

| Anorexia nervosa | Constitutional thinness |
|---|---|
| ***BMI ≤ 17,5 kg/m²*** | |
| Presence of eating disorders | No eating disorders; de-restriction |
| Psychological disorders | No psychological disorders |
| Amenorrhea | Physiological menses |
| Hormonal abnormalities= undernutrition<br>T3 ↓, leptine↓, IGF-1 ↓, GH ↑<br>Cortisol↑, ACTH ↑, 17 β E2 ↓, LH ↓, FSH ↓ | No hormonal abnormalities<br>= no sign of undernutrition |
| Blunted fat mass | Diminished fat mass |
| Negative energy balance | Equilibrated / positive energy metabolism |
| Weight loss / broken weight growth curve | Stable weight within lower percentile of growth curve |

**FIGURE 1 | Psychiatric, hormonal, and energy balance differences between CT and AN**.

the orexigenic neuron and peptide NPY. Conflicting results were reported on pre-prandial plasma NPY (39). A low-circulating NPY could explain the non-efficacy of orexigenic hormones. Recent work showed role of NPY was more complex (44). These results need confirmation by circadian assessment of NPY levels. NPY data are not available in CT yet.

In CT, ghrelin and obestatin are in a normal range (13, 39). Therefore, normal ghrelin levels in a pure R-AN patient are questionable. In this case, misdiagnosis of CT or purging type AN should be discussed (42).

Studies on anorexic appetite regulating hormones as PYY and GLP-1 in AN present with conflicting results (11, 45, 46). PYY level in R-AN was found in normal range in our experience (11), but elevated in another study (47), perhaps due to the misdiagnosis of CT. Indeed, we reported in CT a high-PYY level throughout the day (08, 12, 16, 20, 24, 04 h) (11). CT's leptin was found lower than in controls but higher than in R-AN with preserved circadian variation. Finally, low level of leptin, another anorexic peptide, found in all R-AN studies (13, 48) is probably more informative on the fat mass than on the eating behavior. Discrepancies found in appetite regulating hormones are in line with differences found in questionnaires measuring restrained eating behavior (DEBQ) or shape concern (EDE). Many questionnaires were proposed for eating disorders phenotyping. EDI questionnaire is a self-report questionnaire used to assess the presence of eating disorders, anorexia nervosa both restricting and binge-eating/purging type (49). EDE questionnaire deals with the frequency in which the patient engages in behaviors indicative of an eating disorder over a 28-day period (restraint, eating, shape, weight concern) (50), DEBQ was developed to measure emotional external and restrain eating (51). All of these psychological scales present pathological scores in AN but not in CT. Moreover, we found an unrestrained eating behavior in CT when compared to controls (3).

For the same, low BMI, anorectic behavior, and adaptive appetite regulating hormone characterizes R-AN patients and distinguishes them from CT patients.

Finally, genetic pathophysiologic role in AN was proposed throughout polymorphism gene studies (52) or familial histories of disease (53). Recently, two studies reported a tendency to the gene hypothesis without the statistical significantly in 421 probants for the first and 1606 probants for the second (54, 55). Significant ratio of chromosome 16p11.2 region duplications was previously noticed in lean patients with autism or schizophrenia (56), without any argument to relate these results to leanness rather than to psychiatric disorders. No genetic signature can be proposed at this moment for the CT patients despite the family pedigree (38). Some studies are in progress but no results are available. Along with all these data on genetic approach, it is important to underline the role of lean subjects' phenotyping within these large cohorts' studies.

## DISCUSSION

This review focused on biological differences between R-AN and CT. As CT display specific clinical features and normal hormonal parameters, the misdiagnosis between CT and R-AN is no longer permitted. A very thin girl who claims a normal diet should be heard and not considered as a "liar" AN patient. A biological assessment including leptin, free-T3, and IGF-1 is proposed in order to avoid a misdiagnosis.

This review also focused on conflicting literature data reported in R-AN. Because of the clinical or biological heterogeneity of patients selected for the studies, publications results cannot always be compared and could explained the conflicting data. Therefore, straight and objective classification also called "phenotyping" is required in clinical research in order to obtain better comparable symptoms. For example, the definition shift between DSM IV and DSM5 changes the developing risk of AN, double in a dancer population (57) or in general population (58). Biological assessment including hormonal, nutritional markers, neuroimaging, or questionnaires could help on AN phenotyping. Currently, no biological determination or questionnaire evaluations are required for AN definition in DSM5.

In conclusion, CT represents a well-defined real state of low BMI associating a real weight gain desire, normal nutritional markers except for a mild decreased leptin, a constitutive appetite regulating hormone profile, the presence of menses in young women and low bone mass. While CT diagnosis is still poorly known, the new DSM5 AN definition proposing only psychological traits and no organic symptom is warring. In line with mentioned somatic differences, we advocate complementary biological markers in AN definition in order to avoid the misdiagnosis between AN and CT.

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## REFERENCES

1. Attia E, Becker AE, Bryant-Waugh R, Hoek HW, Kreipe RE, Marcus MD, et al. Feeding and eating disorders in DSM-5. *Am J Psychiatry* (2013) **170**:1237–9. doi:10.1176/appi.ajp.2013.13030326
2. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. Washington, DC: American Psychiatric Association (1994). 943 p.
3. Germain N, Galusca B, Caron-Dorval D, Martin J, Pujos-Guillot E, Boirie Y, et al. Specific appetite, energetic and metabolomics responses to fat overfeeding in resistant-to-bodyweight-gain constitutional thinness. *Nutr Diabetes* (2014) **4**:e126. doi:10.1038/nutd.2014.17
4. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser* (1995) **854**:1–452.
5. Chopra IJ, Smith SR. Circulating thyroid hormones and thyrotropin in adult patients with protein-calorie malnutrition. *J Clin Endocrinol Metab* (1975) **40**:221–7. doi:10.1210/jcem-40-2-221
6. Kiyohara K, Tamai H, Takaichi Y, Nakagawa T, Kumagai LF. Decreased thyroidal triiodothyronine secretion in patients with anorexia nervosa: influence of weight recovery. *Am J Clin Nutr* (1989) **50**:767–72.
7. Cabranes JA, Almoguera I, Santos JL, Hidalgo I, Borque MM, del Olmo J. Somatomedin-C and growth hormone levels in anorexia nervosa in relation to the puberal or post puberal stages. *Prog Neuropsychopharmacol Biol Psychiatry* (1988) **12**:865–71. doi:10.1016/0278-5846(88)90082-6
8. Estour B, Pugeat M, Lang F, Dechaud H, Pellet J, Rousset H. Sex hormone binding globulin in women with anorexia nervosa. *Clin Endocrinol (Oxf)* (1986) **24**:571–6. doi:10.1111/j.1365-2265.1986.tb03287.x
9. Hebebrand J, van der Heyden J, Devos R, Kopp W, Herpertz S, Remschmidt H, et al. Plasma concentrations of obese protein in anorexia nervosa. *Lancet* (1995) **346**:1624–5. doi:10.1016/S0140-6736(95)91955-4
10. Warren MP. Endocrine manifestations of eating disorders. *J Clin Endocrinol Metab* (2011) **96**:333–43. doi:10.1210/jc.2009-2304
11. Germain N, Galusca B, Le Roux CW, Bossu C, Ghatei MA, Lang F, et al. Constitutional thinness and lean anorexia nervosa display opposite concentrations of

peptide YY, glucagon-like peptide 1, ghrelin, and leptin. *Am J Clin Nutr* (2007) **85**:967–71.
12. Estour B, Germain N, Diconne E, Frere D, Cottet-Emard JM, Carrot G, et al. Hormonal profile heterogeneity and short-term physical risk in restrictive anorexia nervosa. *J Clin Endocrinol Metab* (2010) **95**:2203–10. doi:10.1210/jc.2009-2608
13. Tolle V, Kadem M, Bluet-Pajot MT, Frere D, Foulon C, Bossu C, et al. Balance in ghrelin and leptin plasma levels in anorexia nervosa patients and constitutionally thin women. *J Clin Endocrinol Metab* (2003) **88**:109–16. doi:10.1210/jc.2002-020645
14. Hotta M, Shibasaki T, Masuda A, Imaki T, Demura H, Ling N, et al. The responses of plasma adrenocorticotropin and cortisol to corticotropin-releasing hormone (CRH) and cerebrospinal fluid immunoreactive CRH in anorexia nervosa patients. *J Clin Endocrinol Metab* (1986) **62**:319–24. doi:10.1210/jcem-62-2-319
15. Bliss EL, Migeon CJ. Endocrinology of anorexia nervosa. *J Clin Endocrinol Metab* (1957) **17**:766–76. doi:10.1210/jcem-17-6-766
16. Estour B, Pugeat M, Lang F, Lejeune H, Broutin F, Pellet J, et al. Rapid escape of cortisol from suppression in response to i.v. dexamethasone in anorexia nervosa. *Clin Endocrinol (Oxf)* (1990) **33**:45–52. doi:10.1111/j.1365-2265.1990.tb00464.x
17. Liu JP, Clarke IJ, Funder JW, Engler D. Studies of the secretion of corticotropin-releasing factor and arginine vasopressin into the hypophysial-portal circulation of the conscious sheep. II. The central noradrenergic and neuropeptide Y pathways cause immediate and prolonged hypothalamic-pituitary-adrenal activation. Potential involvement in the pseudo-Cushing's syndrome of endogenous depression and anorexia nervosa. *J Clin Invest* (1994) **93**:1439–50.
18. Baranowska B, Zgliczynski S. The role of sex hormones in the mechanism of inhibited LH release in female patients with anorexia nervosa. *Acta Endocrinol (Copenh)* (1982) **99**:334–8.
19. Allouche J, Bennet A, Barbe P, Plantavid M, Caron P, Louvet JP. LH pulsatility and in vitro bioactivity in women with anorexia nervosa-related hypothalamic amenorrhea. *Acta Endocrinol (Copenh)* (1991) **125**:614–20.
20. Valenti S, Giusti M, Guido R, Cavallero D, Giordano G. Opioid tonus and luteinizing hormone secretion in anorexia nervosa: priming effect with serotonin precursor L-5-hydroxytryptophan during pulsatile gonadotropin-releasing hormone administration. *Biol Psychiatry* (1994) **36**:609–15. doi:10.1016/0006-3223(94)90073-6
21. Judd SJ, Wong J, Saloniklis S, Maiden M, Yeap B, Filmer S, et al. The effect of alprazolam on serum cortisol and luteinizing hormone pulsatility in normal women and in women with stress-related anovulation. *J Clin Endocrinol Metab* (1995) **80**:818–23. doi:10.1210/jcem.80.3.7883836
22. Lado-Abeal J, Rey C, Cabezas-Agricola JM, Rodriguez A, Camarero E, Cabezas-Cerrato J. L-hydroxytryptophan amplifies pulsatile secretion of LH in the follicular phase of normal women. *Clin Endocrinol (Oxf)* (1997) **47**:555–63. doi:10.1046/j.1365-2265.1997.3211126.x
23. Hsu LK, Crisp AH, Harding B. Outcome of anorexia nervosa. *Lancet* (1979) **1**:61–5. doi:10.1016/S0140-6736(79)90060-6
24. Viricel J, Bossu C, Galusca B, Kadem M, Germain N, Nicolau A, et al. [Retrospective study of anorexia nervosa: reduced mortality and stable recovery rates]. *Presse Med* (2005) **34**:1505–10. doi:10.1016/S0755-4982(05)84213-7
25. Bergh C, Brodin U, Lindberg G, Södersten P. Randomized controlled trial of a treatment for anorexia and bulimia nervosa. *Proc Natl Acad Sci U S A* (2002) **99**:9486–91. doi:10.1073/pnas.142284799
26. Nillius SJ, Fries H, Wide L. Successful induction of follicular maturation and ovulation by prolonged treatment with LH-releasing hormone in women with anorexia nervosa. *Am J Obstet Gynecol* (1975) **122**:921–8.
27. Rigotti NA, Nussbaum SR, Herzog DB, Neer RM. Osteoporosis in women with anorexia nervosa. *N Engl J Med* (1984) **311**:1601–6. doi:10.1056/NEJM198412203112503
28. Galusca B, Bossu C, Germain N, Kadem M, Frere D, Lafage-Proust MH, et al. Age-related differences in hormonal and nutritional impact on lean anorexia nervosa bone turnover uncoupling. *Osteoporos Int* (2006) **17**:888–96. doi:10.1007/s00198-005-0063-0
29. Faje AT, Fazeli PK, Miller KK, Katzman DK, Ebrahimi S, Lee H, et al. Fracture risk and areal bone mineral density in adolescent females with anorexia nervosa. *Int J Eat Disord* (2014) **47**:458–66. doi:10.1002/eat.22248
30. Stergioti E, Deligeoroglou E, Economou E, Tsitsika A, Dimopoulos KD, Daponte A, et al. Gene receptor polymorphism as a risk factor for BMD deterioration in adolescent girls with anorexia nervosa. *Gynecol Endocrinol* (2013) **29**:716–9. doi:10.3109/09513590.2013.798275
31. Grinspoon S, Baum H, Lee K, Anderson E, Herzog D, Klibanski A. Effects of short-term recombinant human insulin-like growth factor I administration on bone turnover in osteopenic women with anorexia nervosa. *J Clin Endocrinol Metab* (1996) **81**:3864–70. doi:10.1210/jcem.81.11.8923830
32. Miller KK, Meenaghan E, Lawson EA, Misra M, Gleysteen S, Schoenfeld D, et al. Effects of risedronate and low-dose transdermal testosterone on bone mineral density in women with anorexia nervosa: a randomized, placebo-controlled study. *J Clin Endocrinol Metab* (2011) **96**:2081–8. doi:10.1210/jc.2011-0380
33. Fazeli PK, Wang IS, Miller KK, Herzog DB, Misra M, Lee H, et al. Teriparatide increases bone formation and bone mineral density in adult women with anorexia nervosa. *J Clin Endocrinol Metab* (2014). doi:10.1210/jc.2013-4105
34. Misra M, Klibanski A. Bone health in anorexia nervosa. *Curr Opin Endocrinol Diabetes Obes* (2011) **18**:376–82. doi:10.1097/MED.0b013e32834b4bdc
35. Divasta AD, Feldman HA, Giancaterino C, Rosen CJ, Leboff MS, Gordon CM. The effect of gonadal and adrenal steroid therapy on skeletal health in adolescents and young women with anorexia nervosa. *Metabolism* (2012) **61**:1010–20. doi:10.1016/j.metabol.2011.11.016
36. DiVasta AD, Feldman HA, Beck TJ, LeBoff MS, Gordon CM. Does hormone replacement normalize bone geometry in adolescents with anorexia nervosa? *J Bone Miner Res* (2014) **29**:151–7. doi:10.1002/jbmr.2005
37. Galusca B, Zouch M, Germain N, Bossu C, Frere D, Lang F, et al. Constitutional thinness: unusual human phenotype of low bone quality. *J Clin Endocrinol Metab* (2008) **93**:110–7. doi:10.1210/jc.2007-1591
38. Bossu C, Galusca B, Normand S, Germain N, Collet P, Frere D, et al. Energy expenditure adjusted for body composition differentiates constitutional thinness from both normal subjects and anorexia nervosa. *Am J Physiol Endocrinol Metab* (2007) **292**:E132–7. doi:10.1152/ajpendo.00241.2006
39. Germain N, Galusca B, Grouselle D, Frere D, Tolle V, Zizzari P, et al. Ghrelin/obestatin ratio in two populations with low bodyweight: constitutional thinness and anorexia nervosa. *Psychoneuroendocrinology* (2009) **34**:413–9. doi:10.1016/j.psyneuen.2008.10.001
40. Ando T. Ghrelin gene variants and eating disorders. *Vitam Horm* (2013) **92**:107–23. doi:10.1016/B978-0-12-410473-0.00004-0
41. Terashi M, Asakawa A, Harada T, Ushikai M, Coquerel Q, Sinno MH, et al. Ghrelin reactive autoantibodies in restrictive anorexia nervosa. *Nutrition* (2011) **27**:407–13. doi:10.1016/j.nut.2011.01.002
42. Germain N, Galusca B, Grouselle D, Frere D, Billard S, Epelbaum J, et al. Ghrelin and obestatin circadian levels differentiate bingeing-purging from restrictive anorexia nervosa. *J Clin Endocrinol Metab* (2010) **95**:3057–62. doi:10.1210/jc.2009-2196
43. Galusca B, Jeandel L, Germain N, Alexandre D, Leprince J, Anouar Y, et al. Orexigenic neuropeptide 26RFa: new evidence for an adaptive profile of appetite regulation in anorexia nervosa. *J Clin Endocrinol Metab* (2012) **97**:2012–8. doi:10.1210/jc.2011-3396
44. Södersten P, Nergårdh R, Bergh C, Zandian M, Scheurink A. Behavioral neuroendocrinology and treatment of anorexia nervosa. *Front Neuroendocrinol* (2008) **29**:445–62. doi:10.1016/j.yfrne.2008.06.001
45. Sedlackova D, Kopeckova J, Papezova H, Hainer V, Kvasnickova H, Hill M, et al. Comparison of a high-carbohydrate and high-protein breakfast effect on plasma ghrelin, obestatin, NPY and PYY levels in women with anorexia and bulimia nervosa. *Nutr Metab (Lond)* (2012) **9**:52. doi:10.1186/1743-7075-9-52
46. Lawson EA, Eddy KT, Donoho D, Misra M, Miller KK, Meenaghan E, et al. Appetite-regulating hormones cortisol and peptide YY are associated with disordered eating psychopathology, independent of body mass index. *Eur J Endocrinol* (2011) **164**:253–61. doi:10.1530/EJE-10-0523
47. Utz AL, Lawson EA, Misra M, Mickley D, Gleysteen S, Herzog DB, et al. Peptide YY (PYY) levels and bone mineral density (BMD) in women with anorexia nervosa. *Bone* (2008) **43**:135–9. doi:10.1016/j.bone.2008.03.007
48. Grinspoon S, Gulick T, Askari H, Landt M, Lee K, Anderson E, et al. Serum leptin levels in women with anorexia nervosa. *J Clin Endocrinol Metab* (1996) **81**:3861–3. doi:10.1210/jc.81.11.3861
49. Garner DM, Olmstead MP, Polivy J. Development and validation of a multidimensional eating disorder inventory for anorexia nervosa and bulimia. *Int J Eat Disord* (1983) **2**:15–34. doi:10.1002/1098-108X(198321)2:2<15::AID-EAT2260020203>3.0.CO;2-6
50. Cooper Z, Fairburn CG. The eating disorders examination: a semistructured interview for the assessment of the specific psychopathology of eating disorders.

*Int J Eat Disord* (1987) **6**:1–8. doi:10.1002/1098-108X(198701)6:1<1::AID-EAT2260060102>3.0.CO;2-9
51. Van Strien T, Friejters JE, Bergers GPA, Defares PB. The Dutch eating behavior questionnaire for assessment of restrained, emotional and external eating behavior. *Int J Eat Disord* (1986) **5**:295–315. doi:10.1002/1098-108X(198602)5:2<295::AID-EAT2260050209>3.0.CO;2-T
52. Enoch MA, Kaye WH, Rotondo A, Greenberg BD, Murphy DL, Goldman D. 5-HT2A promoter polymorphism -1438G/A, anorexia nervosa, and obsessive-compulsive disorder. *Lancet* (1998) **351**:1785–6. doi:10.1016/S0140-6736(05)78745-6
53. Woodside DB, Field LL, Garfinkel PE, Heinmaa M. Specificity of eating disorders diagnoses in families of probands with anorexia nervosa and bulimia nervosa. *Compr Psychiatry* (1998) **39**:261–4. doi:10.1016/S0010-440X(98)90033-5
54. Pinheiro AP, Bulik CM, Thornton LM, Sullivan PF, Root TL, Bloss CS, et al. Association study of 182 candidate genes in anorexia nervosa. *Am J Med Genet B Neuropsychiatr Genet* (2010) **153B**:1070–80. doi:10.1002/ajmg.b.31082
55. Boraska V, Franklin CS, Floyd JA, Thornton LM, Huckins LM, Southam L, et al. A genome-wide association study of anorexia nervosa. *Mol Psychiatry* (2014). doi:10.1038/mp.2013.187
56. Jacquemont S, Reymond A, Zufferey F, Harewood L, Walters RG, Kutalik Z, et al. Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. *Nature* (2011) **478**:97–102. doi:10.1038/nature10406
57. Arcelus J, Witcomb GL, Mitchell A. Prevalence of eating disorders amongst dancers: a systemic review and meta-analysis. *Eur Eat Disord Rev* (2013) **22**(2):92–101. doi:10.1002/erv.2271
58. Brown TA, Holland LA, Keel PK. Comparing operational definitions of DSM-5 anorexia nervosa for research contexts. *Int J Eat Disord* (2014) **47**:76–84. doi:10.1002/eat.22184

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# Neuropsychiatric comorbidity in obesity: role of inflammatory processes

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Neuropsychiatric symptoms are frequent in obesity. In addition to their substantial economic and health impact, these symptoms significantly interfere with the quality of life and social function of obese individuals. While the pathophysiological mechanisms underlying obesity-related neuropsychiatric symptoms are still under investigation and remain to be clearly identified, there is increasing evidence for a role of inflammatory processes. Obesity is characterized by a chronic low-grade inflammatory state that is likely to influence neuropsychiatric status given the well-known and highly documented effects of inflammation on brain activity/function and behavior. This hypothesis is supported by recent findings emanating from clinical investigations in obese subjects and from experimentations conducted in animal models of obesity. These studies converge to show that obesity-related inflammatory processes, originating either from the adipose tissue or gut microbiota environment, spread to the brain where they lead to substantial changes in neurocircuitry, neuroendocrine activity, neurotransmitter metabolism and activity, and neurogenesis. Together, these alterations contribute to shape the propitious bases for the development of obesity-related neuropsychiatric comorbidities.

**Keywords: obesity, inflammation, neuroinflammation, cytokines, gut-brain axis, mood, cognition, neuropsychiatric symptoms**

## INTRODUCTION

The pandemic of obesity represents a major public health concern, as this disorder is associated with an increased risk of medical comorbidities contributing to a significant rise in mortality. Among those comorbidities related to obesity, neuropsychiatric disorders are particularly preoccupying. Not only neuropsychiatric symptoms affect the quality of life of obese subjects and contribute to their social impairment, but also they represent potent risk factors for aggravation of obesity. Given the growing prevalence of both obesity and neuropsychiatric disorders worldwide, the identification of the mechanisms underlying their comorbid association is urgently needed.

While different mechanisms are likely to be involved in the development of neuropsychiatric comorbidity in obesity, there is increasing evidence for a role of inflammatory processes. Chronic low-grade inflammation is an important characteristic of obesity and inflammatory processes are notorious for modulating brain functions and causing behavioral alterations. Recent clinical findings indicate that the increased systemic expression of inflammatory markers (e.g., cytokines) in obesity correlates with neuropsychiatric status, notably as it relates to mood and cognitive function. Moreover, studies in experimental animal models of obesity contribute to show that obesity-related inflammation manifests not only at the periphery but also within the brain where it modulates neurocircuitry, neurochemistry, and behavior. These findings that provide strong support to the notion that obesity-related inflammation plays an important role in the pathophysiology of neuropsychiatric symptoms will be presented and discussed in the present review.

## NEUROPSYCHIATRIC COMORBIDITY IN OBESITY

Neuropsychiatric comorbidity, including mood and anxiety disorders, binge eating, and mild cognitive impairment, is frequent in obesity and is associated with a significant reduction in the quality of life and social functioning of obese individuals. Among those disorders often seen in obese subjects, depressive symptoms are particularly frequent with a prevalence rate significantly higher (up to 30%) compared to the general age-matched population (1–5). Similarly, reports of cognitive disturbances in obesity are multiple. Those alterations concern primarily planning function, problem solving, mental flexibility, and inhibitory processes, suggestive of frontal lobe alterations (6–9). Impairment in memory, regardless of age, has been also reported (10, 11). Associations between obesity and cognitive impairment have been more often reported in cross-sectional studies comparing performance from overweight/obese subjects to performance from lean participants. Nevertheless, a predictive longitudinal association of obesity with the development of age-related cognitive deficit has also been documented in several reports (12–14).

The directionality of the relationship between obesity and neuropsychiatric symptoms is usually difficult to determine from clinical studies. The significant improvement in mood and cognitive function reported after weight loss induced by bariatric surgery or diet restriction in obese subjects supports the hypothesis

that obesity significantly impacts neuropsychiatric status and contributes to the development of neuropsychiatric comorbidity (15–22). Nevertheless, other reports indicate that preexisting mood and cognitive alterations can promote and/or predict the development of later obesity (23–26), attesting of the bidirectional link between obesity and neuropsychiatric status. To further address this issue, animal models of obesity represent certainly a useful and unique opportunity. Several models of obesity resulting either from genetic manipulations or diet modifications have been developed over the last decades (27). Among those models, diet-induced obesity (DIO) is probably the closest to human obesity with respect to etiological aspects. Moreover, because of its longitudinal characteristic, DIO allows the investigation of the mechanisms and pathophysiological changes preceding the development of obesity-related comorbidities, including neuropsychiatric alterations. Genetic models of obesity, in particular severe obesity, are also of great interest to explore the genetic–metabolic–brain interactions associated with obesity-related comorbidities. In that context, *ob*/*ob* (deficient for leptin) and *db*/*db* (deficient for functional leptin receptor) mice are particularly relevant as, in addition to metabolic disorders, these mice also display brain alterations (28–30). Overall, behavioral changes reported in experimental models of obesity include alterations in emotional reactivity and impairment in learning and memory [for review, see Ref. (31)]. Relevant to neural function, significant decreases in hippocampal-dependent learning together with impaired hippocampal neurogenesis and neuronal plasticity have been documented in animal models of DIO, notably in young mice (32–35). Similar results were described in *db*/*db* mice (28–30), supporting the notion that the hippocampus plays a major role in mediating obesity-associated cognitive impairment. Interestingly, *db*/*db* mice also display anxiety-like behavior (36). In contrast, depressive-like behavior appears to be mostly unchanged in animal models of obesity (36, 37), except in challenging conditions including stimulation of the immune system (38–40). Altogether, these data comfort the notion that neuropsychiatric comorbidities in obesity rely on interactions involving multiple systems, including metabolic characteristics, environmental influences, and immune-related processes.

## OBESITY AND INFLAMMATION

Basal systemic low-grade inflammation is a fundamental characteristic of obesity, which is now considered not only as a metabolic disorder but also as an inflammatory condition affecting both the innate and acquired immune systems (41, 42). Obesity is characterized by increased levels of circulating proinflammatory cytokines [including interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6], accumulation of leukocytes within the adipose tissue and other organs, activation of macrophages in the liver and fat, and activation of proinflammatory signaling pathways in multiple organs (43, 44). Inflammatory markers in obesity correlate more with measures of central adiposity, such as waist circumference and waist-to-hip ratio, rather than with the general measure of body mass index (BMI) (45–48). Interestingly, significant improvement in inflammatory profile is obtained after weight loss induced by low-caloric diet or bariatric surgery in obese individuals (49–54).

Different mechanisms have been identified as playing a major role in the instauration of the chronic low-grade inflammatory state that characterizes obesity (**Figure 1**). One major player is

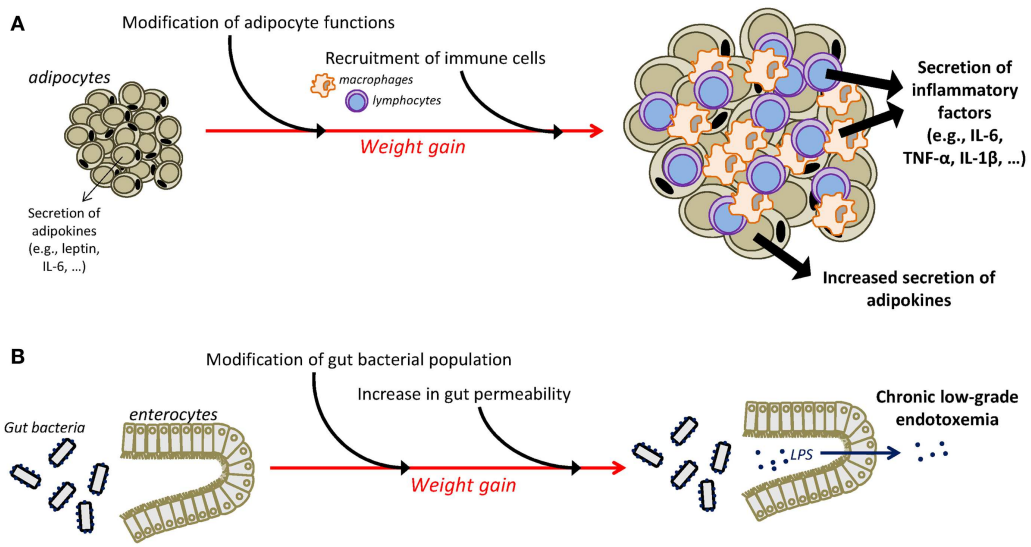


**FIGURE 1 | Mechanisms underlying chronic low-grade inflammatory state in obesity**. The adipose tissue is an important contributor of chronic low-grade inflammation in obesity **(A)**. Weight gain is associated with substantial changes in the function of adipocytes that increase their secretion of adipokines, including inflammatory factors. Moreover, infiltration of immune cells, i.e., macrophages and T cells, in the adipose tissue is also responsible for the adipose secretion of inflammatory cytokines. Additional mechanisms, including alterations in the gut microbiota, contribute also to the instauration of obesity-related inflammation **(B)**. Obesity is associated with modifications in gut microbial population and with an increased permeability of the intestinal wall that promotes the passage of LPS in the circulation, leading thus to the development of chronic low-grade endotoxemia and the increased production of inflammatory factors. IL-1β, interleukin-1β; IL-6, interleukin-6; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α.

the adipose tissue that has the ability to secrete adipokines and in which macrophages accumulate and potently secrete inflammatory factors (55–59). Moreover, an additional role for T cells in the development of adiposity-related inflammation is supported by several recent studies (57, 60–63). Obesity-related inflammation can also be triggered by pathogens, as there is now evidence of gut microbiota alterations associated with inflammatory processes in obesity (64, 65). In obese animals, gut microbial population is altered independently of diet characteristics (66), notably in the form of decreased *Bacteroidetes* and *Bifidobacterium* populations together with increases in the number of *Firmicutes* (64, 66, 67). High-fat diet is also associated with alterations in gut permeability leading to the instauration of a state of chronic low-grade endotoxemia (presence of lipopolysaccharide, LPS, in the blood) that is believed to contribute to obesity-related inflammation by the activation of systemic macrophages through the binding of LPS on TLR4 (68, 69). Similarly, recent clinical data in obese individuals indicate significant associations between gut microbiota modifications, reflected by the reduction in *Bacteroidetes/Firmicutes* ratio, and markers of local and systemic inflammation (67) and document improvement in the intestinal microbiota profile and in serum levels of an endotoxemia marker (LPS binding protein) following weight loss (70–72).

