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Plasma α–melanocyte-stimulating hormone: sex differences and correlations with obesity

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Abstract

Rodent experiments raise the possibility of a regulatory role of peripheral α–melanocyte-stimulating hormone (α-MSH) in obesity and metabolism, but human data on peripheral α-MSH levels remain fragmentary. Because of the possible relationship between α-MSH and obesity, we endeavored to test the hypothesis that higher levels of α-MSH in obese patients would correlate with leptin levels and with other markers of obesity. Sixty normal-weight to obese healthy men and women participated. Weight, measures of body composition, and diet diaries were obtained; fasting blood was analyzed for α-MSH, lipids, glucose, insulin, leptin, and adiponectin. To begin to understand the source of peripherally measured hormones, α-MSH was also measured in serum samples from 5 individuals with untreated Addison disease. Levels of α-MSH were higher in men vs women (10.1 ± 4.3 vs 7.6 ± 3.4 pmol/L, $P = .019$), and α-MSH levels were higher in patients with Addison disease vs controls (17.7 ± 2.3 vs 8.7 ± 0.52 pmol/L, $P < .001$). Measures of adiposity correlated with insulin and leptin in men and women, and with adiponectin in women. α–Melanocyte-stimulating hormone levels did not correlate significantly with any parameter of adiposity or diet composition. The elevated α-MSH levels in patients with untreated Addison disease suggest possible pituitary secretion of α-MSH to the periphery. The lack of correlation between peripheral α-MSH and parameters of adiposity suggests that endogenous plasma α-MSH levels are not a metric for body composition per se.

1. Introduction

Recent investigation of the biological basis of obesity has focused on the role of the centrally located neuroendocrine loop, which regulates appetite in part via leptin and α–melanocyte-stimulating hormone (α-MSH). Briefly, adipocytes secrete leptin in proportion to the percentage of body fat [1]. Leptin then binds to melanocortinergic neurons in the hypothalamus [2], which in turn secrete α-MSH [3]. α–Melanocyte-stimulating hormone then binds to hypothalamic neurons expressing melanocortin 4 receptors, leading to appetite suppression [4]. Mutations in melanocortin 4 receptors result in a severely obese phenotype and may be responsible for up to 4% of severe obesity prevalence in some populations [5].

In addition to these central actions of α-MSH, melanocortin receptors are expressed in a variety of peripheral tissues, including adipocytes [6-9]. It has been shown in animal models that stimulation of peripheral α-MSH receptors appears to enhance utilization of existing fat stores, whereas lack of peripheral α-MSH receptor stimulation appears to lead to increased fat storage [10-15].

Studies designed to further understand the role of peripheral α-MSH in humans have had somewhat conflicting results. For example, 2 studies have shown a correlation between peripheral α-MSH and measures of body composition and insulin resistance in obese male participants compared with lean controls [16,17]. On the other hand, in a large study of young lean participants [18], no correlation between serum α-MSH and body composition parameters

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was found. Obese men have been reported to have higher levels of α-MSH compared with controls [16,17], whereas in women, no difference in plasma α-MSH between obese subjects and controls was found [19]. There are no prior studies in humans that have examined peripheral α-MSH levels in a healthy population, including both men and women, across a diverse range of body weight.

Given the conflicting and limited literature reports on peripheral α-MSH and its potential role in metabolism, data were collected from 60 men and women, spanning across a wide body mass index (BMI) spectrum. Levels of α-MSH were measured and correlated with metabolic parameters relevant to obesity, including percentage of body fat, BMI, waist circumference, insulin sensitivity, leptin, and adiponectin. The primary hypothesis tested was that higher levels of α-MSH in obese patients would correlate with leptin levels, as leptin levels have the strongest correlation with body composition. To gain insight into the origin of the peripheral α-MSH, levels were also measured in 5 patients with known but untreated Addison disease to assess the effects of increased pituitary production of proopiomelanocortin, and thus adrenocorticotropic hormone (ACTH) and α-MSH, on peripheral α-MSH levels.

