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Ghrelin concentration as an indicator of eating-disorder risk in obese women

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1 **Abstract**

2 *Aim.* - Eating disorders (EDs), disordered eating (DE) and obesity are thought to have
3 overlapping aetiological processes. DE in obesity can jeopardize weight-loss results, and acyl
4 ghrelin (AG) is a hormone that stimulates food intake and reward processes. The main study
5 objective was to determine whether higher-than-expected concentrations of AG in common
6 obesity are associated with DE symptoms.

7 *Methods.* - The study population included 84 women, aged 20–55 years, free of established
8 EDs: 55 were severely obese (OB) and 29 were of normal weight (NW). OB participants
9 were stratified into two groups according to their median concentration of fasting AG
10 distribution. The OB women with a high fasting plasma ghrelin concentration (HGC) were
11 compared with both OB women with a low fasting plasma ghrelin concentration (LGC) and
12 NW women. Participants were assessed by the Eating Disorder Inventory (EDI)-2, Three-
13 Factor Eating Questionnaire (TFEQ) and Hospital Anxiety and Depression Scale (HADS).
14 Fasting glucose, insulin, leptin and ghrelin plasma concentrations were also quantified.

15 *Results.* - Between the two AG groups of OB women, there was no statistical difference in
16 either anthropometric or metabolic parameters, HADS, TFEQ or fasting hunger scores.
17 However, the HGC group scored significantly higher than the LGC group on the drive-for-
18 thinness subscale of EDI-2 (9.30 ± 0.99 vs 6.46 ± 0.83 , respectively; $P = 0.033$).

19 *Conclusion.* - Results support the hypothesis of a potential relationship between fasting
20 plasma AG concentrations and ED risk, regardless of mood and anxiety. AG may be
21 considered a potential biomarker of vulnerability for developing EDs.

22

23 **Keywords:** Drive for thinness; Eating disorders; Ghrelin; Impulsive behaviour; Obesity;
24 Vulnerability

25

1 **Introduction**

2 Obesity remains a major concern in patients with diabetes, and the management of obese
3 patients is also still difficult, especially when associated with eating disorders (EDs) or
4 disordered eating (DE). EDs have primarily been considered a psychiatric condition, whereas
5 obesity has been regarded predominantly as a medical concern. However, there is now a
6 widespread movement to consider EDs, DE and obesity as part of the same continuum of
7 weight-related disorders. The basic components necessary for an integrated theory of DE and
8 obesity include sociological and psychological parameters leading to the perception that 'I am
9 too fat' is a problem, maladaptive limitation of food intake (dietary restraint) to correct the
10 problem, hunger (from eating less food) and the physiological reaction to hunger (hormonal
11 control of food intake). To enhance preventative and interventional efforts, there is a strong
12 need for early detection of psychological factors contributing to the development and
13 maintenance of EDs and obesity.

14 Food intake is tightly controlled in humans by homeostatic and non-homeostatic
15 mechanisms that maintain stable body weight and neutral energy balance [1]. Ghrelin, a 28
16 amino-acid peptide synthesized by the stomach, is a leading player in this physiological
17 regulation [2]. The acylated form of ghrelin (AG) stimulates appetite and food intake [2] by
18 activating neuropeptide Y neurons in the arcuate nucleus of the hypothalamus and inhibiting
19 the melanocortin pathway [3]. However, the action of ghrelin in the brain is not restricted to
20 homeostatic control of energy balance within the hypothalamus. Indeed, AG also induces
21 reward-seeking behaviours for food, alcohol and other substance abuse [4–7]. Moreover, AG
22 modulates the dopaminergic and serotonergic pathways involved in the regulation of
23 impulsivity [8,9] and, when injected into the lateral hypothalamus of rodents, it increases
24 both motor and choice impulsivity [10].

1 As ghrelin impacts food intake, food reward and impulsivity, its plasma concentrations may
2 reflect certain behavioural traits. Overeating, increased food reward sensitivity and
3 impulsivity are characteristics shared by many patients with obesity and/or EDs [11] and,
4 contrary to expectations, obese participants usually exhibit lower plasma ghrelin
5 concentrations than their normal-weight counterparts [12–14]. These low plasma ghrelin
6 concentrations have been considered a physiological adaptation to the positive energy
7 balance associated with obesity [15], and the same adaptive phenomenon has been described
8 in some EDs, including binge-eating [16]. However, in clinical practice, a wide range of
9 plasma ghrelin concentrations is observed in obese individuals, with some exhibiting
10 unexpectedly high concentrations approaching those observed in normal-weight individuals.
11 Based on this clinical observation, our hypothesis is that patients with obesity and high
12 concentrations of ghrelin might display greater feelings of hunger, a higher prevalence of DE-
13 related psychopathology/behaviours and, especially, food impulsivity. Thus, the aim of the
14 present study was to determine whether obese patients with unexpectedly high concentrations
15 of ghrelin differ from those with low ghrelin concentrations in terms of hunger and
16 behavioural traits leading to a DE risk.