It is now clear that systemic inflammation in obesity contributes to both increased central inflammatory processes (notably in the hippocampus and hypothalamus) and metabolic dysregulations, including insulin-resistance. While these mechanisms are believed to play a major role in the development and maintenance of obesity (68, 73), their specific contribution to the development of the disorder is still under investigation. With respect to central inflammation, *db*/*db* mice that exhibit immune defects such as increased systemic inflammation and reduced immune competence (64, 74, 75) also show increased hippocampus cytokine expression (36, 37), and these effects are associated. Moreover, there is mounting evidence for enhanced cytokine expression and microglial activation in the hypothalamus in animal models of obesity (76–78). Consistent with these experimental findings, clinical indication of gliosis was recently reported in the mediobasal hypothalamus of obese individuals (79). While it is likely that central inflammation in obesity results from adiposity-related systemic inflammatory processes, the increases in circulating levels of TLR4 ligands and free saturated fatty acids following impaired gut permeability in high-fat diet mice (68, 80) may alternatively contribute to central inflammation, through activation of TLR4/MyD88 signaling (81, 82). Further investigation is needed to determine whether central activation of inflammatory processes occurs as a result of peripheral inflammation or whether it represents an early event promoting the development of obesity following a high-fat diet.

With regard to inflammation-related metabolic dysregulations, the inflammatory cytokine, TNF-α, was found to play an important role in the pathogenesis of insulin-resistance and type 2 diabetes (83–85). Inflammatory factors are also strong modulators of energy balance mainly due to their effects on the brain, in particular the hypothalamus (76), a mechanism that may promote weight gain through impairment of local peptidergic neuronal networks involved in food intake (73, 86). Interestingly, obesity-associated inflammation, notably as it relates to the visceral adipose tissue, was found to impact obesity treatment outcomes, with increased adipose expression of immune cells and inflammatory markers being associated with lower BMI reduction after bariatric surgery in severely obese patients (57).

## CENTRAL EFFECTS OF INFLAMMATION: EVIDENCE AND MECHANISMS

There is clear evidence for a role of proinflammatory cytokines in the development of neuropsychiatric symptoms (87). Proinflammatory cytokines released locally by activated innate immune cells have access to the brain, through different mechanisms that have been reviewed elsewhere (87). These pathways, which include humoral, neural, and cellular routes, ultimately lead to the production of cytokines by activated glial cells, in particular microglia, within the central nervous system (CNS). While microglia activation normally exerts a protective action on the CNS, its unregulated and chronic activation may in contrast become deleterious. Within the brain, proinflammatory cytokines activate the neuroendocrine system, impair neurotransmitter metabolism and function, and alter neural plasticity and brain circuitry (87). These biological alterations are associated with a large number of behavioral changes that have been referred to as sickness behavior (88). Necessary for the recovery of the host to the infection, sickness behavior usually resolves within few days. However, in cases of chronic and unregulated activation of the immune system, sickness behavior may evolve into clinically relevant neuropsychiatric symptoms, including depression and cognitive symptoms (88). At the clinical level, strong evidence for a role of cytokines in the development of neuropsychiatric comorbidity emanates from the model of cytokine therapy, based in particular on interferon (IFN)-α administration. Using this model, we and others have shown that IFN-α induces major depression in up to 45% of treated patients (89, 90) and that this effect relates on inflammation-induced alterations in the hypothalamic–pituitary–adrenal (HPA) axis, neurotransmitter function, and enzymatic pathways involved in the metabolism of monoamines (87, 91). Consistent with this last point, inflammatory factors are able to induce the synthesis of the enzymes indoleamine 2,3-dioxygenase (IDO) and GTP-cyclohydrolase 1 (GTP-CH1) in monocytes/macrophages and dendritic cells, which results in significant alterations in the biosynthesis of key monoamines (e.g., serotonin, dopamine) known to play a major role in mood regulation and cognitive function. Moreover, IDO is the first and rate-limiting enzyme that catabolizes tryptophan along the kynurenine pathway, a pathway leading ultimately to the production of neuroactive metabolites that have been associated with depressive symptoms in IFN-α-treated patients (92). In particular, IDO activation results in an increased production of the glutamatergic metabolites, 3-hydroxykynurenine, and quinolinic acid, which are well-known to induce neuronal death and to whom brain or cerebrospinal fluid concentrations were found to be increased in several neuropsychiatric or neurodegenerative diseases (93–96). Consistent with these data, inflammation-induced depressive- and anxiety-like behaviors in mice can be prevented by pharmacological or genetic inhibition of brain IDO activation (97–101). Moreover, NMDA receptor blockade abrogates cytokine-induced depressive-like behavior in mice (102). Interestingly, the hippocampus was found to play an

important role in cytokine and IDO activation (103–106) and dysregulated activation of hippocampal microglia was associated with sustained IDO activity and protracted depressive-like behavior (104). Moreover, emotional alterations linked to inflammation-induced hippocampus IDO activation in mice was associated with reduced hippocampal expression of the brain-derived neurotrophic factor (BDNF) (107) that contributes to mood regulation and memory function. Altogether, these results point to a pivotal role of IDO activation, particularly in the hippocampus, in mediating cytokine-induced mood and cognitive alterations.

## ROLE OF INFLAMMATORY PROCESSES IN OBESITY-RELATED NEUROPSYCHIATRIC SYMPTOMS: CLINICAL AND EXPERIMENTAL FINDINGS

Recent clinical findings support the hypothesis that inflammatory processes contribute to neuropsychiatric comorbidity in obesity, notably as it relates to mood status and cognitive function [see for review, Ref. (108)]. In support of this notion, concentrations of the inflammatory markers, C reactive protein (CRP) and IL-6, have been associated with depressive symptoms in obese subjects or in patients afflicted with the metabolic syndrome (109–111). Similar associations have been reported with leptin (112). Moreover, it was recently shown that CRP levels explained approximately 20% of the increase in depression scores over time in obese subjects (113). Consistent with the role of adiposity in these associations, reductions in inflammatory markers following weight loss induced by bariatric surgery were found to correlate with significant improvement in the emotional status and depression scores of severely obese individuals (114, 115). Given the bidirectional link reported between obesity and depressive symptoms, it is highly probable that depressive symptoms occurring in the context of obesity-related inflammation may in turn contribute to obesity maintenance, promoting thus the instauration of a vicious circle. Regarding cognitive function, a significant relationship was reported between CRP levels and decreased performance on cognitive tests targeting frontal lobe function in obese and overweight women (116). Moreover, in patients with the metabolic syndrome, higher levels of CRP and IL-6 were found to increase the risk of age-related cognitive decline (117). While these data support a role for obesity-related inflammation in the development of neuropsychiatric symptoms, the literature is still sparse regarding the causality of the events and the mechanisms that specifically underlie these effects in the context of obesity. Result from animal models of obesity may help to start to address this issue. In genetically or diet-induced obese rodents, increased cytokine expression in the hippocampus and cortex is associated with emotional and cognitive alterations (30, 31, 36, 37, 118). Interestingly, hippocampal IL-1β expression in *db*/*db* mice is related to adiposity and its blockade normalizes hippocampal dendritic spine density and prevents synaptic dysfunction and cognitive impairment (30). Associations have also been found between hippocampal microgliosis and obesity-related elevation in plasma glucocorticoids in the same mice (119). Consistent with the role of inflammatory processes, a direct relationship has been recently reported by our group between inflammation-related brain IDO activation and the development of depressive-like behavior in *db*/*db* mice (37). Similarly, we showed that DIO exacerbates both hippocampal induction of cytokines and IDO in response to an immune challenge and related behavioral changes (40). Interestingly, exacerbated depressive-like behavior is also associated in DIO mice with increased hypothalamic inflammation (39, 40). Beyond its impact on energy homeostasis, hypothalamic inflammation might also influence obesity-related emotional alterations. In addition to inflammatory processes, metabolic factors associated with obesity, including insulin or leptin, may also be able to act within the brain and lead to behavioral alterations (120). Nevertheless, several studies suggest that these factors *per se* are not sufficient to explain neuropsychiatric symptoms occurring in contexts of obesity. In support of this, increased emotional behaviors and cognitive impairment have been reported in animal models of obesity in the absence of any significant hyperinsulinemia (32, 36, 40). Reciprocally, the normalization of hyperglycemia in *db*/*db* mice was not effective in reversing spatial cognitive impairment or anxiety-like behavior (28, 29). Moreover, no difference in brain concentrations of glucose and insulin was measured in both *db*/*db* and *db*/+ mice and these concentrations remained the same when peripheral hyperinsulinemia was normalized (28). Altogether, these data point to brain inflammation as a major player in the development of obesity-related neuropsychiatric symptoms, although the pathways linking inflammation to these symptoms still need to be thoroughly studied.

## INFLAMMATION-DRIVEN NEUROPSYCHIATRIC COMORBIDITY IN OBESITY: POTENTIAL UNDERLYING PATHWAYS

There are several pathways by which inflammation may promote the development of neuropsychiatric comorbidity in the context of obesity. Some of these mechanisms may be common to various situations of chronic inflammation and some may be more specific to the condition of obesity. Non-specific mechanisms include diffusion of inflammatory markers from the adipose tissue to the circulation and activation of relevant immune-to-brain pathways including humoral, neural, and cellular routes leading ultimately to the production *de novo* of inflammatory cytokines within the CNS and subsequent alterations in CNS functions (e.g., changes in neuroendocrine function, neurocircuitry, enzymatic pathways, and neurotransmitter metabolism/function) as described above. In particular, similar to other inflammatory conditions, obesity has been often associated with alterations in basal ganglia/reward circuitry and dopamine function (121, 122). Accordingly, several studies have indicated that obesity is associated with reduced striatal dopamine D2 receptor availability together with alterations in the fronto-striatal network (123–125). Moreover, investigations in rodents with DIO have shown significant associations between alterations in striatal circuitry and depressive-like behavior, suggesting a role for dopamine-related disruptions in obesity-associated depressive symptoms (38). Relevant to the contribution of inflammatory processes to these effects, the basal ganglia and dopamine system are highly targeted by inflammatory factors (126). In addition, inflammation-induced neuropsychiatric symptoms, in particular fatigue, anhedonia, psychomotor slowing, decreased motivation, and depressed mood, have been found to relate to alterations in basal ganglia/dopamine

function and striatal circuitry in subjects treated with immune agents (127–129).

Alterations in neuroendocrine function – a mechanism highly described in the neurobiology of mood disorders – represent another common feature of inflammatory conditions, including obesity. In particular, obese subjects have been shown to exhibit an impaired feedback response to cortisol, similar to what is observed in depression (130). The immune system and neuroendocrine system are in constant communication and immune alterations are notorious for causing significant changes in neuroendocrine activity and *vice versa*. While the association between low-grade inflammation and alterations in the neuroendocrine system remains to be determined in obese subjects, it is highly possible that obesity-related neuroendocrine dysfunction contributes to neuropsychiatric comorbidity in obese individuals.

Converging findings have highlighted the consequences of deregulated hippocampal cytokines and neurotrophins expression on mood, learning, and memory (88, 131, 132), and the negative impact of cytokines on neurotrophins and synaptic plasticity (107, 133). Alterations in these mechanisms have been fully documented in models of chronic stress exposure, a well-admitted contributor of mood disorders (134, 135). Several clinical and experimental data strongly suggest that these deregulations may similarly participate to obesity-related neuropsychiatric alterations. Indeed, body weight loss induced by lifestyle intervention program in young obese patients normalizes plasma levels of BDNF (136). Moreover, cognitive impairment and emotional alterations reported in DIO and genetic models of obesity are linked to increased inflammation and reduced BDNF levels in the cortex (118) and the hippocampus (36, 37). Reciprocally, anti-inflammatory interventions in DIO mice reduce body weight, normalize hippocampal levels of BDNF, and prevent hippocampus-mediated cognitive impairments (137).

Alterations in the gut–brain axis represent one mechanism of inflammatory-driven neuropsychiatric comorbidity that may be more relevant to the condition of obesity. As already mentioned, obesity is associated with alterations in the gut microbiota in the form of modifications in microbiota populations, increased gut permeability, and activated inflammatory processes. A rich and complex communication network exists between the gut and the brain that involves endocrine, immune, and neural pathways (138), and there are now multiple evidences that impairment or dysregulation in the gut–brain axis impacts on mood and cognitive function (139). These data suggest that gut microbiota alterations found in obesity may modulate gut-to-brain communication pathways, leading thus to the development of neuropsychiatric comorbidity.

Altogether, these data are in favor of the involvement of inflammation-related complex non-exclusive pathophysiological processes in the development of neuropsychiatric symptoms in obesity. In that context, strategies to reduce inflammation either by pharmacological or non-pharmacological interventions (e.g., diet, surgical weight reduction strategies, exercise) may help in the prevention and management of obesity-related neuropsychiatric comorbidity. In particular, nutritional factors with immunomodulatory properties (i.e., omega-3 fatty acids, antioxidants) may worth being considered.

## CONCLUSION

Data presented in this review strongly support the notion that inflammatory processes represent key players in the development of neuropsychiatric comorbidities in obesity. In addition to clinical investigations that clearly highlight the relationship between

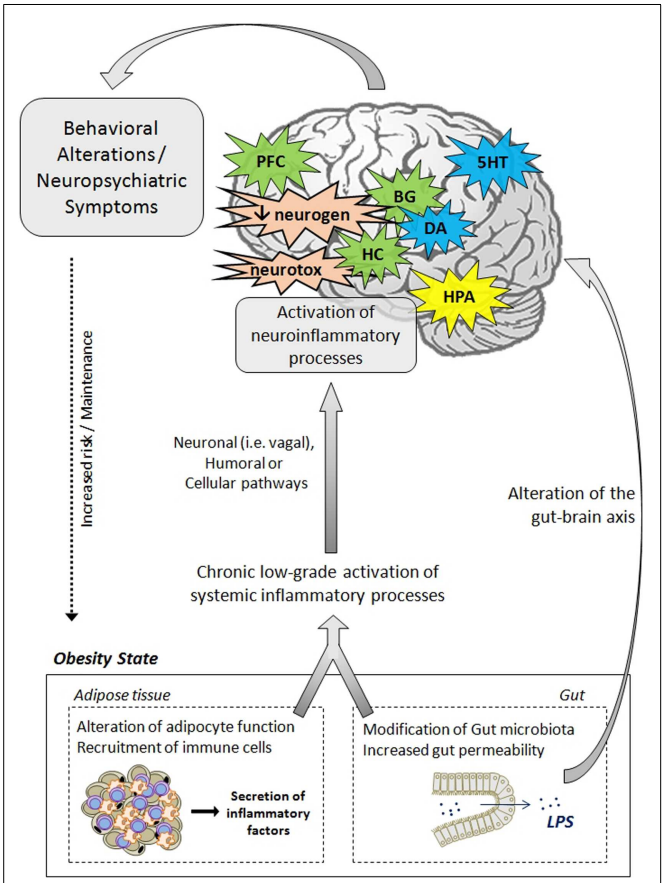


**FIGURE 2 | Pathophysiological mechanisms likely to underlie neuropsychiatric comorbidities associated with obesity**. The activation of systemic inflammatory processes, originating from alterations in adipose tissue and gut functions, can contribute to the development of obesity-associated neuropsychiatric comorbidities. Proinflammatory cytokines released at the periphery can access the brain via several pathways (e.g., neural, humoral, and cellular routes) and induce the activation of neuroinflammatory processes, primarily by activating microglia. In the brain, proinflammatory cytokines impair neuroendocrine activity, neurotransmitter function (e.g., 5HT, DA, glutamate), and neurocircuitry, involving notably the hippocampus, the hypothalamus, the basal ganglia, and the prefrontal cortex. Cytokines can also disturb neurogenesis and induce neurotoxic effects through induction of IDO-derived neuroactive/neurotoxic metabolites. Altogether, these brain alterations lead ultimately to the development of behavioral/neuropsychiatric symptoms. Deregulations of the gut–brain axis, originating from changes in gut microbiota and permeability, may also contribute to mood and cognitive symptoms. These behavioral/neuropsychiatric symptoms can in turn promote the development or maintenance of obesity through risky or unadjusted eating behaviors. 5HT, serotonin; BG, basal ganglia; CNS, central nervous system; DA, dopamine; IDO, indoleamine 2,3-dioxygenase; HC, hippocampus; HPA, hypothalamic–pituitary–adrenal axis; LPS, lipopolysaccharide; PFC, prefrontal cortex; neurogen, neurogenesis; neurotox, neurotoxicity.

adiposity-related inflammation and neuropsychiatric symptoms in obese individuals, animal studies provide strong evidence of the direct effects of obesity-related neuroinflammatory processes on brain function and neurocircuitry and on the development of behavioral symptoms. The mechanisms and pathways leading to neuropsychiatric comorbidities in obesity are also discussed, starting from a general aspect to a viewpoint more specific to the condition of obesity. The effects rely on complex communication networks including the immune system, the gut, the neuroendocrine system, and key brain areas, including the hypothalamus, the hippocampus, and the basal ganglia (**Figure 2**). Alterations in monoamine metabolism and function, impaired neurotransmitter activity together with the occurrence of neurotoxic effects likely to promote neuronal death and decreased neurogenesis appear to represent major pathophysiological pathways to neuropsychiatric morbidity in obese individuals.

## REFERENCES


1. Roberts RO, Geda YE, Knopman DS, Cha RH, Boeve BF, Ivnik RJ, et al. Metabolic syndrome, inflammation, and nonamnestic mild cognitive impairment in older persons: a population-based study. *Alzheimer Dis Assoc Disord* (2010) **24**:11–8. doi:10.1097/WAD.0b013e3181a4485c
2. Goldbacher EM, Bromberger J, Matthews KA. Lifetime history of major depression predicts the development of the metabolic syndrome in middle-aged women. *Psychosom Med* (2009) **71**:266–72. doi:10.1097/PSY.0b013e318197a4d5
3. Lasselin J, Capuron L. Chronic low-grade inflammation in metabolic disorders: relevance for behavioral symptoms. *Neuroimmunomodulation* (2014) **21**:95–101. doi:10.1159/000356535
4. Lin HY, Huang CK, Tai CM, Kao YH, Tsai CC, Hsuan CF, et al. Psychiatric disorders of patients seeking obesity treatment. *BMC Psychiatry* (2013) **13**:1. doi:10.1186/1471-244X-13-1
5. Evans DL, Charney DS, Lewis L, Golden RN, Gorman JM, Krishnan KR, et al. Mood disorders in the medically ill: scientific review and recommendations. *Biol Psychiatry* (2005) **58**:175–89. doi:10.1016/j.biopsych.2005.05.001
6. Boeka AG, Lokken KL. Neuropsychological performance of a clinical sample of extremely obese individuals. *Arch Clin Neuropsychol* (2008) **23**:467–74. doi:10.1016/j.acn.2008.03.003
7. Fagundo AB, de la Torre R, Jimenez-Murcia S, Aguera Z, Granero R, Tarrega S, et al. Executive functions profile in extreme eating/weight conditions: from anorexia nervosa to obesity. *PLoS One* (2012) **7**:e43382. doi:10.1371/journal.pone.0043382
8. Fergenbaum JH, Bruce S, Lou W, Hanley AJ, Greenwood C, Young TK. Obesity and lowered cognitive performance in a Canadian First Nations population. *Obesity (Silver Spring)* (2009) **17**:1957–63. doi:10.1038/oby.2009.161
9. Lokken KL, Boeka AG, Yellumahanthi K, Wesley M, Clements RH. Cognitive performance of morbidly obese patients seeking bariatric surgery. *Am Surg* (2010) **76**:55–9.
10. Elias MF, Elias PK, Sullivan LM, Wolf PA, D'Agostino RB. Lower cognitive function in the presence of obesity and hypertension: the Framingham heart study. *Int J Obes Relat Metab Disord* (2003) **27**:260–8. doi:10.1038/sj.ijo.802225
11. Gunstad J, Paul RH, Cohen RA, Tate DF, Gordon E. Obesity is associated with memory deficits in young and middle-aged adults. *Eat Weight Disord* (2006) **11**:e15–9. doi:10.1007/BF03327747
12. Cournot M, Marquie JC, Ansiau D, Martinaud C, Fonds H, Ferrieres J, et al. Relation between body mass index and cognitive function in healthy middle-aged men and women. *Neurology* (2006) **67**:1208–14. doi:10.1212/01.wnl.0000238082.13860.50
13. Dahl AK, Hassing LB, Fransson EI, Gatz M, Reynolds CA, Pedersen NL. Body mass index across midlife and cognitive change in late life. *Int J Obes (Lond)* (2013) **37**:296–302. doi:10.1038/ijo.2012.37
14. Sabia S, Kivimaki M, Shipley MJ, Marmot MG, Singh-Manoux A. Body mass index over the adult life course and cognition in late midlife: the Whitehall II Cohort Study. *Am J Clin Nutr* (2009) **89**:601–7. doi:10.3945/ajcn.2008.26482
15. Siervo M, Arnold R, Wells JC, Tagliabue A, Colantuoni A, Albanese E, et al. Intentional weight loss in overweight and obese individuals and cognitive function: a systematic review and meta-analysis. *Obes Rev* (2011) **12**:968–83. doi:10.1111/j.1467-789X.2011.00903.x
16. Alosco ML, Spitznagel MB, Strain G, Devlin M, Cohen R, Paul R, et al. Improved memory function two years after bariatric surgery. *Obesity (Silver Spring)* (2014) **22**:32–8. doi:10.1002/oby.20494
17. Miller LA, Crosby RD, Galioto R, Strain G, Devlin MJ, Wing R, et al. Bariatric surgery patients exhibit improved memory function 12 months postoperatively. *Obes Surg* (2013) **23**:1527–35. doi:10.1007/s11695-013-0970-7
18. Brinkworth GD, Buckley JD, Noakes M, Clifton PM, Wilson CJ. Long-term effects of a very low-carbohydrate diet and a low-fat diet on mood and cognitive function. *Arch Intern Med* (2009) **169**:1873–80. doi:10.1001/archinternmed.2009.329
19. Halyburton AK, Brinkworth GD, Wilson CJ, Noakes M, Buckley JD, Keogh JB, et al. Low- and high-carbohydrate weight-loss diets have similar effects on mood but not cognitive performance. *Am J Clin Nutr* (2007) **86**:580–7.
20. Andersen JR, Aasprang A, Bergsholm P, Sletteskog N, Vage V, Natvig GK. Anxiety and depression in association with morbid obesity: changes with improved physical health after duodenal switch. *Health Qual Life Outcomes* (2010) **8**:52. doi:10.1186/1477-7525-8-52
21. Dixon JB, Dixon ME, O'Brien PE. Depression in association with severe obesity: changes with weight loss. *Arch Intern Med* (2003) **163**:2058–65. doi:10.1001/archinte.163.17.2058
22. Thonney B, Pataky Z, Badel S, Bobbioni-Harsch E, Golay A. The relationship between weight loss and psychosocial functioning among bariatric surgery patients. *Am J Surg* (2010) **199**:183–8. doi:10.1016/j.amjsurg.2008.12.028
23. Luppino FS, de Wit LM, Bouvy PF, Stijnen T, Cuijpers P, Penninx BW, et al. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. *Arch Gen Psychiatry* (2010) **67**:220–9. doi:10.1001/archgenpsychiatry.2010.2
24. Guxens M, Mendez MA, Julvez J, Plana E, Forns J, Basagana X, et al. Cognitive function and overweight in preschool children. *Am J Epidemiol* (2009) **170**:438–46. doi:10.1093/aje/kwp140
25. Osika W, Montgomery SM, Longitudinal Birth Cohort Study. Physical control and coordination in childhood and adult obesity: Longitudinal Birth Cohort Study. *BMJ* (2008) **337**:a699. doi:10.1136/bmj.a699
26. Davis C, Levitan RD, Muglia P, Bewell C, Kennedy JL. Decision-making deficits and overeating: a risk model for obesity. *Obes Res* (2004) **12**:929–35. doi:10.1038/oby.2004.113
27. Kanasaki K, Koya D. Biology of obesity: lessons from animal models of obesity. *J Biomed Biotechnol* (2011) **2011**:197636. doi:10.1155/2011/197636
28. Stranahan AM, Arumugam TV, Cutler RG, Lee K, Egan JM, Mattson MP. Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. *Nat Neurosci* (2008) **11**:309–17. doi:10.1038/nn2055
29. Stranahan AM, Lee K, Martin B, Maudsley S, Golden E, Cutler RG, et al. Voluntary exercise and caloric restriction enhance hippocampal dendritic spine density and BDNF levels in diabetic mice. *Hippocampus* (2009) **19**:951–61. doi:10.1002/hipo.20577
30. Erion JR, Wosiski-Kuhn M, Dey A, Hao S, Davis CL, Pollock NK, et al. Obesity elicits interleukin 1-mediated deficits in hippocampal synaptic plasticity. *J Neurosci* (2014) **34**:2618–31. doi:10.1523/JNEUROSCI.4200-13.2014
31. Kanoski SE, Davidson TL. Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. *Physiol Behav* (2011) **103**:59–68. doi:10.1016/j.physbeh.2010.12.003
32. Boitard C, Cavaroc A, Sauvant J, Aubert A, Castanon N, Layé S, et al. Impairment of hippocampal-dependent memory induced by juvenile high-fat diet intake is associated with enhanced hippocampal inflammation in rats. *Brain Behav Immun* (2014). doi:10.1016/j.bbi.2014.03.005
33. Boitard C, Etchamendy N, Sauvant J, Aubert A, Tronel S, Marighetto A, et al. Juvenile, but not adult exposure to high-fat diet impairs relational memory and hippocampal neurogenesis in mice. *Hippocampus* (2012) **22**:2095–100. doi:10.1002/hipo.22032
34. Valladolid-Acebes I, Merino B, Principato A, Fole A, Barbas C, Lorenzo MP, et al. High-fat diets induce changes in hippocampal glutamate metabolism and neurotransmission. *Am J Physiol Endocrinol Metab* (2012) **302**:E396–402. doi:10.1152/ajpendo.00343.2011

