Clinical Endocrinology (2004) **61**, 31–39 doi: 10.1111/j.1365-2265.2004.02056.x

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

Nigel Hoggard*, Alexandra M. Johnstone*, Peter Faber*, Eileen R. Gibney†, Marinos Elia‡, Gerald Lobley*, Vernon Rayner*, Graham Horgan*, Leif Hunter*, Shabina Bashir* and R. James Stubbs*

**Rowett Research Institute, Aberdeen, UK,* †*Department of Biochemistry, Trinity College Dublin, Ireland and* ‡*Institute of Human Nutrition, Southampton General Hospital, UK*

(Received 30 January 2004; returned for revision 24 February 2004; finally revised 12 March 2004; accepted 19 April 2004)

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 ± SEM; $P < 0·001$) and AgRP (49·4 ± 2·4 *vs.* 10·1 ± 0·9 pg/ml ± 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 ± 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

Correspondence: N. Hoggard, Energy Balance and Obesity Division, Aberdeen Centre for Energy Regulation and Obesity, Rowett Research Institute, Greenburn Road, Aberdeen AB21 9SB, UK.
Tel.: + 44 (0) 1224 712751; Fax: + 44 (0) 1224 716686;
E-mail: nh@rowett.ac.uk

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).

Melanocyte-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 (MCR1–5) 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 protocols

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

Table 1 Baseline characteristics of the lean and obese participants (data are mean ± 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)

(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 × 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 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). Chemiluminesence was determined every 5 min until maximum light levels had been achieved in an MLX luminometer (Dynex, Ashford, UK) and analysed using Revelation v3·2 software (Dynex). 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 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).

Table 2 Body composition for the (a) lean and (b) obese men derived from the four-compartment 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	*P*
(a) Lean men				
Fast				
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
VLCD				
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				
Fast				
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
VLCD				
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.

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*	0·41*	–
α-MSH	0·709***	–	–

$^{NS}P > 0·05$; $*P < 0·05$; $**P < 0·01$; $***P < 0·001$.

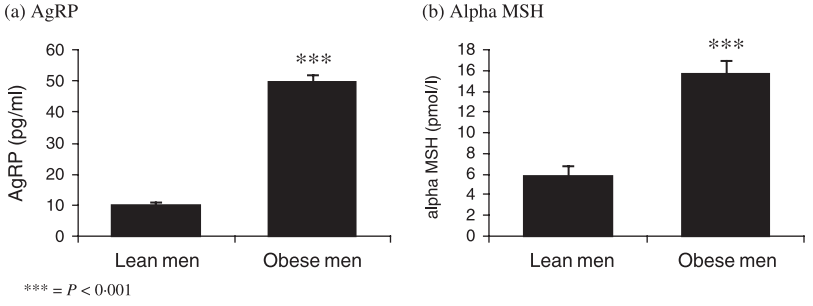

Fig. 1 Plasma concentration at of (a) AgRP and (b) α-MSH at baseline in lean ($n = 11$) and obese men ($n = 18$). Values given are the mean ± SEM.