17

18 **Materials and Methods**

19 *Participants*

20 Women aged between 20 and 55 years and with either normal weight [body mass index
21 (BMI) 20–25 kg.m⁻²] or severe obesity (BMI ≥ 35 kg.m⁻²) were recruited prospectively
22 between July 2013 and June 2015 through printed advertisements on notice boards at various
23 sites of Lyon 1 University, including the cafeteria of the University Hospital and Department
24 of Endocrinology and Nutrition (Groupement Hospitalier Sud, Hospices Civils de Lyon,
25 Lyon). Exclusion criteria included being male, a smoker, psychotropic medication use and

1 substance abuse according to the *Diagnostic and Statistical Manual of Mental Disorders, 4th*
2 *Edition* (DSM-IV), anxiety and depression [Hospital Anxiety and Depression Scale (HADS)
3 subscale anxiety and/or depression scores > 11), medical illness, previous bariatric surgery,
4 diabetes and ongoing pregnancy. Patients with previous or current obvious EDs (defined by
5 DSM-5) as assessed by TFEQ and/or medical interview were also excluded. To avoid
6 metabolic and hormonal disturbances linked to physical activity and diet, participants had to
7 report stable weight and no change in physical activity over the past 3 months.
8 Initially, 108 women were included in the study. Five normal-weight participants were
9 excluded for uncontrolled eating scores > 8 (n = 4) or contraindicated medication use (n = 1),
10 while 13 obese participants were excluded for anxiety scores > 11 (n = 7), unstable body
11 weight (n = 4), unknown diabetes (n = 1) or recent cessation of tobacco use (n = 1). A flow
12 chart detailing participant recruitment is presented in [Fig. 1](#).

13

14 ***Study design***

15 Following their inclusion, participants had to arrive at the study nutrition research centre
16 (Centre de Recherche en Nutrition Humaine Rhône-Alpes, CRNH-RA) at 0700 h after an
17 overnight fast. Anthropometric parameters were assessed, and fasting blood samples drawn
18 for acyl ghrelin (AG) and des-acyl ghrelin (DAG), leptin, glucose and insulin quantification.
19 Participants were then asked to complete three self-administered psychometric
20 questionnaires: the validated French translation of the Eating Disorder Inventory (EDI)-2, the
21 Three-Factor Eating Questionnaire (TFEQ) and the Hospital Anxiety and Depression Scale
22 (HADS). Fasting hunger was evaluated at 0900 h using a 10-cm visual analogue scale (VAS),
23 which rated hunger strength from 0 (not hungry) to 10 (extremely hungry). More precisely,
24 participants had to answer the question ‘How hungry do you feel at this moment?’ by placing
25 a mark along the VAS.

1

2 ***Self-administered questionnaires***

3 The Eating Disorder Inventory (EDI)-2 is a 91-item multidimensional self-reported
4 questionnaire that assesses attitudes, feelings and behaviours typically associated with EDs.

5 Although 11 subscales were used in this study, there was a particular interest in the five
6 subscales that could be affected by appetite, feelings of satiety and food intakes: drive for
7 thinness; bulimia; body dissatisfaction; interoceptive awareness; and impulse regulation.

8 The Three-Factor Eating Questionnaire (TFEQ) is a food-intake-behaviour questionnaire [17]
9 comprising 51 items that measure three dimensions of human eating behaviour: cognitive
10 restraint (21 items); uncontrolled eating (16 items); and emotional eating (14 items). Each
11 item has a binary score (0 or 1) that is summed with the others, and each subscore
12 corresponds to the three dimensions of eating behaviour: the higher the score, the more
13 problematic the related behaviour. Uncontrolled eating scores > 8 were an exclusion criterion
14 for the normal-weight participants in this study.

15 The Hospital Anxiety and Depression Scale (HADS) is a questionnaire [18] of 14 items
16 commonly used to determine the probability of anxiety (seven items) and depression (seven
17 items). Each item has a score between 0 and 3: the higher the score, the higher the probability
18 of exhibiting anxiety or depression. The threshold of each subscore to detect the presence of
19 anxiety or depressive symptoms is 8/21.

20

21 ***Blood samples***

22 Venous blood was collected in EDTA tubes and centrifuged immediately for 10 min at 4° C
23 at 4500 rpm. Plasma glucose was determined by the hexokinase method using an Architect
24 ci8200 clinical chemistry analyzer (Abbott Laboratories, Abbott Park, IL, US). Insulin
25 concentrations were determined by immunoradiometric assay using Bi-Ins-IRMA kits (IBA

1 Molecular – Cis Biointernational, Gif-sur-Yvette, France). For AG determinations, 1 mM p-
2 hydroxymercuribenzoic acid in HCl was added to blood samples to prevent protease
3 degradation of AG (as recommended by Cayman Chemical, Ann Arbor, MI, USA). Plasma
4 samples were then immediately stored at $< -20^{\circ}$ C until needed for analysis. Plasma AG and
5 DAG concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using
6 SPI-Bio kits (Bertin Pharma, Montigny-le-Bretonneux, France), and leptin was also measured
7 by ELISA (Clinical Range, BioVendor, Brno, Czech Republic). Homoeostasis model
8 assessment of insulin resistance (HOMA-IR) was used as a surrogate marker of insulin
9 resistance and calculated as $\text{glucose (mmol.L}^{-1}) \times \text{insulin (mIU.L}^{-1})/22.5$.