35. Molteni R, Barnard RJ, Ying Z, Roberts CK, Gomez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience* (2002) **112**:803–14. doi:10.1016/S0306-4522(02)00123-9
36. Dinel AL, Andre C, Aubert A, Ferreira G, Laye S, Castanon N. Cognitive and emotional alterations are related to hippocampal inflammation in a mouse model of metabolic syndrome. *PLoS One* (2011) **6**:e24325. doi:10.1371/journal.pone.0024325
37. Dinel AL, André C, Aubert A, Ferreira G, Layé S, Castanon N. Lipopolysaccharide-induced brain activation of the indoleamine 2,3-dioxygenase and depressive-like behavior are impaired in a mouse model of metabolic syndrome. *Psychoneuroendocrinology* (2014) **40**:48–59. doi:10.1016/j.psyneuen.2013.10.014
38. Sharma S, Fulton S. Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. *Int J Obes (Lond)* (2013) **37**:382–9. doi:10.1038/ijo.2012.48
39. Aguliar-Valles A, Kim J, Jung S, Woodside B, Luheshi GN. Role of brain transmigrating neutrophils in depression-like behavior during systemic infection. *Mol Psychiatry* (2014) **19**:599–606. doi:10.1038/mp.2013.154
40. André C, Dinel AL, Ferreira G, Layé S, Castanon N. Diet-induced obesity progressively alters cognition, anxiety-like behavior and lipopolysaccharide-induced depressive-like behavior: focus on brain indoleamine 2,3-dioxygenase activation. *Brain Behav Immun* (2014). doi:10.1016/j.bbi.2014.03.012
41. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. *Gastroenterology* (2007) **132**:2169–80. doi:10.1053/j.gastro.2007.03.059
42. Nieman DC, Henson DA, Nehlsen-Cannarella SL, Ekkens M, Utter AC, Butterworth DE, et al. Influence of obesity on immune function. *J Am Diet Assoc* (1999) **99**:294–9. doi:10.1016/S0002-8223(99)00077-2
43. Cancello R, Clement K. Is obesity an inflammatory illness? Role of low-grade inflammation and macrophage infiltration in human white adipose tissue. *BJOG* (2006) **113**:1141–7. doi:10.1111/j.1471-0528.2006.01004.x
44. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. *Annu Rev Immunol* (2011) **29**:415–45. doi:10.1146/annurev-immunol-031210-101322
45. Brooks GC, Blaha MJ, Blumenthal RS. Relation of C-reactive protein to abdominal adiposity. *Am J Cardiol* (2010) **106**:56–61. doi:10.1016/j.amjcard.2010.02.017
46. Hermsdorff HH, Zulet MA, Puchau B, Martinez JA. Central adiposity rather than total adiposity measurements are specifically involved in the inflammatory status from healthy young adults. *Inflammation* (2011) **34**:161–70. doi:10.1007/s10753-010-9219-y
47. Thorand B, Baumert J, Doring A, Herder C, Kolb H, Rathmann W, et al. Sex differences in the relation of body composition to markers of inflammation. *Atherosclerosis* (2006) **184**:216–24. doi:10.1016/j.atherosclerosis.2005.04.011
48. Thewissen MM, Damoiseaux JG, Duijvestijn AM, van Greevenbroek MM, van der Kallen CJ, Feskens EJ, et al. Abdominal fat mass is associated with adaptive immune activation: the CODAM Study. *Obesity (Silver Spring)* (2011) **19**:1690–8. doi:10.1038/oby.2010.337
49. Belza A, Toubro S, Stender S, Astrup A. Effect of diet-induced energy deficit and body fat reduction on high-sensitive CRP and other inflammatory markers in obese subjects. *Int J Obes (Lond)* (2009) **33**:456–64. doi:10.1038/ijo.2009.27
50. Dalmas E, Rouault C, Abdennour M, Rovere C, Rizkalla S, Bar-Hen A, et al. Variations in circulating inflammatory factors are related to changes in calorie and carbohydrate intakes early in the course of surgery-induced weight reduction. *Am J Clin Nutr* (2011) **94**:450–8. doi:10.3945/ajcn.111.013771
51. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *JAMA* (2003) **289**:1799–804. doi:10.1001/jama.289.14.1799
52. Hakeam HA, O'Regan PJ, Salem AM, Bamehriz FY, Jomaa LF. Inhibition of C-reactive protein in morbidly obese patients after laparoscopic sleeve gastrectomy. *Obes Surg* (2009) **19**:456–60. doi:10.1007/s11695-008-9729-y
53. Manco M, Fernandez-Real JM, Equitani F, Vendrell J, Valera Mora ME, Nanni G, et al. Effect of massive weight loss on inflammatory adipocytokines and the innate immune system in morbidly obese women. *J Clin Endocrinol Metab* (2007) **92**:483–90. doi:10.1210/jc.2006-0960
54. Viana EC, Araujo-Dasilio KL, Miguel GP, Bressan J, Lemos EM, Moyses MR, et al. Gastric bypass and sleeve gastrectomy: the same impact on IL-6 and TNF-alpha. Prospective clinical trial. *Obes Surg* (2013) **23**:1252–61. doi:10.1007/s11695-013-0894-2
55. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr* (2004) **92**:347–55. doi:10.1079/BJN20041213
56. Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest* (2003) **112**:1785–8. doi:10.1172/JCI200320514
57. Lasselin J, Magne E, Beau C, Ledaguenel P, Dexpert S, Aubert A, et al. Adipose inflammation in obesity: relationship with circulating levels of inflammatory markers and association with surgery-induced weight loss. *J Clin Endocrinol Metab* (2014) **99**:E53–61. doi:10.1210/jc.2013-2673
58. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* (2003) **112**:1796–808. doi:10.1172/JCI19246
59. Cancello R, Henegar C, Viguerie N, Taleb S, Poitou C, Rouault C, et al. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes* (2005) **54**:2277–86. doi:10.2337/diabetes.54.8.2277
60. Kintscher U, Hartge M, Hess K, Foryst-Ludwig A, Clemenz M, Wabitsch M, et al. T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. *Arterioscler Thromb Vasc Biol* (2008) **28**:1304–10. doi:10.1161/ATVBAHA.108.165100
61. Deiuliis J, Shah Z, Shah N, Needleman B, Mikami D, Narula V, et al. Visceral adipose inflammation in obesity is associated with critical alterations in tregulatory cell numbers. *PLoS One* (2011) **6**:e16376. doi:10.1371/journal.pone.0016376
62. Zeyda M, Huber J, Prager G, Stulnig TM. Inflammation correlates with markers of T-cell subsets including regulatory T cells in adipose tissue from obese patients. *Obesity (Silver Spring)* (2011) **19**:743–8. doi:10.1038/oby.2010.123
63. Goossens GH, Blaak EE, Theunissen R, Duijvestijn AM, Clement K, Tervaert JW, et al. Expression of NLRP3 inflammasome and T cell population markers in adipose tissue are associated with insulin resistance and impaired glucose metabolism in humans. *Mol Immunol* (2012) **50**:142–9. doi:10.1016/j.molimm.2012.01.005
64. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* (2009) **58**:1091–103. doi:10.1136/gut.2008.165886
65. Flint HJ. Obesity and the gut microbiota. *J Clin Gastroenterol* (2011) **45**(Suppl):S128–32. doi:10.1097/MCG.0b013e31821f44c4
66. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A* (2005) **102**:11070–5. doi:10.1073/pnas.0504978102
67. Verdam FJ, Fuentes S, de Jonge C, Zoetendal EG, Erbil R, Greve JW, et al. Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. *Obesity (Silver Spring)* (2013) **21**:E607–15. doi:10.1002/oby.20466
68. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* (2007) **56**:1761–72. doi:10.2337/db06-1491
69. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* (2008) **57**:1470–81. doi:10.2337/db07-1403
70. Furet JP, Kong LC, Tap J, Poitou C, Basdevant A, Bouillot JL, et al. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes* (2010) **59**:3049–57. doi:10.2337/db10-0253
71. Aron-Wisnewsky J, Dore J, Clement K. The importance of the gut microbiota after bariatric surgery. *Nat Rev Gastroenterol Hepatol* (2012) **9**:590–8. doi:10.1038/nrgastro.2012.161
72. Yang PJ, Lee WJ, Tseng PH, Lee PH, Lin MT, Yang WS. Bariatric surgery decreased the serum level of an endotoxin-associated marker: lipopolysaccharide-binding protein. *Surg Obes Relat Dis* (2014). doi:10.1016/j.soard.2014.02.022
73. Velloso LA, Araújo EP, de Souza CT. Diet-induced inflammation of the hypothalamus in obesity. *Neuroimmunomodulation* (2008) **15**:189–93. doi:10.1159/000153423

74. Lawrence CB, Brough D, Knight EM. Obese mice exhibit an altered behavioural and inflammatory response to lipopolysaccharide. *Dis Model Mech* (2012) **5**:649–59. doi:10.1242/dmm.009068
75. Rummel C, Inoue W, Poole S, Luheshi GN. Leptin regulates leukocyte recruitment into the brain following systemic LPS-induced inflammation. *Mol Psychiatry* (2010) **15**:523–34. doi:10.1038/mp.2009.98
76. Cai D, Liu T. Inflammatory cause of metabolic syndrome via brain stress and NF-kappaB. *Aging (Albany NY)* (2012) **4**:98–115.
77. De Souza CT, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boschero AC, et al. Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. *Endocrinology* (2005) **146**:4192–9. doi:10.1210/en.2004-1520
78. Gao Y, Ottaway N, Schriever SC, Legutko B, García-Cáceres C, de la Fuente E, et al. Hormones and diet, but not body weight, control hypothalamic microglial activity. *Glia* (2014) **62**:17–25. doi:10.1002/glia.22580
79. Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, et al. Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest* (2012) **122**:153–62. doi:10.1172/JCI59660
80. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* (2006) **116**:3015–25. doi:10.1172/JCI28898
81. Milanski M, Degasperi G, Coope A, Morari J, Denis R, Cintra DE, et al. Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. *J Neurosci* (2009) **29**:359–70. doi:10.1523/JNEUROSCI.2760-08.2009
82. Kleinridders A, Schenten D, Könner AC, Belgardt BF, Mauer J, Okamura T, et al. MyD88 signaling in the CNS is required for development of fatty acid-induced leptin resistance and diet-induced obesity. *Cell Metab* (2009) **10**:249–59. doi:10.1016/j.cmet.2009.08.013
83. Lann D, LeRoith D. Insulin resistance as the underlying cause for the metabolic syndrome. *Med Clin North Am* (2007) **91**:1063–77, viii. doi:10.1016/j.mcna.2007.06.012
84. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* (2006) **444**:860–7. doi:10.1038/nature05485
85. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* (2003) **112**:1821–30. doi:10.1172/JCI19451
86. Thaler JP, Choi SJ, Schwartz MW, Wisse BE. Hypothalamic inflammation and energy homeostasis: resolving the paradox. *Front Neuroendocrinol* (2010) **31**:79–84. doi:10.1016/j.yfrne.2009.10.002
87. Capuron L, Miller AH. Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacol Ther* (2011) **130**:226–38. doi:10.1016/j.pharmthera.2011.01.014
88. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* (2008) **9**:46–56. doi:10.1038/nrn2297
89. Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS, et al. Paroxetine for the prevention of depression induced by high-dose interferon alfa. *N Engl J Med* (2001) **344**:961–6. doi:10.1056/NEJM200103293441303
90. Capuron L, Gumnick JF, Musselman DL, Lawson DH, Reemsnyder A, Nemeroff CB, et al. Neurobehavioral effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. *Neuropsychopharmacology* (2002) **26**:643–52. doi:10.1016/S0893-133X(01)00407-9
91. Capuron L, Schroecksnadel S, Feart C, Aubert A, Higueret D, Barberger-Gateau P, et al. Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: role in neuropsychiatric symptoms. *Biol Psychiatry* (2011) **70**:175–82. doi:10.1016/j.biopsych.2010.12.006
92. Raison CL, Dantzer R, Kelley KW, Lawson MA, Woolwine BJ, Vogt G, et al. CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. *Mol Psychiatry* (2010) **15**:393–403. doi:10.1038/mp.2009.116
93. Myint AM, Kim YK, Verkerk R, Scharpe S, Steinbusch H, Leonard B. Kynurenine pathway in major depression: evidence of impaired neuroprotection. *J Affect Disord* (2007) **98**:143–51. doi:10.1016/j.jad.2006.07.013
94. Stone TW, Forrest CM, Stoy N, Darlington LG. Involvement of kynurenines in Huntington's disease and stroke-induced brain damage. *J Neural Transm* (2012) **119**:261–74. doi:10.1007/s00702-011-0676-8
95. Schwarcz R, Rassoulpour A, Wu HQ, Medoff D, Tamminga CA, Roberts RC. Increased cortical kynurenate content in schizophrenia. *Biol Psychiatry* (2001) **50**:521–30. doi:10.1016/S0006-3223(01)01078-2
96. Gulaj E, Pawlak K, Bien B, Pawlak D. Kynurenine and its metabolites in Alzheimer's disease patients. *Adv Med Sci* (2010) **55**:204–11. doi:10.2478/v10039-010-0023-6
97. Henry CJ, Huang Y, Wynne AM, Godbout JP. Peripheral lipopolysaccharide (LPS) challenge promotes microglial hyperactivity in aged mice that is associated with exaggerated induction of both pro-inflammatory IL-1beta and anti-inflammatory IL-10 cytokines. *Brain Behav Immun* (2009) **23**:309–17. doi:10.1016/j.bbi.2008.09.002
98. O'Connor JC, Lawson MA, Andre C, Moreau M, Lestage J, Castanon N, et al. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol Psychiatry* (2009) **14**:511–22. doi:10.1038/sj.mp.4002148
99. O'Connor JC, Lawson MA, Andre C, Briley EM, Szegedi SS, Lestage J, et al. Induction of IDO by bacille Calmette-Guerin is responsible for development of murine depressive-like behavior. *J Immunol* (2009) **182**:3202–12. doi:10.4049/jimmunol.0802722
100. O'Connor JC, Andre C, Wang Y, Lawson MA, Szegedi SS, Lestage J, et al. Interferon-gamma and tumor necrosis factor-alpha mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus Calmette-Guerin. *J Neurosci* (2009) **29**:4200–9. doi:10.1523/JNEUROSCI.5032-08.2009
101. Salazar A, Gonzalez-Rivera BL, Redus L, Parrott JM, O'Connor JC. Indoleamine 2,3-dioxygenase mediates anhedonia and anxiety-like behaviors caused by peripheral lipopolysaccharide immune challenge. *Horm Behav* (2012) **62**:202–9. doi:10.1016/j.yhbeh.2012.03.010
102. Walker AK, Budac DP, Bisulco S, Lee AW, Smith RA, Beenders B, et al. NMDA receptor blockade by ketamine abrogates lipopolysaccharide-induced depressive-like behavior in C57BL/6J mice. *Neuropsychopharmacology* (2013) **38**:1609–16. doi:10.1038/npp.2013.71
103. Andre C, O'Connor JC, Kelley KW, Lestage J, Dantzer R, Castanon N. Spatio-temporal differences in the profile of murine brain expression of proinflammatory cytokines and indoleamine 2,3-dioxygenase in response to peripheral lipopolysaccharide administration. *J Neuroimmunol* (2008) **200**:90–9. doi:10.1016/j.jneuroim.2008.06.011
104. Corona AW, Huang Y, O'Connor JC, Dantzer R, Kelley KW, Popovich PG, et al. Fractalkine receptor (CX3CR1) deficiency sensitizes mice to the behavioral changes induced by lipopolysaccharide. *J Neuroinflammation* (2010) **7**:93. doi:10.1186/1742-2094-7-93
105. Frenois F, Moreau M, O'Connor J, Lawson M, Micon C, Lestage J, et al. Lipopolysaccharide induces delayed FosB/DeltaFosB immunostaining within the mouse extended amygdala, hippocampus and hypothalamus, that parallel the expression of depressive-like behavior. *Psychoneuroendocrinology* (2007) **32**:516–31. doi:10.1016/j.psyneuen.2007.03.005
106. Fu X, Zunich SM, O'Connor JC, Kavelaars A, Dantzer R, Kelley KW. Central administration of lipopolysaccharide induces depressive-like behavior in vivo and activates brain indoleamine 2,3 dioxygenase in murine organotypic hippocampal slice cultures. *J Neuroinflammation* (2010) **7**:43. doi:10.1186/1742-2094-7-43
107. Gibney SM, McGuinness B, Prendergast C, Harkin A, Connor TJ. Poly I:C-induced activation of the immune response is accompanied by depression and anxiety-like behaviours, kynurenine pathway activation and reduced BDNF expression. *Brain Behav Immun* (2013) **28**:170–81. doi:10.1016/j.bbi.2012.11.010
108. Shelton RC, Miller AH. Eating ourselves to death (and despair): the contribution of adiposity and inflammation to depression. *Prog Neurobiol* (2010) **91**:275–99. doi:10.1016/j.pneurobio.2010.04.004
109. Ladwig KH, Marten-Mittag B, Lowel H, Doring A, Koenig W. Influence of depressive mood on the association of CRP and obesity in 3205 middle aged healthy men. *Brain Behav Immun* (2003) **17**:268–75. doi:10.1016/S0889-1591(03)00056-4
110. Dixon JB, Hayden MJ, Lambert GW, Dawood T, Anderson ML, Dixon ME, et al. Raised CRP levels in obese patients: symptoms of depression have an

independent positive association. *Obesity (Silver Spring)* (2008) **16**:2010–5. doi:10.1038/oby.2008.271
111. Capuron L, Su S, Miller AH, Bremner JD, Goldberg J, Vogt GJ, et al. Depressive symptoms and metabolic syndrome: is inflammation the underlying link? *Biol Psychiatry* (2008) **64**:896–900. doi:10.1016/j.biopsych.2008.05.019
112. Chirinos DA, Goldberg R, Gellman M, Mendez AJ, Gutt M, McCalla JR, et al. Leptin and its association with somatic depressive symptoms in patients with the metabolic syndrome. *Ann Behav Med* (2013) **46**:31–9. doi:10.1007/s12160-013-9479-5
113. Daly M. The relationship of C-reactive protein to obesity-related depressive symptoms: a longitudinal study. *Obesity (Silver Spring)* (2013) **21**:248–50. doi:10.1002/oby.20051
114. Capuron L, Poitou C, Machaux-Tholliez D, Frochot V, Bouillot JL, Basdevant A, et al. Relationship between adiposity, emotional status and eating behaviour in obese women: role of inflammation. *Psychol Med* (2011) **41**:1517–28. doi:10.1017/S0033291710001984
115. Emery CF, Fondow MD, Schneider CM, Christofi FL, Hunt C, Busby AK, et al. Gastric bypass surgery is associated with reduced inflammation and less depression: a preliminary investigation. *Obes Surg* (2007) **17**:759–63. doi:10.1007/s11695-007-9140-0
116. Sweat V, Starr V, Bruehl H, Arentoft A, Tirsi A, Javier E, et al. C-reactive protein is linked to lower cognitive performance in overweight and obese women. *Inflammation* (2008) **31**:198–207. doi:10.1007/s10753-008-9065-3
117. Yaffe K, Kanaya A, Lindquist K, Simonsick EM, Harris T, Shorr RI, et al. The metabolic syndrome, inflammation, and risk of cognitive decline. *JAMA* (2004) **292**:2237–42. doi:10.1001/jama.292.18.2237
118. Pistell PJ, Morrison CD, Gupta S, Knight AG, Keller JN, Ingram DK, et al. Cognitive impairment following high fat diet consumption is associated with brain inflammation. *J Neuroimmunol* (2010) **219**:25–32. doi:10.1016/j.jneuroim.2009.11.010
119. Dey A, Hao S, Erion JR, Wosiski-Kuhn M, Stranahan AM. Glucocorticoid sensitization of microglia in a genetic mouse model of obesity and diabetes. *J Neuroimmunol* (2014) **269**:20–7. doi:10.1016/j.jneuroim.2014.01.013
120. Yates KF, Sweat V, Yau PL, Turchiano MM, Convit A. Impact of metabolic syndrome on cognition and brain: a selected review of the literature. *Arterioscler Thromb Vasc Biol* (2012) **32**:2060–7. doi:10.1161/ATVBAHA.112.252759
121. Volkow ND, Wang GJ, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. *Trends Cogn Sci* (2011) **15**:37–46. doi:10.1016/j.tics.2010.11.001
122. Volkow ND, Wang GJ, Tomasi D, Baler RD. The addictive dimensionality of obesity. *Biol Psychiatry* (2013) **73**:811–8. doi:10.1016/j.biopsych.2012.12.020
123. de Weijer BA, van de Giessen E, van Amelsvoort TA, Boot E, Braak B, Janssen IM, et al. Lower striatal dopamine D2/3 receptor availability in obese compared with non-obese subjects. *EJNMMI Res* (2011) **1**:37. doi:10.1186/2191-219X-1-37
124. Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, et al. Brain dopamine and obesity. *Lancet* (2001) **357**:354–7. doi:10.1016/S0140-6736(00)03643-6
125. Tomasi D, Volkow ND. Brain activation and neurochemistry. *Proc Natl Acad Sci U S A* (2013) **110**:10888–9. doi:10.1073/pnas.1309471110
126. Felger JC, Miller AH. Cytokine effects on the basal ganglia and dopamine function: the subcortical source of inflammatory malaise. *Front Neuroendocrinol* (2012) **33**:315–27. doi:10.1016/j.yfrne.2012.09.003
127. Capuron L, Pagnoni G, Drake DF, Woolwine BJ, Spivey JR, Crowe RJ, et al. Dopaminergic mechanisms of reduced basal ganglia responses to hedonic reward during interferon alfa administration. *Arch Gen Psychiatry* (2012) **69**:1044–53. doi:10.1001/archgenpsychiatry.2011.2094
128. Capuron L, Pagnoni G, Demetrashvili MF, Lawson DH, Fornwalt FB, Woolwine B, et al. Basal ganglia hypermetabolism and symptoms of fatigue during interferon-alpha therapy. *Neuropsychopharmacology* (2007) **32**:2384–92. doi:10.1038/sj.npp.1301362
129. Brydon L, Harrison NA, Walker C, Steptoe A, Critchley HD. Peripheral inflammation is associated with altered substantia nigra activity and psychomotor slowing in humans. *Biol Psychiatry* (2008) **63**:1022–9. doi:10.1016/j.biopsych.2007.12.007
130. Mattsson C, Reynolds RM, Simonyte K, Olsson T, Walker BR. Combined receptor antagonist stimulation of the hypothalamic-pituitary-adrenal axis test identifies impaired negative feedback sensitivity to cortisol in obese men. *J Clin Endocrinol Metab* (2009) **94**:1347–52. doi:10.1210/jc.2008-2054
131. Martinowich K, Manji H, Lu B. New insights into BDNF function in depression and anxiety. *Nat Neurosci* (2007) **10**:1089–93. doi:10.1038/nn1971
132. Yamada K, Nabeshima T. Brain-derived neurotrophic factor/TrkB signaling in memory processes. *J Pharmacol Sci* (2003) **91**:267–70. doi:10.1254/jphs.91.267
133. Barrientos RM, Sprunger DB, Campeau S, Watkins LR, Rudy JW, Maier SF. BDNF mRNA expression in rat hippocampus following contextual learning is blocked by intrahippocampal IL-1beta administration. *J Neuroimmunol* (2004) **155**:119–26. doi:10.1016/j.jneuroim.2004.06.009
134. Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* (2006) **59**:1116–27. doi:10.1016/j.biopsych.2006.02.013
135. Goshen I, Kreisel T, Ben-Menachem-Zidon O, Licht T, Weidenfeld J, Ben-Hur T, et al. Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. *Mol Psychiatry* (2008) **13**:717–28. doi:10.1038/sj.mp.4002055
136. Corripio R, Gonzalez-Clemente JM, Jacobo PS, Silvia N, Lluis G, Joan V, et al. Plasma brain-derived neurotrophic factor in prepubertal obese children: results from a 2-year lifestyle intervention programme. *Clin Endocrinol (Oxf)* (2012) **77**:715–20. doi:10.1111/j.1365-2265.2012.04431.x
137. Moy GA, McNay EC. Caffeine prevents weight gain and cognitive impairment caused by a high-fat diet while elevating hippocampal BDNF. *Physiol Behav* (2013) **109**:69–74. doi:10.1016/j.physbeh.2012.11.008
138. Grenham S, Clarke G, Cryan JF, Dinan TG. Brain-gut-microbe communication in health and disease. *Front Physiol* (2011) **2**:94. doi:10.3389/fphys.2011.00094
139. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* (2012) **13**:701–12. doi:10.1038/nrn3346

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# Ghrelin-derived peptides: a link between appetite/reward, GH axis, and psychiatric disorders?

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Psychiatric disorders are often associated with metabolic and hormonal alterations, including obesity, diabetes, metabolic syndrome as well as modifications in several biological rhythms including appetite, stress, sleep–wake cycles, and secretion of their corresponding endocrine regulators. Among the gastrointestinal hormones that regulate appetite and adapt the metabolism in response to nutritional, hedonic, and emotional dysfunctions, at the interface between endocrine, metabolic, and psychiatric disorders, ghrelin plays a unique role as the only one increasing appetite. The secretion of ghrelin is altered in several psychiatric disorders (anorexia, schizophrenia) as well as in metabolic disorders (obesity) and in animal models in response to emotional triggers (psychological stress . . .) but the relationship between these modifications and the physiopathology of psychiatric disorders remains unclear. Recently, a large literature showed that this key metabolic/endocrine regulator is involved in stress and reward-oriented behaviors and regulates anxiety and mood. In addition, preproghrelin is a complex prohormone but the roles of the other ghrelin-derived peptides, thought to act as functional ghrelin antagonists, are largely unknown. Altered ghrelin secretion and/or signaling in psychiatric diseases are thought to participate in altered appetite, hedonic response and reward. Whether this can contribute to the mechanism responsible for the development of the disease or can help to minimize some symptoms associated with these psychiatric disorders is discussed in the present review. We will thus describe (1) the biological actions of ghrelin and ghrelin-derived peptides on food and drugs reward, anxiety and depression, and the physiological consequences of ghrelin invalidation on these parameters, (2) how ghrelin and ghrelin-derived peptides are regulated in animal models of psychiatric diseases and in human psychiatric disorders in relation with the GH axis.

**Keywords: ghrelin, eating disorders, food reward, alcohol and drug addiction, anxiety, depression, growth hormone**

## INTRODUCTION

Psychiatric disorders are often associated with metabolic and hormonal alterations, including obesity, diabetes, metabolic syndrome as well as modifications in several biological rhythms including appetite, stress, sleep–wake cycles, and secretion of their corresponding endocrine regulators.

At the interface between endocrine, metabolic, and psychiatric disorders, ghrelin plays a unique role as the only one increasing appetite. Ghrelin was initially identified as a gastrointestinal peptide originating from the stomach (1, 2) and as an endogenous ligand for the GH secretagogue receptor (GHS-R1a), the only ghrelin receptor identified so far. In addition to its primary effect as a GH secretagogue, ghrelin modulates many other neuroendocrine and metabolic functions: it is a powerful orexigenic and adipogenic peptide and a long-term regulator of energy homeostasis (3, 4). The actions of ghrelin on GH secretion and food intake require the addition of an eight-carbon fatty acid that is attached on a serine in position 3 by the enzyme ghrelin-*O*-acyltransferase (GOAT) (5, 6) and are exclusively mediated through the GHS-R1a. In addition, acyl ghrelin is processed from a 117 amino-acid prohormone that is unique in that it encodes other proghrelin-derived peptides with structural and functional heterogeneity. A naturally occurring molecule encoded by the same prohormone is desacyl ghrelin, which is the most abundant form in plasma (7) and initially thought to be an inactive peptide resulting from deacylation of ghrelin in tissues and blood (8). Another bioactive 23 amino-acid peptide is also derived from the same precursor and was originally proposed as the endogenous ligand for the GPR-39 (9). Although non-endocrine biological activities, such as regulation of glucose or lipid metabolism have been attributed to desacyl ghrelin or obestatin through receptors that still need to be characterized (10–12), some studies suggest that these derived peptides also antagonize the effects of acylated ghrelin on food intake and GH secretion (13, 14) (**Figure 1**).

The GHS-R1a is highly expressed in the hypothalamus, a region that controls neuroendocrine functions such as GH secretion, stress, and appetite, in the dorsal vagal complex that receives inputs from gut vagal afferents and also in the mesolimbic dopaminergic system of the ventral tegmental area (VTA) involved in addiction and reward (15, 16). Consistently, a recent literature shows that

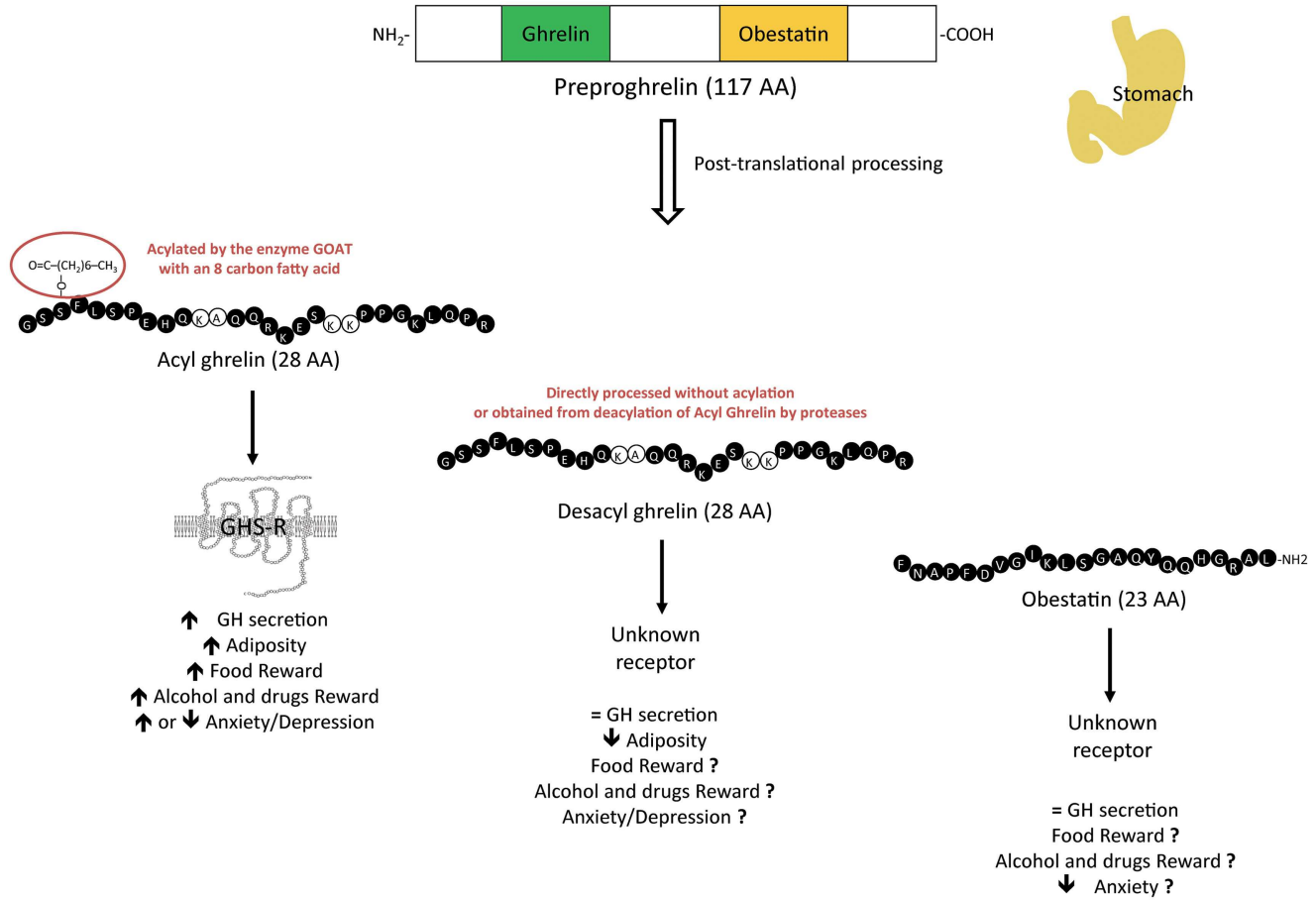



**FIGURE 1 | Physiological effects of the different preproghrelin-derived peptides on GH secretion, adiposity, food reward, drugs reward, and anxiety/depression**. Preproghrelin is a unique prohormone as it encodes several preproghrelin-derived peptides with structural and functional heterogeneity. Whereas the contribution of acyl ghrelin, which binds to the GHS-R1a, has been well explored, the roles of the other variants, desacyl ghrelin, and obestatin, through receptors that still need to be identified, in these regulations need to be clarified.

ghrelin is involved in stress and reward-oriented behaviors and regulates anxiety and mood. Thus, ghrelin is thought to adapt metabolism in response to emotional dysfunctions. Consistent with such hypothesis, the secretion of the gastrointestinal hormone and its derived peptides is altered in several psychiatric disorders (anorexia, schizophrenia) as well as in metabolic disorders (obesity) and in animal models in response to emotional triggers (such as psychological stress) but the relationship between these modifications and the physiopathology of psychiatric disorders remains unclear, especially since contradictory results arise from the literature. Interestingly, although the prevalence of most psychiatric disorders is not higher in obese individuals, the incidence of depression and anxiety is increased in this pathology (17).

