

Plasma concentrations of alpha-MSH, AgRP and leptin in lean and obese men and their relationship to differing states of energy balance perturbation

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Introduction

This study investigates the association between being overweight and brain centric hunger hormones.

Conclusions

There is a difference in hunger hormones (Melanocyte Stimulating Hormone and Agouti Related Peptide) between lean and overweight individuals.

Having more body fat is associated with higher overall AgRP.

Fasting increases AgRP and decreases Leptin, which is not seen with short term calorie restriction.

Study Design & Additional Information

Two groups of people were recruited for this study (20 - 55 years of age) and underwent separate weight loss regimens (fasting for 4-6 days or eating a very low calorie diet for 12 days). Participants were lean or obese and separated into groups as such. So, 4 conditions:

1. Lean and Fasting.
2. Lean and Very Low Calorie Diet (VLCD)
3. Obese and Fasting
4. Obese and VLCD

Fasting group consumed nothing but water until they lost about 5% of their body weight.
VLCD group consumed a little over 600 kcalories until they lost about 5% of their bodyweight (meals were the same macronutrient composition).

Amendments

Plasma concentrations of α -MSH, AgRP and leptin in lean and obese men and their relationship to differing states of energy balance perturbation

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Summary

OBJECTIVE A great deal of attention has focused on the central role of alpha melanocyte-stimulating hormone (α -MSH) and its antagonism at the melanocortin-4 receptor (MC4R) by agouti related protein (AgRP) in the regulation of energy balance. However, very little is known regarding the function of circulating AgRP and α -MSH in humans. We aimed to determine whether circulating α -MSH and AgRP are responsive to long-term perturbations in energy balance, in a manner consistent with their central putative functions.

DESIGN AND MEASUREMENTS Circulating α -MSH, AgRP and leptin were measured in both lean ($n = 11$) and obese ($n = 18$) male volunteers, some of whom (lean $n = 11$, obese $n = 12$) were then allocated one of two weight-loss dietary strategies to achieve about 5% weight loss. This was achieved by either total starvation (for 4–6 days) for rapid weight loss or a very low calorie diet (VLCD, 2.6 MJ/day) (11–12 days) for less rapid weight loss, in both the lean and obese volunteers.

RESULTS At baseline, prior to any weight loss both plasma α -MSH (15.8 ± 1.2 vs. 5.8 ± 1.0 pmol/l \pm SEM; $P < 0.001$) and AgRP (49.4 ± 2.4 vs. 10.1 ± 0.9 pg/ml \pm SEM; $P < 0.001$) were elevated in obese subjects compared with lean. In both cases this correlated closely with fat mass ($P < 0.001$), percentage body fat ($P < 0.001$)

and leptin ($P < 0.05$). Plasma AgRP increased significantly during a 6-day fast in lean individuals (11.1 ± 1.6 vs. 21.6 ± 3.1 pg/ml \pm SEM; $P < 0.05$) but not in the VLCD subjects or in the obese, while α -MSH was not affected by any changes in energy balance in either the lean or the obese volunteers.

CONCLUSION We show a difference in α -MSH and AgRP in lean and obese subjects that correlates closely with body fat at baseline. We demonstrate an increase in plasma AgRP during a 6-day fast in lean individuals that is coincident with a decrease in plasma leptin. This increase in AgRP was not due to weight loss per se as there was no change in AgRP as a result of the same weight loss in the VLCD intervention in lean individuals. The source of the increase in plasma AgRP and its physiological function in the periphery remains to be elucidated but we suggest that the dynamics of the change in plasma leptin may determine the elevation in fasting plasma AgRP in lean subjects.

Introduction

Leptin secreted by adipose tissue influences energy balance principally through its hypothalamic receptors. Although leptin receptors in the hypothalamus are known to interact with pathways involving a number of neuropeptides, neurones originating in the arcuate nucleus that release α -melanocyte stimulating hormone (α -MSH) in the paraventricular nucleus form an important part of this system. The melanocortin receptors thought to be involved in the energy balance signalling are the melanocortin-4 receptor (MC4R) and the melanocortin-3 receptor (MC3R). An integral part of this system appears to be another leptin-sensitive pathway releasing agouti related protein (AgRP), an MC4/3R antagonist. Leptin thus stimulates α -MSH and inhibits AgRP in a coordinated manner to regulate energy balance by inhibiting food intake and stimulating energy expenditure. For this reason much attention has focused on the role of the melanocortin system in obesity (Zimanyi & Pelleymounter, 2003). Melanocortin peptides including α -MSH are generated from a common precursor glycoprotein, pro-opiomelanocortin (POMC), by post-translational processing. The POMC gene is expressed at high levels in the arcuate nucleus of the hypothalamus. It is also