10

11 *Stratification of obese participants according to fasting plasma AG concentrations*

12 Plasma AG values were available for 84/90 participants, and the six participants lacking AG
13 values were excluded from the analysis. The severely obese participants were stratified into
14 two groups according to the median AG value obtained in this sample.

15

16 *Ethics*

17 All participants gave their written informed consent to participate. The study promoter was
18 the Hospices Civils de Lyon (University Hospital), and the study was performed after a
19 favourable opinion from the Committee for the protection of persons concerned (CPP) in
20 accordance with French law (Data Protection and Civil Liberties) and the ethical standards
21 laid down by the 1964 Declaration of Helsinki. The ClinicalTrials.gov identifier is
22 NCT01948414.

23

24 *Statistical analysis*

1 Data were analyzed using SPSS version 19.0 software (IBM Corp., Armonk, NY, USA) and
2 expressed as the mean \pm SEM (standard error of mean). Normality of each variable was
3 checked using the Shapiro–Wilk test. Differences between normal-weight (NW) and obese
4 (OB) participants were assessed by a parametric (*t*) test or a non-parametric test (Mann–
5 Whitney U test, for non-normal distributions). Anthropometric, biological and psychometric
6 differences among the three groups (NW and two OB groups) were assessed using one-way
7 analysis of variance (ANOVA) or by Kruskal–Wallis test. Multiple comparisons were
8 performed using Tukey’s post-hoc test, while effect sizes were assessed by Cohen’s *d*. Linear
9 correlations between AG and drive-for-thinness (DT) scores were assessed by the Pearson
10 correlation coefficient. For all statistical analyses, the level of significance was the
11 conventional two-sided 5% alpha error.

12

13 **Results**

14 *Participants’ characteristics*

15 These are presented in [Table I](#). The population included 29 NW and 55 severely OB (BMI \geq
16 35 kg.m⁻²) women. The severely OB women were stratified into two groups according to the
17 median AG value observed in this sample (19.0 pg.mL⁻¹; 95% CI: 12.0–44.0; Fig. 1): 28
18 participants were allocated to the low ghrelin concentration (LGC) group (mean AG value:
19 7.51 \pm 1.11 pg.mL⁻¹); and 27 were allocated to the high ghrelin concentration (HGC) group
20 (mean AG value: 65.77 \pm 8.67 pg.mL⁻¹).

21 The three groups did not differ in either age or height, although OB participants exhibited
22 higher systolic and diastolic blood pressures than did NW participants. Stratification of the
23 OB group according to fasting plasma AG concentration revealed no anthropometric
24 differences between the LGC and HGC groups.

25

1 ***Biological profiles***

2 These profiles are presented in Table I and, compared with those in the NW group, women in
3 the OB groups had higher fasting glucose and insulin values, and higher levels of insulin
4 resistance, whereas there were no significant differences in metabolic parameters between the
5 HGC and LGC subgroups. Leptin was higher, whereas fasting AG, DAG and total ghrelin
6 levels were lower in the OB vs NW groups, although leptin did not differ between the LGC
7 and HGC groups. In the OB groups, mean fasting plasma AG was $36.11 \pm 5.8 \text{ pg.mL}^{-1}$ and
8 the median was 19.0 pg.mL^{-1} . AG values in these OB groups were not normally distributed
9 and, as shown in Fig. 2, a bimodal distribution of ghrelin in this sample corresponded to the
10 HGC and LGC subgroups. Also in keeping with the study design, significant differences in
11 plasma ghrelin concentrations were observed between LGC, HGC and NW groups (ANOVA-
12 1; $P < 0.001$). The HGC subgroup had a fasting plasma AG concentration approaching that of
13 the NW group, but nevertheless remained significantly lower than that of the NW group
14 (Table I).

15

16 ***Hunger scores***

17 Fasting hunger scores were similar between OB and NW groups, although a trend was
18 observed towards higher hunger scores in the HGC vs LGC groups (4.51 ± 0.46 vs $3.38 \pm$
19 0.43 , respectively; $P = 0.08$) with a medium effect size ($d = 0.49$).

20

21 ***Self-administered questionnaire scores***

22 The EDI-2, TFEQ and HADS scores are presented in Table II. Psychological states assessed
23 by HADS showed similar anxiety scores in both the NW and OB groups, but a significantly
24 greater depression score in the OB groups ($P = 0.008$). However, no difference in anxiety and
25 depression scores was found between the HGC and LGC subgroups.