2. Methods

2.1. Study design/subjects

The study was approved by the Colorado Multiple Institutional Review Board. Sixty healthy men (n = 26) and women (n = 34) aged 18 to 65 years were recruited from the Denver metropolitan area and were included in the study after they gave informed consent. Subjects had a BMI between 18.6 and 42.7 kg/m^2. The only exclusions from participation were pregnancy, heart disease, cancer, diabetes, or untreated thyroid disease. All subjects were weight stable for a minimum of 3 months. Because of difficulty with phlebotomy on 1 normal-weight female participant, a measurement of plasma α-MSH could not be obtained. Five women were taking oral contraceptive pills or hormone replacement therapy, and 3 were treated with thyroid hormone. All participants had normal thyrotropin levels. The patients with Addison disease, additional to the n = 60 just described, consisted of 4 men and 1 woman at initial diagnosis (ie, before glucocorticoid or mineralocorticoid treatment). Only α-MSH was measured in these 5 individuals.

Subjects were seen in the Outpatient Clinic of the Adult General Clinical Research Center (GCRC) at the University of Colorado Denver. The same investigator performed all waist circumference measurements at the level of the umbilicus using a standardized tape measure with tension regulator (Novel Products, Rockton, IL). Within 3 days of the visit, dual-energy x-ray absorptiometry (DXA) scans were performed in the Center for Human Nutrition at the University of Colorado Denver.

After a 12-hour fast, all subjects had blood samples for measurement of glucose, thyroid-stimulating hormone, insulin, leptin, adiponectin, α-MSH, C-peptide, and free fatty acids (FFAs). With the instruction and oversight of a registered dietitian, subjects also completed a 3-day diet diary. Dietary macronutrient composition and other dietary components were analyzed using ProNutra software (Viocare Technologies, Princeton, NJ).

2.2. Laboratory measurements

α–Melanocyte-stimulating hormone was measured in plasma using the Euria-α-MSH radioimmunoassay kit (RIA; Alpco Diagnostics, American Laboratory Products, Windham, NH; range, 7.4-25.2 pmol/L). Briefly, this assay used an antiserum to an α-MSH conjugate; and to increase the sensitivity of the assay, a sequential assay with delayed addition of ^{125}I-α-MSH was performed per the instructions included with the kit. Antibody-bound ^{125}I-α-MSH was separated from the free fraction using the double antibody polyethylene glycol precipitation technique, and the radioactivity of the precipitates was quantified. The antiserum used in this kit was directed to the C-terminal region of the α-MSH molecule and showed no cross-reactivity with ACTH. The intraassay coefficient of variation is 6.47%, and the interassay coefficient of variation is 8.47%. The method is sensitive to 3 pmol/L.

Thyroid-stimulating hormone was measured in serum using a chemiluminescence immunoassay (Nichol's Institute, San Juan Capistrano, CA). Serum insulin was measured by RIA (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Serum FFAs were measured enzymatically with a colorimetric end point (Wako Chemicals, Richmond, VA). Plasma adiponectin and leptin levels were measured by RIA (Linco Research, St Charles, MO), as was C-peptide (Diagnostic Products, Los Angeles, CA). Glucose was measured using a hexokinase methodology (Roche Diagnostic Systems, Indianapolis, IN). Insulin sensitivity was determined from fasting glucose, insulin, and FFA levels using the revised quantitative insulin sensitivity check index (QUICKI) formula, as follows: insulin sensitivity = 1/[log(glucose) + log(insulin) + log(FFAs)] [20].

2.3. Dual-energy x-ray absorptiometry

Body composition was measured by DXA using a Lunar DPX-IQ scanner (Madison, WI). Daily phantom scanning was performed routinely to check the accuracy of the detector throughout the course of the study [21].

2.4. Statistics

The sample size for this study was based on data reported by Katsuki and colleagues [16]. Using a *t* test with significance level at .05 and 80% power, it was determined that a sample of n = 60 would be needed to detect a difference in means of 6.4 ± 6.67 pmol/L (mean ± SD) in lean vs obese participants. Statistical analyses were carried out using SigmaStat software, version 2.03 (SPSS, Chicago,

