CLINICAL STUDY

Association of acylated ghrelin profiles with chronic inflammatory markers in overweight and obese postmenopausal women: a MONET study

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Abstract

Objective: Recent reports have suggested that the existence of associations between hormonal dysregulation and chronic upregulation of inflammatory markers, which may cause obesity-related disturbances. Thus, we examined whether acylated ghrelin (AcylG) and total ghrelin (TotG) levels could be associated with the following inflammatory markers: C-reactive protein (CRP), tumor necrosis factor α (TNF- α), and soluble TNF receptor 1 (sTNF-R1).

Design: Cross-sectional study consisting of 50 overweight and obese postmenopausal women.

Methods: AcylG and TotG levels were assessed at 0, 60, 160, 170, and 180 min of the euglycemic/hyperinsulinemic clamp (EHC). We evaluated insulin sensitivity, body composition, and blood lipid profiles as well as fasting concentrations of CRP, TNF- α , and sTNF-R1.

Results: In fasting conditions, sTNF-R1 was negatively correlated with AcylG ($r = -0.48$, $P < 0.001$) levels. In addition, AcylG/TotG was associated negatively with sTNF-R1 ($r = -0.44$, $P = 0.002$) and positively with TNF- α ($r = 0.38$, $P = 0.009$) values. During the EHC, TotG (at all time points) and AcylG (at 60 and 160 min) values were significantly decreased from fasting concentrations. AcylG maximal reduction and area under the curve (AUC) values were correlated to sTNF-R1 ($r = -0.35$, $P = 0.02$ and $r = -0.34$, $P = 0.02$, respectively). Meanwhile, the AcylG/TotG AUC ratio was associated negatively with sTNF-R1 ($r = -0.29$, $P < 0.05$) and positively with TNF- α ($r = 0.36$, $P = 0.02$). Following adjustments for total adiposity, sTNF-R1 remained correlated with fasting and maximal reduction AcylG values. Similarly, AcylG/TotG ratios remained significantly correlated with sTNF-R1 and TNF- α . Importantly, 23% of the variation in sTNF-R1 was independently predicted by fasting AcylG.

Conclusion: These results are the first to suggest that both fasting and EHC-induced AcylG profiles are correlated with fasting values of sTNF-R1, a component of the TNF- α system. Thus, AcylG may act, at least in part, as one mediator of chronic inflammatory activity in human obesity.

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Introduction

Ghrelin, a gut-derived peptide (1), has been shown to be associated with insulin sensitivity (2–6). Total ghrelin (TotG) includes acylated (~10% of TotG) and non-acylated (~90% of TotG) ghrelin (AcylG and NAcylG respectively), two distinct forms that were previously identified in circulation (1, 7). The physiology of AcylG and NAcylG suggests that the existence of intimate relationships with insulin secretion/sensitivity. For

instance, under euglycemic/hyperinsulinemic clamp (EHC) conditions, a model of hyperinsulinemia, TotG levels are reduced to a greater extent in normal individuals when compared with insulin resistant or diabetic subjects (8–10). Moreover, postprandial reduction of AcylG is positively associated with insulin sensitivity in children with Prader–Willi syndrome (11). Furthermore, at pharmacological concentrations, AcylG increases the degree of insulin resistance, whereas co-administration of AcylG and NAcylG

improves insulin sensitivity (12, 13). Thus, AcylG and NAcylG forms may induce different physiological and/or metabolic effects.

There is growing evidence suggesting that the development of obesity-related disturbances may be closely associated with gut-derived hormonal dysregulation and chronic inflammation (14–17). There is presently conflicting information regarding the association between TotG and markers of chronic inflammation. For instance, both *in vivo* and *in vitro* studies have provided evidence that ghrelin can exert anti-inflammatory effects by reducing pro-inflammatory cytokine expression (18, 19). In addition, TotG concentrations are negatively correlated with tumor necrosis factor α (TNF- α) and C-reactive protein (CRP) levels in patients with chronic obstructive pulmonary diseases (20). On the other hand, TotG levels are positively associated with TNF- α in subjects with chronic heart failure (21). Although indications suggest that possible interactions with the TNF- α system discrepancies still remain regarding metabolic outcomes of the relation between ghrelin and TNF- α . Moreover, recent evidence have suggested that the soluble TNF receptor 1 (sTNF-R1) is a better indicator of metabolic dysfunctions than TNF- α itself (16). Furthermore, the relationship between ghrelin and chronic inflammatory markers has not been evaluated in an exhaustively phenotyped population of sedentary overweight and obese postmenopausal women with high risks to develop metabolic disturbances. Therefore, the purpose of the present study was to investigate whether fasting levels of chronic inflammatory markers, such as CRP, TNF- α , and sTNF-R1 are associated with a complete profile of AcylG, TotG, and AcylG/TotG values characterized before and during an EHC in overweight and obese postmenopausal women.