1 As for the TFEQ, both OB groups scored significantly higher in uncontrolled and emotional
2 eating ($P < 0.001$; Fig. 3A, Table II) than did the NW group, although there was no
3 significant difference in cognitive restraint. Also, no significant difference was found in the
4 three subscales of the TFEQ between the LGC and HGC subgroups (Fig. 3B), although a
5 small effect size was observed for cognitive restraint ($d = 0.19$; Table II). Behavioural
6 profiles frequently associated with EDs were assessed by EDI-2 (Table II), and almost all
7 subscales, especially the five of particular interest (Fig. 3C), differed between the OB and
8 NW groups, except for the ‘maturity fears’ subscale, albeit not significantly ($P > 0.05$; Table
9 II). The LGC and HGC subgroups differed significantly only for the DT subscale (Table II,
10 Fig. 3D): OB participants in the HGC group had a higher DT score than the LGC group (9.30
11 ± 0.99 vs 6.46 ± 0.83 , respectively; $P < 0.05$). A medium effect size was observed between
12 groups for DT ($d = 0.63$), whereas there were small effect sizes for body dissatisfaction ($d =$
13 0.39) and interpersonal distrust ($d = 0.36$), although both were higher in the HGC group. In
14 addition, a positive and significant linear correlation was observed between AG values and
15 DT scores in the entire OB population ($\rho = 0.297$, $P = 0.03$).

16

17 Discussion

18 The present study confirms our assumption, based on clinical experience, that fasting AG
19 values are wide-ranging in OB participants, with some of them having unexpectedly high
20 concentrations of ghrelin approaching those observed in their NW counterparts. This
21 observation might be a reflection of the variability in central ghrelin resistance associated
22 with obesity, a feature that has also been demonstrated in animal models of obesity [19,20].
23 However, high fasting concentrations of AG in OB participants were indicative of higher DT
24 subscale scores in the EDI-2 and a trend towards higher hunger scores.

1 The concept of DT includes aspiring to a thin body ideal, thereby placing excessive
2 importance on thinness while experiencing extreme fear of becoming fat. It is considered a
3 key motivational variable underlying dieting/weight-loss efforts [21,22]. While DT may
4 reflect a healthy motivation to lose weight in obesity, other studies of OB binge-eaters, using
5 the EDI-2, have found that DT is also a strong predictor of ED severity [23,24].

6 In the present study, all EDI-2 scores remained under the cut-off values usually considered
7 indicative of the presence of an ED (DT > 14, body dissatisfaction > 16, bulimia > 8) [25–
8 27]. However, it has been shown that DT scores are more relevant to binge-eating disorder
9 (BED) than to obesity [28]. Yet, little data are available in the scientific literature on the links
10 between DT and obesity other than BED. Nevertheless, it has been reported in previous
11 research that a higher DT is associated with bigger body size [29,30] and with more
12 intentional weight-loss behaviours [31]. Obesity is associated with body dissatisfaction,
13 leading some patients to have a high desire for thinness which can, in turn, trigger intentional
14 weight-loss behaviours with repetitive food restrictions that can induce EDs such as BED
15 [32]. Given this possibility, it is not surprising to find an association between DT and BED.
16 Indeed, in a 2016 study by Mustelin et al. [33], it was proposed that the DT and other EDI-2
17 subscales could be useful in combination for the diagnosis of BED, in particular, the drive for
18 thinness, bulimia and body dissatisfaction subscales. The fact that our study focused on
19 patients without EDs and our final sample size might explain why those dimensions did not
20 differ significantly between our HGC and LGC subgroups.

21 In the available literature thus far, only a few studies have focused on ghrelin concentrations
22 and the DT subscale. In women who exercise and in women with anorexia, high DT scores
23 have been associated with significantly higher ghrelin concentrations in comparison to
24 normal DT groups, independently of BMI [22,23]. Those authors considered that high ghrelin
25 concentrations were adaptations for chronic energy deficiencies.

1 Although ghrelin has been a topic of DE research for > 10 years, its causal involvement has
2 still not been resolved. It remains unclear whether ghrelin plays a functionally important role
3 in the aetiology of DE and EDs. Common polymorphisms in human ghrelin and ghrelin
4 receptor genes are unlikely to represent major contributors to the pathogenesis of obesity
5 [34]. In one large case–control study, three common single nucleotide polymorphisms (SNPs)
6 in the human ghrelin gene were studied in 692 Caucasian cases with EDs (366 with anorexia
7 nervosa and 326 with bulimia nervosa) and found no specific associations [35]. Concerning
8 binge-eating, a positive correlation between fasting concentrations of plasma ghrelin and
9 frequency of binge/vomiting episodes in bulimic women was reported, but not confirmed in
10 later studies [36–38]. In addition, the majority of studies reported lower plasma ghrelin
11 concentrations in OB participants with BED, which was contrary to the expected higher
12 concentrations [39].