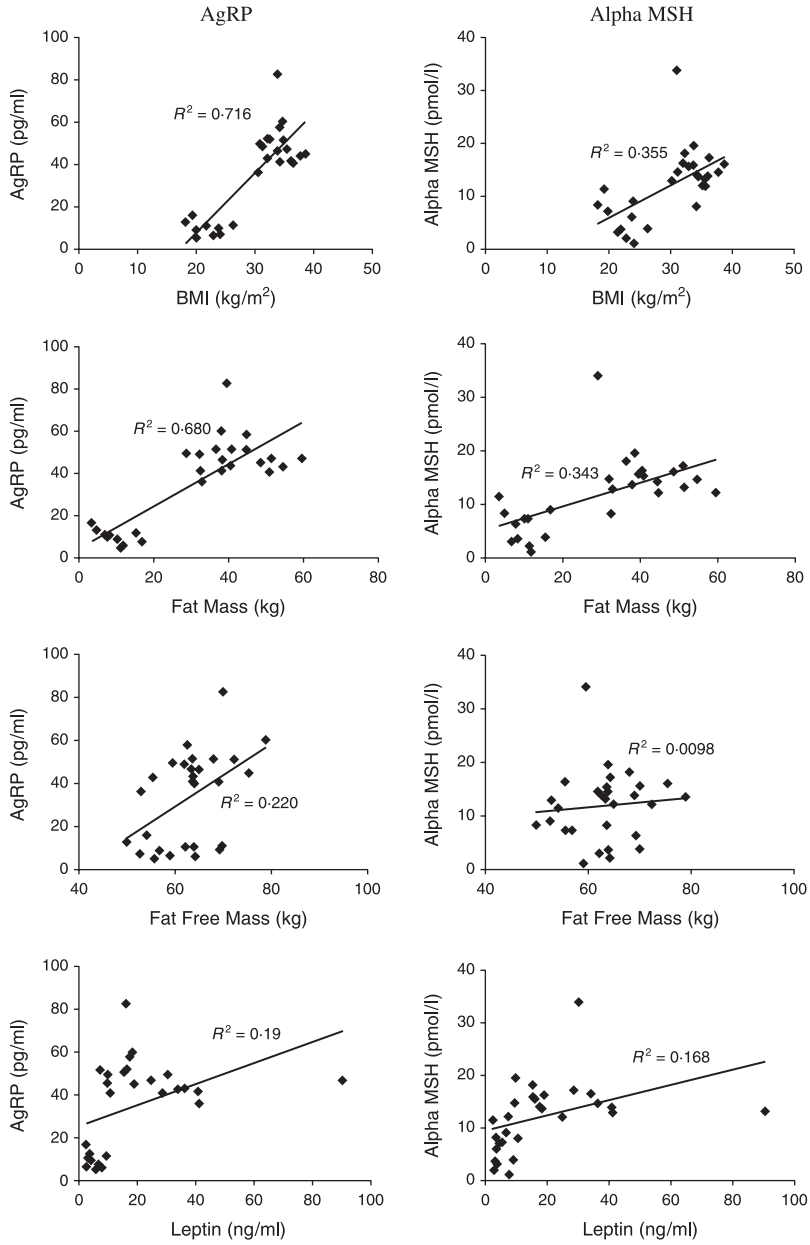

Fig. 2 Body mass index, fat mass, fat-free mass and leptin at baseline correlated with either AgRP or α-MSH.

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; $P < 0{\cdot}05$) and decreased leptin (Fig. 3; $P < 0{\cdot}05$). 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; $P < 0{\cdot}05$).

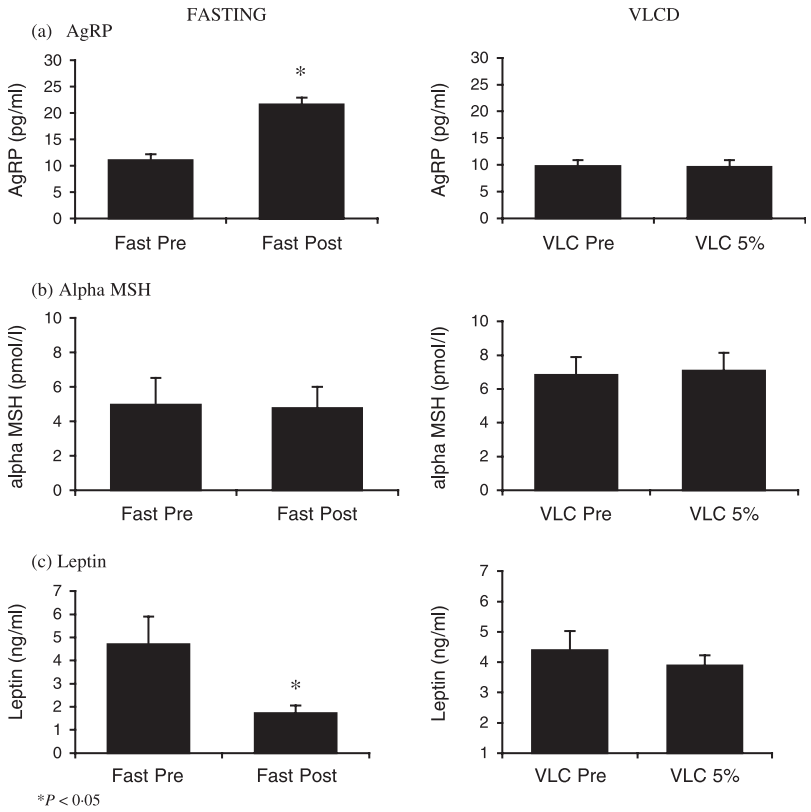

Fig. 3 Plasma concentrations of either (a) AgRP, (b) α-MSH or (c) leptin in lean men who were subject to either fasting for 5–6 days to lose approximately 5% body weight [six volunteers: each volunteer has two samples; baseline (Fast Pre) and following weight loss (Fast Post)] or to a very low calorie 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 ± 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/m^2) 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*.,