1. Leptin controls energy balance mainly through receptors it binds on the hypothalamus of the brain. Melanocortin Receptors in the hypothalamus also interact with other neuropeptides like melanocyte stimulating hormone (MSH), which is thought to be part of the energy balance signaling. Another neuropeptide that is stimulated by leptin binding the hypothalamus is Agouti Related Protein (AgRP), which binds the Melanocortin receptors and stops them from signaling. Leptin stimulates MSH and inhibits AgRP.

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expressed in the anterior and intermediate lobes of the pituitary, and in addition at lower level in a wide variety of peripheral tissues in mammals. The importance of the melanocortin signalling pathway in humans in the control of appetite and energy balance has been suggested by a number of monogenetic mutations identified in genes involved in the synthesis or processing of the glycoprotein POMC or in mutations leading to defects in POMC signalling via the melanocortin receptors; all mutations result in profound obesity. A number of reports have shown that mutations in MC4R are the most common single gene mutation identified to date in the obese population, being associated with more than 4% of obesity in some populations (Farooqi *et al.*, 2000; Yeo *et al.*, 2000).

Melanocortin-stimulating hormones are now recognized to have a broad array of physiological functions coincident with the wide distribution of the corresponding melanocortin receptors (MCRs) (Eberle, 1988; Zimanyi & Pelleymounter, 2003). Five subtypes of melanocortin receptors have been identified so far (MC1–5) with discrete pharmacological properties and tissue distribution (Boston & Cone, 1996; Chagnon *et al.*, 1997). Two endogenous antagonists of MCRs have also been identified, agouti and agouti-related protein (AgRP), that act in a paracrine manner to regulate MCR function (Ahima *et al.*, 2000).

α -MSH and AgRP are also present at significant levels in the human systemic circulation (Catania *et al.*, 2000; Li *et al.*, 2000). Very little is known with regard to the function of α -MSH and AgRP in the circulation particularly in relation to energy balance. However, it has been demonstrated that both plasma α -MSH (Katsuki *et al.*, 2000) and AgRP (Katsuki *et al.*, 2001) are elevated in obese men compared with nonobese men, suggesting some peripheral involvement in energy balance regulation. To investigate this further we hypothesized that circulating α -MSH and AgRP are responsive to long-term perturbations in

energy balance, in a manner consistent with their putative functions in the brain. To test this we determined whether plasma α -MSH and AgRP are responsive to an energy deficit amounting to 5% of weight loss. This was achieved either rapidly by total starvation (for 4–6 days) or more slowly by a very low calorie diet (VLCD, 2.6 MJ/day) (11–12 days), in both lean and obese men.

Methods

Subjects and protocol

Subjects were only included if they were not on any special religious or prescribed diet; were nonsmokers; had stable weight (weight change of no more than 2 kg in the previous 3 months); had normal medical examination; screening blood tests (full blood count, renal, liver and thyroid function) and electrocardiogram; and took no regular prescribed medication, vitamin or mineral supplements. These inclusion criteria were also checked with the participant's primary care physician. The study was approved by either the Joint Ethical Committee of Grampian Health Board and The University of Aberdeen (obese) or the Ethical Committee at the Dunn Clinical Nutrition Centre, Cambridge (lean). Written informed consent was obtained from all volunteers. Subjects were given a gratuity and travelling expenses on completion of the study.

Two groups of subjects were recruited, all healthy and aged between 20 and 55 years (Table 1). The study comprised a lean vs. obese comparative study, as well as two separate weight-loss studies described below (Table 1). In Cambridge, at the Dunn Clinical Nutrition Centre, 12 healthy, lean males were recruited, 11 of whom completed the study. In Aberdeen, at the Rowett Research Institute, 18 obese but healthy males were recruited

2. Melanocortin Receptor mutations are the most common mutations in obese population, affecting 4% of the obese population.

3. MSH binds a number of different melanocortin receptors, and agouti and agouti related proteins bind the same receptors, antagonizing (blocking/stopping their function) them from functioning with MSH. They function in the brain in a paracrine way (localized) and in an endocrine way (into the blood system).