Subjects and methods

Subjects

The study population consisted of 53 non-diabetic overweight and obese postmenopausal women aged between 48 and 73 years old. Women were included in the study if they met the following criteria: 1) body mass index $> 27 \text{ kg/m}^2$, 2) follicle-stimulating hormone levels $\geq 30 \text{ U/l}$, 3) sedentary ($< 2 \text{ h}$ a week of structured exercise), 4) non-smokers, 5) low to moderate alcohol consumption (< 2 drinks/day), 6) absence of any known inflammatory disease, and 7) no use of hormone replacement therapy within the last 3 months. On physical examination or biological testing, no participant had a history or evidence of: 1) cardiovascular diseases, peripheral vascular diseases, or stroke, 2) diabetes evaluated by a 2 h 75 g oral glucose tolerance test, 3) orthopedic limitations, 4) uncontrolled thyroid or pituitary diseases, 5) infection (medical questionnaire

examination and complete blood count), or 6) medication that could affect cardiovascular function and/or metabolism. The study was approved by the University of Montreal ethics committee.

Sequence of tests

After reading and signing the consent form each participant was invited to the Metabolic Unit for a series of tests. After a 4-week period of weight stabilization, patients underwent a 3 h EHC. Body composition was determined by dual energy X-ray absorptiometry (DXA) and body fat distribution by the computed tomography (CT) technique.

Euglycemic/hyperinsulinemic clamp (EHC)

The test began at 0730 h after a 12-h overnight fast following the procedure described by DeFronzo *et al.* (22). A catheter was introduced in the antecubital vein for the infusion of 20% dextrose and insulin (Actrapid, Novo-Nordisk, Toronto, Canada). Another catheter was inserted into the other arm for blood sampling. Three fasting samples of plasma glucose and insulin were collected over 40 min. Then, insulin was infused at the rate of 75 mU/m^2 per min for 180 min. Plasma glucose was measured every 10 min with a glucose analyzer (Beckman Instruments, Fullerton, CA, USA) and maintained at fasting level with a variable infusion rate of 20% dextrose. Glucose disposal (insulin sensitivity) was calculated as the mean rate of glucose infusion measured during the last 30 min of the clamp steady state and was expressed as mg/min per kg of fat-free mass.

Blood samples

After an overnight fast (12 h) blood samples were collected at times 0, 60, 160, 170, and 180 min during the EHC. Fasting serum samples of triglyceride, high density lipoprotein (HDL-cholesterol), low density lipoprotein (LDL-cholesterol), insulin, glucose, CRP, TNF- α , and sTNF-R1 concentrations were analyzed at 0 min. Moreover, AcylG and TotG levels were measured at 0, 60, 160, 170, and 180 min during the EHC. Blood samples were centrifuged at 3900 g for 10 min at 4°C and kept at -80°C until further analyses were achieved. For AcylG samples, plasma was treated with $50 \text{ }\mu\text{l/ml}$ HCL (1 M) and $10 \text{ }\mu\text{l/ml}$ phenylmethylsulfonyl fluoride (57.4 mM) (Sigma-Aldrich) to prevent peptidic degradation and/or loss of the acyl group on Ser³. Plasma immunoreactive AcylG and TotG levels were measured in duplicate with a commercial RIA procedure using ^{125}I -labeled bioactive human AcylG and TotG as tracers and a rabbit polyclonal antibody raised against full-length peptides (Linco Research,

St-Charles, MO, USA). As indicated by the manufacturer, inter- and intra-assay percent coefficients of variation were respectively under 18 and 10%. Blood glucose and the lipid profile were analyzed on the day of collection while insulin samples were kept at -80°C until they were analyzed. Analyses were done on the COBAS INTEGRA 400 (Roche Diagnostic) analyzer for total cholesterol, HDL-cholesterol, and triglycerides. Total cholesterol, HDL-cholesterol, and triglycerides were used in the Friedewald formula (23) to calculate LDL-cholesterol concentration. Insulin levels were determined by RIA (Linco Research). Serum levels of CRP were assessed by immunonephelometry on IMMAGE analyzer (Beckman-Coulter, Villepinte, France), the inter- and intra-assay variations were below 5%. Circulating TNF- α and sTNF-R1 levels were measured by ELISA (R&D Systems, Minneapolis, MN, USA), the inter- and intra-assay variations were respectively below 9 and 5%. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated according to the formula of Matthews *et al.* (24).