Table 1
Characteristics of 60 subjects

	Men	Women
n	26	34
Age (y)	42 ± 13	41 ± 11
BMI (kg/m^2)	27.5 ± 4	26.0 ± 7*
Waist circumference (cm)	95 ± 11	85 ± 16*
Weight (kg)	86.8 ± 14.6	71.4 ± 20.3*
Body fat (%)	25.4 ± 7.7	35.5 ± 10.6*
Glucose (mg/dL)	90.9 ± 6.7	87.3 ± 7.7
Insulin (μU/mL)	8.4 ± 7.6	9.1 ± 9.3
Leptin (ng/mL)	5.9 ± 6	16.7 ± 12.4*
α-MSH (pmol/L)	10.1 ± 4.3	7.6 ± 3.4*
C-peptide (ng/mL)	2.0 ± 0	1.97 ± 1
FFAs (μEq/L)	438 ± 149	513 ± 218
S_I (rev_QUICKI)	0.425 ± 0.05	0.419 ± 0.06
Adiponectin (μg/mL)	9.4 ± 4.4	12.5 ± 4.9*
TSH (μU/mL)	1.5 ± 0.8	1.8 ± 0.9

Values are expressed as mean ± SD. Comparisons are between men and women. TSH indicates thyroid-stimulating hormone.
* $P < .05$.

IL). Significance tests were 2-tailed, and α was set at .05. Comparisons were made using an unpaired Student *t* test. In the case of comparisons between patients with and without Addison disease, the Mann-Whitney rank sum test was used. Pearson *r* was used to examine correlations between pairs of variables that were both normally distributed. Spearman ρ was used to examine correlations between variables where at least one of the pair was nonnormal. Leptin levels were natural log transformed to approximate a normal distribution for each variable. All data are presented as mean ± SD.

3. Results

Characteristics of the 60 subjects are represented in Table 1. Men and women differed in weight ($P = .002$), waist circumference ($P = .012$), leptin ($P < .001$), adiponectin ($P = .016$), BMI ($P = .049$), and percentage of body fat ($P < .001$). Levels of α-MSH were significantly higher for men compared with women (Fig. 1; α-MSH for men = 10.1 ± 4.3 pmol/L, α-MSH for women = 7.6 ± 3.4 pmol/L, $t = 2.42$, $P = .019$). Of interest, the median level of α-MSH in 5 patients with Addison disease was significantly higher than the median α-MSH level in the sample of 59 men and women combined (median, 17.19 pmol/L [25%, 75%: 13.56, 20.29] in the group with Addison disease; median, 8.37 pmol/L [25%, 75%: 6.1, 9.9] in the normal cohort; $t = 289.0$; $P < .002$; mean ± SD = 17.74 ± 5.2 pmol/L in the group with Addison disease; mean ± SD = 8.69 ± 4.0 pmol/L in the normal cohort).

Table 2
Relationships between plasma α-MSH and metabolic parameters tested in men (n = 26) and women (n = 33)

	Men		Women		Combined	
	Correlation	*P*	Correlation	*P*	Correlation	*P*
BMI (kg/m^2)	0.22	.28	0.01	.94	0.09	.52
% Body fat	0.003	.98	0.04	.82	−0.14	.29
Body fat (g)	0.01	.96	−0.15	.40	−0.15	.27
Waist circumference (cm)	0.08	.72	0.03	.85	0.12	.39
Log(leptin [ng/mL])	−0.21	.29	−0.01	.93	−0.22	.09
Log(insulin [μU/mL])	−0.11	.60	0.01	.96	−0.02	.88
S_I (QUICKI)	0.01	.96	−0.11	.53	−0.05	.69
Adiponectin (μg/mL)	0.24	.24	−0.03	.86	−0.03	.81

Of the 34 women, 20 were normal weight (BMI <24.9 kg/m^2; α-MSH range, 3.15-19.03 pmol/L), 4 were overweight (BMI, 25-29.9 kg/m^2; α-MSH range, 6.22-7.5 pmol/L), and 10 were obese (BMI ≥30 kg/m^2; α-MSH range, 3.16-13.09 pmol/L). Out of the 26 men, 6 were normal weight (α-MSH range, 5.06-14.71 pmol/L), 13 were overweight (α-MSH range, 5.14-19.02 pmol/L), and 7 were obese (α-MSH range, 3.63-18.3 pmol/L). The groups were divided