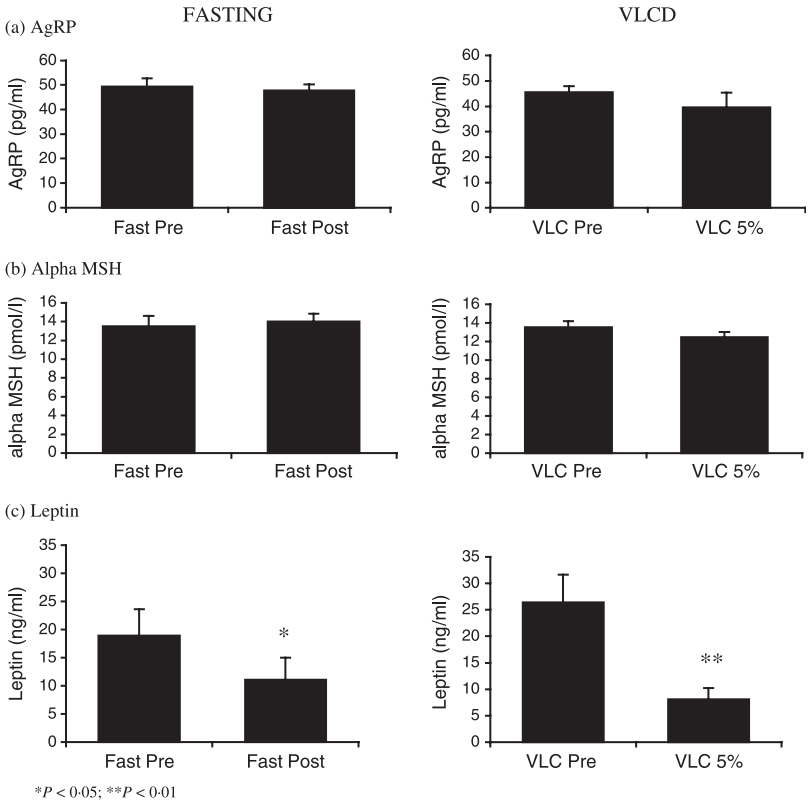

Fig. 4 Plasma concentrations of either (a) AgRP, (b) α-MSH or (c) leptin in obese men who were subject to either fasting for 5–6 days to lose approximately 5% body weight [six volunteers: each volunteer has two samples; baseline (Fast Pre) and following weight loss (Fast Post)] or a very low calorie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

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). Alternately, 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 pM) and that which has been reported previously (Li *et al*., 2000; Shen *et al*., 2002) are below the IC 50 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.

Acknowledgements

This work was supported by Slimming World, Alfreton, UK, and SEERAD (Scottish Executive Environment and Rural Affairs Department).

References

Ahima, R.S., Saper, C.B., Flier, J.S. & Elmquist, J.K. (2000) Leptin regulation of neuroendocrine systems. *Frontiers in Neuroendocrinology*, **21**, 263–307.

Bicknell, A.B., Lomthaisong, K., Gladwell, R.T. & Lowry, P.J. (2000) Agouti related protein in the rat adrenal cortex: implications for novel autocrine mechanisms modulating the actions of pro-opiomelanocortin peptides. *Journal of Neuroendocrinology*, **12**, 977–982.

Boston, B.A. & Cone, R.D. (1996) Characterization of melanocortin receptor subtype expression in murine adipose tissues and in the 3T3-L1 cell line. *Endocrinology*, **137**, 2043–2050.

Catania, A., Airaghi, L., Colombo, G. & Lipton, J.M. (2000) Alpha-melanocyte-stimulating hormone in normal human physiology and disease states. *Trends in Endocrinology and Metabolism*, **11**, 304–308.