4. Two groups of people were recruited for this study (20–55 years of age) and underwent separate weight loss regimens (fasting for 4–6 days or eating a very low calorie diet for 12 days). Participants were lean or obese and separated into groups as such. So, 4 conditions:

1. Lean and Fasting.
2. Lean and Very Low Calorie Diet (VLCD)
3. Obese and Fasting
4. Obese and VLCD

Fasting group consumed nothing but water until they lost about 5% of their body weight. VLCD group consumed a little over 600 kcalories until they lost about 5% of their bodyweight (meals were the same macronutrient composition).

Table 1 Baseline characteristics of the lean and obese participants (data are mean \pm SD)

	Lean study			Obese study		
	Fasting study	VLCD study	All lean participants	Fasting study	VLCD study	All obese participants
Number of men	6	5	11	6	6	18
Age (years)	40 (12)	47 (7)	44 (8)	39 (13)	46 (10)	43 (10)
Height (m)	1.79 (0.05)	1.77 (0.05)	1.78 (0.05)	1.76 (0.06)	1.75 (0.05)	1.77 (0.04)
Body weight (kg)	71.0 (9.1)	67.0 (8.1)	69.2 (8.5)	107.2 (11.5)	107.3 (15.0)	106.7 (11.6)
Body mass index (kg/m ²)	22.2 (2.3)	21.4 (2.5)	21.9 (2.5)	34.7 (2.5)	34.9 (3.5)	34.0 (2.0)
Fat-free mass (kg)	61.2 (5.8)	57.8 (7.8)	59.6 (6.6)	68.4 (7.2)	62.0 (7.0)	65.0 (6.4)
Fat mass (kg)	9.8 (3.9)	9.3 (4.6)	9.6 (4.0)	38.8 (6.5)	45.3 (9.9)	41.6 (8.5)
Systolic blood pressure (mmHg)	116 (23)	114 (16)	115 (19)	118 (4)	137 (14)	131 (14)
Diastolic blood pressure (mmHg)	67 (7)	69 (13)	68 (8)	77 (7)	86 (10)	86 (12)
Resting metabolic rate (MJ/24 h)	6.57 (0.4)	6.09 (0.6)	6.35 (0.5)	8.50 (1.2)	8.12 (1.0)	8.18 (0.8)
Fasting cholesterol (mmol/l)	4.2 (0.20)	1.9 (1.2)	4.5 (0.9)	5.0 (1.1)	5.3 (1.2)	5.2 (0.9)
Fasting plasma glucose (mmol/l)	5.1 (0.3)	5.1 (1.0)	5.1 (0.7)	5.3 (0.5)	5.8 (0.4)	5.6 (0.4)

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(Table 1). All of these subjects participated in the lean vs. obese comparative study; however, only 12 of these males participated in one or other of the two weight-loss studies (Table 1).

Each of the two weight-loss studies had a similar design. For the first week (weight maintenance period or baseline), subjects consumed a mandatory maintenance diet (13% protein, 30% fat and 57% carbohydrate), calculated to meet energy requirements [estimated at $1.6 \times$ measured resting metabolic rate (RMR)]. This was followed by a period of weight loss (WL) described below.

In the fasted group, subjects were deprived of food (but allowed water) for 4–6 days to lose approximately 5% of their original body weight. The VLCD subjects were provided with a very low calorie diet (2.6 MJ/day) for 11–12 days to also lose about 5% of their original body weight. Diets and recipes are available upon request. All food was provided for the subjects, for the duration of the study, being prepared daily by the dietetic research assistant. Compliance with the dietary regime was monitored by daily body weight loss, respiratory quotient (RQ) and plasma concentrations of β -hydroxybutyrate and glucose (data not shown). In addition, body weight was progressively lost in all subjects during the study and the rate and extent of weight loss were consistent with previous studies of this type. At the weight maintenance period (baseline) and following 5% weight loss, fasted (overnight) blood samples were collected into heparin tubes, centrifuged, and the plasma stored in aliquots at -70°C until analysis.

Participants were resident in the Human Nutrition Unit at the Rowett Research Institute, Aberdeen and the Dunn Clinical Nutrition Centre, Cambridge. They were resident in the Unit at night for the duration of the study (5–7 weeks), and during the daytime when blood samples and other intensive measurements were being performed, but were allowed to leave the Unit during the day at other times to attend their workplace or home. Although this was a two-centre study, all blood samples were analysed at one site, the Rowett Research Institute.