The focus of this article is to review recent data concerning (1) the biological actions of ghrelin and ghrelin-derived peptides on food and drugs reward, anxiety, and depression by exploring pharmacological actions of the peptides in both rodents and humans and consequences of genetic or pharmacological blockade of the ghrelin/GHS-R system on these parameters, and (2) how ghrelin and ghrelin-derived peptides are regulated in human psychiatric disorders and animal models of psychiatric diseases such as eating disorders, anxio-depressive disorders, addiction to alcohol, and drugs of abuse. The link between ghrelin-derived peptides, GH axis, and psychiatric disorders will also be discussed.

## PREPROGHRELIN-DERIVED PEPTIDES IN EATING DISORDERS

### PREPROGHRELIN-DERIVED PEPTIDES AND FOOD REWARD

Many psychiatric disorders are associated with an alteration in reward-related behaviors linked to the consumption of either natural reward (food) or drugs of abuse (alcohol, cocaine, nicotine, . . .). Both behaviors involve the meso-cortico-limbic system, including a dopaminergic projection from the VTA to the nucleus accumbens (NAcc).

#### *Pharmacological actions of ghrelin in animals and humans*

A significant number of studies suggest that ghrelin acts directly on the VTA to increase preference for and motivation to obtain highly palatable food but no data are available on the actions of the other ghrelin-derived peptides (**Figure 1**). In animal models, motivation to obtain rewarding food (like sucrose) is tested in an operant

system by measuring lever pressing or nose poking in a progressive ratio paradigm. In such behavioral tests, either intraperitoneal (i.p), intracerebroventricular (i.c.v) or intra-VTA but not intra-NAcc ghrelin administration induces motivation for palatable food by increasing operant lever pressing for sucrose pellets or for 5% sucrose solution in rats (18–20). Similarly, reinforcement in satiated rats and motivation for highly palatable food even in food-restricted animals are increased after intra-VTA chronic ghrelin infusion (21). Interestingly, this operant response to sucrose pellets following acute intra-VTA ghrelin is prevented by forebrain dopamine depletion (6-OHDA) (22). In addition, ghrelin-induced increases in lever presses for a 5% sucrose solution is inhibited by pre-treatment with the D1-R antagonist, SCH-23390, but not D2-R antagonist, indicating that ghrelin-induced motivation for food is mediated via D1-R dependent mechanism. However, the significant increase for food motivation/reward behavior observed after ghrelin injection in the VTA is abolished by a pre-treatment with D1- or D2-receptor antagonist, injected in the NAcc, a main target of VTA dopaminergic neurons (23).

Ghrelin can also modify food preference, that can be measured in rodents by a free choice feeding paradigm, where animals are given a free choice of either chow or highly palatable food. Indeed, acute bilateral intra-VTA injection of ghrelin in rats increases consumption of rewarding food (peanut butter) but not standard chow and i.c.v ghrelin-induced motivation for this rewarding food is reduced in VTA-lesioned animals (24). Furthermore, in a two-bottle open access paradigm, i.c.v ghrelin injections in rats increases consumption of a 5% sucrose solution and this is prevented by i.p administration of 18-methoxycoronaridine, a selective antagonist of α3β4 nicotinic receptors, known to reduce operant response to sucrose (25). However, whether the palatability or the energetic value of energy-rich food is increased by ghrelin is still unresolved. In a single bottle test, mice injected i.p with ghrelin show a significant increase in saccharin intake, in the presence or in the absence of regular food. In addition, in a free-choice preference test, preference for the non-nutritive saccharin-flavored jelly is enhanced by i.p ghrelin injection in mice, demonstrating that ghrelin can augment the overconsumption of a sweet palatable non-nutritive solution, even when presented without any source of calories (26). However, when rats are given the choice between a palatable yet low-calorie sucrose solution or a calorically dense chow, i.c.v or intra-PVN ghrelin administration results in an increased intake of chow but not sucrose, suggesting that the primary effect of ghrelin is to stimulate food to satisfy energy needs (27).

Hedonic and rewarding effects of ghrelin are also observed in humans. Indeed, ghrelin administered intravenously to healthy volunteers during fMRI increases the neural response to high-energy- and low-energy-food pictures evaluation task in brain regions involved in reward processing and hedonic feeding, including amygdala, orbitofrontal cortex, anterior insula, striatum and/or hippocampus (28, 29), and ghrelin effects in the amygdala are correlated with self-rated hunger score (28).

#### *Genetic or pharmacological blockade of the ghrelin/GHS-R pathway*

Suppressed intake of rewarding food in a free-choice food paradigm, lack of cue-potentiated feeding and suppressed motivation for food in an operant responding model in *ghsr*$^{-/-}$ mice support a role of the endogenous peptide in hedonic eating [for review, see Ref. (30)]. Attenuated motivation for food in an operant responding model and decreased hedonic feeding response for a palatable high-fat dessert has also been described in mice invalidated for GOAT, the enzyme that acylates ghrelin, suggesting that a specific role for acyl ghrelin in this response (31).

## REGULATION OF PREPROGHRELIN-DERIVED PEPTIDES IN ANIMAL AND HUMAN PATHOPHYSIOLOGY

### *Animal pathophysiology*

In obesity, changes in food intake and reward-associated behaviors can be observed, often accompanied by alterations in eating patterns and increased intake of foods with high fat and sugar content [for review, see Ref. (32)]. Studies in animal models evidenced a dysregulation of the ghrelinergic pathway in obesity. Indeed, the long-term exposure to high-fat diet in the diet-induced obese mouse model leads to lower plasma acyl ghrelin (AG) and total ghrelin (TG) levels (**Table 1**), reduced hypothalamic GHS-R1a expression and suppressed feeding response to ghrelin injections (33, 34), indicating central ghrelin resistance. Interestingly, the modulatory action of ghrelin on reward on a progressive ratio paradigm is blunted in C57BL/6 mice with diet-induced obesity (35), supporting a dysfunction of the reward system and ghrelin resistance at the level of the reward circuit as well in obesity.

### *Human pathophysiology*

Dysregulation of the ghrelinergic system is also observed in human obesity [for review, see Ref. (43)] in relation with the alteration in the reward system (44) (**Table 2**). In most obese syndromes, low plasma ghrelin levels is due to a reduction in the desacyl form of ghrelin whereas acyl ghrelin is either increased, decreased or unchanged (45–47) (**Figure 2**). In contrast, in Prader–Willi syndrome, a rare genetic disorder characterized by multiple symptoms including severe binge-eating, growth retardation as well as learning disabilities, anxiety and depression, hyperghrelinemia correlates positively with hyperphagia (48, 49) and is mostly due to an increase in acyl ghrelin levels whereas desacyl ghrelin and obestatin are unchanged (50, 51). Although the origin of these elevated ghrelin levels remain unclear, it could result from undernutrition due to a failure to thrive during infancy.

Anorexia nervosa is a major cause of undernutrition in young women. In most of the studies, plasma acyl ghrelin and obestatin levels are elevated in anorexic patients with a pure restrictive-type (AN-R) compared to control or obese subjects (52–54, 87) and an inverse correlation between acyl ghrelin or obestatin and body mass index (BMI) is found (52). In contrast to AN-R, in patients with binge-purging (AN-BP), acyl ghrelin is increased and obestatin levels reduced or unchanged (53, 54), suggesting different abilities to adapt to starvation in AN-R and AN-BP despite similar BMI (**Table 2**). Inability of ghrelin to induce appetite during intravenous infusion may suggest some resistance to the orexigenic peptide in AN-R patients (55). However, pharmacological effects of ghrelin in AN have not been conclusive yet as some studies also reported increased hunger sensations after ghrelin treatment (56, 57). Data should be interpreted with caution due to the small number of patients and the difficulty to evaluate

**Table 1 | Regulation of plasma ghrelin concentrations in different mouse models, nutritional status, and experimental conditions**.

| Type of study | Reference | Sex | Animal model or strain | Experimental conditions/ stimulus | Nutritional status | Ghrelin secretion |
|---|---|---|---|---|---|---|
| Eating disorders/ obesity | (33) | M | Diet-induced obese C57BL/6 mice | 12 weeks on HFD | NA | ↘ AG and TG in DIO mice<br>Hypothalamic ghrelin resistance |
| | (34) | M | Diet-induced obese C57BL/6 mice | 12 weeks on HFD response to fasting | 20 h Fasting | ↗ AG in response to fasting in control and DIO mice |
| | (35) | M | Diet-induced obese C57BL/6 mice | 13 weeks on HFD response to sucrose reward | Free access to food | Resistance to ghrelin-induced sucrose reward |
| Alcohol addiction | (36) | M | Wistar, Wistar high preferring (WHP), Wistar low preferring (WLP) | Naïve (no alcohol) | 12 h fasting | ↘ AG and TG in WHP compared to WLP and Wistar ethanol-naïve rats |
| | (36) | M | Wistar, Wistar low preferring (WLP), Wistar high preferring (WHP) ethanol naive rat | Acute ip ethanol injection | Free access to food | ↘ AG and TG in Wistar and WLP rats |
| | (36) | M | Wistar alcohol-preferring (PR), Wistar non-preferring (NP), Wistar high preferring (WHP), Wistar low preferring (WLP) | Chronic alcohol consumption | 12 h Fasting | ↘↘ AG and TG in PR and WHP rats<br>↘ AG and TG in NP and WLP rats |
| | (37) | M | Voluntary chronic alcohol consumption in high-alcohol (alko, alcohol: AA) and low-alcohol (alko, non-alcohol: ANA) consuming rats | Continuous then limited access to increasing alcohol concentrations in a two-bottle-choice drinking paradigm for 14 weeks | Free access to food | ↘ TG in AA rats<br>↘↘ TG in ANA rats |
| | (38) | M | Voluntary chronic alcohol consumption in Wistar rats | Access to 20% alcohol in a two-bottle-choice drinking paradigm during 10 months | Free access to food | Plasma ghrelin not assayed<br>Negative correlation between GHS-R expression in the VTA and alcohol intake |
| Drug addiction | (39) | M | Drug self-administration in Lister hooded rats | Trained to self-administer cocaine iv | Restricted diet regime | Positive correlation between plasma ghrelin and cocaine-seeking behavior |
| Stress/anxiety/depression | (40) | NA | Caloric restriction | 60% Caloric restriction during 10 days | 60% Caloric restriction | ↗ GA |
| | (40) | M | Chronic social defeat stress (CSDS) | 10 days of CSDS | Free access to food | ↗ GA associated with increased caloric intake and weight gain |
| | (41) | M | Chronic social defeat stress (CSDS) | 10 days of CSDS | Free access to food | ↗ GA and = GNA in both WT and KO<br>Attenuated weight gain and feeding in GHS-R compared to WT |
| | (42) | M | High-anxiety Wistar Kyoto and low-anxiety SD rats | Unstressed | Free access to food | ↘ TG in high-anxiety compared to low-anxiety rats |
| | (42) | F | High-anxiety Wistar Kyoto (WKY) and low-anxiety SD (SPD) rats | Acute exposure to water avoidance | Free access to food | ↗ TG in WKY<br>↗↗ TG in SPD |

*M, male; F, female; NA, not available; TG, total ghrelin; AG, acyl ghrelin; Ghrelin, no information on isoform measured.*

**Table 2 | Regulation of plasma ghrelin/obestatin concentrations in different human pathologies, health and nutritional status, and experimental conditions**.

| Type of study | Reference | Sex | Subjects and health status | Experimental conditions/ stimulus | Nutritional status/time of sampling | Ghrelin/obestatin secretion |
|---|---|---|---|---|---|---|
| Eating disorders/obesity | (52) | 32 F | 10 Obese (OB)<br>11 Anorexia nervosa (AN)<br>11 Healthy controls (HC) | Basal conditions | Morning after overnight fast | ↘ G, ↘ AG, ↘ obestatin (OB)<br>↗ AG, ↘ DAG, ↗ obestatin (AN) |
| | (53) | 41 F | 10 Constitutional thinness (CT) 15 AN-R<br>7 AN-R recovered (PRAN)<br>9 HC | Basal conditions | Morning after overnight fast<br>Circadian pattern (every 4 h) | ↗ AG and TG, ↗ obestatin (AN) = AG, = obestatin (CT) |
| | (54) | 57 F | 22 AN-R<br>10 AN-BP<br>16 AN-BN<br>9 HC | Basal conditions | Morning after overnight fast<br>Circadian pattern | ↗ AG and TG, ↗ obestatin (AN-R)<br>↘ AG and TG, ↘ obestatin (AN-BP)<br>↘ AG and TG, ↘ obestatin (BN) |
| | (55) | 25 F | 9 AN, 6 AN recovered, 10 CT | Basal conditions | Morning after overnight fast | ↗ TG (AN) |
| | (55) | 25 F | 9 AN<br>6 AN recovered<br>10 CT | Ghrelin infusion (5 pmol/kg × min) during 300 min | Morning after overnight fast | ↘ GH response to ghrelin = Feeding response to ghrelin |
| | (56) | 16 F | 9 AN-R<br>7 HC | Basal conditions | Morning after overnight fast | ↗ TG (AN) |
| | (56) | 16 F | 9 AN-R<br>7 HC | Acute ghrelin administration (1.0 μg/kg) | Morning after overnight fast | ↘ GH response to ghrelin = Glucose response to ghrelin |
| | (57) | 5 F | 5 AN-R | Ghrelin infusion for 24 days: 3 μg/kg for 5 min during 14 days before breakfast and dinner | Morning after overnight fasting | ↗ Hunger sensation evaluated as VAS score |
| | (45) | M<br>F | Normal weight<br>Obese, no metabolic syndrome (no-MS)<br>Obese, metabolic syndrome (MS) | Basal conditions | Morning after overnight fast | ↘ TG and DAG, = AG (MS)<br>↘ TG and ↗ AG (non-MS) |
| | (46) | 34 M | Normal weight (17 M)<br>Overweight (17 M) | Basal conditions | Fasting | ↘ TG and DAG (in OW)<br>= AG (in OW) |
| | (47) | 101 M<br>79 F | Normal weight (31 M, 34 F)<br>Obese, no metabolic syndrome (no-MS) (40 M, 20 F)<br>Obese, metabolic syndrome (MS) (30 M, 25 F) | Basal conditions | Overnight fasting | ↗ AG and ↘ DAG (non-MS)<br>↗↗ AG and ↘↘ DAG (MS) |
| | (48) | 21 M<br>27 F | Prader–Willi syndrome (PWS) (10 M, 8 F)<br>Obese (4 M, 10 F)<br>Lean (7 M, 9 F) | Basal conditions | Overnight fasting | ↗ TG (in PWS) |

*(Continued)*

**Table 2 | Continued**

| Type of study | Reference | Sex | Subjects and health status | Experimental conditions/ stimulus | Nutritional status/time of sampling | Ghrelin/obestatin secretion |
|---|---|---|---|---|---|---|
| | (49) | M<br>F | Prader–Willi syndrome (PWS)<br>obese controls | Basal conditions | Fasting | ↗ TG (in PWS) |
| | (50) | 21 | Prader–Willi syndrome (PWS) (11)<br>Obese control children (10)<br>Identical BMI | Basal conditions | Fasting | ↗ AG<br>= DAG |
| | (51) | | Prader–Willi syndrome (PWS) (15)<br>Obese control children (18)<br>Identical BMI | Basal conditions | NA | ↗ AG and TG<br>= Obestatin |
| Alcohol addiction | (58) | 4 M<br>4 F | Healthy subjects<br>Non-obese (moderate social drinkers) | Acute oral (ethanol versus drinking water) | Morning | ↘ TG |
| | (59) | 6 M<br>6 F | Healthy subjects<br>Normal BMI (moderate social drinkers) | Acute oral (ethanol versus drinking water) | Overnight fasting before and after ethanol | ↘ AG and TG |
| | (60) | 9 M | Healthy subjects<br>Normal BMI | Acute oral (ethanol versus non-ethanol drink) + stress exposure | Early afternoon before and after ethanol | ↘ TG |
| | (61) | 5 M<br>5 F | Healthy subjects<br>Non-obese (moderate social drinkers) | Acute oral (ethanol versus drinking water) | Morning fed before and after ethanol | ↘ TG<br>= Obestatin |
| | (62) | 22 M<br>22 F | Healthy subjects<br>Normal BMI (moderate social drinkers) | Acute intravenous (ethanol versus saline) | Morning fed | ↘ TG, = obestatin<br>No gender effect |
| | (63) | 20 M | Healthy subjects: lean (11) or overweight (9) | Moderate alcohol during 3 weeks | Overnight fasting | ↗ Ghrelin |
| | (64, 65) | 142 | Healthy control (24)<br>Alcohol-dependent (early abstainers, 21)<br>Alcohol-dependent (active drinkers, 97)<br>Normal BMI | Chronic alcoholism | Overnight fasting | ↗ Ghrelin in alcoholic compared to HC<br>↗ Ghrelin in early abstainers compared to active drinkers |
| | (66) | 44<br>(M + F) | Healthy control (20) versus alcohol-dependent (24)<br>Non-obese | Chronic alcoholism | Overnight fasting | ↘ Ghrelin |
| | (67) | 30 M | Healthy control (15 M) versus alcohol-dependent (15 M)<br>Non-obese | Chronic alcoholism | Overnight fasting | ↘ Ghrelin<br>Positive correlation between ghrelin levels and alcohol craving |
| | (68) | 115 M<br>39 F | Healthy control (12 F + 33 M) versus alcohol-dependent (27 F + 82 H)<br>Normal BMI | Chronic alcoholism | NA | ↗ Ghrelin (females)<br>= Ghrelin (males)<br>Positive association between ghrelin and alcohol craving |

*(Continued)*

**Table 2 | Continued**

| Type of study | Reference | Sex | Subjects and health status | Experimental conditions/ stimulus | Nutritional status/time of sampling | Ghrelin/obestatin secretion |
|---|---|---|---|---|---|---|
| | (69) | 97 M | Healthy control (50 M) versus alcohol-dependent abstainers (47 M)<br>Normal BMI | >30 days of abstinence | Overnight fasting | ↗ Ghrelin<br>Positive correlation between ghrelin and duration of abstinence |
| | (70) | 111 M | Healthy control (50 M) versus alcohol-dependent abstainers (61 M) | 14 days of abstinence | Overnight fasting | ↗ AG and = TG<br>Positive association between AG and alcohol craving |
| | (71) | 64 M | Alcohol-dependent abstainers (64 M) classified in normal glucose tolerance (NGT), pre-diabetes (pre-DM) and diabetes (DM)<br>Normal BMI | >30 days of abstinence with rehabilitation treatment | Overnight fasting | ↗ Ghrelin in NGT<br>↗↗ Ghrelin in pre-DM and DM |
| **Drug addiction** | (72) | 11 | Heavy smokers | Acute 24 h nicotine withdrawal | Fed a light meal 2 h before | No association between TG and craving or withdrawal symptoms |
| | (73) | 123 M<br>143 F | Young adults<br>Normal BMI | Intrauterine exposure to prenatal smoke | Smoking and food *ad libitum* | ↗ TG |
| | (74) | 54 M | Smokers (31 M) and non-smokers (23 M)<br>Slightly overweight | Chronic smoking | Overnight fasting and abstinence from smoking | = TG |
| | (74) | 54 M | Smokers (31 M) and non-smokers (23 M)<br>Slightly overweight | Acute smoking (2 cigarettes) | Overnight fasting and abstinence from smoking | = TG (smokers)<br>↘ TG (non-smokers) |
| | (75) | 24 M<br>26 F | Healthy non-smokers<br>Normal BMI | Nicotine administration | Overnight fasting | = DAG |
| **Stress/anxiety/depression** | (60) | 9 M | Healthy subjects | Psychological stress = public speaking stressor | NA | = TG |
| | (76) | 8 M<br>16 F | Normal weight (8)<br>Obese (8)<br>Binge-eating (8) | Psychological stress = standardized trial social stress test (TSST) = public speaking | Morning after light breakfast | = TG after psychological stress Positive correlation between the change in TG and the change in cortisol |
| | (77) | 103 F | Healthy subjects | Stress = public speaking | Food provided | ↘ AG in emotional eaters<br>↗ AG in women anticipating the stressor compared to those not subjected to the stressor<br>↘ AG in non-emotional eaters following food consumption<br>= AG in non-emotional eaters following food consumption |
| | (78) | 68 M<br>61 F | Major-depressive disorder (MDD) (44 M, 39 F)<br>Healthy subjects (24 M, 22 F)<br>MDD slightly overweight | Basal conditions | Overnight fast | = TG in MDD<br>Positive correlation between ghrelin and eating behavior scales TFEQ |

*(Continued)*

**Table 2 | Continued**

| Type of study | Reference | Sex | Subjects and health status | Experimental conditions/ stimulus | Nutritional status/time of sampling | Ghrelin/obestatin secretion |
|---|---|---|---|---|---|---|
| Stress/anxiety/depression | (79) | 18 M<br>22 F | MDD patients (9 M, 11 F)<br>Healthy subjects (9 M, 11 F)<br>Normal BMI | Basal conditions | Fed<br>Overnight sampling | = TG in MDD |
| | (80) | M + F | MDD patients (9 M, 6 F)<br>Healthy subjects (16) | Basal conditions | | = AG or TG in MDD |
| | (81) | 48 M + F | MDD patients (24)<br>Healthy subjects (24) | Basal conditions | Overnight fasting | = AG in MDD<br>Positive correlation between AG and the severity of reduced appetite in MMD |
| | (82) | 64 F | Anorexic patients (15)<br>Normal weight (32)<br>Overweight (17) | Basal conditions | Overnight fasting | No relationship between TG and symptoms of depression or anxiety |
| | (83) | 245<br>M + F | MDD or panic disorders<br>Treatment responders (89)<br>Treatment non-responders (59)<br>Healthy subjects (97) | Basal conditions | Early afternoon<br>No eating 60 min before | ↗ AG in treatment-resistant patients |
| | (84) | 12 M<br>12 F | MDD patients (24)<br>Healthy subjects (22) | Basal conditions<br>citalopram treatment (3 months) | Overnight fasting | ↘ AG and TG in MDD<br>↘ AG and TG after treatment |
| | (85) | 40 M | MDD lean patients (40 M) | Basal conditions<br>maprotiline treatment (30 days) | Overnight fasting | ↗ TG and weight gain after treatment |
| | (86) | M + F | Major-depressive episode (MDE, 16)<br>Bipolar disorder manic episode (BD-me, 12)<br>Healthy subjects (25) | Electroconvulsive therapy (ECT) | Overnight fasting | ↘ AG after treatment in all subgroups but BMI unchanged |

*M, male; F, female; BMI, body mass index; OB, obese; AN-R, anorexia nervosa restrictive-type; AN-BN, anorexia nervosa with episodes of bulimia; AN-BP, anorexia nervosa with episodes of binge-purging; HC, healthy control; CT, constitutional thinness; MDD, major-depressive disorder; NA, not available; AG, acyl ghrelin; DAG, desacyl ghrelin; TG, total ghrelin; Ghrelin, either TG or AG (?).*

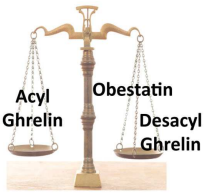
the degree of hunger in this pathology. Other factors that may counterbalance hyperghrelinemia should be taken into account. Interestingly, it has been hypothesized that higher obestatin levels in the restrictive type may also contribute to the reduced hunger and/or reduced motivation to eat in this pathology (53, 54) (**Figure 2**).

Alterations in the serotonergic/dopaminergic signaling are described in AN patients (88, 89) and avoidance for food is positively correlated with the increased striatal D2/D3 receptor binding. The altered impact of ghrelin on the reward system could modify the integration of information related to emotional processes, as suggested by connections between the prefrontal cortex and the NAcc. Interestingly, it has been demonstrated that the heterodimeric formation of ghrelin receptor and dopamine D2-receptor is required for the anorexigenic effects of dopamine in hypothalamic neurons (90). The co-expression of these receptors in the mesolimbic dopaminergic system may also be important to modulate motivation and reward.

## PREPROGHRELIN-DERIVED PEPTIDES IN ALCOHOL ADDICTION

### PREPROGHRELIN-DERIVED PEPTIDES AND ALCOHOL CONSUMPTION

Several reviews already focused on the role of the ghrelin/GHS-R signaling in the rewarding properties of alcohol in animal models or humans (91, 92). A large number of studies demonstrate that ghrelin is involved in alcohol intake, showing altered plasma ghrelin levels in alcoholic patients or high-alcohol consuming rat strains as well as reduced alcohol intake in animal with disrupted ghrelin signaling. However, very little pharmacological data exist so far.




| | Total Ghrelin | Acyl Ghrelin | Desacyl Ghrelin | Obestatin |
|---|---|---|---|---|
| Anorexia nervosa (Restrictive) | ↑ | ↑ | ? | ↑↑ |
| Anorexia nervosa (Binge-purging) | ↓ | ↓ | ? | ↓ |
| Obesity syndromes | ↑↓ | ↑=↓ | = or ↓ | ↑↓ |
| Alcohol-dependent | ↓↑ | ↓↑ | ? | = or ? |
| Anxiety/Stress | ? | ↑ or = | ? | ? |
| Depressive disorder | = | = | ? | ? |

**FIGURE 2 | Plasma levels of preproghrelin-derived peptides in metabolic and psychiatric disorders**. In anorexic patients, total ghrelin, acyl ghrelin, and obestatin are increased in the restrictive type and reduced in binge-purging. This may represent different abilities to adapt to starvation. In obesity syndromes, total ghrelin, acyl ghrelin and obestatin are either found reduced, unchanged, or increased. Discrepancies may be due to the variety of obesity syndromes (monogenic obesity, nutritionally induced obesity, presence of a metabolic syndrome, multifactorial disorders such as the Prader-Willi syndrome). In alcohol-dependent patients, ghrelin is found either reduced or increased compared to healthy subjects but there might be differences in gender, body mass index, nutritional/metabolic status and/or time after alcohol withdrawal from one study to another that can explain the contradictory results. In major depressive disorders, the majority of studies report no differences in plasma total or acyl ghrelin levels compared with healthy controls. Whether ghrelin can contribute to the degree of food craving, alcohol craving or depression is not clearly demonstrated as treatments and metabolic modifications can interfere with the results.

### *Pharmacological actions of ghrelin in animals and humans*

Ghrelin, delivered either peripherally, centrally or directly in specific brain nuclei, increases reward-relevant behaviors such as alcohol consumption (92) (**Figure 1**). Central ghrelin administrations, i.c.v or directly in the VTA or laterodorsal tegmental area (LDTg), increase alcohol intake in a two-bottle (alcohol/water) free-choice paradigm where access to alcohol was limited to 90 min/day for 2 weeks in C57BL/6 mice, a strain with high-alcohol preference (93). Pre-clinical models termed "drinking in the dark" (DID) are developed to examine binge-like ethanol consumption in rodent populations (94). In this procedure where animals have a 2 h access to a 20% ethanol solution during the beginning of the dark period, neither food deprivation nor i.p administration of ghrelin altered drinking in C57BL/6J mice (95). In humans, intravenous ghrelin administration increases alcohol craving in alcohol-dependent heavy drinkers (96).

### *Genetic or pharmacological blockade of the ghrelin/GHS-R pathway*

Genetic or pharmacological blockade of the GHS-R1a also reveals the importance of ghrelin signaling in alcohol-related behaviors. Pharmacological blockade with GHS-R1a antagonists reduces voluntary alcohol consumption and preference and suppresses reward induced by alcohol in both rats and mice [reviewed in Ref. (91)]. For example, JMV2959, a selective GHS-R1a antagonist, impacts both acute and chronic alcohol consumption. Acute injections of the antagonist reduce the operant self-administration of alcohol in rats, decreases high-alcohol consumption in two strains of alcohol-preferring rats, Long–Evans and alko alcohol (AA) preferring rats (97) as well as alcohol consumption after several months of exposure to alcohol (38). Chronic administrations of the antagonist decrease alcohol intake without inducing tolerance or rebound (38). In mice, in a two-bottle choice paradigm, the non-selective antagonist [D-Lys3]-GHRP-6 reduces preference to alcohol (98). In addition, GHS-R1a blockade with JMV2959 either peripherally or centrally reduces voluntary alcohol consumption and preference, alcohol-induced locomotor stimulation, accumbal DA release, and conditioned-place preference (CPP) (93, 99). Similarly, when compared to wild-type mice, *ghrelin*$^{-/-}$ mice display lower ethanol-induced CPP and locomotor stimulation and reduced voluntary alcohol consumption and preference in a two-bottle choice test (99). The ability of alcohol to increase NAcc dopamine release is absent in

*ghrelin*$^{-/-}$ mice (100). The rewarding properties of alcohol are also reduced in the *ghsr*$^{-/-}$ mice (93). However, Spiegelmer-neutralization of circulating ghrelin, thereby preventing its access to the brain, does not attenuate alcohol-induced locomotor activity, NAcc DA release, and CPP in mice, neither modifies alcohol consumption in a two-bottle free-choice paradigm in rats, suggesting that central rather than peripheral ghrelin signaling is preferentially involved in alcohol consumption (101). In contrast, Roux-en-Y gastric bypass (RYGB), which reduces circulating ghrelin levels, decreases ethanol intake and the reinforcing properties of ethanol in ethanol-preferring rats. In this model, pharmacological replacement of ghrelin restores drinking behavior (31), suggesting that endogenous circulating ghrelin is important in alcohol preference. It should also be noted that, in RYGB rats, a GHS-R1a antagonist, [D-Lys3]-GHRP-6, reduces operant performance to earn alcohol reward and alcohol consumption, suggesting that increased ghrelin might contribute to increased alcohol reward in such animals (102). Altogether, these data suggest that selective blockade of the ghrelin/GHS-R pathway could be a potential treatment for pathological alcohol consumption.