Body composition

Body weight was measured using an electronic scale (Balances Industrielles, Montreal, Canada) and standing height was measured using a wall stadiometer (Perspective Enterprises, Portage, MI, USA). Lean body mass and total adiposity were measured by DXA (General Electric Lunar Corporation version 6.10.019, Madison, WI, USA). Percent coefficient of variation was 0.8% for lean body mass and 1.1% for total adiposity.

Computed tomography (CT)

A GE High Speed Advantage CT scanner (General Electric Medical Systems, Milwaukee, WI, USA) was used to evaluate visceral and subcutaneous fat areas at the L4–L5 using a scout image of the body. Subjects were examined in the supine position with both arms stretched above their head. Visceral adipose tissue area was quantified by delineating the intra-abdominal cavity at the most internal aspect of the abdominal and oblique muscle walls surrounding the cavity and the posterior aspect of the vertebral body. Test–retest measures of the different body fat distribution compartments on ten CT scans yielded a mean absolute difference of 1% in obese postmenopausal women (25).

Statistical analysis

The data are expressed as mean $\pm$ s.d. A repeated measures ANOVA was used to detect a significant effect of time within the EHC (0 vs 60, 160, 170, and 180 min). If a significant time interaction was observed, a Bonferroni test was used to identify the differences

between fasting and other values throughout the clamp. Insulin-induced AcylG and TotG maximal reductions were evaluated for each subject using the fasting value (0 min) minus the lowest value during the hyperinsulinemic state. Area under the curve values (AUC) were assessed to provide an overall index of hormonal profiles throughout the EHC and were calculated by the trapezoidal method. The relationships of AcylG or TotG with CRP, TNF- α , or sTNF-R1 were evaluated by Pearson's correlations. Partial correlation analyses were evaluated in order to correct for the effect of total adiposity. Statistical analyses were performed with SPSS for Windows version 11.5 (SPSS Inc., Chicago, IL, USA). Significance was accepted at $P < 0.05$.

Results

Fifty-three overweight and obese postmenopausal women were enrolled in this study. These women displayed a broad range of body weights, body composition, blood lipid, insulin resistance, and inflammatory profiles as presented in Tables 1 and 2. One month before testing, weight stability (within ± 1 kg) was verified by monitoring body weight for each subject on a weekly basis. Due to this weight stabilization period, two subjects displayed a BMI slightly under 27 kg/m^2 on testing day and were therefore excluded from statistical analyses. The average BMI of this population was $32.8 \pm 4.2\text{ kg/m}^2$ with 16 overweight (average: $28.6 \pm 0.9\text{ kg/m}^2$) and 32 obese (average: $34.9 \pm 3.6\text{ kg/m}^2$) individuals. Three subjects displayed supra-physiological levels of either CRP or sTNF-R1 and were not taken into consideration for statistical analyses.

CRP and sTNF-R1 levels were positively associated with total adiposity ($r = 0.41$, $P = 0.004$ and $r = 0.44$, $P = 0.002$ respectively), subcutaneous adipose tissue ($r = 0.42$, $P = 0.003$ and $r = 0.43$, $P = 0.003$ respectively), and visceral adiposity ($r = 0.54$, $P < 0.001$ and $r = 0.39$, $P = 0.006$ respectively). On the other hand, AcylG profiles were significantly correlated with total adiposity ($r = -0.35$, $P = 0.02$) but not with subcutaneous

Table 1 Physical characteristics of 48 overweight and obese postmenopausal women.