Clinical measurements

Each subject's height, weight and RMR were measured using standard protocols as described elsewhere (Johnstone *et al.*, 2002). Body weight was measured daily. Body composition was assessed by a four-compartment model (Fuller *et al.*, 1992), which required measurement of body density, total body water and bone mineral content. Density was determined using air-displacement whole-body plethysmography (BodPod[®] Body Composition System, Life Measurement Instruments, Concord, CT, USA). Fat and fat-free mass (FFM) was calculated from body density using the Siri equation. Total body water was determined by deuterium dilution (Pullicino *et al.*, 1990). Bone mass was estimated by dual-energy X-ray absorptiometry (DEXA), using a Norland XR-26, Mark II

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high-speed pencil beam scanner (Norland Corporation, Wisconsin, USA) for the obese subjects and a Hologic QDR-1000 W instrument (Hologic Inc., Waltham, MA, USA) for the lean subjects.

Laboratory measurements

Measurement of α -MSH. Plasma α -MSH was detected using a commercial kit according to the manufacturer's instructions (Euro-Diagnostica IDS Ltd, Tyne & Wear, UK). All samples were run in duplicate. The minimum level of detection of α -MSH was 3 pmol/l and the intra- and inter-assay coefficients of variation (CVs) of the assay were 11.8% and 13.0%, respectively. The cross-reactivity with other POMC peptides (ACTH 1–24, ACTH 1–39, β -MSH and γ -MSH) was stated to be < 0.002%.

Measurement of AgRP. The biological actions of AgRP have been shown to reside in the C-terminal fragment of AgRP (residues 83–132), suggesting that this region is the functional domain of the molecule. Plasma AgRP was detected using a commercial kit according to the manufacturer's instructions (Phoenix Peptide, Belmont, CA, USA). All samples were run in duplicate. The detection range for AgRP was 1–128 pg/ml. Cross-reactivities with AgRP(83–131)-NH₂(mouse), AgRP(83–132)-NH₂(human) and AgRP form C are 100%. Cross-reactivities with orexin A, orexin B (human), orexin B (rat, mouse), leptin (human), leptin (mouse), α -MSH, GLP1 (7–37), GLP2 and NPY (human) were zero.

Measurement of leptin. Leptin concentration of the samples were determined in a solid-phase chemiluminescent sandwich enzyme-linked immunosorbent assay (ELISA) as described previously (Hoggard *et al.*, 2001). Chemiluminescence was determined every 5 min until maximum light levels had been achieved in an MLX luminometer (Dynek, Ashford, UK) and analysed using Revelation v3.2 software (Dynek). Leptin levels were expressed as recombinant human leptin equivalents by comparison to recombinant human leptin standards on each plate using a log-log cubic regression plot. Dose-response curves were linear between 0.02 and 80 ng leptin/ml but thereafter the signal was observed to plateau. The detection limit was 0.02 ng/ml human leptin equivalents and the intra- and interassay CVs were 5.6% and 8.7%, respectively. Serum spiked with recombinant human leptin gave recoveries of 99.5 ± 4.3% ($n = 9$) from expected values. All samples were assayed in duplicate.

Statistical analysis

Changes in measurements after weight loss were assessed by paired *t*-tests, and differences between lean and obese subjects by unpaired *t*-tests. Linear regression (simple and multiple) was

used to assess associations between variables. All variables were normally distributed.

Results

Anthropometry

Within the lean subjects, the fasted group reduced body weight by 3.6 kg (5.1% of the original body weight); the VLCD group by 2.9 kg (4.3%) over 11–12 days (Table 2). Within the obese

Table 2 Body composition for the (a) lean and (b) obese men derived from the four-component model, within each weight-loss (WL) treatment by period. The values are reported for each WL treatment separately, along with the *P*-value and standard error of the difference of the means (SED), for effects within each treatment

Body composition	Baseline	5% weight loss	SED	<i>P</i>
(a) Lean men				
<i>Fast</i>				
Body weight (kg)	71.0	67.4	0.18	< 0.001
FFM (kg)	61.2	59.3	0.28	< 0.001
FM (kg)	9.8	8.1	0.34	0.002
% Body fat	13.8	11.8	0.52	0.009
<i>VLCD</i>				
Body weight (kg)	67.0	64.1	0.38	< 0.001
FFM (kg)	57.8	55.9	0.56	0.02
FM (kg)	9.3	8.2	0.64	0.022
% Body fat	14.1	12.8	0.77	0.018
(b) Obese men				
<i>Fast</i>				
Body weight (kg)	107.2	101.1	1.04	< 0.001
FFM (kg)	68.4	65.6	0.66	0.002
FM (kg)	38.8	35.5	0.59	< 0.001
% Body fat	36.1	35.0	0.41	< 0.001
<i>VLCD</i>				
Body weight (kg)	107.3	102.1	0.80	< 0.001
FFM (kg)	62.0	60.4	0.58	0.035
FM (kg)	45.3	41.7	0.62	< 0.001
% Body fat	41.9	40.5	0.41	< 0.001