## REGULATION OF PREPROGHRELIN-DERIVED PEPTIDES IN ANIMAL AND HUMAN PATHOPHYSIOLOGY

Although ghrelin induces addictive behaviors, including alcohol consumption in both rodents and humans, an important question is whether hyperghrelinemia is associated with addictive behaviors, such as alcohol drinking. Conflicting data concerning the involvement of ghrelin in the physiopathology of alcohol dependence have been reported (103, 104).

### *Animal pathophysiology*

In rodents, chronic alcohol consumption leads to reduced total and acylated ghrelin levels in rats of different strains (PR: Wistar alcohol preferring, NP: Wistar non-preferring, and WHP: Wistar high preferring) but the lowest ghrelin levels are observed in PR and WHP strains (**Table 1**). Furthermore, there is an inverse relationship between ghrelin levels and alcohol intake (36). In high-alcohol (AA) consuming rats, reduction in plasma total ghrelin levels after alcohol exposure during several weeks is of lower amplitude as compared to low-alcohol (ANA) consuming rats (37). Interestingly, GHS-R1a expression in NAcc, VTA, amygdala, prefrontal cortex, and hippocampus is higher in AA rats, suggesting that the ghrelin pathway may be involved in alcohol susceptibility. But, after 10 months of voluntary alcohol consumption induced by intermittent access to alcohol in a two-bottle choice drinking paradigm, GHS-R expression in the VTA is significantly down-regulated in high- compared to low-alcohol consuming Wistar rats and a negative correlation is observed between GHS-R expression in the VTA and alcohol intake (38).

### *Human pathophysiology*

In healthy humans, acute alcohol consumption induces significant declines in total and acylated ghrelin concentrations as early as 15 min following alcohol ingestion (58–61), whereas obestatin levels are unchanged (61). Interestingly, fasting-induced increase in ghrelin levels is also reduced after intravenous alcohol administration in both males and females (62). In contrast, after moderate alcohol consumption during 3 weeks in healthy men, ghrelin concentrations are increased but both lean and overweight subjects were included in the analyses and plasma ghrelin levels tended to be lower in overweight associated with reduced insulin sensitivity (63). Data concerning the effect of chronic alcohol intake in alcohol-dependent subjects are conflicting. Indeed, alcohol dependence is associated with either increases or decreases in plasma ghrelin levels compared to healthy control subjects (65–67) (**Figure 2**) but higher ghrelin levels correlate with higher measurements of alcohol craving using the obsessive compulsive drinking scale (OCDS) (67, 105, 106) (**Figure 3**). Some difficulties in comparing data from the literature are that explorations are sometimes performed in different genders, various BMI, early abstainers versus active drinkers and at different times after alcohol withdrawal. The small number of subjects in some studies is also a factor to take into account (**Table 2**). Interestingly, a study comparing both genders found that ghrelin levels are higher in female alcohol-dependent patients only when compared to appropriate gender controls while no differences are found in males (68), suggesting that ghrelin response may be gender dependent. In addition, among alcohol-dependent subjects, ghrelin levels are higher in early abstainers compared to active drinkers (65). Although most hormonal explorations are performed after an overnight fast, nutritional status at the time of sampling may also contribute to differences observed. Finally, differences in ghrelin secretion observed in alcohol-dependent patients from one study to another may also be a consequence of the loss of metabolic control as alcohol intake is compensated by a decrease in non-alcoholic nutrient intake (66). Consumption of other substances of abuse can also interfere with plasma ghrelin levels and although smoking is an exclusion factor in some studies (62), others have to take into account the smoking status of the participants (70).

Whereas alcohol intake seems to reduce plasma ghrelin levels, alcohol withdrawal induces elevation in plasma concentrations of the hormone. Indeed, fasting plasma ghrelin levels are higher in alcohol abstainers than in controls and ghrelin levels are positively correlated to the duration of abstinence (64, 69–71). Ghrelin elevation, however, is greater in subjects with prediabetes and diabetes mellitus than in normoglucose-tolerant subjects (71), suggesting that metabolic factors impact the hormone levels.

Finally, only one study to date reported that a *GHSR* gene polymorphism was more frequent in heavy drinkers than in moderate drinkers and abstinents (107), suggesting genetic association between ghrelin and heavy alcohol consumption.

In conclusion, reduced plasma ghrelin levels with alcohol consumption and elevated levels with abstinence, as well as correlation between ghrelin and alcohol craving suggest that elevated ghrelin might contribute to cravings for alcohol (**Figures 2** and **3**). However, plasma concentrations of DAG and obestatin, regarding alcohol status, are unknown and the contribution of these other ghrelin-derived peptides in alcohol addiction would need to be explored as well (**Figure 2**).

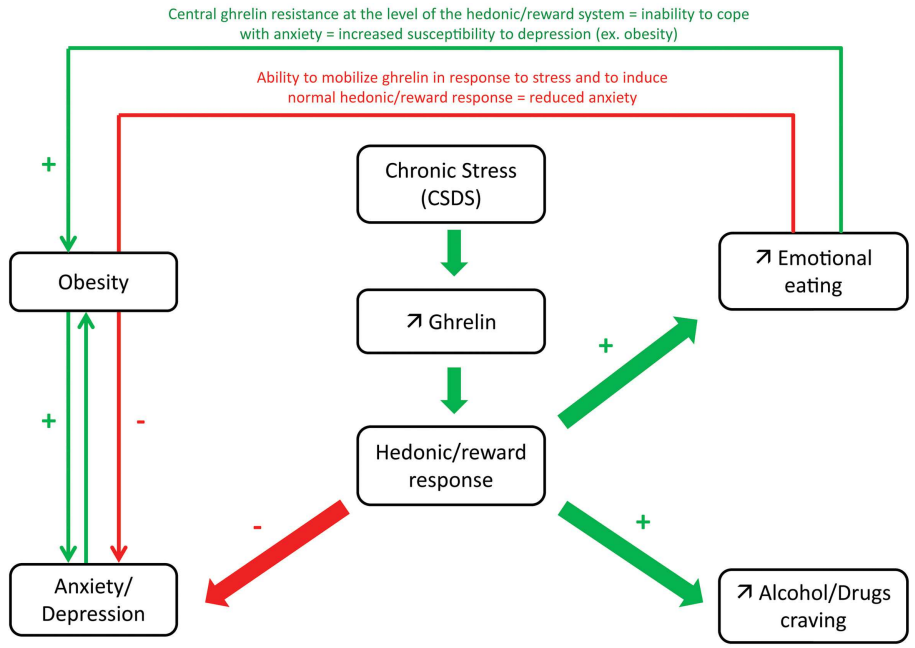



**FIGURE 3 | Proposed model of hedonic/reward response to ghrelin after chronic stress in relation with anxio-depressive symptoms**. During chronic stress, increased ghrelin secretion has been shown to induce emotional eating by acting at the level of the hedonic/reward system. As ghrelin has been shown to have anxiolytic actions in response to stress, this adaptative response may contribute to control excessive anxiety and prevent depression. In obesity, lower ability to mobilize ghrelin in response to stress or central ghrelin resistance at the level of the hedonic/reward system may explain the inability to cope with anxiety and increased susceptibility to depression. Reciprocally, depressed subjects have increased susceptibility to obesity or eating disorders (due to altered hedonic/reward response). Elevated ghrelin may also contribute to alcohol/drug craving: higher ghrelin levels correlate with higher measurements of alcohol craving.

## PREPROGHRELIN-DERIVED PEPTIDES IN ADDICTION TO DRUGS OF ABUSE

### PREPROGHRELIN-DERIVED PEPTIDES AND CONSUMPTION OF DRUGS OF ABUSE

#### *Pharmacological actions of ghrelin in animals and humans*

An emerging literature demonstrates that ghrelin modulates the action of psychostimulants such as nicotine, amphetamine, cocaine, and heroin but no data is available on the effects of the other ghrelin-derived peptides (**Figure 1**). In animal models, these rewarding properties of drugs of abuse are often measured by locomotor stimulation, NAcc dopamine release and/or CPP. Indeed, systemic ghrelin infusion increases cocaine-induced hyperlocomotion as well as CPP in rats [reviewed in Ref. (108)]. In addition, i.c.v infusion of ghrelin induces an increase in heroin reinforcement breakpoint on a progressive ratio schedule when animals have to work to obtain a reward (109). Bilateral micro-injection of ghrelin into the NAcc, where cocaine induces locomotor activation, increases cocaine-induced hyperactivity and these effects are blocked by a GHS-1a antagonist, [D-Lys3]-GHRP-6, in rats (110). Finally, ghrelin also amplifies nicotine-induced striatal dopamine release in this species (111).

In healthy non-smokers, after a caloric load of glucose, nicotine increases the modulatory effect of ghrelin on food-cue reactivity, measured by magnetic resonance imaging, in the ventromedial prefrontal cortex (112). No other clinical data are currently available and observations in rodents will need to be confirmed in humans.

#### *Genetic or pharmacological blockade of the ghrelin/GHS-R pathway*

As for alcohol consumption, GHS-R1a antagonists have been shown to suppress reward induced by cocaine and amphetamine. This was partly reviewed previously [reviewed in Ref. (91)] and recent data corroborate these findings. In rats, inactivation of ghrelin signaling by JMV2959 reduces nicotine-induced locomotor sensitization (108). In mice, the ability of nicotine, amphetamine, and cocaine to induce hyperlocomotion, to stimulate NAcc DA release and to condition a place preference is reduced after treatment with the same GHS-R1a antagonist (100, 113). In addition, in rats, the ability of morphine to induce behavioral stimulation, including stereotyped behavior and DA release in the NAcc is reduced by JMV2959 injections (114). Genetic ablation of GHS-R in mice similarly attenuates hyperlocomotion and CPP induced by nicotine, cocaine, and amphetamine (108). However, although exogenous ghrelin administration increases heroin self-reinforcement as described above, central administration of the GHS-R1a antagonist [D-Lys3]-GHRP-6, has no effect on heroin self-administration or food-deprivation induced reinstatement of heroin seeking behavior (109). Whether this lack of effect is due to the specificity of the antagonist or the nature of the psychostimulant (i.e., heroin or other) is unknown.

### REGULATION OF PREPROGHRELIN-DERIVED PEPTIDES IN ANIMAL AND HUMAN PATHOPHYSIOLOGY

A positive correlation between plasma ghrelin levels and cocaine-seeking behavior is observed in rats trained to self-administer cocaine i.v (39).

Very little pre-clinical and clinical data are available concerning substance of abuse and ghrelin levels (**Table 2**). In humans, circulating total ghrelin levels are not associated with craving and withdrawal symptoms in heavy smokers suffering from acute 24 h nicotine withdrawal (72). Young adults exposed to prenatal smoke have higher plasma total ghrelin levels (73). No differences in total ghrelin levels are found between smokers and non-smokers and smoking two cigarettes acutely does not provoke any short-term changes in total ghrelin levels in smokers but induces a decline in non-smokers (74). However, nicotine administration in healthy non-smokers does not alter plasma non-acylated ghrelin levels (75). Genetic association studies only report variation of the ghrelin signaling system in individuals with amphetamine dependence (38).

## CENTRAL MECHANISM OF ACTION MEDIATING REWARD-RELEVANT BEHAVIORS

### MESOLIMBIC CHOLINERGIC-DA SYSTEM

All major drugs of abuse acutely activate the mesolimbic dopamine system. Several lines of evidence converge to show that ghrelin activates the cholinergic –dopaminergic reward link, including a dopaminergic projection from the VTA to the NAcc to increase the consumption of rewarding foods and alcohol after i.v and i.c.v administrations in rodents (91). Ghrelin administration peripherally or locally into the LDTg concomitantly increases VTA acetylcholine as well as DA release in rats. In contrast, a GHS-R1a antagonist blocks this synchronous neurotransmitter release induced by peripheral ghrelin. In addition, local perfusion of a non-selective nicotinic antagonist mecamylamine into the VTA blocks the ability of ghrelin to increase NAcc dopamine but not VTA acetylcholine (115). The ability of alcohol to increase accumbal DA release in wild-type mice is not observed in *ghr*$^{-/-}$ mice, suggesting that endogenous ghrelin may be required for the ability of alcohol to activate the mesolimbic DA system (116).

The mesolimbic GHS-R also plays an important role in the response to drugs of abuse. Indeed, JMV2959, a GHS-R1a antagonist, reduces morphine-induced DA release in the NAcc and behavioral stimulation, including stereotyped behavior (114). Amphetamine- and cocaine-induced locomotor stimulation and NAcc DA release, as well as the ability of these drugs to condition a place preference, are reduced in mice treated with JMV2959 in the mouse (113).

Central pathways involved in increased alcohol or other drugs consumption potentially involve several other structures and neurotransmitters, beside dopamine release in the NAcc. For example, ghrelin-induced locomotor stimulation is attenuated by VTA administration of AP5, a selective NMDA receptor antagonist, but not orexin-A antagonist or peripheral opioid receptor antagonist (naltrexone) in mice (117).

### GLUTAMATERGIC/GABAergic SYSTEM

In central amygdala (CeA) GABAergic neurons, critical in regulating ethanol consumption and the response to ethanol withdrawal, ghrelin attenuates ethanol-increased IPSP amplitude and superfusion of GHS-R1a antagonists decreases IPSC and mIPSC frequency and block ghrelin-induced increases in GABAergic responses (118). Ghrelin regulation of alcohol consumption may also involve the perioculomotor urocortin neurons (pIIIu) as [D-Lys3]-GHRP-6 reduces the induction of cFos by i.p ethanol in this population of neurons but not in the VTA or arcuate nucleus of the hypothalamus (98).

### SEROTONIN SYSTEM

The serotonin (5-HT) system is also involved in the response to ghrelin. Indeed, acute central ghrelin injections in mice increase 5-HT turnover in the amygdala and 5-HT-R mRNA in the amygdala and dorsal raphe (119). The serotonin system is also regulated by endogenous ghrelin/GHS-R signaling. Indeed in *ghsr*$^{-/-}$ mice, decreased expression of 5-HT-R is observed in the amygdala and dorsal raphe (119).

## PREPROGHRELIN-DERIVED PEPTIDES IN STRESS/ANXIETY/DEPRESSION

### PREPROGHRELIN-DERIVED PEPTIDES AND STRESS-RESPONSE/ANXIETY/DEPRESSION

#### *Pharmacological actions of ghrelin in animals and humans*

Ghrelin is involved in neuroendocrine and behavioral responses to stress through activation of the HPA axis: peripheral ghrelin indeed increases hypothalamic CRH mRNA and serum corticosterone. In addition, ghrelin-induced anxiogenic effects are inhibited by a CRH receptor antagonist (120). Behavioral responses include anxiety-like behaviors like exploration in the open field, elevated plus maze (EPM), light/dark box, and social interactions.

In the mouse, both i.c.v and i.p administration of ghrelin induce anxiogenic behavior in the EPM test (120). Intracerebroventricular injections or direct injections in specific nuclei, including the hippocampus, amygdala, dorsal raphe nucleus or in several hypothalamic nuclei (Arc, PVN, VMH, PFH) induce anxiogenic responses in the open-field or EPM in both male and ovariectomized female rats (121–125), whereas i.c.v injection of obestatin elicits an anxiolytic effect in the EPM test (**Figure 1**) (126).

In rats, chronic i.c.v treatment with ghrelin also reveals an increase in anxiety- and depression-like behaviors that are associated with modifications in the expression of key markers involved in these behavioral changes in the amygdala (127). Interestingly, the anxiogenic actions of ghrelin are inhibited by a CRH antagonist, suggesting that anxiety response may be relayed by hyperactivity of the HPA stress axis (120). Electrophysiological responses to ghrelin challenges in the dorsal raphe, the main region expressing serotonergic neurons, which are key mediators of emotional reactivity further support a key role of ghrelin in emotional responses (127).

Despite a large literature supporting a pro-anxiety action of ghrelin in rodents, contradictory data arise from the effects of ghrelin on anxiety response (**Figure 1**): one study demonstrates that a subcutaneous injection of the 28-AA peptide produces anxiolytic- and antidepressant-like responses in the EPM

and forced-swim test in mice (40) while in another study in food-deprived rats during 1 h between ghrelin injection and testing, a decrease in anxiety-like behavior was observed after intra-amygdala injections (122). This discrepancy needs to be clarified and may reflect differences in contextual environment during the testing period (i.e., situation where stress in present or not, see below in the next paragraph differential response in basal conditions or in response to stress).

So far, human data on the effects of ghrelin are missing and do not corroborate observations in rodents. One study demonstrates that, in male and female patients with mood depressive disorders (MDD), ghrelin administration (50 μg between 11 p.m. and 1 a.m.) induces transient GH and cortisol secretion, increases the time of sleeping in males only but has no significant effect on depressive symptoms (128). Ghrelin affects the sleep/wake pattern in healthy subjects and may have also the same effect on MDD patients but it seems independent of the etiology of the depression.

#### *Genetic or pharmacological blockade of the ghrelin/GHS-R pathway*

Data are currently missing concerning the effects of GHS-R1a pharmacological blockade on anxiety- and depression-like behaviors but exploration of knock-out models give some insights about endogenous ghrelin function. *ghsr*$^{-/-}$ mice have reduced latency to leave center in the open-field test, suggesting increased anxiety in this model (129). Interestingly, a decreased expression of serotonin receptors (5 HT-R) is observed in the amygdala and dorsal raphe of *ghsr*$^{-/-}$ mice (119). Although *ghrelin*$^{-/-}$ mice have lower anxiety in basal unstressed conditions in three different behavioral tests (open field, EPM, light/dark box), they are also more anxious in response to acute restraint stress and show exacerbated central responses to stress as well (130). This stress response involves, not only the hypothalamus and amygdala, but also urocortin 1 neurons in the Edinger-Westphal nucleus. Interestingly, exogenous ghrelin reverses the exacerbated neuronal activation in the hypothalamic PVN and medial nucleus of the amygdala in *ghrelin*$^{-/-}$ mice after acute restraint stress, supporting an anxiolytic action of ghrelin (130). The differential response in basal and in stress conditions suggests that the role of the ghrelin system may be different depending on the context. Ghrelin may also prevent excessive anxiety under conditions of chronic stress (see next paragraph for animal models of chronic social defeat stress). Indeed, *ghsr*$^{-/-}$ mice display enhanced deleterious effects of chronic exposure to stress. Interestingly, increased plasma ghrelin levels induced by caloric restriction also produces anxiolytic and anti-depressant-like effects (40). Thus, stress-induced elevated ghrelin may help to control excessive anxiety and prevent depression in conditions of chronic stress exposure (**Figure 3**).

Finally, administration of antisense DNA for ghrelin into the lateral ventricle induces anxiolytic and antidepressant responses in the forced-swim test, EPM as well as in the black and white test and conditioned fear test in rats (131), which is in favor of an anxiogenic role of endogenous ghrelin.

## REGULATION OF PREPROGHRELIN-DERIVED PEPTIDES IN ANIMAL AND HUMAN PATHOPHYSIOLOGY

### *Animal pathophysiology*

Several studies suggest that ghrelin plays an important role in metabolic adaptations following chronic stress, which function would be to defend against depressive-like symptoms of chronic stress but which can also lead to metabolic dysfunctions in the long-term.

Animal models of anxiety/depression can be induced by chronic exposure to social stress (CSDS), a model of prolonged psychosocial stress in humans. CSDS is based on a resident intruder paradigm in which a test mouse is introduced into the cage of an aggressive CD1 mouse for a few minutes each day during several days and is a model of prolonged social defeat stress in rodents. Acylated ghrelin levels are increased in CSDS mice (40) (**Table 1**). Elevated ghrelin levels induced by chronic exposure to social stress are associated with increased caloric intake and body weight gain in male C57BL/6 mice and minimizes CSDS-associated depression-like behavior whereas stressed mice lacking ghrelin receptors (*ghsr*$^{-/-}$ mice) or treated i.c.v with ghrelin receptor antagonist [D-Lys3]-GHRP-6 show attenuated weight gain and feeding responses under the same social stress paradigm (41, 132). In high-anxiety Wistar Kyoto rats, lower total ghrelin levels compared to SD low-anxiety rats in both fasted and fed states were reported (42). In rats, exposure to water avoidance stress, an acute psychological stress, mobilizes total ghrelin. Interestingly, higher plasma ghrelin levels are induced after stress in low-anxiety SD rats than in high-anxiety Wistar Kyoto rats (133), suggesting that animals with low anxiety have a greater ability to mobilize ghrelin in response to stress.

### *Human pathophysiology*

***Anxiety/stress.*** Although stress elevates plasma ghrelin levels in animal models, which was proposed by some studies to increase emotional eating and help defend against some depressive symptoms induced by stress, there are farther less evidence in human pathophysiology that ghrelin is involved in the eating/metabolic response to stress (**Figure 2**).

Psychological stress, induced by public speaking over 2 days, does not modify plasma total ghrelin levels (60). In another study performed in normal weight, obese patients and subjects with binge-eating, social stress test does not modify ghrelin levels. However, when subjects are analyzed according to their cortisol response, ghrelin levels are found increased in cortisol responders subjects following the stress whereas no change occurred in cortisol non-responders and positive correlations are found between ghrelin and cortisol change (76). In emotional eaters, evaluated by the "emotional eating subscale of the Dutch eating behaviors questionnaire," anticipation of a psychological stressor (public speaking) leads to a greater food consumption than in-non-emotional eaters and ghrelin levels are more elevated in women anticipating the stressor compared to those not subjected to the stressor. Interestingly, the normal decline in ghrelin concentrations following food consumption is lower in non-emotional eaters (77), suggesting that ghrelin may contribute to emotional eating following a stressor (**Figure 3**).

***Depression.*** In the majority of studies, no difference in plasma total or acylated ghrelin levels were reported between patients with major-depressive disorder (MDD) and healthy subjects (**Figure 2**; **Table 2**) (78–81). However, in MMD patients, ghrelin levels correlate positively with the severity of reduced appetite and

negatively with gray matter volume of the VTA (81), and correlate with eating behavior scales like the three-factor eating questionnaire (TFEQ), suggesting that ghrelin may be associated with increased susceptibility to eating disorders observed in psychiatric patients (78).

Whether ghrelin can contribute to the degree of depression is not clearly demonstrated. In women across the weight spectrum, there was no relationship between ghrelin and symptoms of depression or anxiety (82). Interestingly, however, plasma acyl ghrelin levels were higher in treatment-resistant patients than responsive patients or in controls (83). One bias of these studies is that some of them do not discriminate ghrelin levels according to patients with or without medication (either neuroleptics, anti-depressant, antipsychotics or hypnotics). However, treatments impact plasma ghrelin levels and metabolic modifications may be secondary to the treatment. For example, in one study with MDD patients, treatment reduced plasma levels of acyl and desacyl ghrelin, as well as BMI, and ghrelin levels were lower than in controls (84). In contrast, treatment of lean patients with MDD with maprotiline, an anti-depressant, resulted in a minor increase in total ghrelin levels and WG (85). In both major-depressive episode (MDE) and bipolar disorder manic episode (BD-me), acylated ghrelin levels are decreased by electroconvulsive therapy (ECT), although BMI is unchanged (86), but remains higher than in controls.

***Schizophrenia.*** Weight gain is a common side effect of the atypical antipsychotics (AAPs) used to treat schizophrenia and it has been related with the orexigenic effect of elevated serum ghrelin rather than leptin deficit (134). Among five widely used AAPs (clozapine, olanzapine, risperidone, amisulpride, or quetiapine), only the later did not elevate the ghrelin level. Ghrelin gene polymorphisms have been associated with pathogenic variations in plasma lipid concentrations, blood pressure, plasma glucose, and BMI. Four SNPs (Leu72Met, −501A/C, −604 G/A, and −1062 G > C) were genotyped in 634 schizophrenia patients and 606 control Chinese Han subjects (135). These four *GHRL* gene SNPs were not associated with SZ in this Chinese Han population. However, the −604 G/A polymorphism was associated with significant BW and BMI increases during AAP treatment.

The effect of a 16 week-treatment with olanzapine was studied by functional magnetic resonance imaging in conjunction with a task requiring visual processing of appetitive stimuli in schizophrenic patients and healthy controls (136). Neuronal activity in the fusiform gyrus was brought back to "normal" after olanzapine treatment and this change was positively correlated with the restoration in ghrelin and leptin levels, following the treatment. However, others have reported that serum leptin levels might be a more sensitive biomarker than ghrelin or adiponectin levels to differentiate schizophrenic patients and healthy controls (137).

In some of the cited disorders, we need to be precautious with the interpretation because they concern small groups of subjects and inter-individual variability in the ghrelin pattern has to be considered (**Table 2**).

## GHRELIN-GH AXIS AND PSYCHIATRIC DISORDERS

As ghrelin has initially been described as a powerful GH secretagogue and a number of studies have focused on its role as a regulator of the GH/IGF-1 axis (1, 13, 138), the link between ghrelin, GH axis, and psychiatric disorders needs to be questioned. The GH/IGF-1 axis is deregulated in anorexia nervosa and the evolution of GH levels during renutrition is predictive of short-term outcome in AN-R patients (139, 140). More specifically, low GH levels at admission and absence of GH reduction after weight recovery could be predictive of short-term relapse. In patients with depression or post-traumatic stress disorder (PTSD), sleep-related GH secretion is lower compared to healthy controls (141). Abnormal IGF-1 levels have also been reported in several psychiatric disorders like schizophrenia and major depression (142). Interestingly, in GH-deficient patients symptoms of depression and cognitive impairments are improved after GH therapy (143).

Several components of the GH/IGF-1 axis also modulate anxiety and depression. At the hypothalamic level, GH secretion is directly regulated by two antagonistic neurohormones: GHRH stimulates whereas somatostatin inhibits GH secretion. Intracerebroventricular and intra-amygdala administrations of somatostatin induces anxiolytic and anti-depressant effects in rats (144, 145) while somatostatin KO mice show hyperactivity and anxiety-like behavior (146). GHRH KO mice display anxiety and depression-related behaviors (147). In addition, the GHRH agonist, JI-34, induces anxiety and depression (148) whereas the GHRH antagonist, MZ-4-71, elicits anxiolytic and anti-depressant effects (149).

Interestingly, deficiency in circulating and hippocampal IGF-1 induced by virus-mediated IGF-1 KO is associated with depressive symptoms measured in the forced-swim test in mice (150). A recent study brings an interesting point of view on the link between ghrelin–GH axis and stress-associated mental diseases. In a rodent model of PTSD, in which rats are repeatedly exposed to a stressor and display enhanced fear, stress-related increases in circulating ghrelin are necessary and sufficient for stress-associated vulnerability to exacerbated fear learning. These actions of ghrelin require GH in the amygdala to exert fear-enhancing effects, suggesting that ghrelin–GH axis can mediate maladaptive changes following prolonged stress (151).

## CONCLUSION

Ghrelin interacts with the GHS-R to modulate GH secretion, natural and artificial reward as well as stress and anxiety. Anhedonic symptoms, which include loss of pleasure, appetite, and motivation, are often observed in these disorders in association with altered ghrelin secretion and/or signaling. Whether dysfunction of the ghrelin/GHS-R signal contributes to the mechanism responsible for the development of the disease or can help to minimize some symptoms associated with these psychiatric disorders is still debated.

The recent demonstration of the heterodimerization of the GHS-R and the dopamine D2-receptor requested for appetite regulation in animal is a novel avenue for future studies deciphering the role of ghrelin in reward and addictions that are both impaired in psychiatric disorders. The multiple links between the

ghrelin/GHS-R system and other biological pathways, via the functional heterodimerization with other receptors that could play a role in psychiatric disorders, would be interesting to explore.

Finally, although ghrelin has been proposed to be a pharmacological target for treatment of psychiatric disorders, only a few data on the involvement of the other ghrelin-derived peptides, DAG, and obestatin, in psychiatric disorders are available. This would require further investigations.