13 Ghrelin is also involved in the regulation of impulsivity [8,9,40]. Injecting ghrelin into the
14 lateral ventricle of rodents increased both motor and choice impulsivity [40]. However, in the
15 present study, impulsivity in the HGC subgroup, as assessed by the impulse regulation
16 subscale of EDI-2 and the uncontrolled and emotional eating subscales of TFEQ, did not
17 differ from that in the LGC group. An obesity-induced central ghrelin resistance, a
18 phenomenon that has yet to be demonstrated in humans, might partly explain the absence of a
19 strong association between plasma ghrelin concentrations and eating behaviours, especially
20 food impulsivity, in the present study [20].

21 One limitation of our study is that the findings refer to a single morning measurement of
22 plasma AG. As circulating concentrations of this orexigenic peptide fluctuate throughout the
23 day [41], the pathophysiological relevance of just this one measurement could be questioned.
24 In addition, it is possible that the measurement of ghrelin in peripheral blood is not an
25 accurate reflection of its intracerebral concentrations or activity. Moreover, the use of self-

1 administered questionnaires may have introduced a disorder underestimation bias through
2 either the participants' underreporting or lack of sensitivity. The use of neurocognitive tests
3 may have been more efficient in detecting subthreshold impairments in eating or impulsivity,
4 but such tests are difficult to access in routine care. Finally, the unknown menstrual status of
5 our participants is a potential limitation when interpreting our data.

6

7 **Conclusion**

8 The present results support the hypothesis of a potentially positive relationship between
9 fasting plasma AG concentrations and the risk of developing EDs, regardless of mood and
10 anxiety, in OB women. While no inferences can be made as regards the direction of causality,
11 it can be concluded that OB woman with high fasting plasma AG may be prone to display the
12 problematic traits commonly associated with EDs. Thus, it is possible to postulate that fasting
13 plasma ghrelin might be a biomarker of vulnerability for developing DE and, potentially, EDs
14 in obesity. This vulnerability should be assessed and managed to prevent the weight cycling
15 wherein the excessive importance of eating, body shape and weight leads to strict dieting that,
16 in turn, results in binge-eating and weight regain.

17

18

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1

2 **Conflict of Interest (COI) Statement:** None to declare

3

4 **Authors' contributions:** ED, SLP and PC designed the research; BJ, BS, EB and SLP
5 conducted the research; BJ, ED and KS analyzed the data or performed the statistical
6 analyses; SI, BJ and ED wrote the paper; BS, SLP and ML performed a critical revision of
7 the manuscript for important intellectual content. ED is primarily responsible for its final
8 content; all authors read and approved the final version of the manuscript.

9

10

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Figure legends

Fig. 1. Flow chart of participant recruitment into the study.

Fig. 2. Fasting plasma ghrelin distribution in obese women stratified into two groups according to the median acyl ghrelin value (19.0 pg.mL^{-1} , indicated by the vertical line in the boxplot). LGL/HGL: low/high ghrelin levels.

Fig. 3. Kiviat diagrams of the results of self-administered questionnaires: (A, B) results of the Three-Factor Eating Questionnaire (TFEQ) and (C, D) results for the five subscales of interest in the Eating Disorder Inventory (EDI)-2 in obese and normal-weight (control) groups and in obese groups with low (LGL) and high (HGL) ghrelin levels. * $P < 0.05$.

Fig. 1.