FFM, fat-free mass; FM, fat mass; *P*, difference between baseline.

subjects, the fasted group reduced body weight by 6.1 kg (5.6% of original body weight); the VLCD group lost 5.2 kg (4.9%) over 11–12 days (Table 2).

Plasma levels of AgRP and α -MSH in obese and lean men

To determine whether either plasma AgRP or α -MSH are elevated in obesity we compared their circulating concentrations in lean and obese men, at baseline, prior to any weight loss. The plasma concentrations of both AgRP and α -MSH at baseline, prior to any weight loss, were elevated in obese compared with lean men (*P* < 0.001) (Fig. 1a,b). AgRP was fivefold higher (Fig. 1a) and α -MSH was threefold higher (Fig. 1b) in obese subjects compared with lean subjects. Plasma leptin was also found to be greater in the obese compared with the lean men (25.6 ± 4.57 vs. 4.56 ± 0.68 ng/ml ± SEM; *P* < 0.001).

Both α -MSH and AgRP correlated with body weight (*P* < 0.001; Table 3), body mass index (BMI; *P* < 0.01; Fig. 2 and Table 3), fat mass (*P* < 0.001; Fig. 2 and Table 3), percentage body fat (*P* < 0.001; Table 3) and leptin (*P* < 0.05; Fig. 2 and Table 3). In addition, AgRP showed a correlation with FFM (*P* < 0.05; Fig. 2 and Table 3) and α -MSH (*P* < 0.001; Table 3). Regression analysis shows that the variation in fat mass can account for the correlation between AgRP and FFM at baseline.

Table 3 Correlation (*R*-value) of AgRP, α -MSH or leptin at weight maintenance (baseline) with various anthropometric parameters

	AgRP	α -MSH	Leptin
Body weight	0.825***	0.511***	0.524**
Height	-0.058 ^{NS}	-0.303 ^{NS}	-0.130 ^{NS}
Body mass index	0.846***	0.596***	0.572**
Fat mass	0.824***	0.586***	0.619***
Percentage body fat	0.816***	0.628***	0.638***
Fat-free mass	0.469*	0.099 ^{NS}	0.057 ^{NS}
Bone mineral content	0.215 ^{NS}	-0.129 ^{NS}	-0.053 ^{NS}
Leptin	0.437*	0.415*	—
α -MSH	0.709***	—	—

^{NS}*P* > 0.05; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Table 3

This table shows the association between a variety of variables (weight, body fat, etc.) and the change in AgRP, MSH, and Leptin.

Primary Results:

- Bodyweight is associated with AgRP, MSH, and Leptin.
- Fat Mass is associated with all three, as well.

Take Away: There is an association between bodyweight and body fat mass and the three hormones being investigated here.

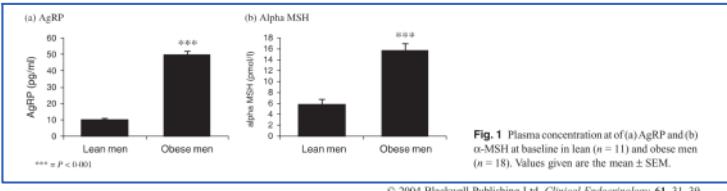


Fig. 1 Plasma concentration at of (a) AgRP and (b) α -MSH at baseline in lean (*n* = 11) and obese men (*n* = 18). Values given are the mean ± SEM.

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Figure 1

This data shows the amount of blood AgRP and MSH found in lean and obese individuals at baseline (before weight loss).

Primary Results:

- Obese men have higher AgRP and MSH than lean men.

Take Away: There is dysregulated levels of AgRP and MSH in obese individuals.