## REFERENCES


1. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* (1999) **402**:656–60. doi:10.1038/45230
2. Tomasetto C, Karam SM, Ribieras S, Masson R, Lefèbvre O, Staub A, et al. Identification and characterization of a novel gastric peptide hormone: the motilin-related peptide. *Gastroenterology* (2000) **119**:395–405. doi:10.1053/gast.2000.9371
3. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* (2000) **407**:908–13. doi:10.1038/35038090
4. Méquinion M, Langlet F, Zgheib S, Dickson S, Dehouck B, Chauveau C, et al. Ghrelin: central and peripheral implications in anorexia nervosa. *Front Endocrinol* (2013) **4**:15. doi:10.3389/fendo.2013.00015
5. Gutierrez JA, Solenberg PJ, Perkins DR, Willency JA, Knierman MD, Jin Z, et al. Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc Natl Acad Sci U S A* (2008) **105**:6320–5. doi:10.1073/pnas.0800708105
6. Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* (2008) **132**:387–96. doi:10.1016/j.cell.2008.01.017
7. Kojima M, Kangawa K. Ghrelin: structure and function. *Physiol Rev* (2005) **85**:495–522. doi:10.1152/physrev.00012.2004
8. Satou M, Nakamura Y, Ando H, Sugimoto H. Understanding the functional significance of ghrelin processing and degradation. *Peptides* (2011) **32**:2183–90. doi:10.1016/j.peptides.2011.06.029
9. Zhang JV, Ren P-G, Avsian-Kretchmer O, Luo C-W, Rauch R, Klein C, et al. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science* (2005) **310**:996–9. doi:10.1126/science.1117255
10. Delhanty PJ, Neggers SJ, Van Der Lely AJ. Mechanisms in endocrinology: Ghrelin: the differences between acyl- and des-acyl ghrelin. *Eur J Endocrinol* (2012) **167**:601–8. doi:10.1530/EJE-12-0456
11. Gargantini E, Grande C, Trovato L, Ghigo E, Granata R. The role of obestatin in glucose and lipid metabolism. *Horm Metab Res* (2013) **45**:1002–8. doi:10.1055/s-0033-1351325
12. Gesmundo I, Gallo D, Favaro E, Ghigo E, Granata R. Obestatin: a new metabolic player in the pancreas and white adipose tissue. *IUBMB Life* (2013) **65**:976–82. doi:10.1002/iub.1226
13. Zizzari P, Longchamps R, Epelbaum J, Bluet-Pajot MT. Obestatin partially affects ghrelin stimulation of food intake and growth hormone secretion in rodents. *Endocrinology* (2007) **148**:1648–53. doi:10.1210/en.2006-1231
14. Inhoff T, Monnikes H, Noetzel S, Stengel A, Goebel M, Dinh QT, et al. Desacyl ghrelin inhibits the orexigenic effect of peripherally injected ghrelin in rats. *Peptides* (2008) **29**:2159–68. doi:10.1016/j.peptides.2008.09.014
15. Guan XM, Yu H, Palyha OC, Mckee KK, Feighner SD, Sirinathsinghji DJ, et al. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res Mol Brain Res* (1997) **48**:23–9. doi:10.1016/S0169-328X(97)00071-5
16. Zigman JM, Jones JE, Lee CE, Saper CB, Elmquist JK. Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J Comp Neurol* (2006) **494**:528–48. doi:10.1002/cne.20823
17. De Wit LM, Fokkema M, Van Straten A, Lamers F, Cuijpers P, Penninx BWJH. Depressive and anxiety disorders and the association with obesity, physical, and social activities. *Depress Anxiety* (2010) **27**:1057–65. doi:10.1002/da.20738
18. Skibicka KP, Hansson C, Alvarez-Crespo M, Friberg PA, Dickson SL. Ghrelin directly targets the ventral tegmental area to increase food motivation. *Neuroscience* (2011) **180**:129–37. doi:10.1016/j.neuroscience.2011.02.016
19. Overduin J, Figlewicz DP, Bennett-Jay J, Kittleson S, Cummings DE. Ghrelin increases the motivation to eat, but does not alter food palatability. *Am J Physiol Regul Integr Comp Physiol* (2012) **303**:R259–69. doi:10.1152/ajpregu.00488.2011
20. Skibicka KP, Hansson C, Egecioglu E, Dickson SL. Role of ghrelin in food reward: impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor gene expression. *Addict Biol* (2012) **17**:95–107. doi:10.1111/j.1369-1600.2010.00294.x
21. King SJ, Isaacs AM, O'Farrell E, Abizaid A. Motivation to obtain preferred foods is enhanced by ghrelin in the ventral tegmental area. *Horm Behav* (2011) **60**:572–80. doi:10.1016/j.yhbeh.2011.08.006
22. Weinberg ZY, Nicholson ML, Currie PJ. 6-Hydroxydopamine lesions of the ventral tegmental area suppress ghrelin's ability to elicit food-reinforced behavior. *Neurosci Lett* (2011) **499**:70–3. doi:10.1016/j.neulet.2011.05.034
23. Skibicka KP, Shirazi RH, Rabasa-Papio C, Alvarez-Crespo M, Neuber C, Vogel H, et al. Divergent circuitry underlying food reward and intake effects of ghrelin: dopaminergic VTA-accumbens projection mediates ghrelin's effect on food reward but not food intake. *Neuropharmacology* (2013) **73**:274–83. doi:10.1016/j.neuropharm.2013.06.004
24. Egecioglu E, Jerlhag E, Salomé N, Skibicka KP, Haage D, Bohlooly-Y M, et al. Ghrelin increases intake of rewarding food in rodents. *Addict Biol* (2010) **15**:304–11. doi:10.1111/j.1369-1600.2010.00216.x
25. Mccallum SE, Taraschenko OD, Hathaway ER, Vincent MY, Glick SD. Effects of 18-methoxycoronaridine on ghrelin-induced increases in sucrose intake and accumbal dopamine overflow in female rats. *Psychopharmacology* (2011) **215**:247–56. doi:10.1007/s00213-010-2132-0
26. Disse E, Bussier A-L, Veyrat-Durebex C, Deblon N, Pfluger PT, Tschöp MH, et al. Peripheral ghrelin enhances sweet taste food consumption and preference, regardless of its caloric content. *Physiol Behav* (2010) **101**:277–81. doi:10.1016/j.physbeh.2010.05.017
27. Bomberg EM, Grace MK, Wirth MM, Levine AS, Olszewski PK. Central ghrelin induces feeding driven by energy needs not by reward. *Neuroreport* (2007) **18**:591–5. doi:10.1097/WNR.0b013e3280b07bb5
28. Malik S, Mcglone F, Bedrossian D, Dagher A. Ghrelin modulates brain activity in areas that control appetitive behavior. *Cell Metab* (2008) **7**:400–9. doi:10.1016/j.cmet.2008.03.007
29. Goldstone AP, Prechtl CG, Scholtz S, Miras AD, Chhina N, Durighel G, et al. Ghrelin mimics fasting to enhance human hedonic, orbitofrontal cortex, and hippocampal responses to food. *Am J Clin Nutr* (2014) **99**:1319–30. doi:10.3945/ajcn.113.075291
30. Uchida A, Zigman JM, Perelló M. Ghrelin and eating behavior: evidence and insights from genetically-modified mouse models. *Front Neurosci* (2013) **7**:121. doi:10.3389/fnins.2013.00121
31. Davis JF, Schurdak JD, Magrisso IJ, Mul JD, Grayson BE, Pfluger PT, et al. Gastric bypass surgery attenuates ethanol consumption in ethanol-preferring rats. *Biol Psychiatry* (2012) **72**:354–60. doi:10.1016/j.biopsych.2012.01.035
32. Vucetic Z, Reyes TM. Central dopaminergic circuitry controlling food intake and reward: implications for the regulation of obesity. *Wiley Interdiscip Rev Syst Biol Med* (2010) **2**:577–93. doi:10.1002/wsbm.77
33. Briggs DI, Enriori PJ, Lemus MB, Cowley MA, Andrews ZB. Diet-induced obesity causes ghrelin resistance in arcuate NPY/AgRP neurons. *Endocrinology* (2010) **151**:4745–55. doi:10.1210/en.2010-0556
34. Briggs DI, Lemus MB, Kua E, Andrews ZB. Diet-induced obesity attenuates fasting-induced hyperphagia. *J Neuroendocrinol* (2011) **23**:620–6. doi:10.1111/j.1365-2826.2011.02148.x
35. Finger BC, Dinan TG, Cryan JF. Diet-induced obesity blunts the behavioural effects of ghrelin: studies in a mouse-progressive ratio task. *Psychopharmacology* (2012) **220**:173–81. doi:10.1007/s00213-011-2468-0
36. Szulc M, Mikolajczak PL, Geppert B, Wachowiak R, Dyr W, Bobkiewicz-Kozlowska T. Ethanol affects acylated and total ghrelin levels in peripheral blood of alcohol-dependent rats. *Addict Biol* (2013) **18**:689–701. doi:10.1111/adb.12025
37. Landgren S, Engel JA, Hyytiä P, Zetterberg H, Blennow K, Jerlhag E. Expression of the gene encoding the ghrelin receptor in rats selected for differential alcohol preference. *Behav Brain Res* (2011) **221**:182–8. doi:10.1016/j.bbr.2011.03.003
38. Suchankova P, Steensland P, Fredriksson I, Engel JA, Jerlhag E. Ghrelin receptor (GHS-R1A) antagonism suppresses both alcohol consumption and the alcohol deprivation effect in rats following long-term voluntary alcohol consumption. *PLoS One* (2013) **8**:e71284. doi:10.1371/journal.pone.0071284
39. Tessari M, Catalano A, Pellitteri M, Di Francesco C, Marini F, Gerrard PA, et al. Correlation between serum ghrelin levels and cocaine-seeking behaviour

triggered by cocaine-associated conditioned stimuli in rats. *Addict Biol* (2007) **12**:22–9. doi:10.1111/j.1369-1600.2007.00052.x
40. Lutter M, Sakata I, Osborne-Lawrence S, Rovinsky SA, Anderson JG, Jung S, et al. The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat Neurosci* (2008) **11**:752–3. doi:10.1038/nn.2139
41. Chuang J-C, Perello M, Sakata I, Osborne-Lawrence S, Savitt JM, Lutter M, et al. Ghrelin mediates stress-induced food-reward behavior in mice. *J Clin Invest* (2011) **121**:2684–92. doi:10.1172/JCI57660
42. Kristensson E, Sundqvist M, Håkanson R, Lindström E. High gastrin cell activity and low ghrelin cell activity in high-anxiety Wistar Kyoto rats. *J Endocrinol* (2007) **193**:245–50. doi:10.1677/JOE-07-0028
43. Yi C-X, Heppner K, Tschöp MH. Ghrelin in eating disorders. *Mol Cell Endocrinol* (2011) **340**:29–34. doi:10.1016/j.mce.2011.03.001
44. Yildiz BO, Suchard MA, Wong M-L, Mccann SM, Licinio J. Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. *Proc Natl Acad Sci U S A* (2004) **101**:10434–9. doi:10.1073/pnas.0403465101
45. Barazzoni R, Zanetti M, Ferreira C, Vinci P, Pirulli A, Mucci M, et al. Relationships between desacylated and acylated ghrelin and insulin sensitivity in the metabolic syndrome. *J Clin Endocrinol Metab* (2007) **92**:3935–40. doi:10.1210/jc.2006-2527
46. Mackelvie KJ, Meneilly GS, Elahi D, Wong AC, Barr SI, Chanoine JP. Regulation of appetite in lean and obese adolescents after exercise: role of acylated and desacyl ghrelin. *J Clin Endocrinol Metab* (2007) **92**:648–54. doi:10.1210/jc.2006-1028
47. Rodriguez A, Gomez-Ambrosi J, Catalan V, Becerril S, Sainz N, Gil MJ, et al. Association of plasma acylated ghrelin with blood pressure and left ventricular mass in patients with metabolic syndrome. *J Hypertens* (2010) **28**:560–7. doi:10.1097/HJH.0b013e328334327c
48. Cummings DE, Clement K, Purnell JQ, Vaisse C, Foster KE, Frayo RS, et al. Elevated plasma ghrelin levels in Prader Willi syndrome. *Nat Med* (2002) **8**:643–4. doi:10.1038/nm0702-643
49. DelParigi A, Tschop M, Heiman ML, Salbe AD, Vozarova B, Sell SM, et al. High circulating ghrelin: a potential cause for hyperphagia and obesity in Prader-Willi syndrome. *J Clin Endocrinol Metab* (2002) **87**:5461–4. doi:10.1210/jc.2002-020871
50. Paik KH, Choe YH, Park WH, Oh YJ, Kim AH, Chu SH, et al. Suppression of acylated ghrelin during oral glucose tolerance test is correlated with whole-body insulin sensitivity in children with Prader-Willi syndrome. *J Clin Endocrinol Metab* (2006) **91**:1876–81. doi:10.1210/jc.2005-2168
51. Park WH, Oh YJ, Kim GY, Kim SE, Paik KH, Han SJ, et al. Obestatin is not elevated or correlated with insulin in children with Prader-Willi syndrome. *J Clin Endocrinol Metab* (2007) **92**:229–34. doi:10.1210/jc.2006-0754
52. Nakahara T, Harada T, Yasuhara D, Shimada N, Amitani H, Sakoguchi T, et al. Plasma obestatin concentrations are negatively correlated with body mass index, insulin resistance index, and plasma leptin concentrations in obesity and anorexia nervosa. *Biol Psychiatry* (2008) **64**:252–5. doi:10.1016/j.biopsych.2007.08.005
53. Germain N, Galusca B, Grouselle D, Frere D, Tolle V, Zizzari P, et al. Ghrelin/obestatin ratio in two populations with low bodyweight: constitutional thinness and anorexia nervosa. *Psychoneuroendocrinology* (2009) **34**:413–9. doi:10.1016/j.psyneuen.2008.10.001
54. Germain N, Galusca B, Grouselle D, Frere D, Billard S, Epelbaum J, et al. Ghrelin and obestatin circadian levels differentiate bingeing-purging from restrictive anorexia nervosa. *J Clin Endocrinol Metab* (2010) **95**(6):3057–62. doi:10.1210/jc.2009-2196
55. Miljic D, Pekic S, Djurovic M, Doknic M, Milic N, Casanueva FF, et al. Ghrelin has partial or no effect on appetite, growth hormone, prolactin, and cortisol release in patients with anorexia nervosa. *J Clin Endocrinol Metab* (2006) **91**:1491–5. doi:10.1210/jc.2005-2304
56. Broglio F, Gianotti L, Destefanis S, Fassino S, Abbate Daga G, Mondelli V, et al. The endocrine response to acute ghrelin administration is blunted in patients with anorexia nervosa, a ghrelin hypersecretory state. *Clin Endocrinol* (2004) **60**:592–9. doi:10.1111/j.1365-2265.2004.02011.x
57. Hotta M, Ohwada R, Akamizu T, Shibasaki T, Takano K, Kangawa K. Ghrelin increases hunger and food intake in patients with restricting-type anorexia nervosa: a pilot study. *Endocr J* (2009) **56**:1119–28. doi:10.1507/endocrj.K09E-168
58. Calissendorff J, Danielsson O, Brismar K, Röjdmark S. Inhibitory effect of alcohol on ghrelin secretion in normal man. *Eur J Endocrinol* (2005) **152**:743–7. doi:10.1530/eje.1.01905
59. Calissendorff J, Danielsson O, Brismar K, Röjdmark S. Alcohol ingestion does not affect serum levels of peptide YY but decreases both total and octanoylated ghrelin levels in healthy subjects. *Metabolism* (2006) **55**:1625–9. doi:10.1016/j.metabol.2006.08.003
60. Zimmermann US, Buchmann A, Steffin B, Dieterle C, Uhr M. Alcohol administration acutely inhibits ghrelin secretion in an experiment involving psychosocial stress. *Addict Biol* (2007) **12**:17–21. doi:10.1111/j.1369-1600.2006.00026.x
61. Calissendorff J, Gustafsson T, Holst JJ, Brismar K, Röjdmark S. Alcohol intake and its effect on some appetite-regulating hormones in man: influence of gastroprotection with sucralfate. *Endocr Res* (2012) **37**:154–62. doi:10.3109/07435800.2012.662662
62. Leggio L, Schwandt ML, Oot EN, Dias AA, Ramchandani VA. Fasting-induced increase in plasma ghrelin is blunted by intravenous alcohol administration: a within-subject placebo-controlled study. *Psychoneuroendocrinology* (2013) **38**:3085–91. doi:10.1016/j.psyneuen.2013.09.005
63. Beulens JWJ, De Zoete EC, Kok FJ, Schaafsma G, Hendriks HFJ. Effect of moderate alcohol consumption on adipokines and insulin sensitivity in lean and overweight men: a diet intervention study. *Eur J Clin Nutr* (2008) **62**:1098–105. doi:10.1038/sj.ejcn.1602821
64. Kraus CL, Salazar NC, Mitchell JR, Florin WD, Guenther B, Brady D, et al. Inconsistencies between actual and estimated blood alcohol concentrations in a field study of college students: do students really know how much they drink? *Alcohol Clin Exp Res* (2005) **29**:1672–6. doi:10.1097/01.alc.0000179205.24180.4a
65. Kraus T, Schanze A, Groschl M, Bayerlein K, Hillemacher T, Reulbach U, et al. Ghrelin levels are increased in alcoholism. *Alcohol Clin Exp Res* (2005) **29**:2154–7. doi:10.1097/01.alc.0000191753.82554.7e
66. De Timary P, Cani PD, Duchemin J, Neyrinck AM, Gihousse D, Laterre P-F, et al. The loss of metabolic control on alcohol drinking in heavy drinking alcohol-dependent subjects. *PLoS One* (2012) **7**:e38682. doi:10.1371/journal.pone.0038682
67. Addolorato G, Capristo E, Leggio L, Ferrulli A, Abenavoli L, Malandrino N, et al. Relationship between ghrelin levels, alcohol craving, and nutritional status in current alcoholic patients. *Alcohol Clin Exp Res* (2006) **30**:1933–7. doi:10.1111/j.1530-0277.2006.00238.x
68. Wurst FM, Graf I, Ehrenthal HD, Klein S, Backhaus J, Blank S, et al. Gender differences for ghrelin levels in alcohol-dependent patients and differences between alcoholics and healthy controls. *Alcohol Clin Exp Res* (2007) **31**:2006–11. doi:10.1111/j.1530-0277.2007.00527.x
69. Kim DJ, Yoon SJ, Choi B, Kim TS, Woo YS, Kim W, et al. Increased fasting plasma ghrelin levels during alcohol abstinence. *Alcohol Alcohol* (2005) **40**:76–9. doi:10.1093/alcalc/agh108
70. Koopmann A, Von Der Goltz C, Grosshans M, Dinter C, Vitale M, Wiedemann K, et al. The association of the appetitive peptide acetylated ghrelin with alcohol craving in early abstinent alcohol dependent individuals. *Psychoneuroendocrinology* (2012) **37**:980–6. doi:10.1016/j.psyneuen.2011.11.005
71. Kim JH, Kim SJ, Lee WY, Cheon YH, Lee SS, Ju A, et al. The effects of alcohol abstinence on BDNF, ghrelin, and leptin secretions in alcohol-dependent patients with glucose intolerance. *Alcohol Clin Exp Res* (2013) **37**(Suppl 1):E52–8. doi:10.1111/j.1530-0277.2012.01921.x
72. Mutschler J, Graf N, Spanaus KS, Rössler W, Hergan K, Binkert CA, et al. Circulating ghrelin levels are not associated with craving and withdrawal symptoms in acute nicotine withdrawal. *Psychiatr Danub* (2012) **24**:229–30.
73. Paslakis G, Buchmann AF, Westphal S, Banaschewski T, Hohm E, Zimmermann US, et al. Intrauterine exposure to cigarette smoke is associated with increased ghrelin concentrations in adulthood. *Neuroendocrinology* (2014) **99**:123–9. doi:10.1159/000363325
74. Kokkinos A, Tentolouris N, Kyriakaki E, Argyrakopoulou G, Doupis J, Psallas M, et al. Differentiation in the short- and long-term effects of smoking on plasma total ghrelin concentrations between male nonsmokers and habitual smokers. *Metabolism* (2007) **56**:523–7. doi:10.1016/j.metabol.2006.11.012
75. Pilhatsch M, Scheuing H, Kroemer N, Kobiella A, Bidlingmaier M, Farger G, et al. Nicotine administration in healthy non-smokers reduces appetite but does not alter plasma ghrelin. *Hum Psychopharmacol* (2014) **29**:384–7. doi:10.1002/hup.2405
76. Rouach V, Bloch M, Rosenberg N, Gilad S, Limor R, Stern N, et al. The acute ghrelin response to a psychological stress challenge does not predict

the post-stress urge to eat. *Psychoneuroendocrinology* (2007) **32**:693–702. doi:10.1016/j.psyneuen.2007.04.010
77. Raspopow K, Abizaid A, Matheson K, Anisman H. Anticipation of a psychosocial stressor differentially influences ghrelin, cortisol and food intake among emotional and non-emotional eaters. *Appetite* (2014) **74**:35–43. doi:10.1016/j.appet.2013.11.018
78. Schanze A, Reulbach U, Scheuchenzuber M, Groschl M, Kornhuber J, Kraus T. Ghrelin and eating disturbances in psychiatric disorders. *Neuropsychobiology* (2008) **57**:126–30. doi:10.1159/000138915
79. Kluge M, Schussler P, Schmid D, Uhr M, Kleyer S, Yassouridis A, et al. Ghrelin plasma levels are not altered in major depression. *Neuropsychobiology* (2009) **59**:199–204. doi:10.1159/000223731
80. Gimenez-Palop O, Coronas R, Cobo J, Gallart L, Barbero JD, Parra I, et al. Fasting plasma peptide YY concentrations are increased in patients with major depression who associate weight loss. *J Endocrinol Invest* (2012) **35**:645–8. doi:10.3275/8180
81. Matsuo K, Nakano M, Nakashima M, Watanuki T, Egashira K, Matsubara T, et al. Neural correlates of plasma acylated ghrelin level in individuals with major depressive disorder. *Brain Res* (2012) **1473**:185–92. doi:10.1016/j.brainres.2012.07.027
82. Lawson EA, Miller KK, Blum JI, Meenaghan E, Misra M, Eddy KT, et al. Leptin levels are associated with decreased depressive symptoms in women across the weight spectrum, independent of body fat. *Clin Endocrinol* (2012) **76**:520–5. doi:10.1111/j.1365-2265.2011.04182.x
83. Ishitobi Y, Kohno K, Kanehisa M, Inoue A, Imanaga J, Maruyama Y, et al. Serum ghrelin levels and the effects of antidepressants in major depressive disorder and panic disorder. *Neuropsychobiology* (2012) **66**:185–92. doi:10.1159/000339948
84. Barim AO, Aydin S, Colak R, Dag E, Deniz O, Sahin I. Ghrelin, paraoxonase and arylesterase levels in depressive patients before and after citalopram treatment. *Clin Biochem* (2009) **42**:1076–81. doi:10.1016/j.clinbiochem.2009.02.020
85. Pinar M, Gulsun M, Tasci I, Erdil A, Bolu E, Acikel C, et al. Maprotiline induced weight gain in depressive disorder: changes in circulating ghrelin and adiponectin levels and insulin sensitivity. *Prog Neuropsychopharmacol Biol Psychiatry* (2008) **32**:135–9. doi:10.1016/j.pnpbp.2007.07.028
86. Kurt E, Guler O, Serteser M, Cansel N, Ozbulut O, Altinbas K, et al. The effects of electroconvulsive therapy on ghrelin, leptin and cholesterol levels in patients with mood disorders. *Neurosci Lett* (2007) **426**:49–53. doi:10.1016/j.neulet.2007.08.018
87. Koyama KI, Yasuhara D, Nakahara T, Harada T, Uehara M, Ushikai M, et al. Changes in acyl ghrelin, des-acyl ghrelin, and ratio of acyl ghrelin to total ghrelin with short-term refeeding in female inpatients with restricting-type anorexia nervosa. *Horm Metab Res* (2010) **42**:595–8. doi:10.1055/s-0030-1252017
88. Bailer UF, Frank GK, Henry SE, Price JC, Meltzer CC, Mathis CA, et al. Exaggerated 5-HT1A but normal 5-HT2A receptor activity in individuals ill with anorexia nervosa. *Biol Psychiatry* (2007) **61**:1090–9. doi:10.1016/j.biopsych.2006.07.018
89. Bailer UF, Frank GK, Price JC, Meltzer CC, Becker C, Mathis CA, et al. Interaction between serotonin transporter and dopamine D2/D3 receptor radioligand measures is associated with harm avoidant symptoms in anorexia and bulimia nervosa. *Psychiatry Res* (2013) **211**:160–8. doi:10.1016/j.pscychresns.2012.06.010
90. Kern A, Albarran-Zeckler R, Walsh HE, Smith RG. Apo-ghrelin receptor forms heteromers with DRD2 in hypothalamic neurons and is essential for anorexigenic effects of DRD2 agonism. *Neuron* (2012) **73**:317–32. doi:10.1016/j.neuron.2011.10.038
91. Dickson SL, Egecioglu E, Landgren S, Skibicka KP, Engel JA, Jerlhag E. The role of the central ghrelin system in reward from food and chemical drugs. *Mol Cell Endocrinol* (2011) **340**:80–7. doi:10.1016/j.mce.2011.02.017
92. Revitsky AR, Klein LC. Role of ghrelin in drug abuse and reward-relevant behaviors: a burgeoning field and gaps in the literature. *Curr Drug Abuse Rev* (2013) **6**:231–44. doi:10.2174/18744737070666140205200532
93. Jerlhag E, Egecioglu E, Landgren S, Salomé N, Heilig M, Moechars D, et al. Requirement of central ghrelin signaling for alcohol reward. *Proc Natl Acad Sci U S A* (2009) **106**:11318–23. doi:10.1073/pnas.0812809106
94. Sprow GM, Thiele TE. The neurobiology of binge-like ethanol drinking: evidence from rodent models. *Physiol Behav* (2012) **106**:325–31. doi:10.1016/j.physbeh.2011.12.026
95. Lyons AM, Lowery EG, Sparta DR, Thiele TE. Effects of food availability and administration of orexigenic and anorectic agents on elevated ethanol drinking associated with drinking in the dark procedures. *Alcohol Clin Exp Res* (2008) **32**:1962–8. doi:10.1111/j.1530-0277.2008.00784.x
96. Leggio L, Zywiak WH, Fricchione SR, Edwards SM, De La Monte SM, Swift RM, et al. Intravenous ghrelin administration increases alcohol craving in alcohol-dependent heavy drinkers: a preliminary investigation. *Biol Psychiatry* (2014) **76**:734–41. doi:10.1016/j.biopsych.2014.03.019
97. Landgren S, Simms JA, Hyytiä P, Engel JA, Bartlett SE, Jerlhag E. Ghrelin receptor (GHS-R1A) antagonism suppresses both operant alcohol self-administration and high alcohol consumption in rats. *Addict Biol* (2012) **17**:86–94. doi:10.1111/j.1369-1600.2010.00280.x
98. Kaur S, Ryabinin AE. Ghrelin receptor antagonism decreases alcohol consumption and activation of perioculomotor urocortin-containing neurons. *Alcohol Clin Exp Res* (2010) **34**:1525–34. doi:10.1111/j.1530-0277.2010.01237.x
99. Bahi A, Tolle V, Fehrentz J-A, Brunel L, Martinez J, Tomasetto C-L, et al. Ghrelin knockout mice show decreased voluntary alcohol consumption and reduced ethanol-induced conditioned place preference. *Peptides* (2013) **43**:48–55. doi:10.1016/j.peptides.2013.02.008
100. Jerlhag E, Engel JA. Ghrelin receptor antagonism attenuates nicotine-induced locomotor stimulation, accumbal dopamine release and conditioned place preference in mice. *Drug Alcohol Depend* (2011) **117**:126–31. doi:10.1016/j.drugalcdep.2011.01.010
101. Jerlhag E, Ivanoff L, Vater A, Engel JA. Peripherally circulating ghrelin does not mediate alcohol-induced reward and alcohol intake in rodents. *Alcohol Clin Exp Res* (2014) **38**:959–68. doi:10.1111/acer.12337
102. Hajnal A, Zharikov A, Polston JE, Fields MR, Tomasko J, Rogers AM, et al. Alcohol reward is increased after Roux-en-Y gastric bypass in dietary obese rats with differential effects following ghrelin antagonism. *PLoS One* (2012) **7**:e49121. doi:10.1371/journal.pone.0049121
103. Addolorato G, Leggio L, Hillemacher T, Kraus T, Jerlhag E, Bleich S. Hormones and drinking behaviour: new findings on ghrelin, insulin, leptin and volume-regulating hormones. An ESBRA Symposium report. *Drug Alcohol Rev* (2009) **28**:160–5. doi:10.1111/j.1465-3362.2008.00023.x
104. Kenna GA, Swift RM, Hillemacher T, Leggio L. The relationship of appetitive, reproductive and posterior pituitary hormones to alcoholism and craving in humans. *Neuropsychol Rev* (2012) **22**:211–28. doi:10.1007/s11065-012-9209-y
105. Hillemacher T, Kahl KG, Heberlein A, Muschler MA, Eberlein C, Frieling H, et al. Appetite- and volume-regulating neuropeptides: role in treating alcohol dependence. *Curr Opin Investig Drugs* (2010) **11**:1097–106.
106. Leggio L. Role of the ghrelin system in alcoholism: acting on the growth hormone secretagogue receptor to treat alcohol-related diseases. *Drug News Perspect* (2010) **23**:157–66. doi:10.1358/dnp.2010.23.3.1429490
107. Landgren S, Jerlhag E, Zetterberg H, Gonzalez-Quintela A, Campos J, Olofsson U, et al. Association of pro-ghrelin and GHS-R1A gene polymorphisms and haplotypes with heavy alcohol use and body mass. *Alcohol Clin Exp Res* (2008) **32**:2054–61. doi:10.1111/j.1530-0277.2008.00793.x
108. Wellman PJ, Clifford PS, Rodriguez JA. Ghrelin and ghrelin receptor modulation of psychostimulant action. *Front Neurosci* (2013) **7**:171. doi:10.3389/fnins.2013.00171
109. Maric T, Sedki F, Ronfard B, Chafetz D, Shalev U. A limited role for ghrelin in heroin self-administration and food deprivation-induced reinstatement of heroin seeking in rats. *Addict Biol* (2012) **17**:613–22. doi:10.1111/j.1369-1600.2011.00396.x
110. Jang JK, Kim WY, Cho BR, Lee JW, Kim J-H. Microinjection of ghrelin in the nucleus accumbens core enhances locomotor activity induced by cocaine. *Behav Brain Res* (2013) **248**:7–11. doi:10.1016/j.bbr.2013.03.049
111. Palotai M, Bagosi Z, Jászberényi M, Csabafi K, Dochnal R, Manczinger M, et al. Ghrelin amplifies the nicotine-induced dopamine release in the rat striatum. *Neurochem Int* (2013) **63**:239–43. doi:10.1016/j.neuint.2013.06.014
112. Kroemer NB, Wuttig F, Bidlingmaier M, Zimmermann US, Smolka MN. Nicotine enhances modulation of food-cue reactivity by leptin and ghrelin in the ventromedial prefrontal cortex. *Addict Biol* (2014). doi:10.1111/adb.12167
113. Jerlhag E, Egecioglu E, Dickson SL, Engel JA. Ghrelin receptor antagonism attenuates cocaine- and amphetamine-induced locomotor stimulation, accumbal dopamine release, and conditioned place preference. *Psychopharmacology* (2010) **211**:415–22. doi:10.1007/s00213-010-1907-7