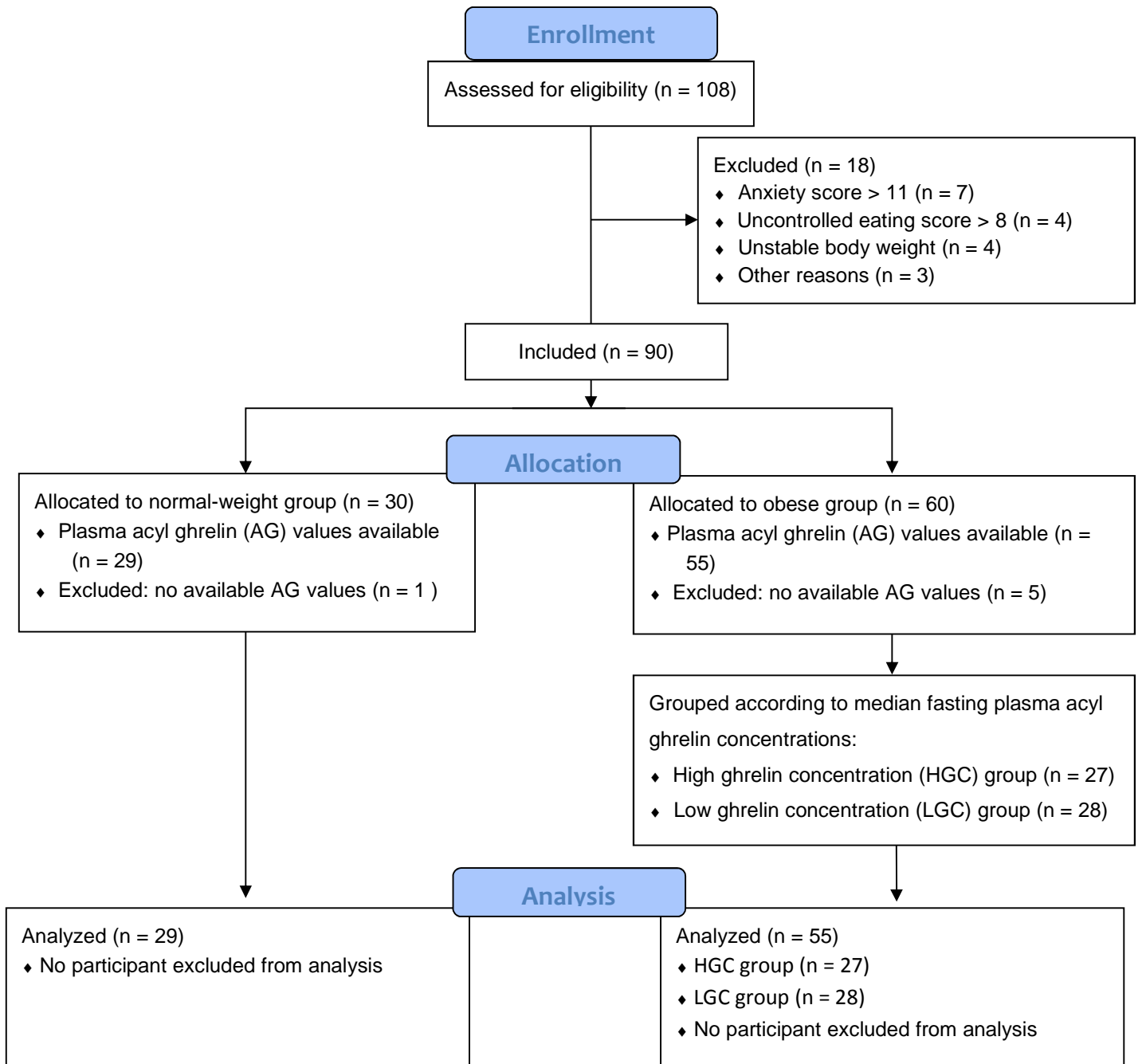


Figure 3: Kiviati diagrams representing the results of self-administered questionnaires.

Panels A and B present the results of TFEQ and Panels C and D present the results of the 5 subscales of interest of EDI-2 in obese and control groups (Panels A and C) and in the low ghrelin concentration (LGL) and high ghrelin concentration HGL groups (Panels B and D). * $P < 0.05$.