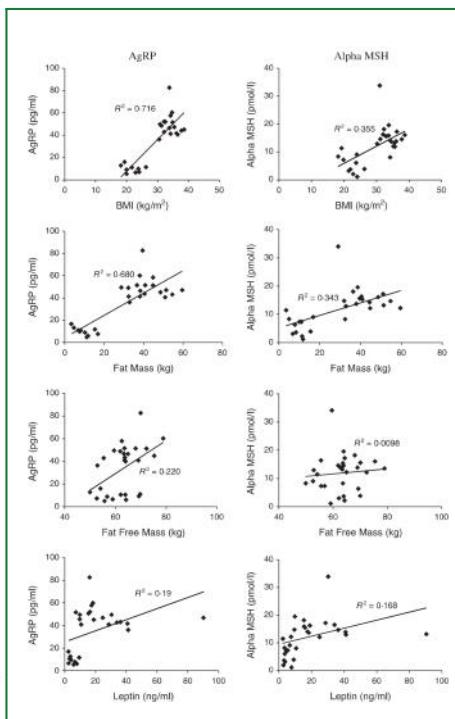


Fig. 2 Body mass index, fat mass, fat-free mass and leptin at baseline correlated with either AgRP or α -MSH.

There was no correlation with either AgRP or α -MSH and height or bone mineral content (Table 3).

Plasma levels of AgRP, α -MSH and leptin in lean and obese men who were subject to long-term perturbations of energy balance

To determine the effect of long-term perturbations in energy balance, we examined the same relationships after 5% weight loss

at two different rates in all the lean and 12 out of 18 of the obese subjects discussed above.

Fasting for 6 days in lean men increased plasma AgRP (Fig. 3; $P < 0.05$) and decreased leptin (Fig. 3; $P < 0.05$). No change in plasma AgRP was observed in lean subjects that were placed on the VLCD and nor was there any significant change in plasma leptin (Fig. 3). In contrast, plasma AgRP was unchanged by either form of weight loss in the obese men, but there was a decrease in plasma leptin in both groups (Fig. 4; $P < 0.05$).

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Figure 2

This data shows the association between a variable (BMI, Fat Mass, Fat Free Mass, Leptin) and the levels of AgRP and MSH.

Primary Results:

- There is a strong association between increasing fat mass and elevated AgRP.
- There is a moderate association between fat mass and elevated MSH.
- There is a weak association between increasing leptin and increasing AgRP (same for MSH).

Take Away: Having more body fat is associated with higher AgRP.

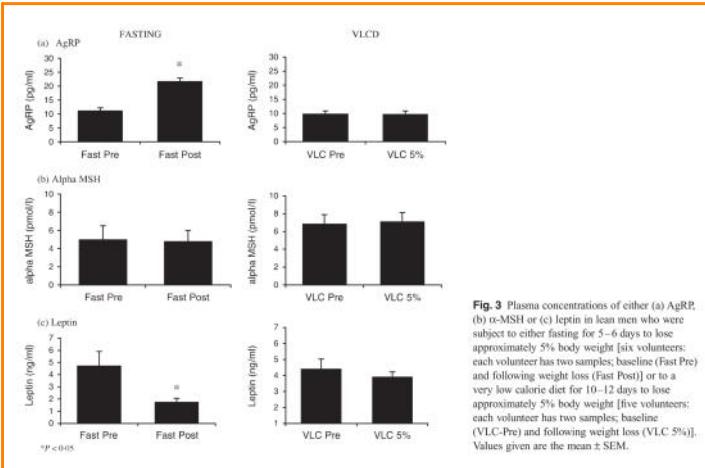


Fig. 3 Plasma concentrations of either (a) AgRP, (b) α -MSH or (c) leptin in lean men who were subject to either fasting for 5–6 days to lose approximately 5% body weight [six volunteers: each volunteer has two samples; baseline (Fast Pre) and following weight loss (Fast Post)] or to a very low calorie diet for 10–12 days to lose approximately 5% body weight [five volunteers: each volunteer has two samples; baseline (VLC Pre) and following weight loss (VLC 5%)]. Values given are the mean \pm SEM.

No change in α -MSH was observed in either lean or obese subjects placed on either of these two food restriction paradigms (see Figs 3 and 4).

There was no correlation between AgRP or α -MSH and changes in body weight, BMI, fat mass, percentage body fat, leptin or FFM as a result of weight loss.

Discussion

We hypothesized that circulating α -MSH and AgRP are responsive to long-term perturbations in energy balance. Supporting this, we have demonstrated that circulating AgRP and α -MSH are markedly elevated in obese subjects compared with lean. Both these hormones showed a significant correlation with various parameters of obesity including BMI, body fat and leptin at baseline. The elevation in circulating AgRP and α -MSH in obese subjects when compared with lean confirms two previous studies showing an elevation in α -MSH (Katsuki *et al.*, 1999) or AgRP (Katsuki *et al.*, 2001); however, this contrasts with a second study that showed no change in α -MSH (Nam *et al.*, 2001). In addition, in keeping with a previous finding (Katsuki *et al.*, 2001), we also show a correlation at baseline prior to weight loss between plasma α -MSH and AgRP.