114. Sustkova-Fiserova M, Jerabek P, Havlickova T, Kacer P, Krsiak M. Ghrelin receptor antagonism of morphine-induced accumbens dopamine release and behavioral stimulation in rats. *Psychopharmacology* (2014) **231**:2899–908. doi:10.1007/s00213-014-3466-9
115. Jerlhag E, Janson AC, Waters S, Engel JA. Concomitant release of ventral tegmental acetylcholine and accumbal dopamine by ghrelin in rats. *PLoS One* (2012) **7**:e49557. doi:10.1371/journal.pone.0049557
116. Jerlhag E, Landgren S, Egecioglu E, Dickson SL, Engel JA. The alcohol-induced locomotor stimulation and accumbal dopamine release is suppressed in ghrelin knockout mice. *Alcohol* (2011) **45**:341–7. doi:10.1016/j.alcohol.2010.10.002
117. Jerlhag E, Egecioglu E, Dickson SL, Engel JA. Glutamatergic regulation of ghrelin-induced activation of the mesolimbic dopamine system. *Addict Biol* (2011) **16**:82–91. doi:10.1111/j.1369-1600.2010.00231.x
118. Cruz MT, Herman MA, Cote DM, Ryabinin AE, Roberto M. Ghrelin increases GABAergic transmission and interacts with ethanol actions in the rat central nucleus of the amygdala. *Neuropsychopharmacology* (2013) **38**:364–75. doi:10.1038/npp.2012.190
119. Hansson C, Alvarez-Crespo M, Taube M, Skibicka KP, Schmidt L, Karlsson-Lindahl L, et al. Influence of ghrelin on the central serotonergic signaling system in mice. *Neuropharmacology* (2014) **79**:498–505. doi:10.1016/j.neuropharm.2013.12.012
120. Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Fujimiya M, et al. A role of ghrelin in neuroendocrine and behavioral responses to stress in mice. *Neuroendocrinology* (2001) **74**:143–7. doi:10.1159/000054680
121. Carlini VP, Varas MM, Cragnolini AB, Schiöth HB, Scimonelli TN, De Barioglio SR. Differential role of the hippocampus, amygdala, and dorsal raphe nucleus in regulating feeding, memory, and anxiety-like behavioral responses to ghrelin. *Biochem Biophys Res Commun* (2004) **313**:635–41. doi:10.1016/j.bbrc.2003.11.150
122. Alvarez-Crespo M, Skibicka KP, Farkas I, Molnár CS, Egecioglu E, Hrabovszky E, et al. The amygdala as a neurobiological target for ghrelin in rats: neuroanatomical, electrophysiological and behavioral evidence. *PLoS One* (2012) **7**:e46321. doi:10.1371/journal.pone.0046321
123. Currie PJ, Khelemsky R, Rigsbee EM, Dono LM, Coiro CD, Chapman CD, et al. Ghrelin is an orexigenic peptide and elicits anxiety-like behaviors following administration into discrete regions of the hypothalamus. *Behav Brain Res* (2012) **226**:96–105. doi:10.1016/j.bbr.2011.08.037
124. Kajbaf F, Ahmadi R, Fatemi Tabatabaie R, Safarpoor E. Effect of intrahippocampal ghrelin agonist administration on passive avoidance learning and anxiety in rats. *Pak J Biol Sci* (2012) **15**:1063–8. doi:10.3923/pjbs.2012.1063.1068
125. Currie PJ, Schuette LM, Wauson SER, Voss WN, Angeles MJ. Activation of urocortin 1 and ghrelin signaling in the basolateral amygdala induces anxiogenesis. *Neuroreport* (2014) **25**:60–4. doi:10.1097/WNR.0000000000000047
126. Carlini VP, Schiöth HB, Debarioglio SR. Obestatin improves memory performance and causes anxiolytic effects in rats. *Biochem Biophys Res Commun* (2007) **352**:907–12. doi:10.1016/j.bbrc.2006.11.112
127. Hansson C, Haage D, Taube M, Egecioglu E, Salomé N, Dickson SL. Central administration of ghrelin alters emotional responses in rats: behavioural, electrophysiological and molecular evidence. *Neuroscience* (2011) **180**:201–11. doi:10.1016/j.neuroscience.2011.02.002
128. Kluge M, Schüssler P, Dresler M, Schmidt D, Yassouridis A, Uhr M, et al. Effects of ghrelin on psychopathology, sleep and secretion of cortisol and growth hormone in patients with major depression. *J Psychiatr Res* (2011) **45**:421–6. doi:10.1016/j.jpsychires.2010.09.002
129. Albarran-Zeckler RG, Brantley AF, Smith RG. Growth hormone secretagogue receptor (GHS-R1a) knockout mice exhibit improved spatial memory and deficits in contextual memory. *Behav Brain Res* (2012) **232**:13–9. doi:10.1016/j.bbr.2012.03.012
130. Spencer SJ, Xu L, Clarke MA, Lemus M, Reichenbach A, Geenen B, et al. Ghrelin regulates the hypothalamic-pituitary-adrenal axis and restricts anxiety after acute stress. *Biol Psychiatry* (2012) **72**:457–65. doi:10.1016/j.biopsych.2012.03.010
131. Kanehisa M, Akiyoshi J, Kitaichi T, Matsushita H, Tanaka E, Kodama K, et al. Administration of antisense DNA for ghrelin causes an antidepressant and anxiolytic response in rats. *Prog Neuropsychopharmacol Biol Psychiatry* (2006) **30**:1403–7. doi:10.1016/j.pnpbp.2006.05.005
132. Patterson ZR, Khazall R, Mackay H, Anisman H, Abizaid A. Central ghrelin signaling mediates the metabolic response of C57BL/6 male mice to chronic social defeat stress. *Endocrinology* (2013) **154**:1080–91. doi:10.1210/en.2012-1834
133. Kristenssson E, Sundqvist M, Astin M, Kjerling M, Mattsson H, Dornonville De La Cour C, et al. Acute psychological stress raises plasma ghrelin in the rat. *Regul Pept* (2006) **134**:114–7. doi:10.1016/j.regpep.2006.02.003
134. Esen-Danaci A, Sarandöl A, Taneli F, Yurtsever F, Ozlen N. Effects of second generation antipsychotics on leptin and ghrelin. *Prog Neuropsychopharmacol Biol Psychiatry* (2008) **32**:1434–8. doi:10.1016/j.pnpbp.2008.03.015
135. Yang Y, Li W, Zhao J, Zhang H, Song X, Xiao B, et al. Association between ghrelin gene (GHRL) polymorphisms and clinical response to atypical antipsychotic drugs in Han Chinese schizophrenia patients. *Behav Brain Funct* (2012) **8**:11. doi:10.1186/1744-9081-8-11
136. Stip E, Lungu OV, Anselmo K, Letourneau G, Mendrek A, Stip B, et al. Neural changes associated with appetite information processing in schizophrenic patients after 16 weeks of olanzapine treatment. *Transl Psychiatry* (2012) **2**:e128. doi:10.1038/tp.2012.53
137. Tsai M-C, Chang C-M, Liu C-Y, Chang P-Y, Huang T-L. Association of serum levels of leptin, ghrelin, and adiponectin in schizophrenic patients and healthy controls. *Int J Psychiatry Clin Pract* (2011) **15**:106–11. doi:10.3109/13651501.2010.550400
138. Hassouna R, Zizzari P, Tomasetto C, Veldhuis JD, Fiquet O, Labarthe A, et al. An early reduction in GH peak amplitude in preproghrelin deficient male mice has a minor impact on linear growth. *Endocrinology* (2014) **155**(9):3561–71. doi:10.1210/en.2014-1126
139. Tolle V, Kadem M, Bluet-Pajot M-T, Frere D, Foulon C, Bossu C, et al. Balance in ghrelin and leptin plasma levels in anorexia nervosa patients and constitutionally thin women. *J Clin Endocrinol Metab* (2003) **88**:109–16. doi:10.1210/jc.2002-020645
140. Nogueira JP, Valéro R, Maraninchi M, Lorec AM, Samuelian-Massat C, Bégu-Le Corroller A, et al. Growth hormone level at admission and its evolution during refeeding are predictive of short-term outcome in restrictive anorexia nervosa. *Br J Nutr* (2013) **109**:2175–81. doi:10.1017/S000711451200431X
141. Voderholzer U, Laakmann G, Wittmann R, Daffner-Bujia C, Hinz A, Haag C, et al. Profiles of spontaneous 24-hour and stimulated growth hormone secretion in male patients with endogenous depression. *Psychiatry Res* (1993) **47**:215–27. doi:10.1016/0165-1781(93)90080-Z
142. Aleman A, Torres-Alemán I. Circulating insulin-like growth factor I and cognitive function: neuromodulation throughout the lifespan. *Prog Neurobiol* (2009) **89**:256–65. doi:10.1016/j.pneurobio.2009.07.008
143. Maric NP, Doknic M, Pavlovic D, Pekic S, Stojanovic M, Jasovic-Gasic M, et al. Psychiatric and neuropsychological changes in growth hormone-deficient patients after traumatic brain injury in response to growth hormone therapy. *J Endocrinol Invest* (2010) **33**:770–5. doi:10.3275/7045
144. Engin E, Stellbrink J, Treit D, Dickson CT. Anxiolytic and antidepressant effects of intracerebroventricularly administered somatostatin: behavioral and neurophysiological evidence. *Neuroscience* (2008) **157**:666–76. doi:10.1016/j.neuroscience.2008.09.037
145. Yeung M, Treit D. The anxiolytic effects of somatostatin following intra-septal and intra-amygdalar microinfusions are reversed by the selective sst2 antagonist PRL2903. *Pharmacol Biochem Behav* (2012) **101**:88–92. doi:10.1016/j.pbb.2011.12.012
146. Albrecht A, Thiere M, Bergado-Acosta JR, Poranzke J, Muller B, Stork O. Circadian modulation of anxiety: a role for somatostatin in the amygdala. *PLoS One* (2013) **8**:e84668. doi:10.1371/journal.pone.0084668
147. Leone S, Shohreh R, Manippa F, Recinella L, Ferrante C, Orlando G, et al. Behavioural phenotyping of male growth hormone-releasing hormone (GHRH) knockout mice. *Growth Horm IGF Res* (2014) **24**:192–7. doi:10.1016/j.ghir.2014.06.004
148. Nair D, Ramesh V, Li RC, Schally AV, Gozal D. (2013). Growth hormone releasing hormone (GHRH) signaling modulates intermittent hypoxia-induced oxidative stress and cognitive deficits in mouse. *J Neurochem* **127**:531–40. doi:10.1111/jnc.12360
149. Tanaka M, Schally AV, Telegdy G. (2012). Neurotransmission of the antidepressant-like effects of the growth hormone-releasing hormone antagonist MZ-4-71. *Behav Brain Res* **228**:388–91. doi:10.1016/j.bbr.2011.12.022
150. Mitschelen M, Yan H, Farley JA, Warrington JP, Han S, Hereñú CB, et al. Long-term deficiency of circulating and hippocampal insulin-like growth factor

I induces depressive behavior in adult mice: a potential model of geriatric depression. *Neuroscience* (2011) **185**:50–60. doi:10.1016/j.neuroscience.2011.04.032

151. Meyer RM, Burgos-Robles A, Liu E, Correia SS, Goosens KA. A ghrelin-growth hormone axis drives stress-induced vulnerability to enhanced fear. *Mol Psychiatry* (2013). doi:10.1038/mp.2013.135

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# Apo-ghrelin receptor (apo-GHSR1a) regulates dopamine signaling in the brain

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The orexigenic peptide hormone ghrelin is synthesized in the stomach and its receptor growth hormone secretagogue receptor (GHSR1a) is expressed mainly in the central nervous system (CNS). In this review, we confine our discussion to the physiological role of GHSR1a in the brain. Paradoxically, despite broad expression of GHSR1a in the CNS, other than trace amounts in the hypothalamus, ghrelin is undetectable in the brain. In our efforts to elucidate the function of the ligand-free ghrelin receptor (apo-GHSR1a), we identified subsets of neurons that co-express GHSR1a and dopamine receptors. In this review, we focus on interactions between apo-GHSR1a and dopamine-2 receptor (DRD2) and formation of GHSR1a:DRD2 heteromers in hypothalamic neurons that regulate appetite, and discuss implications for the treatment of Prader–Willi syndrome (PWS). GHSR1a antagonists of distinct chemical structures, a quinazolinone and a triazole, respectively, enhance and inhibit dopamine signaling through GHSR1a:DRD2 heteromers by an allosteric mechanism. This finding illustrates a potential strategy for designing the next generation of drugs for treating eating disorders as well as psychiatric disorders caused by abnormal dopamine signaling. Treatment with a GHSR1a antagonist that enhances dopamine/DRD2 activity in GHSR1a:DRD2 expressing hypothalamic neurons has the potential to inhibit the uncontrollable hyperphagia associated with PWS. DRD2 antagonists are prescribed for treating schizophrenia, but these block dopamine signaling in all DRD2 expressing neurons and are associated with adverse side effects, including enhanced appetite and excessive weight gain. A GHSR1a antagonist of structural class that allosterically blocks dopamine/DRD2 action in GHSR1a:DRD2 expressing neurons would have no effect on neurons expressing DRD2 alone; therefore, the side effects of DRD2 antagonists would potentially be reduced thereby enhancing patient compliance.

**Keywords: ghrelin, growth hormone secretagogue receptor, dopamine, heterodimers, hypothalamus**

## INTRODUCTION

The orexigenic hormone ghrelin is produced in stomach and influences feeding behavior, metabolism, pulsatile growth hormone (GH) release, and immune function (1–4). Ghrelin's action is regulated by the GH secretagogue receptor (GHSR1a, *aka* the ghrelin receptor) that was originally identified by reverse pharmacology using a small molecule, MK-0677, developed to rejuvenate the GH/insulin-like growth factor axis in elderly subjects (5, 6). GHSR1a belongs to the Class A G-protein coupled receptor (GPCR) family. In isolation, under defined conditions, GHSR1a couples to $G\alpha_q$ resulting in activation of phospholipase C (PLC), inositol trisphosphate ($IP_3$), and mobilization of $[Ca^{2+}]_i$ (7).

The precise physiological function of ghrelin remains to be defined. Traditionally ghrelin was believed to control appetite and facilitate excessive weight gain in response to a high-fat diet, but recent findings question these conclusions. Studies in congenic C57BL/6J *ghrelin* knockout (KO) and *ghsr* KO mice showed food intake is independent of ghrelin signaling, and that the absence of ghrelin fails to protect mice from diet-induced obesity (8–10). Indeed, recent results in transgenic mice where ghrelin producing cells were selectively ablated confirm these findings (11). Acute stimulation of food intake in ghrelin-cell ablated mice requires doses of exogenous ghrelin that produce plasma ghrelin concentrations many-fold higher than the endogenous concentrations found in wildtype mice, suggesting endogenous ghrelin is not a critical regulator of food intake. With prolonged calorie restriction ghrelin-cell ablated mice exhibit profound hypoglycemia (11). Similarly, profound hypoglycemia was reported by the same group in calorie-restricted ghrelin-deficient mice generated by ablating medium chain fatty acid acyl-transferase that is essential for converting the inactive 28-aminoacid ghrelin peptide into its biologically active form (12). Injection of ghrelin or GH rescued the hypoglycemia. Based on the results from these two transgenic mouse models, the authors concluded that ghrelin's major metabolic role is to regulate blood glucose under conditions of famine.

GHSR1a is expressed broadly in the brain and localized mainly in the hippocampal structures, hypothalamus, midbrain, cortex, and amygdala (13). These findings led us to investigate possible interactions of GHSR1a with dopamine receptors. By employing *Ghsr-IRES-tau-GFP* mice, we showed that

subsets of neurons in the midbrain and hippocampus co-express GHSR1a and dopamine receptor-1 (DRD1) (14), and in the hypothalamus subsets co-express GHSR1a and dopamine receptor-2 (DRD2) (15). *In vitro* studies illustrated the formation of GHSR1a:DRD1 heteromers, and treatment with ghrelin and dopamine in combination augmented cAMP accumulation (14). Again, using *Ghsr-IRES-tau-GFP* mice, we identified subsets of hypothalamic neurons that co-express GHSR1a and dopamine-2 receptor (DRD2) (15). DRD2 signaling influences feeding frequency and volume, and mutations in *DRD2* are associated with human obesity (16–20). In this review, we describe the co-expression of GHSR1a with dopamine receptors in neurons of the CNS, the dependence on GHSR1a for dopamine/DRD2 suppression of appetite and implications for the uncontrollable hyperphagia associated with Prader–Willi syndrome (PWS).

## GHRELIN ACTIVATION OF HYPOTHALAMIC NEURONS ENHANCES DOPAMINE RELEASE FROM MIDBRAIN DOPAMINERGIC NEURONS

Pharmacological doses of ghrelin activate extra-hypothalamic neurons implicating a role for ghrelin in memory, addiction, depression, and neuroprotection (21–27). Indeed, $ghsr^{-/-}$ mice exhibit impaired contextual memory (28). In addition, improvements in spatial memory induced by exogenous ghrelin are inhibited by a dopamine-1 receptor (DRD1) antagonist (29). Ghrelin also augments cocaine-induced locomotor activity and the rewarding effects of alcohol, consistent with effects on dopamine signaling (30, 31). Although ghrelin administration induces dopamine release (30), there is no evidence that ghrelin has direct access to midbrain dopaminergic neurons (32). Exogenously applied ghrelin activates c-Fos expression in the arcuate nucleus, paraventricular nucleus (PVN), and lateral hypothalamus (LH), but not in GHSR1a expressing neurons of the midbrain or hippocampus (33, 34). Tracing studies with inactivated rabies virus show that neurons in the ventral tegmental area (VTA) and pars compacta of the substantia nigra (SNpc) receive projections from the PVN and LH (33–35). Furthermore, infusion of ghrelin into the LH, but not in the VTA, stimulates orexin release, which activates orexin receptors on VTA neurons causing release of dopamine (36). Neuroanatomy, neuropharmacology, and cell biology studies indicate VTA dopaminergic neurons innervate the hippocampus (37–41). These collective observations are consistent with exogenous ghrelin inducing hippocampal plasticity indirectly by first activating neurons in the LH and PVN that in turn enhance dopamine release from the VTA resulting in activation of hippocampal neurons. However, these results obtained using pharmacological doses of ghrelin cannot simply be extrapolated to predict the physiological role of endogenous ghrelin. A recent series of studies in mice and sheep indicate that other than trace amounts in the hypothalamus, endogenous ghrelin is not present in the CNS (42–45). This is remarkable given the broad distribution of GHSR1a in the brain and underlines a potential role for GHSR1a independent of ghrelin, which led us to speculate that apo-GHSR1a regulates neuronal function through protein–protein interactions.

## GHSR1a AND DRD2 ARE CO-EXPRESSED IN HYPOTHALAMIC NEURONS RESULTING IN MODIFICATION OF CANONICAL DOPAMINE SIGNALING

Since endogenous ghrelin is undetectable in the brain, we addressed the question of what biological role un-liganded GHSR1a (apo-GHSR1a) might play by focusing on the function of hypothalamic neurons that co-express GHSR1a and DRD2. To determine the impact of expression of apo-GHSR1a on DRD2 signaling, we initially used HEK293 cells where we could control the relative expression levels of the two receptors and investigate signal transduction pathways. Dopamine activation of DRD2 in HEK293 cells results in canonical DRD2 coupling to $G\alpha_{i/o}$ and suppression of intracellular cAMP accumulation without mobilization of intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$) (46). However, when GHSR1a is expressed with DRD2, treatment with dopamine, or the DRD2 agonist quinpirole results in $[Ca^{2+}]_i$ mobilization. When DRD2 is expressed with the motilin receptor, which is closely related to GHSR1a and is also coupled to $G\alpha_q$, neither dopamine nor quinpirole treatment mobilizes $[Ca^{2+}]_i$, illustrating the significance of specific interactions between GHSR1a and DRD2. When GHSR1a is co-expressed with DRD2, dopamine-induced $[Ca^{2+}]_i$ release is blocked by: pertussis toxin, $G\beta\gamma$ antagonists, PLC inhibitor, thapsigargin, and $IP_3$ receptor blocker. We conclude that dopamine activation causes liberation of $G\beta\gamma$ subunits from $G\alpha_{i/o}$, activation of PLC, $IP_3$ mobilization, and release of $[Ca^{2+}]_i$ (**Figure 1**). Importantly, this mechanism is activated in the complete absence of ghrelin. A mechanism involving downstream cross-talk between GHSR1a and DRD2 caused by GHSR1a basal activity was ruled out. Blocking GHSR1a basal activity with $G\alpha_q$ siRNA or GHSR1a point mutants that lack constitutive activity failed to inhibit dopamine-induced $[Ca^{2+}]_i$ release. The lack of cross-talk between the two receptors supports the hypothesis that GHSR1a and DRD2 co-expression results in non-canonical DRD2 signal transduction that is dependent on allosteric interactions between GHSR1a and DRD2 protomers. These results were recapitulated in DRD2 expressing SH-SY5Y neuroblastoma cells engineered to stably express GHSR1a, and in primary cultures of hypothalamic neurons (15), confirming that non-canonical DRD2 signaling is dependent upon interactions between GHSR1a and DRD2.

## DRD2 AGONIST SUPPRESSION OF FEEDING BEHAVIOR IS DEPENDENT ON GHSR1a AND FORMATION OF GHSR1a:DRD2 HETEROMERS IN HYPOTHALAMIC NEURONS

The change in canonical to non-canonical DRD2 signal transduction when GHSR1a is co-expressed with DRD2 implicates allosteric interactions between GHSR1a and DRD2 as a result of GHSR1a:DRD2 heteromer formation. It is well documented, based mainly on *in vitro* experiments, that GPCRs form homomers and heteromers, and that in heteromers one protomer in the complex allosterically modifies signaling of the other (47, 48). To test if modification of signal transduction is a consequence of a physical interaction between GHSR1a and DRD2, time resolved fluorescence resonance energy transfer (Tr-FRET) experiments were conducted in HEK293 cells (49, 50). The high sensitivity of Tr-FRET where SNAP- or CLIP-tags are introduced at the N-terminus of GHSR1a and DRD2 allowed monitoring

of GHSR1a:GHSR1a and GHSR1a:DRD2 heteromer formation at physiological concentrations on the surface of living cells (15).

When SNAP-GHSR1a is expressed in the presence of untagged DRD2 at a 1:1 ratio the Tr-FRET signal generated by GHSR1a homomers was reduced by ~50%, consistent with formation of GHSR1a:DRD2 heteromers. As further confirmation of direct interactions and heteromer formation, Tr-FRET assays were performed using SNAP-GHSR1a and CLIP-DRD2 in a receptor titration assay. High Tr-FRET signals were detected over a wide range of receptor expression, confirming GHSR1a:DRD2 heteromerization. To test if formation of heteromers was functionally relevant, Tr-FRET results were compared to the magnitude of dopamine-induced $[Ca^{2+}]_i$ mobilization at various ratios of GHSR1a to DRD2. Dopamine-induced $[Ca^{2+}]_i$ release correlated with the concentration of GHSR1a:DRD2 heteromers. Indeed, in support of an allosteric mechanism, the GHSR1a neutral antagonist, JMV2959, inhibited dopamine-induced $[Ca^{2+}]_i$ mobilization without disrupting heteromer formation; similarly, a DRD2 antagonist attenuated ghrelin signaling (15). Collectively, these data show that in cell lines modification of canonical DRD2 signaling is dependent on formation of GHSR1a:DRD2 heteromers (15).

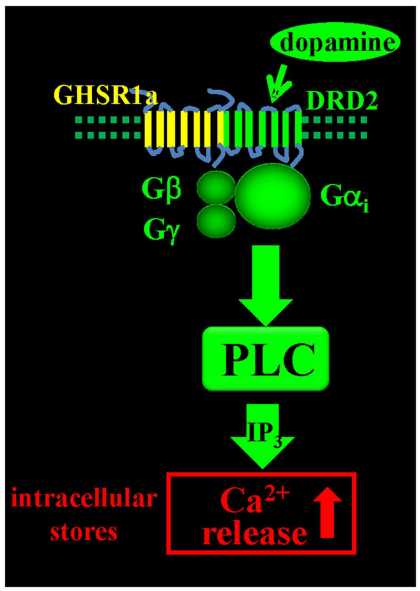


**FIGURE 1 | Dopamine-induced $[Ca^{2+}]_i$ mobilization via GHSR1a:DRD2 heteromers**. Dopamine activation of GHSR1a:DRD2 causes release of $Ca^{2+}$ from intracellular stores by Gβγ dependent PLC activation and mobilization of $IP_3$.

To test if GHSR1a:DRD2 heteromer formation has physiological relevance, we sought evidence for the presence of the heteromers in native brain tissue. We applied confocal FRET microscopy on mouse hypothalamic neurons using a fluorescently labeled ghrelin analog to visualize GHSR1a, and a fluorescently tagged DRD2 monoclonal antibody to localize DRD2. In hypothalamic neurons from *Ghsr*$^{+/+}$ mice, confocal FRET microscopy analysis shows GHSR1a and DRD2 in close proximity within 5 nm, consistent with heteromer formation (**Figure 2**). In striatal neurons of *Ghsr*$^{+/+}$ mice, the confocal FRET signal is very weak indicating the absence of heteromers in the striatum. In hypothalamic neurons of *Ghsr*$^{-/-}$ mice, a confocal FRET signal is not detected confirming the specificity of imaging GHSR1a:DRD2 heteromers in *Ghsr* + + mice (15).

The physiological importance of GHSR1a:DRD2 interactions was tested by monitoring food intake of fasted *Ghsr*$^{+/+}$ and *Ghsr*$^{-/-}$ mice following treatment with the selective DRD2 agonist cabergoline. In *Ghsr*$^{+/+}$ mice, food intake was markedly inhibited by cabergoline compared to vehicle treated animals, but *Ghsr*$^{-/-}$ mice were refractory to cabergoline-induced anorexia; hence, the anorexigenic activity of DRD2 is dependent upon GHSR1a. To test if endogenous ghrelin played a role, food intake was compared in *Ghrelin*$^{+/+}$ and *Ghrelin*$^{-/-}$ mice treated with cabergoline. Cabergoline inhibited food intake in both genotypes,

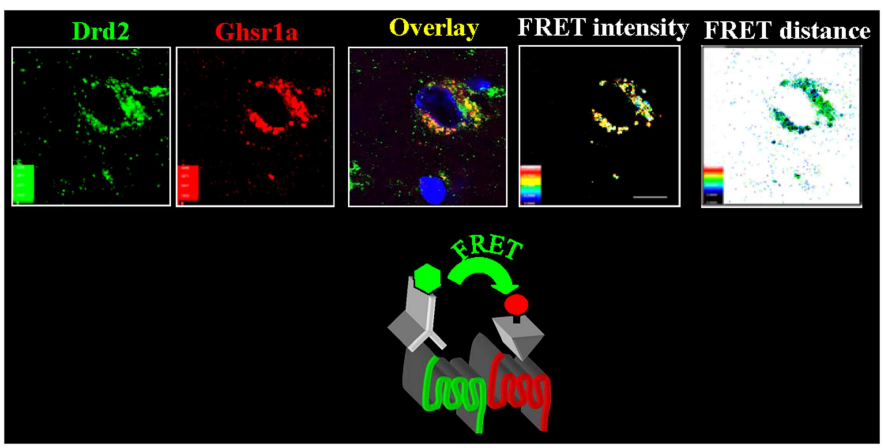


**FIGURE 2 | In hypothalamic neurons GHSR1a and DRD2 form heteromers**. Hypothalamic brain slices from *Ghsr*$^{+/+}$ mice were used to identify GHSR1a:DRD2 heteromers. Confocal FRET microscopy show that GHSR1a [identified using red fluorescent ghrelin (Cy5)] and DRD2 [in green, identified by immunofluorescent DRD2 monoclonal antibody (Cy3)] are in close proximity at a distance of 5–6 nm and FRET intensity 0.4–0.6 (15).

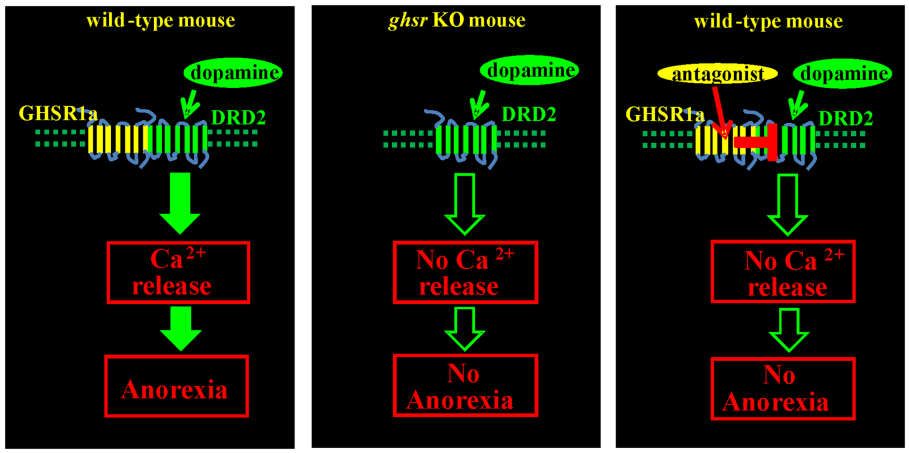


**FIGURE 3 | Anorexigenic effect of a DRD2 agonist is mediated by GHSR1a:DRD2 heteromers**. Treatment of fasted *Ghsr*$^{+/+}$ mice with the DRD2 agonist cabergoline inhibits food intake. *Ghsr*$^{-/-}$ mice, and *Ghsr*$^{+/+}$ mice treated with the ghrelin receptor neutral antagonist, JMV2959, are refractory to the anorexigenic effects of cabergoline; hence, inhibition of food intake by cabergoline is dependent upon allosteric interactions between GHSR1a and DRD2.

showing that the anorexigenic activity of the DRD2 agonist is dependent on GHSR1a but not on ghrelin. Having shown in cells co-expressing GHSR1a and DRD2 that the GHSR1a neutral antagonist, JMV2959, inhibits dopamine-induced $[Ca^{2+}]_i$ mobilization, mice were pretreated with JMV2959 before cabergoline injection. JMV2959 inhibited the anorexigenic effect of cabergoline in *Ghsr*$^{+/+}$ mice, but not in *Ghsr*$^{-/-}$ mice; thus the antagonistic action of JMV2959 on DRD2 activity is dependent on GHSR1a. These data illustrate that allosteric interactions between GHSR1a and DRD2 as a consequence of heteromer formation are physiologically relevant mediators of feeding behavior (**Figure 3**) (15).