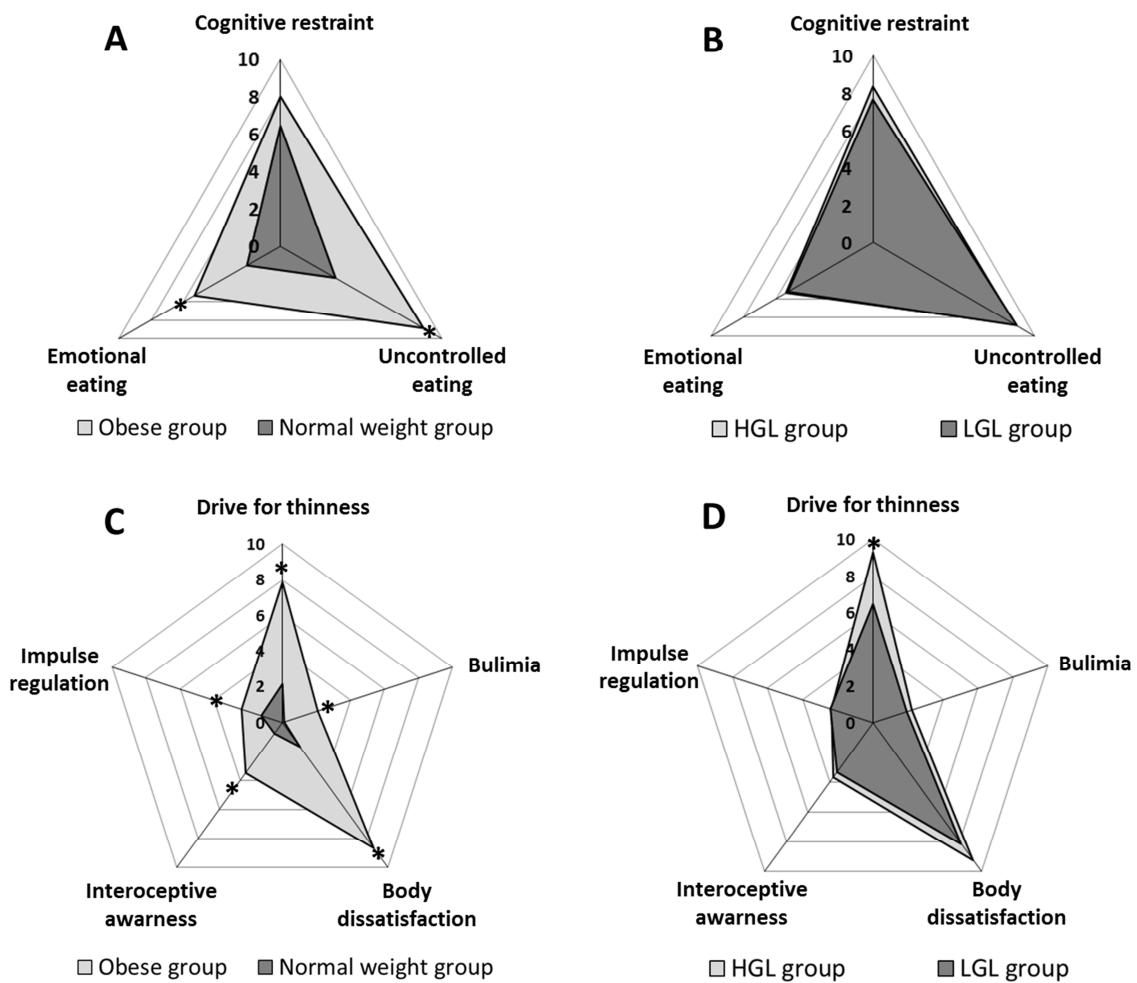


Figure 2: Fasting plasma ghrelin distribution among the obese group. Obese participants were stratified into two groups according to the median acyl ghrelin value (vertical line within the boxplot; median value = 19.0 pg.mL^{-1}). LGL=low ghrelin concentration group; HGL= high ghrelin concentration group.

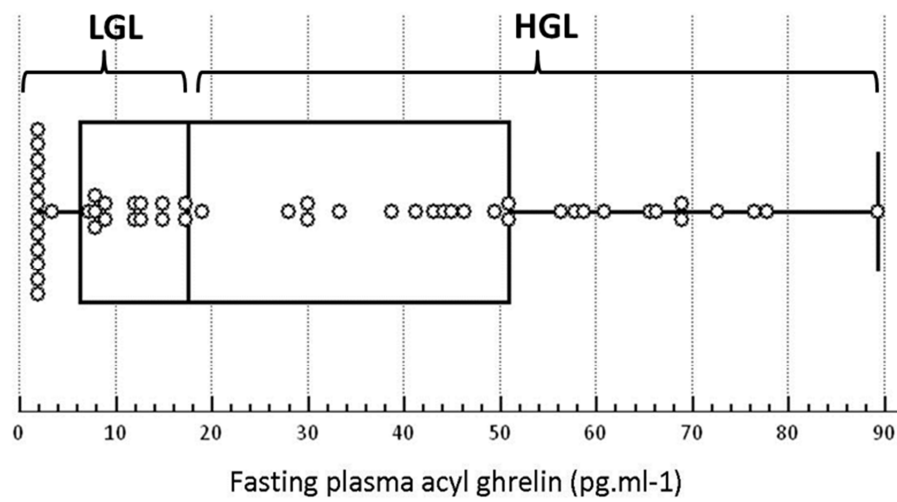


Table I

Clinical and biological characteristics and fasting hunger scores in the study population

	Normal-weight (NW) women	Obese (OB) women	OB vs NW	OB women grouped by fasting plasma acyl ghrelin concentration		LGC vs HGC
	(n = 29)	(n = 55)		(n = 28)	(n = 27)	
Clinical characteristics	Mean ± SEM	Mean ± SEM	<i>P</i>	Mean ± SEM	Mean ± SEM	<i>P</i>
Age (years)	37 ± 2.0	38 ± 1.5	NS	37 ± 2.2	38 ± 2.1	NS
Height (cm)	163.9 ± 1.0	163.8 ± 0.8	NS	162.9 ± 1.2	164.7 ± 1.1	NS
Weight (kg)	57.8 ± 1.1	111.5 ± 2.5	< 0.001	113.4 ± 4.0*	109.5 ± 3.1*	NS
Body mass index (kg.m ⁻²)	21.5 ± 0.4	41.5 ± 0.8	< 0.001	42.6 ± 1.3*	40.3 ± 0.9*	NS
Waist circumference (cm)	78 ± 1.2	119 ± 2.1	< 0.001	122 ± 2.5*	116 ± 3.5*	NS
Hip circumference (cm)	94 ± 0.8	129 ± 1.9	< 0.001	129 ± 2.8*	128 ± 2.5*	NS
Waist-to-hip ratio	0.83 ± 0.01	0.91 ± 0.01	< 0.001	0.94 ± 0.02*	0.89 ± 0.02*	NS
SBP (mmHg)	110 ± 1.7	124 ± 1.5	< 0.001	126 ± 2.2*	123 ± 2.1*	NS
DBP (mmHg)	71 ± 1.2	75 ± 1.2	0.042	76 ± 1.9	74 ± 1.6	NS
Heart rate (bpm)	65 ± 1.9	70 ± 1.6	0.046	74 ± 2.0*	66 ± 2.4	NS
Biological characteristics						
Glucose (mmol.L ⁻¹)	4.8 ± 0.1	5.1 ± 0.1	0.002	5.1 ± 0.1*	5.1 ± 0.1*	NS
Insulin (mIU.L ⁻¹)	4.5 ± 0.4	9.7 ± 0.7	< 0.001	10.8 ± 1.0*	8.6 ± 0.8*	NS
HOMA-IR	0.95 ± 0.1	2.21 ± 0.2	< 0.001	2.45 ± 0.25*	1.96 ± 0.18*	NS
Leptin (ng.mL ⁻¹)	12.5 ± 1.3	57.7 ± 2.9	< 0.001	61.2 ± 4.3*	54 ± 3.8*	NS
Acyl ghrelin (pg.mL ⁻¹)	107 ± 12	36 ± 6	< 0.001	8 ± 1*	66 ± 9*	< 0.05
Des-acyl ghrelin (pg.mL ⁻¹)	365 ± 32	145 ± 15	< 0.001	101 ± 8*	190 ± 27*	< 0.05
Total ghrelin (pg.mL ⁻¹)	471 ± 40	181 ± 20	< 0.001	109 ± 8*	256 ± 33*	< 0.05
Acyl ghrelin/total ghrelin	0.23 ± 1.7	0.18 ± 1.8	0.049	0.083 ± 1.5*	0.27 ± 2.0	< 0.05
Visual analogue scale						
Hunger scale	3.86 ± 0.45	3.93 ± 0.32	NS	3.38 ± 0.43	4.51 ± 0.46	NS