Physical characteristics	Mean $\pm$ s.d.	Range
Age (years)	58.0 ± 5.8	48.8–72.9
Height (m)	1.60 ± 0.06	1.50–1.73
Body mass (kg)	84.1 ± 13.8	62.6–123.6
BMI (kg/m^2)	32.8 ± 4.2	27.0–42.8
Total adiposity (kg)	39.14 ± 8.8	25.2–61.2
Lean body mass (kg)	42.57 ± 6.9	32.6–61.3
Percent body fat (%)	46.3 ± 4.7	37.4–56.9
Subcutaneous adipose tissue (cm^2)	480 ± 125	289–776
Visceral adipose tissue (cm^2)	191 ± 61.8	93.7–345.6

Values are the mean $\pm$ s.d. BMI, body mass index; total adiposity and fat-free mass were measured by dual energy X-ray absorptiometry.

Table 2 Metabolic and inflammatory profile characteristics of 48 overweight and obese postmenopausal women.

Variable	Mean $\pm$ s.d.	Range
Total cholesterol (mmol/l)	5.72 $\pm$ 0.82	4.01–7.45
Triglycerides (mmol/l)	1.77 $\pm$ 0.76	0.7–4.5
HDL-cholesterol (mmol/l)	1.51 $\pm$ 0.32	0.99–2.36
LDL-cholesterol (mmol/l)	3.40 $\pm$ 0.75	1.43–5.09
Fasting glucose (mmol/l)	4.99 $\pm$ 0.44	4.00–5.83
Fasting insulin (μ U/ml)	15.22 $\pm$ 7.11	4.87–44.48
HOMA-IR	3.40 $\pm$ 1.68	1.00–9.50
Insulin sensitivity (mg/min per kg FFM)	11.76 $\pm$ 3.55	4.76–19.04
Fasting CRP (mg/l)	3.98 $\pm$ 3.43 ($n=47$)	0.40–14.60
Fasting TNF- α (pg/ml)	0.68 $\pm$ 0.63 ($n=46$)	0.40–14.60
Fasting sTNF-R1 (pg/ml)	995 $\pm$ 209 ($n=47$)	612–1607

Values are mean $\pm$ s.d. HDL-cholesterol, high density lipoprotein; LDL-cholesterol, low density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; FFM, fat free mass; Mm value, insulin sensitivity at steady state; CRP, high sensitivity C-reactive protein; TNF- α , high sensitivity tumor necrosis factor α ; sTNF-R1, soluble tumor necrosis factor receptor 1.

adiposity ($r = -0.26$; not significant) nor visceral adiposity (AcylG: $r = -0.22$; not significant). Therefore, the different associations were adjusted for the effect of total adiposity. Meanwhile, sTNF-R1 concentrations were also positively associated with body weight, fasting insulinemia, and HOMA-IR ($r = 0.39$, $P = 0.007$; $r = 0.44$, $P = 0.002$ and $r = 0.44$, $P = 0.002$ respectively) but negatively associated with total and LDL-cholesterol ($r = -0.31$, $P = 0.04$ and $r = -0.41$, $P = 0.005$ respectively). However, no significant interaction was observed with TNF- α .

The relationships between ghrelin (acylated and total) and the inflammatory markers are presented in Table 3. In fasting conditions, sTNF-R1 was negatively linked with both fasting AcylG ($r = -0.48$, $P = 0.001$; see Fig. 1) and AcylG/TotG ($r = -0.44$, $P = 0.002$). Following adjustments for total adiposity, sTNF-R1 remained significantly associated with both fasting AcylG ($r = -0.38$, $P = 0.009$) and AcylG/TotG ($r = -0.32$; $P = 0.03$) values. TNF- α was positively correlated with AcylG/TotG ratio ($r = 0.38$, $P = 0.009$)

Table 3 Associations between ghrelin profiles and chronic inflammatory markers in overweight and obese postmenopausal women.

	Pearson's correlations (r)		
	CRP ($n=47$)	TNF- α ($n=46$)	sTNF-R1 ($n=47$)
Fasting TotG	-0.21	-0.07	-0.14
Fasting AcylG	-0.21	0.25	-0.48 [†]
Fasting AcylG/TotG	-0.06	0.38 [†]	-0.44 [†]
TotG AUC	-0.14	-0.15	-0.05
AcylG AUC	-0.14	0.21	-0.34*
AcylG/TotG AUC	-0.03	0.36*	-0.29*
TotG maximal reduction	-0.33*	0.05	-0.25
AcylG maximal reduction	-0.19	0.11	-0.35*

TotG, total ghrelin; AUC, area under the curve; AcylG, acylated ghrelin; AcylG/TotG, acylated ghrelin over total ghrelin ratio. * $P < 0.05$; [†] $P < 0.01$.