We investigated this further in two different weight-loss interventions in both the lean and obese subjects in which the subjects lost 5% of their body weight. We have shown that there was no significant change in plasma α -MSH in response to either the food deprivation or the food restriction interventions in both lean and obese subjects. Our findings in both the obese and lean male subjects are supported by a previous report in which obese Korean women (mean BMI 35.6 kg/m²) who were subjected to a 2-week VLCD (800 kcal/day) showed no change in plasma α -MSH as a result of the diet (Nam *et al.*, 2001). These data are the first in lean subjects of this kind as far as we are aware, and are contrary to our original hypothesis and do not support a role for circulating α -MSH in the long-term regulation of energy balance.

The source of the increase in plasma α -MSH in obese subjects is unknown. It is possible that α -MSH originates from the hypothalamus. This would appear unlikely because in rodent models of obesity, hypothalamic POMC is downregulated, which would suggest a decrease in circulating α -MSH. The α -MSH may originate from the pituitary, where POMC expression has been shown to increase with obesity in rodents (Renz *et al.*, 2000). Although the human pituitary gland does not have a neurointermediate lobe, it is generally accepted that α -MSH is produced by this gland in cells of the pars distalis (Catania *et al.*,

Figure 3

This data shows the effect of fasting or calorie restriction on AgRP, MSH and Leptin in lean men.

Primary Results:

- Calorie restriction had no effect on any hormonal outcome.
- Fasting increased AgRP.
- Fasting decreased Leptin.

Take Away: Fasting leads to an increase in AgRP and decrease in leptin in lean men, which is not seen in calorie restriction - potentially implying a greater difficulty of fasting.

5. Elevated MSH could be coming from the pituitary gland.

5

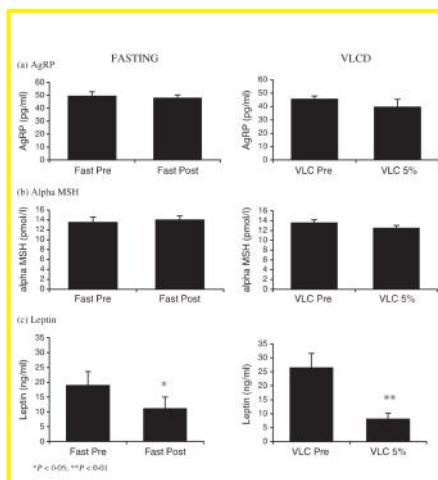


Fig. 4 Plasma concentrations of either (a) AgRP, (b) α -MSH or (c) leptin in obese men who were subject to either fasting for 5–6 days to lose approximately 5% body weight [six volunteers: each volunteer has two samples, baseline (Fast Pre) and following weight loss (Fast Post)] or a very low calorie diet for 10–12 days to lose approximately 5% body weight [six volunteers; each volunteer has two samples, baseline (VLC Pre) and following weight loss (VLC 5%)]. Values given are the mean \pm SEM.

2000). Another possible source is the increase in immune cells associated with a greater fat mass. In humans these cells have been shown to secrete α -MSH (Catania *et al.*, 2000).

The exact function of the increase in plasma α -MSH with obesity remains to be determined. However, previous studies, particularly in rodents, have shown that peripheral α -MSH has a number of functions; for example, α -MSH has been shown to have potent lipolytic activity in a number of mammals. In addition, α -MSH has been reported to regulate insulin secretion and leptin secretion, inhibit the effects of cytokines and apoptosis, stimulate corticosterone release, and to have a role in thermogenesis (Vinson *et al.*, 1983; Shimizu *et al.*, 1995; Rajora *et al.*, 1997; Forbes *et al.*, 2001; Jo *et al.*, 2001; Hoggard *et al.*, 2004). It should, however, be noted that the level of circulating α -MSH reported here (approximately 16 pmol/l in obese subjects) is very low, below the EC50 values for melanocortin receptors, and therefore its physiological function in humans remains to be determined (Zimanyi & Pelleymounter, 2003).