* *P* < 0.05 vs NW group;

NS: not significant; LGC/HGC: low/high ghrelin concentration; SBP/DBP: systolic/diastolic blood pressure; HOMA-IR: homoeostasis model assessment of insulin resistance

Table II

Eating Disorder Inventory (EDI)-2, Three-Factor Eating Questionnaire (TFEQ) and Hospital Anxiety and Depression Scale (HADS) scores on psychometric evaluation of the study population

	Normal-weight (NW) women	Obese (OB) women	OB vs CT	OB women grouped by fasting plasma acyl ghrelin concentration		LGC vs HGC	LGC vs HGC
	(n = 29)	(n = 55)		LGC (n = 28)	HGC (n = 27)		
EDI-2	Mean ± SEM	Mean ± SEM	<i>P</i>	Mean ± SEM	Mean ± SEM	Cohen's d	<i>P</i>
Drive for thinness	2.14 ± 0.46	7.85 ± 0.67	< 0.001	6.46 ± 0.83*	9.30 ± 0.99*	0.63	0.032
Bulimia	0.10 ± 0.08	2.07 ± 0.40	< 0.001	1.93 ± 0.61*	2.22 ± 0.53*	0.09	0.72
Body dissatisfaction	4.21 ± 0.83	21.67 ± 0.86	< 0.001	20.32 ± 1.32*	23.07 ± 1.06*	0.39	0.11
Ineffectiveness	1.83 ± 0.69	3.13 ± 0.52	0.011	3.39 ± 0.78*	2.85 ± 0.69*	0.13	0.61
Perfectionism	1.31 ± 0.38	5.29 ± 0.57	< 0.001	5.71 ± 0.91*	4.85 ± 0.70*	0.18	0.45
Interpersonal distrust	1.76 ± 0.42	3.51 ± 0.46	0.019	3.00 ± 0.54	4.04 ± 0.75*	0.36	0.26
Interoceptive awareness	0.76 ± 0.24	3.49 ± 0.58	0.001	3.32 ± 0.88*	3.67 ± 0.76*	0.07	0.77
Maturity fears	4.93 ± 0.38	5.20 ± 0.31	0.59	5.25 ± 0.45	5.15 ± 0.44	0.04	0.87
Asceticism	2.83 ± 0.39	5.22 ± 0.45	< 0.001	5.46 ± 0.64*	4.96 ± 0.64*	0.15	0.58
Impulse regulation	1.24 ± 0.54	2.40 ± 0.49	0.031	2.43 ± 0.61	2.37 ± 0.79	0.02	0.95
Social insecurity	1.79 ± 0.53	3.49 ± 0.44	0.002	3.64 ± 0.60*	3.33 ± 0.65*	0.10	0.72
TFEQ							
Cognitive restraint	6.45 ± 0.75	8.02 ± 0.43	0.055	7.68 ± 0.67	8.37 ± 0.55	0.19	0.43
Uncontrolled eating	3.41 ± 0.39	8.84 ± 0.44	< 0.001	8.89 ± 0.65*	8.78 ± 0.62*	0.03	0.90
Emotional eating	2.07 ± 0.32	5.31 ± 0.47	< 0.001	5.25 ± 0.68*	5.37 ± 0.66*	0.03	0.89
HADS							
Anxiety score	5.72 ± 0.38	6.65 ± 0.37	0.11	6.75 ± 0.47	6.56 ± 0.57	0.08	0.79
Depression score	2.52 ± 0.30	4.07 ± 0.38	0.008	4.61 ± 0.55*	3.52 ± 0.50	0.37	0.15

* $P < 0.05$ vs NW group;

LGC/HGC: low/high ghrelin concentration