Figure 1 Correlation between fasting acylated ghrelin and sTNF-R1 levels. Open squares indicate overweight and obese postmenopausal women. The line of best fit is indicated. The partial r value remained significant after statistical control for total adiposity.

and the association was still significant after correcting for total adiposity ($r = 0.40$, $P = 0.007$). However, no significant relationship was observed between CRP and AcylG or AcylG/TotG.

The effect of a prolonged hyperinsulinemic state on ghrelin (acylated and total) circulating levels is described in Fig. 2. When compared with baseline, AcylG, and TotG concentrations were reduced throughout the EHC, while the AcylG/TotG ratio remained

Figure 2 Acylated and total ghrelin circulation profile throughout EHC in overweight and obese postmenopausal women. Values are means $\pm$ s.d. *Significantly different from fasting values ($P < 0.05$).

Table 4 Independent predictors of soluble tumor necrosis factor receptor 1 (sTNF-R1) in overweight and obese postmenopausal women.

Dependent variable	Step	Independent variables	Relationship	Partial r^2	Total r^2 cumulative	P value
sTNF-R1 ^a	1	Fasting AcylG	—	0.23	0.23	0.001
	2	LDL-cholesterol	—	0.15	0.38	0.002
	3	Fasting insulin	+	0.09	0.47	0.01

^aIndependent variables used in this model were: fasting AcylG, fasting LDL-cholesterol; fasting insulin; and total adiposity

constant in fasting (0.09 ± 0.04) and EHC-induced (0.09 ± 0.05) conditions.

Maximal reduction of TotG and AcylG levels was observed between 60 and 180 min of the EHC. Insulin-stimulated maximal reduction of TotG and AcylG values were negatively linked to CRP ($r = -0.33$, $P = 0.03$) and sTNF-R1 ($r = -0.35$, $P = 0.02$) respectively. After controlling for the effect of total adiposity, only insulin-stimulated maximal reduction of AcylG remained negatively linked to sTNF-R1 ($r = -0.31$, $P = 0.04$). We also observed that sTNF-R1 was associated with AUC values of AcylG ($r = -0.34$, $P = 0.02$) and the AcylG/Total ratio ($r = -0.34$, $P = 0.02$), but these associations did not remain significant after correcting for total adiposity ($P > 0.05$). Finally, TNF- α was correlated with AcylG/TotG AUC ($r = 0.36$, $P = 0.02$) and the association persisted after correcting for total adiposity ($r = 0.37$, $P = 0.01$).

As presented in Table 4, a stepwise regression analysis showed that fasting levels of AcylG, LDL-cholesterol, and insulinemia explained 47% (23, 15, and 9% respectively) of the variation in sTNF-R1 levels. On the other hand, neither AcylG nor TotG were independent predictors of CRP and TNF- α (data not shown).

Discussion

There is growing evidence suggesting the existence of a relationship between GI tract-derived hormones and chronic inflammatory markers, which may contribute to the development of obesity-related disturbances such as insulin resistance and type 2 diabetes (17). Recent studies have provided conflicting information regarding interactions of fasting TotG with CRP and TNF- α in patients with different chronic diseases (20, 21). However, these relationships were never evaluated in a model of obesity. Previous investigations have indicated that fasting levels of CRP, TNF- α , and sTNF-R1 are not significantly modulated during the EHC (26–31). Therefore, in the present study, CRP, TNF- α , and sTNF-R1 concentrations were only evaluated in a fasted state. On the other hand, our group and several others have reported that the amplitude of ghrelin reduction during postprandial and hyperinsulinemic states is representative of the metabolic status (8, 9, 32, 33). Therefore, the present study provides novel information regarding associations between chronic

inflammatory markers and complete EHC-induced ghrelin (AcylG and TotG) profiles in a model of overweight and obese individuals characterized by a broad range of body composition and insulin sensitivity.

To our knowledge, the relationships between ghrelin profiles and markers of chronic inflammation were not evaluated in any other study involving overweight and obese individuals with increased risks of developing insulin resistance. Moreover, the present results indicate that under fasting and EHC-induced conditions, AcylG profiles were negatively associated with sTNF-R1 concentrations while AcylG/TotG ratios (fasting and AUC) were positively correlated with TNF- α levels. These interactions persisted after controlling for the effect of total adiposity. Moreover, fasting AcylG independently predicted 23% of the variation in sTNF-R1 concentrations.