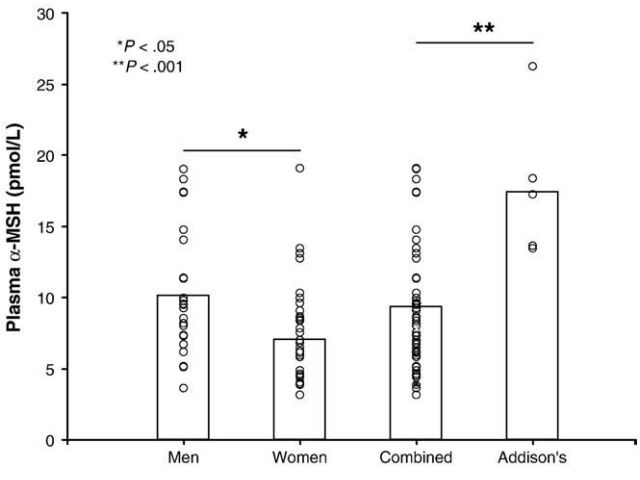

Fig. 1. Fasting plasma α-MSH levels demonstrate a sex dimorphism in that the levels are elevated in men compared with women. The source of the peripheral α-MSH is not defined, but untreated patients with Addison disease have significantly higher α-MSH than the healthy control population. Data are presented as mean ± SD.

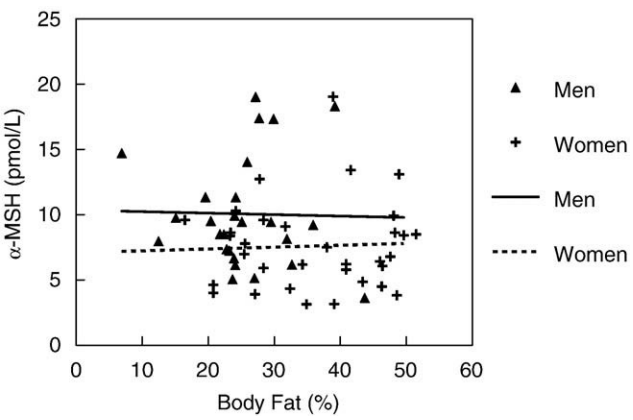

Fig. 2. There was no relationship between plasma α-MSH and body fat as measured by DXA in either men or women.

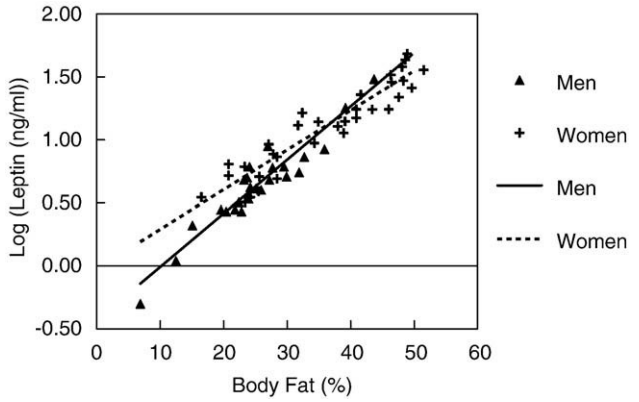

Fig. 3. There was a significant positive correlation between plasma leptin levels (log transformed) and body fat as measured by DXA in both men (r = 0.90, P < .001) and women (ρ = 0.94, P < .001).

to assess for differences between levels of α-MSH strictly between normal-weight (BMI <25 kg/m^2) and obese participants (BMI >27.5 kg/m^2) (n = 52 included). There was no significant difference in plasma α-MSH in women (8.0 ± 3.9 pmol/L in lean [n = 20] vs 7.2 ± 2.9 pmol/L in obese [n = 11] participants). However, there was a trend toward increased levels in obese men (9.4 ± 3.8 pmol/L in lean [n = 10] vs 11.7 ± 4.7 pmol/L in obese [n = 11, P = .07] participants).