Our results support the concept that heteromerization of GPCRs is an important mechanism for regulating GPCR function. Many GPCRs function as oligomeric complexes, where receptors physically interact to form homo- or heteromers. These protein–protein interactions stabilize specific conformations of GPCRs to activate specific down-stream effectors and signaling pathways (51–54). For example, ghrelin inhibition of insulin release from pancreatic beta-cells is mediated by GHSR1a coupling to $G\alpha_i$ rather than canonical coupling through $G\alpha_q$ (55); this modification in G-protein coupling is a consequence of formation of heteromers formed between GHSR1a and somatostatin receptor-5 (56). Heterodimers formed between GHSR1a and melanocortin-3 receptor has also been reported, and a combination of *in situ* hypridization and immunohistochemistry indicated co-localization of the receptors in hypothalamic neurons (57).

Examples of dimerization of GPCRs *in vitro* are well documented, but the physiological relevance is repeatedly questioned because of the paucity of data illustrating dimerization in native tissues. However, we show by applying FRET confocal microscopy the presence of GHSR1a:DRD2 heteromers in native hypothalamic neurons and further that inhibition of food intake by a DRD2 agonist is dependent on GHSR1a. Formation of the heteromeric complex results in non-canonical signaling dependent on $G\beta\gamma$ activation of PLC and mobilization of $[Ca^{2+}]_i$. This occurs in the absence of ghrelin, showing apo-GHSR1a is capable of allosterically modifying DRD2 signaling. The allosteric interaction within the GHSR1a:DRD2 heteromer is altered by the presence of a neutral GHSR1a antagonist (15); hence, the conformation of one protomer influences the signaling properties of the other. These findings demonstrate an important role for apo-GHSR1a in the brain and resolve the paradox that GHSR1a is expressed in brain areas not accessible to peripherally produced ghrelin, and where there is no evidence of ghrelin production.

## IMPLICATIONS OF GHSR1:DRD2 HETEROMERS IN OBSESSIVE EATING ASSOCIATED WITH PRADER–WILLI SYNDROME

Prader–Willi syndrome is a genetic disorder occurring in approximately 1 in 10,000 births and is associated with parent of origin imprinting. In normal subjects, maternal 15q11.2 is imprinted, but the paternal chromosome has a working copy of 15q11.2. Patients with PWS lack a working copy because of paternal deletion (70% of cases), maternal uniparental disomy (mUPD, 25% of cases), or imprinting errors in the imprinting center (IC, 5% of cases) (58). The deletion or lack of expression of 15q11.2 on the paternal chromosome results in a complex multi-systemic disorder. The phenotype includes short stature, low muscle tone, uncontrollable appetite, incomplete sexual development, and impaired cognition. The behavioral characteristics fall into the spectrum of autism spectrum disorders. In narrowing down the DNA region responsible for PWS, it was found that individuals with paternal deletion of the *SNORD116* gene cluster had all the major characteristic features of PWS (59). This locus encompasses a long non-coding RNA transcript that is processed into multiple small nuclear RNAs, as well as spliced exons of host gene *116G* (60). The expression of *Snord116* in mice becomes progressively more prominent in the arcuate nucleus, PVN, and ventromedial nucleus of the hypothalamus as the mice mature (61); these particular areas express GHSR1a and are involved in regulation of appetite mediated by DRD2.

While GH replacement is frequently used to treat impaired growth and hypotonia associated with PWS, the most distinguishing feature, for which there is no medical therapy, is the involuntary and uncontrollable chronic feeling of hunger, combined with a slow metabolism that leads to excessive eating and life-threatening obesity (62–64). The major theory proposed for explaining the insatiable appetite and excessive weight gain associated with PWS is elevated circulating levels of the orexigenic hormone ghrelin in the blood (65–67). Despite this, the direct approach of treating PWS patients with a GHSR1a antagonist, to our knowledge, has not been tested; this is likely because early clinical studies where GHSR1a antagonists were tested as antiobesity agents in normal subjects proved disappointing. The age-dependent (7 months to 5 years) transition to hyperphagia in PWS does not correlate with a change in ghrelin levels (68). However, as PWS children progress into adulthood hyperphagia becomes more severe; notably, the postprandial decrease in ghrelin observed in normal subjects is absent (69). Indeed, this is a most important distinction between normal and PWS adults. The postprandial nadir in endogenous ghrelin observed in normal subjects provides a mechanism for GHSR1a re-sensitization, and a satiety signal to tell the brain – stop eating! Indeed, the overall phenotype of PWS can be interpreted as impaired GHSR1a re-sensitization and as consequence low levels of active GHSR1a.

Prader–Willi syndrome patients present with high ghrelin and low GH levels; however, the anterior pituitary gland appears structurally normal and produces GH, suggesting impaired hypothalamic-pituitary signaling. Since GHSR1a agonists, including ghrelin, stimulate GH release, a possible explanation, other than GHSR1a desensitization, is that *GHSR* expression is attenuated. Expression of *GHSR* is positively regulated by thyroid hormone and estradiol, and negatively by cortisol (70). Indeed, hypothyroidism, hypogonadism, and elevated cortisol are all associated with PWS (71). Although not assessed quantitatively, GHSR1a expression appears normal in brains from deceased PWS patients (72). Nevertheless, compared to normal subjects, PWS patients elicit attenuated GH release in response to a GHSR1a agonist (73). High plasma concentrations of ghrelin should result in hyperglycemia and insulin resistance, but these metabolic changes are not consistently observed in PWS (73, 74). Lowering ghrelin levels by administering somatostatin or octreotide had no effect on appetite, weight gain, or behavior in PWS subjects (75–77). However, this does not preclude a role for ghrelin, because these compounds also suppress secretion of gastrointestinal anorexigenic hormones (76).

A recent report on the *Snord116*$^{\pm}$ mouse model of PWS showed that different inhibitors of GHSR1a failed to inhibit food intake (78) and that the involvement of some new pathway is linked to changes in feeding and psychiatric behavior (79). The authors concluded that ghrelin signaling is not involved in PWS and perhaps the elevated plasma ghrelin concentration is playing a compensatory role in PWS subjects. These findings support our suggestion that elevated endogenous ghrelin results in GHSR1a desensitization and lower levels of active GHSR1a. Low GHSR1a levels on the plasma membrane of hypothalamic neurons would predictably cause hyperphagia because as described above the appetite suppressing effect of DRD2 agonists is dependent on molecular interactions with apo-GHSR1a and DRD2 (15). Indeed, *ghsr*$^{-/-}$ mice are completely resistant to the anorexigenic effect of the DRD2 agonist cabergoline. Down-stream, cabergoline increases expression and signaling of brain-derived neurotrophic factor (BDNF). BDNF is one of the most important suppressors of food intake, and BDNF is low in PWS (80, 81).

Endogenous dopamine signaling via DRD2 inhibits excessive food intake and is dependent upon apo-GHSR1a and formation of GHSR1a:DRD2 heteromers. Formation of heteromers results in non-canonical DRD2 signal transduction and suppression of food intake. We suggest that persistently elevated circulating ghrelin in PWS reduces accumulation of GHSR1a on the plasma membrane of hypothalamic neurons. Low concentrations of GHSR1a contribute to hyperphagia by lowering the concentration of GHSR1a:DRD2, thereby attenuating satiety signals regulated by dopamine. The feasibility of targeting heterodimers formed between GHSR1a and DRD2 to modify non-canonical dopamine signaling was illustrated by us previously (15). This finding provides an approach to suppress or augment dopamine signaling selectively in neurons that express GHSR1a:DRD2 through an allosteric mechanism. Indeed, we have identified GHSR1a antagonists belonging to distinct structural classes, quinazolinones and triazoles that respectively enhance and inhibit DRD2 signaling through GHSR1a:DRD2 heteromers. Since these heteromers are found in a hypothalamic regions involved in development of PWS, GHSR1a antagonists that enhance DRD2 signaling are potential candidates for therapeutic intervention to suppress appetite. Targeting dopamine signaling in GHSR1a:DRD2 expressing neurons with GHSR1a antagonists provides the opportunity to design drugs that selectively target neurons co-expressing GHSR1a and DRD2 without affecting neurons expressing DRD2 alone.

## SUMMARY

In subsets of hypothalamic neurons that co-express GHSR1a and DRD2, GHSR1a:DRD2 heteromers are formed. Formation of these heteromers results in allosteric modification of the conformation of DRD2, thereby causing non-canonical signal transduction in response to dopamine. In contrast to canonical DRD2 signal transduction that involves suppression of cAMP accumulation, the non-canonical pathway results in $G\beta\gamma_{\gamma}$ subunit-dependent activation of PLC and mobilization of $[Ca^{2+}]_i$. The physiological relevance of GHSR1a:DRD2 heteromers is illustrated by experiments in *Ghsr*$^{+/+}$, *Ghsr*$^{-/-}$, and *Ghrelin*$^{-/-}$ mice. DRD2 agonism produces anorexia in *Ghsr*$^{+/+}$ and *Ghrelin*$^{-/-}$ mice, but not in *Ghsr*$^{-/-}$ mice. Hence, the anorexigenic effects of DRD2 agonists are dependent on GHSR1a, but not ghrelin. Further, *Ghsr*$^{+/+}$ mice treated with a neutral GHSR1a antagonist (the triazole, JMV2959) are resistant to DRD2 agonist-induced anorexia. Hence, pharmacological intervention with a GHSR1a antagonist according to structure, triazole vs. quinazolinone will respectively allosterically block or enhance dopamine signaling in neurons expressing GHSR1a:DRD2 heteromers without affecting signaling in neurons expressing DRD2 alone. These results show the potential of developing drugs that selectively act on subsets of neurons that express GHSR1a:DRD2 heteromers for treating obsessive eating disorders such as in PWS and for psychiatric disorders associated with irregularities in dopamine signaling.

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## REFERENCES

1. Dixit VD, Yang H, Sun Y, Weeraratna AT, Youm YH, Smith RG, et al. Ghrelin promotes thymopoiesis during aging. *J Clin Invest* (2007) **117**:2778–90. doi:10.1172/JCI30248
2. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* (1999) **402**:656–60. doi:10.1038/45230
3. Sun Y, Wang P, Zheng H, Smith RG. Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. *Proc Natl Acad Sci U S A* (2004) **101**:4679–84. doi:10.1073/pnas.0305930101
4. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, et al. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* (2001) **86**:5992. doi:10.1210/jcem.86.12.8111
5. Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, et al. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* (1996) **273**:974–7. doi:10.1126/science.273.5277.974
6. Smith RG. Development of growth hormone secretagogues. *Endocr Rev* (2005) **26**:346–60. doi:10.1210/er.2004-0019
7. Smith RG, Van der Ploeg LH, Howard AD, Feighner SD, Cheng K, Hickey GJ, et al. Peptidomimetic regulation of growth hormone secretion. *Endocr Rev* (1997) **18**:621–45. doi:10.1210/edrv.18.5.0316
8. Sun Y, Ahmed S, Smith RG. Deletion of ghrelin impairs neither growth nor appetite. *Mol Cell Biol* (2003) **23**:7973–81. doi:10.1128/MCB.23.22.7973-7981.2003
9. Sun Y, Asnicar M, Saha PK, Chan L, Smith RG. Ablation of ghrelin improves the diabetic but not obese phenotype of ob/ob mice. *Cell Metab* (2006) **3**:379–86. doi:10.1016/j.cmet.2006.04.004
10. Sun Y, Butte NF, Garcia JM, Smith RG. Characterization of adult ghrelin and ghrelin receptor knockout mice under positive and negative energy balance. *Endocrinology* (2008) **149**:843–50. doi:10.1210/en.2007-0271
11. McFarlane MR, Brown MS, Goldstein JL, Zhao TJ. Induced ablation of ghrelin cells in adult mice does not decrease food intake, body weight, or response to high-fat diet. *Cell Metab* (2014) **20**:54–60. doi:10.1016/j.cmet.2014.04.007
12. Zhao TJ, Liang G, Li RL, Xie X, Sleeman MW, Murphy AJ, et al. Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. *Proc Natl Acad Sci U S A* (2010) **107**:7467–72. doi:10.1073/pnas.1002271107
13. Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, et al. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res Mol Brain Res* (1997) **48**:23–9. doi:10.1016/S0169-328X(97)00071-5
14. Jiang H, Betancourt L, Smith RG. Ghrelin amplifies dopamine signaling by crosstalk involving formation of GHS-R/D1R heterodimers. *Mol Endocrinol* (2006) **20**:1772–85. doi:10.1210/me.2005-0084
15. Kern A, Albarran-Zeckler R, Walsh HE, Smith RG. Apo-ghrelin receptor forms heteromers with DRD2 in hypothalamic neurons and is essential for anorexigenic effects of DRD2 agonism. *Neuron* (2012) **73**:317–32. doi:10.1016/j.neuron.2011.10.038
16. Fetissov SO, Meguid MM, Sato T, Zhang LH. Expression of dopaminergic receptors in the hypothalamus of lean and obese Zucker rats and food intake. *Am J Physiol Regul Integr Comp Physiol* (2002) **283**:R905–10.
17. Johnson PM, Kenny PJ. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat Neurosci* (2010) **13**:635–41. doi:10.1038/nn.2519
18. Palmiter RD. Is dopamine a physiologically relevant mediator of feeding behavior? *Trends Neurosci* (2007) **30**:375–81. doi:10.1016/j.tins.2007.06.004
19. Pijl H. Reduced dopaminergic tone in hypothalamic neural circuits: expression of a "thrifty" genotype underlying the metabolic syndrome? *Eur J Pharmacol* (2003) **480**:125–31. doi:10.1016/j.ejphar.2003.08.100
20. Volkow ND, Wang G-J, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. *Trends Cogn Sci* (2011) **15**:37–46. doi:10.1016/j.tics.2010.11.001
21. Andrews ZB. The extra-hypothalamic actions of ghrelin on neuronal function. *Trends Neurosci* (2011) **34**:31–40. doi:10.1016/j.tins.2010.10.001
22. Atcha Z, Chen WS, Ong AB, Wong FK, Neo A, Browne ER, et al. Cognitive enhancing effects of ghrelin receptor agonists. *Psychopharmacology (Berl)* (2009) **206**:415–27. doi:10.1007/s00213-009-1620-6
23. Carlini VP, Varas MM, Cragnolini AB, Schiöth HB, Scimonelli TN, de Barioglio SR. Differential role of the hippocampus, amygdala, and dorsal raphe nucleus in regulating feeding, memory, and anxiety-like behavioral responses to ghrelin. *Biochem Biophys Res Commun* (2004) **313**:635–41. doi:10.1016/j.bbrc.2003.11.150
24. Chen L, Xing T, Wang M, Miao Y, Tang M, Chen J, et al. Local infusion of ghrelin enhanced hippocampal synaptic plasticity and spatial memory through activation of phosphoinositide 3-kinase in the dentate gyrus of adult rats. *Eur J Neurosci* (2011) **33**:266–75. doi:10.1111/j.1460-9568.2010.07491.x
25. Davis JF, Choi DL, Clegg DJ, Benoit SC. Signaling through the ghrelin receptor modulates hippocampal function and meal anticipation in mice. *Physiol Behav* (2011) **103**:39–43. doi:10.1016/j.physbeh.2010.10.017
26. Diano S, Farr SA, Benoit SC, McNay EC, da Silva I, Horvath B, et al. Ghrelin controls hippocampal spine synapse density and memory performance. *Nat Neurosci* (2006) **9**:381–8. doi:10.1038/nn1656
27. Jerlhag E, Egecioglu E, Landgren S, Salomé N, Heilig M, Moechars D, et al. Requirement of central ghrelin signaling for alcohol reward. *Proc Natl Acad Sci U S A* (2009) **106**:11318–23. doi:10.1073/pnas.0812809106
28. Albarran-Zeckler RG, Brantley AF, Smith RG. Growth hormone secretagogue receptor (GHS-R1a) knockout mice exhibit improved spatial memory and deficits in contextual memory. *Behav Brain Res* (2012) **232**:13–9. doi:10.1016/j.bbr.2012.03.012
29. Jacoby SM, Currie PJ. SKF 83566 attenuates the effects of ghrelin on performance in the object location memory task. *Neurosci Lett* (2011) **504**:316–20. doi:10.1016/j.neulet.2011.09.056
30. Jerlhag E, Egecioglu E, Dickson SL, Engel JA. Ghrelin receptor antagonism attenuates cocaine- and amphetamine-induced locomotor stimulation, accumbal dopamine release, and conditioned place preference. *Psychopharmacology (Berl)* (2010) **211**:415–22. doi:10.1007/s00213-010-1907-7
31. Wellman PJ, Davis KW, Nation JR. Augmentation of cocaine hyperactivity in rats by systemic ghrelin. *Regul Pept* (2005) **125**:151–4. doi:10.1016/j.regpep.2004.08.013
32. Schaeffer M, Langlet F, Lafont C, Molino F, Hodson DJ, Roux T, et al. Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying neurons. *Proc Natl Acad Sci U S A* (2013) **110**:1512–7. doi:10.1073/pnas.1212137110
33. Lawrence CB, Snape AC, Baudoin FM, Luckman SM. Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. *Endocrinology* (2002) **143**:155–62. doi:10.1210/endo.143.1.8561
34. Pirnik Z, Bundziková J, Holubová M, Týchová M, Fehrentz JA, Martinez J, et al. Ghrelin agonists impact on Fos protein expression in brain areas related to food intake regulation in male C57BL/6 mice. *Neurochem Int* (2011) **59**:889–95. doi:10.1016/j.neuint.2011.08.001
35. Watabe-Uchida M, Zhu L, Ogawa SK, Vamanrao A, Uchida N. Whole-brain mapping of direct inputs to midbrain dopamine neurons. *Neuron* (2012) **74**:858–73. doi:10.1016/j.neuron.2012.03.017
36. Cone JJ, McCutcheon JE, Roitman MF. Ghrelin acts as an interface between physiological state and phasic dopamine signaling. *J Neurosci* (2014) **34**:4905–13. doi:10.1523/JNEUROSCI.4404-13.2014
37. Gasbarri A, Sulli A, Packard MG. The dopaminergic mesencephalic projections to the hippocampal formation in the rat. *Prog Neuropsychopharmacol Biol Psychiatry* (1997) **21**:1–22. doi:10.1016/S0278-5846(96)00157-1
38. Gasbarri A, Verney C, Innocenzi R, Campana E, Pacitti C. Mesolimbic dopaminergic neurons innervating the hippocampal formation in the rat: a combined retrograde tracing and immunohistochemical study. *Brain Res* (1994) **668**:71–9. doi:10.1016/0006-8993(94)90512-6
39. Swanson LW. The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res Bull* (1982) **9**:321–53. doi:10.1016/0361-9230(82)90145-9
40. Lisman JE, Grace AA. The hippocampal-VTA loop: controlling the entry of information into long-term memory. *Neuron* (2005) **46**:703–13. doi:10.1016/j.neuron.2005.05.002

41. Rossato JI, Bevilaqua LR, Izquierdo I, Medina JH, Cammarota M. Dopamine controls persistence of long-term memory storage. *Science* (2009) **325**:1017–20. doi:10.1126/science.1172545
42. Banks WA, Tschop M, Robinson SM, Heiman ML. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. *J Pharmacol Exp Ther* (2002) **302**:822–7. doi:10.1124/jpet.102.034827
43. Furness JB, Hunne B, Matsuda N, Yin L, Russo D, Kato I, et al. Investigation of the presence of ghrelin in the central nervous system of the rat and mouse. *Neuroscience* (2011) **193**:1–9. doi:10.1016/j.neuroscience.2011.07.063
44. Grouselle D, Chaillou E, Caraty A, Bluet-Pajot MT, Zizzari P, Tillet Y, et al. Pulsatile cerebrospinal fluid and plasma ghrelin in relation to growth hormone secretion and food intake in the sheep. *J Neuroendocrinol* (2008) **20**:1138–46. doi:10.1111/j.1365-2826.2008.01770.x
45. Sakata I, Nakano Y, Osborne-Lawrence S, Rovinsky SA, Lee CE, Perello M, et al. Characterization of a novel ghrelin cell reporter mouse. *Regul Pept* (2009) **155**:91–8. doi:10.1016/j.regpep.2009.04.001
46. Missale C, Nash S, Robinson S, Jaber M, Caron M. Dopamine receptors: from structure to function. *Physiol Rev* (1998) **78**:189–225.
47. Milligan G. G protein-coupled receptor hetero-dimerization: contribution to pharmacology and function. *Br J Pharmacol* (2009) **158**:5–14. doi:10.1111/j.1476-5381.2009.00169.x
48. Smith NJ, Milligan G. Allostery at G protein-coupled receptor homo- and heteromers: uncharted pharmacological landscapes. *Pharmacol Rev* (2010) **62**:701–25. doi:10.1124/pr.110.002667
49. Albizu L, Cottet M, Kralikova M, Stoev S, Seyer R, Brabet I, et al. Time-resolved FRET between GPCR ligands reveals oligomers in native tissues. *Nat Chem Biol* (2010) **6**:587–94. doi:10.1038/nchembio.396
50. Maurel D, Comps-Agrar L, Brock C, Rives ML, Bourrier E, Ayoub MA, et al. Cell-surface protein-protein interaction analysis with time-resolved FRET and snap-tag technologies: application to GPCR oligomerization. *Nat Methods* (2008) **5**:561–7. doi:10.1038/nmeth.1213
51. González S, Moreno-Delgado D, Moreno E, Pérez-Capote K, Franco R, Mallol J, et al. Circadian-related heteromerization of adrenergic and dopamine $D_4$ receptors modulates melatonin synthesis and release in the pineal gland. *PLoS Biol* (2012) **10**:e1001347. doi:10.1371/journal.pbio.1001347
52. Hasbi A, Fan T, Alijaniaram M, Nguyen T, Perreault ML, O'Dowd BF, et al. Calcium signaling cascade links dopamine D1-D2 receptor heteromer to striatal BDNF production and neuronal growth. *Proc Natl Acad Sci U S A* (2009) **106**:21377–82. doi:10.1073/pnas.0903676106
53. Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, et al. GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. *Nature* (1998) **396**:674–9. doi:10.1038/25460
54. Rashid AJ, So CH, Kong MM, Furtak T, El-Ghundi M, Cheng R, et al. D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. *Proc Natl Acad Sci U S A* (2007) **104**:654–9. doi:10.1073/pnas.0604049104
55. Dezaki K, Kakei M, Yada T. Ghrelin uses Galphai2 and activates voltage-dependent K+ channels to attenuate glucose-induced Ca2+ signaling and insulin release in islet beta-cells: novel signal transduction of ghrelin. *Diabetes* (2007) **56**:2319–27. doi:10.2337/db07-0345
56. Park S, Jiang H, Zhang H, Smith RG. Modification of ghrelin receptor signaling by somatostatin receptor-5 regulates insulin release. *Proc Natl Acad Sci* (2012) **109**:19003–8. doi:10.1073/pnas.1209590109
57. Rediger A, Piechowski CL, Yi CX, Tarnow P, Strotmann R, Gruters A, et al. Mutually opposite signal modulation by hypothalamic heterodimerization of ghrelin and melanocortin-3 receptors. *J Biol Chem* (2011) **286**:39623–31. doi:10.1074/jbc.M111.287607
58. Whittington JE, Butler JV, Holland AJ. Changing rates of genetic subtypes of Prader-Willi syndrome in the UK. *Eur J Hum Genet* (2007) **15**:127–30. doi:10.1038/sj.ejhg.5201716
59. Duker AL, Ballif BC, Bawle EV, Person RE, Mahadevan S, Alliman S, et al. Paternally inherited microdeletion at 15q11.2 confirms a significant role for the SNORD116 C/D box snoRNA cluster in Prader-Willi syndrome. *Eur J Hum Genet* (2010) **18**:1196–201. doi:10.1038/ejhg.2010.102
60. Powell WT, Coulson RL, Crary FK, Wong SS, Ach RA, Tsang P, et al. A Prader-Willi locus lncRNA cloud modulates diurnal genes and energy expenditure. *Hum Mol Genet* (2013) **22**:4318–28. doi:10.1093/hmg/ddt281
61. Zhang Q, Bouma GJ, McClellan K, Tobet S. Hypothalamic expression of snoRNA Snord116 is consistent with a link to the hyperphagia and obesity symptoms of Prader-Willi syndrome. *Int J Dev Neurosci* (2012) **30**:479–85. doi:10.1016/j.ijdevneu.2012.05.005
62. Driscoll DJ, Miller JL, Schwartz S, Cassidy SB. Prader-Willi Syndrome. In: Pagon RA, Adam MP, Ardinger HH, Bird TD, Dolan CR, Fong CT, et al. editors. *GeneReviews®* [Internet]. Seattle WA: University of Washington; 1993–2014 (1993). Available from http://www.ncbi.nlm.nih.gov/books/NBK1330/
63. Schulze AH, Petersen MB, Blichfeldt SS, Kastrup KW, Brondum-Nielsen K. [Prader-Willi syndrome – clinical picture and genetics]. *Ugeskr Laeger* (1995) **157**:1513–9.
64. Whittington J, Holland A. Neurobehavioral phenotype in Prader-Willi syndrome. *Am J Med Genet C Semin Med Genet* (2010) **154C**:438–47. doi:10.1002/ajmg.c.30283
65. Cummings DE, Clement K, Purnell JQ, Vaisse C, Foster KE, Frayo RS, et al. Elevated plasma ghrelin levels in Prader-Willi syndrome. *Nat Med* (2002) **8**:643–4. doi:10.1038/nm0702-643
66. Haqq AM, Farooqi IS, O'Rahilly S, Stadler DD, Rosenfeld RG, Pratt KL, et al. Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. *J Clin Endocrinol Metab* (2003) **88**:174–8. doi:10.1210/jc.2002-021052
67. Yi CX, Heppner K, Tschop MH. Ghrelin in eating disorders. *Mol Cell Endocrinol* (2011) **340**:29–34. doi:10.1016/j.mce.2011.03.001
68. Goldstone AP, Holland AJ, Butler JV, Whittington JE. Appetite hormones and the transition to hyperphagia in children with Prader-Willi syndrome. *Int J Obes (Lond)* (2012) **36**:1564–70. doi:10.1038/ijo.2011.274
69. DelParigi A, Tschop M, Heiman ML, Salbe AD, Vozarova B, Sell SM, et al. High circulating ghrelin: a potential cause for hyperphagia and obesity in Prader-Willi syndrome. *J Clin Endocrinol Metab* (2002) **87**:5461–4. doi:10.1210/jc.2002-020871
70. Petersenn S, Rasch AC, Penshorn M, Beil FU, Schulte HM. Genomic structure and transcriptional regulation of the human growth hormone secretagogue receptor. *Endocrinology* (2001) **142**:2649–59. doi:10.1210/en.142.6.2649
71. Vaiani E, Herzovich V, Chaler E, Chertkoff L, Rivarola MA, Torrado M, et al. Thyroid axis dysfunction in patients with Prader-Willi syndrome during the first 2 years of life. *Clin Endocrinol* (2010) **73**:546–50. doi:10.1111/j.1365-2265.2010.03840.x
72. Talebizadeh Z, Kibiryeva N, Bittel DC, Butler MG. Ghrelin, peptide YY and their receptors: gene expression in brain from subjects with and without Prader-Willi syndrome. *Int J Mol Med* (2005) **15**:707–11.
73. Grugni G, Guzzaloni G, Morabito F. Impairment of GH responsiveness to GH-releasing hexapeptide (GHRP-6) in Prader-Willi syndrome. *J Endocrinol Invest* (2001) **24**:340–8. doi:10.1007/BF03343871
74. Choe YH, Jin DK, Kim SE, Song SY, Paik KH, Park HY, et al. Hyperghrelinemia does not accelerate gastric emptying in Prader-Willi syndrome patients. *J Clin Endocrinol Metab* (2005) **90**:3367–70. doi:10.1210/jc.2004-1651
75. De Waele K, Ishkanian SL, Bogarin R, Miranda CA, Ghatei MA, Bloom SR, et al. Long-acting octreotide treatment causes a sustained decrease in ghrelin concentrations but does not affect weight, behaviour and appetite in subjects with Prader-Willi syndrome. *Eur J Endocrinol* (2008) **159**:381–8. doi:10.1530/EJE-08-0462
76. Haqq AM, Stadler DD, Rosenfeld RG, Pratt KL, Weigle DS, Frayo RS, et al. Circulating ghrelin levels are suppressed by meals and octreotide therapy in children with Prader-Willi syndrome. *J Clin Endocrinol Metab* (2003) **88**:3573–6. doi:10.1210/jc.2003-030205
77. Tan TM, Vanderpump M, Khoo B, Patterson M, Ghatei MA, Goldstone AP. Somatostatin infusion lowers plasma ghrelin without reducing appetite in adults with Prader-Willi syndrome. *J Clin Endocrinol Metab* (2004) **89**:4162–5. doi:10.1210/jc.2004-0835
78. Lin D, Wang Q, Ran H, Liu K, Wang Y, Wang J, et al. Abnormal response to the anorexic effect of GHS-R inhibitors and exenatide in male Snord116 deletion mouse model for Prader-Willi syndrome. *Endocrinology* (2014) **155**:2355–62. doi:10.1210/en.2013-2083
79. Tauber M, Diene G, Mimoun E, Cabal-Berthoumieu S, Mantoulan C, Molinas C, et al. Prader-Willi syndrome as a model of human hyperphagia. *Front Horm Res* (2014) **42**:93–106. doi:10.1159/000358317

80. Chiba S, Numakawa T, Ninomiya M, Yoon HS, Kunugi H. Cabergoline, a dopamine receptor agonist, has an antidepressant-like property and enhances brain-derived neurotrophic factor signaling. *Psychopharmacology (Berl)* (2010) **211**:291–301. doi:10.1007/s00213-010-1894-8
81. Han JC, Muehlbauer MJ, Cui HN, Newgard CB, Haqq AM. Lower brain-derived neurotrophic factor in patients with Prader-Willi syndrome compared to obese and lean control subjects. *J Clin Endocrinol Metab* (2010) **95**:3532–6. doi:10.1210/jc.2010-0127

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