In this study, elevated AcylG/TotG values are associated with increased TNF- α concentration. This is in line with recent observations by our group and others suggesting that increased AcylG concentrations/proportions are associated with a detrimental metabolic profile (8, 12, 13). The TNF- α system (including TNF- α , its soluble receptors sTNF-R1 and sTNF-R2) is recognized as an inflammatory component for the development of metabolic disturbances in overweight subjects (34–36). Moreover, recent advances suggest that TNF- α increases insulin resistance in skeletal muscle, hepatocytes, and adipose tissues by decreasing the expression of glucose transporter-4 and insulin receptor substrate-2 (37–39). Previous authors have reported that fasting AcylG levels are negatively correlated with circulating TNF- α concentrations in women with gestational diabetes mellitus and TotG in patients with chronic pulmonary diseases (20, 40). In contrast, a positive association was observed between fasting TotG and TNF- α concentrations in patients with bowel disease, post-operative abdominal sepsis, or chronic heart failure (21, 41, 42). However, because of its autocrine effects (43), blood samples may not be representative of localized TNF- α levels/activities in specific organs or tissues. Therefore, it could be relevant to evaluate individual and combined effects of ghrelin and TNF- α in isolated tissues (i.e., adipose and skeletal muscle tissues) collected from animal models with metabolic abnormalities. Moreover, the present results suggest that the existence of a relationship between AcylG/TotG and TNF- α in overweight and obese

postmenopausal women, this association could be further investigated in larger populations with specific metabolic disturbances (such as different levels of insulin sensitivity). In turn, these studies could provide novel information regarding the physiological implications of ghrelin and TNF- α in chronic inflammatory states.

To our knowledge, interactions between ghrelin and sTNF-R1 were never reported in any other study. As previously suggested sTNF-R1 binds to TNF- α and therefore prevents the sustained immune priming resulting from the activation of the functional membrane-bound TNF-R1 (44, 45). In humans and rats, it was previously observed that weight loss suppresses sTNF-R1 levels but not TNF- α concentrations (46–48). Moreover, the amplitude of sTNF-R1 reduction is an independent predictor of insulin sensitivity improvements after weight loss (47). Thus, sTNF-R1 may be a better predictor of insulin resistance and metabolic dysfunctions than TNF- α (16). Our results indicate that AcylG levels are negatively associated with and are independent predictors of sTNF-R1 concentrations in overweight and obese postmenopausal women. Furthermore, AcylG/TotG ratios are positively associated with TNF- α . The present observations suggest that AcylG could be a two-way mediator of TNF- α system activation. Consequently, higher AcylG concentrations/proportions may play a dual role on potentiating TNF- α system activity, and could therefore increase the incidence of obesity-related disturbances in overweight and obese postmenopausal women.

We observed that insulin-induced maximal reduction of TotG levels was negatively correlated with fasting levels of CRP. Similar results were obtained by Luo *et al.*, in patients suffering from chronic obstructive pulmonary disease (20). However, this association did not remain significant after controlling for total adiposity. Numerous groups have reported that the development of metabolic dysfunctions is closely associated with deleterious ghrelin profiles (10, 49) and with elevated fasting CRP levels (50–55). Thus, the present results suggest that decreased EHC-stimulated reduction of ghrelin levels are associated with elevated CRP concentrations in a population highly susceptible to develop obesity-related disturbances.

The current study is limited by several factors. First, our cohort is composed of overweight and obese sedentary postmenopausal women who display a wide range of severity in their metabolic phenotypes. Therefore, our findings were limited to this population. Secondly, we used a cross-sectional approach, which does not allow us to make conclusions on any causal associations between the inflammatory state and the hormonal profile in our cohort. Despite these limitations, our results are strengthened by the use of gold standard techniques for the evaluation of various metabolic risk factors in a relatively large well-characterized cohort.

In conclusion, the present results provide new evidence of the association between AcylG profiles and inflammatory markers, more specifically the TNF- α system, in a population of overweight and obese postmenopausal women. Further investigations will be needed to elucidate the mechanisms underlying the regulation of AcylG, TNF- α , and sTNF-R1 in normal subjects and in patients suffering from obesity-related complications. Ultimately, a better understanding of the relationship between ghrelin and the TNF- α system may lead to new clinical targets for the development of novel therapies against metabolic disturbances.

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