There were no statistically significant relationships found between α-MSH and any of the metabolic or dietary parameters tested in men, women, or combined data (total energy intake, percentage of fat intake, percentage of carbohydrate intake, calcium intake [in milligrams]) (Table 2). There was also no relationship between peripheral α-MSH levels and body fat in either men or women (Fig. 2). Conversely, significant positive relationships were found in men and women between leptin and both BMI (r = 0.57, P = .002 in men and ρ = 0.86, P < .001 in women) and percentage of body fat (r = 0.90, P < .001 in men and ρ = 0.94, P < .001 in women) (Fig. 3). A significant negative correlation was found between adiponectin and body fat in women (ρ = −0.53, P = .002) but not in men (r = −0.177, P = .39) (Fig. 4).

4. Discussion

This study was designed to examine relationships between peripheral α-MSH, leptin, and other metabolic parameters relevant to obesity. It was hypothesized that levels of α-MSH in healthy obese adults would be associated with leptin levels. The study was designed in light of evidence from rodent models showing a role of peripheral α-MSH in regulating metabolism and body weight, and of inconsistent results in the literature describing plasma α-MSH levels in humans.

Recent research from rodent models shows that α-MSH, in addition to its central nervous system role in regulating appetite, plays a role in the peripheral circulation regulating metabolism and both fat storage and glucagon secretion [10,13,22]. Although in rodents α-MSH levels appear to be affected by leptin levels, they are not strictly dependent on leptin. Although there is a suggestion that peripheral α-MSH can affect body weight in rodents, the relationship between adiposity and plasma α-MSH levels in humans remains unclear [13]. Extrapolation of peripheral α-MSH function from rodents to humans is further complicated by the absence of melanotrophs in the human pituitary. In rodents, the pituitary is known to be the source of much of the α-MSH found in the peripheral circulation, although a number of peripheral tissues are also known to express α-MSH [23,24]. Our finding that patients with Addison disease (hypertrophy of corticotrophs) have elevated levels of α-MSH in their peripheral circulation suggests that human corticotrophs may secrete α-MSH in addition to ACTH. This was proposed previously by Coates and colleagues [25], who reported increased numbers of cells containing immunoreactive α-MSH in the anterior lobe in patients with untreated Addison disease. Alternatively, the hyperactivity of corticotrophs may have a stimulatory effect on another tissue secreting α-MSH.

In this study, the range of levels of α-MSH in plasma was similar in the normal-weight (3.2-19.0 pmol/L), overweight (6.2-7.5 pmol/L), and obese (3.2-13.1 pmol/L) participants, although there was a weak trend toward increased α-MSH in obese vs lean men, consistent with prior literature [16,17]. In the current cohort of men and women, α-MSH levels also did not correlate significantly with weight, BMI, body composition, leptin, adiponectin, insulin sensitivity, dietary calcium, and macronutrient intake (Table 2) when the groups were analyzed by sex. There were higher levels of plasma α-MSH in men compared with women. The sex difference in α-MSH levels is consistent with, albeit opposite in direction to, sex differences in other hormones involved in energy homeostasis including leptin and adiponectin [26]. When the groups were analyzed together for any correlations between α-MSH levels and parameters

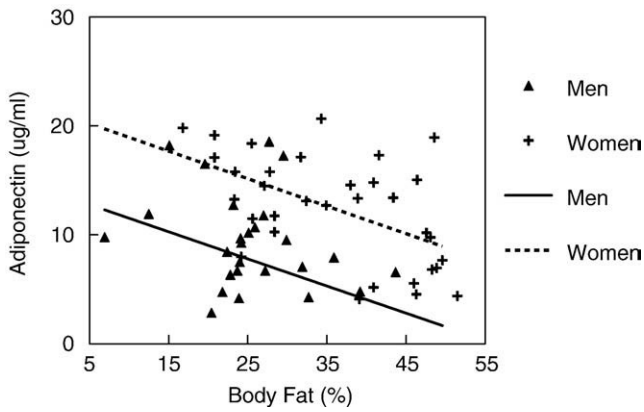

Fig. 4. There was a significant negative correlation between plasma adiponectin levels and body fat as measured by DXA in women (ρ = −0.53, P = .002), but not men (r = −0.18, P = .39).

including weight, BMI, body composition, leptin, adiponectin, insulin sensitivity, dietary calcium, and macronutrient intake, the correlations did improve modestly; but this was probably due to the fact that women had significantly lower levels of α-MSH than men.