It has previously been shown in humans (Shen *et al.*, 2002) that plasma AgRP is increased following a short-term fast (2 h) and decreased following a meal. Thus it was proposed that AgRP acts as a short-term biomarker for the transition between the fed and fasted state (Shen *et al.*, 2002). However, there is no information

on the effect of long-term perturbations on the regulation of plasma AgRP in humans. We have shown that plasma AgRP is significantly increased in response to a 6-day fast in the lean but not the obese subjects. This is consistent with fasting studies (48 h) in rodents, where plasma AgRP is also increased (Li *et al.*, 2000). All blood samples were taken following an overnight fast to avoid any changes as a result of short-term food intake discussed above (Shen *et al.*, 2002).

In rodents, AgRP is normally suppressed by leptin, and the release of this suppression during fasting leads to an increase in AgRP mRNA in the hypothalamus and a resultant increase in food intake (Wilson *et al.*, 1999). AgRP mRNA has been shown to increase in the adrenal gland as a result of fasting in rodents (Bicknell *et al.*, 2000), where leptin receptors have also been identified (Hoggard *et al.*, 1997). We speculate that the fall in leptin as a result of fasting regulates the secretion of AgRP from either the hypothalamus or more probably from the adrenal gland. This is consistent with the data from the lean studies, where AgRP only increases in the fasting study, which shows a coincident fall in plasma leptin, and not in the VLCD study where no change in plasma leptin was observed as a result of the weight loss. However, this does not explain why there is no change in circulating AgRP in the obese subjects in response to weight loss,

Figure 4

This data shows the effect of fasting or calorie restriction on AgRP, MSH and Leptin in obese men.

Primary Results:

- Calorie restriction reduced leptin levels.
- Fasting decreased Leptin.

Take Away: Fasting and calorie deficit reduced leptin in obese individuals, but has no effect on AgRP or MSH. This implies AgRP secretion/excretion is potentially insensitive in obese individuals

6. MSH is thought to be lipolytic (breaks down fat), regulates insulin and leptin release, and stimulate cortisol release, as well as affect thermogenesis (heat production).

7. AgRP is thought to be secreted from the hypothalamus and the adrenal glands when leptin is low, which would normally inhibit AgRP release. The speculation for why AgRP did not change in obese is thought to be because of leptin resistance.

where there was a decrease in plasma leptin as a result of weight loss. This may be due to some form of leptin resistance in the obese, but clearly requires further investigation.

AgRP expression has been detected in a range of human tissues, not only in the brain and adrenal glands, thought to be the main sites of AgRP expression, but also in the testis, lung and kidney (Shutter *et al.*, 1997), and therefore the source of plasma AgRP remains to be clarified. The function of systemically circulating AgRP is also currently unknown. AgRP is known to antagonize the action of α -MSH centrally and may have a similar function at several peripheral tissues where melanocortin receptors, in particular MC3R, MC4R and MC5R, are present, including adipose tissue, pancreas, muscle, adrenal and the gastrointestinal tract (Yang *et al.*, 1999; Zimanyi & Pelleymounter, 2003). Alternatively, it may act as a signal from the periphery regulating central control of energy balance. In support of this, AgRP has been shown to cross the blood brain barrier into the brain (Kastin *et al.*, 2000).

It should be noted that the plasma concentration of AgRP we observed (50 pg/ml) and that which has been reported previously (Li *et al.*, 2000; Shen *et al.*, 2002) are below the IC₅₀ of AgRP, as determined in pharmacological studies of the cloned human MC3R (1 nM) (Yang *et al.*, 1999). However, the mahogany protein has been shown to concentrate agouti, and agouti has very close homology to AgRP at its site of action (He *et al.*, 2001). It has been suggested that a mahogany-like protein, syndecan-3, may also bind AgRP to enhance its biological activity (Reizes *et al.*, 2001; Shen *et al.*, 2002). More recently, a novel attractin-like protein (ALP) has been identified that may act as a coreceptor modulating the AgRP-MC4R interaction (Haqq *et al.*, 2003).

In conclusion, we have shown a difference in plasma α -MSH and AgRP in lean and obese subjects that correlates closely with body fat prior to weight loss. We have demonstrated an increased concentration of plasma AgRP during a 6-day fast in lean individuals, which is coincident with a decrease in plasma leptin. This increase in AgRP was not due to weight loss per se, as there was no change in AgRP as a result of similar weight loss in the VLCD intervention. The source of the increase in plasma AgRP and its physiological function in the periphery remains to be elucidated but we speculate that the fall in leptin may regulate the release of AgRP from peripheral tissue, in particular the adrenal into the plasma.

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