In other studies that have examined the correlation of plasma α-MSH with body composition, a correlation between α-MSH and obesity was seen in men but not women. Katsuki et al [16] found that, in 30 Japanese men, there was a statistically significant relationship between α-MSH levels and visceral adiposity ($r = 0.716$, $P < .01$), as well as insulin resistance measured by the hyperinsulinemic euglycemic clamp technique ($r = -0.625$, $P < .02$). In another study from the United Kingdom of 11 lean and 18 obese men, there was a correlation between peripheral α-MSH and both BMI ($R^2 = 0.355$) and fat mass ($R^2 = 0.343$) [17]. However, in a study from Korea with 16 obese and 14 normal-weight women, no significant difference between the levels of plasma α-MSH in lean and obese women was found at baseline or after a 5% weight loss in the obese women [19]. Although not reported, a relationship between plasma α-MSH and body composition would be unexpected. No relationships between peripheral levels of α-MSH and body composition parameters were found in 108 healthy normal-weight Greek adults [18]. Our findings on the lack of relationship between body composition and plasma α-MSH add to the published literature.

Examining the prior published work, the average α-MSH levels in lean and obese men were reported to be 6 to 9 pmol/L vs approximately 16 pmol/L, respectively, whereas the levels in lean and obese men in our study were approximately 9 vs 12 pmol/L, respectively; thus, the levels were in the same range [16,17]. In lean and obese women, Nam and colleagues [19] reported that plasma α-MSH levels were approximately 14 pmol/L, whereas levels were approximately 8 pmol/L in the present study. Although interassay variability could contribute to this discrepancy, the assays used in the published studies in men were commercially available with similar methodology, cross-reactivity, and sensitivity to the assay used in this study [16,17]. The specificity and methodology of the assay used in the study evaluating plasma α-MSH levels in women [19] are not apparent; thus, the increased levels in that study could be due in part to cross-reactivity with ACTH or other peptides.

The lack of a strong correlation between plasma α-MSH and measures of obesity and insulin resistance, as is seen with leptin and adiponectin, suggests a regulation of plasma α-MSH levels beyond body composition. In support of this concept, one of the studies in men that found a correlation of α-MSH with increased fat mass failed to find a change in α-MSH after acute caloric restriction or weight loss [17]. Likewise, the study in women failed to find a change in plasma or cerebrospinal fluid α-MSH after weight loss [19]. As only weight-stable individuals were included in this study, any correlation with weight change would be outside of the analyses.

Plasma α-MSH in humans appears to be more complexly regulated than by the level of adiposity, insulin sensitivity, or energy balance. Thus, other possible sources of regulation were examined, including the consumption of dietary macronutrients. In this population, no correlation between dietary macronutrient consumption and α-MSH was found. Given the potential role of α-MSH in adipose tissue lipolysis, the described role of acute dietary intake of calcium on fatty acid oxidation [27], and the proposed interaction between the melanocortin system and calcium [28], a possible relationship between plasma α-MSH and dietary calcium intake was examined. Again, no relationship was evident.

To better understand peripheral α-MSH levels, additional characterization of sources of plasma α-MSH is necessary. The increase in α-MSH levels in untreated addisonian patients supports a role of the hypothalamic-pituitary axis as a contributor to peripheral α-MSH levels, although the contribution of the hypothalamic-pituitary axis to peripheral α-MSH levels in the nondisease state has yet to be explored. Because a limited number of patients with Addison disease were studied, more research is needed in this population. α–Melanocyte-stimulating hormone is also produced by peripheral cells including keratinocytes and other dermal cells [23], as well as in monocytes and macrophages [24].

In summary, plasma α-MSH levels were found to be elevated in men compared with women. In addition, the elevated α-MSH in the patients with untreated Addison disease suggests the possible role of hypothalamic-pituitary secretion of α-MSH in the periphery. The etiology of the elevated α-MSH levels in healthy men vs women does not appear to be due to differences in insulin sensitivity or body composition, given the lack of correlation between these parameters and peripheral α-MSH. Although the α-MSH system is critical to the control of energy metabolism in humans, as is evident from humans with mutations in the α-MSH receptor [29] or in proopiomelanocortin [30], peripheral α-MSH levels are not a simple reflection of adiposity or insulin resistance in healthy humans.

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