Chagnon, Y.C., Chen, W.J., Perusse, L., Chagnon, M., Nadeau, A., Wilkison, W.O. & Bouchard, C. (1997) Linkage and association studies between the melanocortin receptors 4 and 5 genes and obesity-related phenotypes in the Quebec Family Study. *Molecular Medicine*, **3**, 663–673.

Eberle, A.N. (1988) *The Melanotropins: Chemistry, Physiology, and Mechanisims of Action.* Karger, Basel.

Farooqi, I.S., Yeo, G.S., Keogh, J.M., Aminian, S., Jebb, S.A., Butler, G., Cheetham, T. & O'Rahilly, S. (2000) Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. *Journal of Clinical Investigation*, **106**, 271–279.

Forbes, S., Bui, S., Robinson, B.R., Hochgeschwender, U. & Brennan, M.B. (2001) Integrated control of appetite and fat metabolism by the leptin–proopiomelanocortin pathway. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 4233–4237.

Fuller, N.J., Jebb, S.A., Laskey, M.A., Coward, W.A. & Elia, M. (1992) Four-component model for the assessment of body composition in humans: comparison with alternative methods, and evaluation of the density and hydration of fat-free mass. *Clinical Science (London)*, **82**, 687–693.

Haqq, A.M., Rene, P., Kishi, T., Khong, K., Lee, C.E., Liu, H., Friedman, J.M., Elmquist, J.K. & Cone, R.D. (2003) Characterization of a novel binding partner of the melanocortin-4 receptor: attractin-like protein. *Biochemical Journal*, **376**, 595–605.

He, L., Gunn, T.M., Bouley, D.M., Lu, X.Y., Watson, S.J., Schlossman, S.F., Duke-Cohan, J.S. & Barsh, G.S. (2001) A biochemical function for attractin in agouti-induced pigmentation and obesity. *Nature Genetics*, **27**, 40–47.

Hoggard, N., Mercer, J.G., Rayner, D.V., Moar, K., Trayhurn, P. & Williams, L.M. (1997) Localization of leptin receptor mRNA splice variants in murine peripheral tissues by RT-PCR and in situ hybridization. *Biochemical and Biophysical Research Communications*, **232**, 383–387.

Hoggard, N., Crabtree, J., Allstaff, S., Abramovich, D.R. & Haggarty, P. (2001) Leptin secretion to both the maternal and fetal circulation in the ex vivo perfused human term placenta. *Placenta*, **22**, 347–352.

Hoggard, N., Hunter, L., Duncan, J.S. & Rayner, D.V. (2004) Regulation of adipose tissue leptin secretion by alpha-melanocyte-stimulating hormone and agouti-related protein: further evidence of an interaction between leptin and the melanocortin signalling system. *Journal of Molecular Endocrinology*, **32**, 145–153.

Jo, S.K., Yun, S.Y., Chang, K.H., Cha, D.R., Cho, W.Y., Kim, H.K. & Won, N.H. (2001) alpha-MSH decreases apoptosis in ischaemic acute renal failure in rats: possible mechanism of this beneficial effect. *Nephrology Dialysis Transplantation*, **16**, 1583–1591.

Johnstone, A.M., Faber, P., Gibney, E.R., Elia, M., Horgan, G., Golden, B.E. & Stubbs, R.J. (2002) Effect of an acute fast on energy compensation and feeding behaviour in lean men and women. *International Journal of Obesity and Related Metabolic Disorders*, **26**, 1623–1628.

Kastin, A.J., Akerstrom, V. & Hackler, L. (2000) Agouti-related protein (83–132) aggregates and crosses the blood–brain barrier slowly. *Metabolism*, **49**, 1444–1448.

Katsuki, A., Sumida, Y., Furuta, M., Ito, K., Tsuchihashi, K., Hori, Y., Nakatani, K., Yano, Y., Gabazza, E.C. & Adachi, Y. (1999) Elevated serum levels of tumour necrosis factor-alpha are correlated with serum levels of leptin in obese healthy subjects and in obese male patients with type 2 diabetes. *Medical Science Research*, **27**, 519–520.

Katsuki, A., Sumida, Y., Murashima, S., Furuta, M., Araki-Sasaki, R., Tsuchihashi, K., Hori, Y., Yano, Y. & Adachi, Y. (2000) Elevated plasma levels of alpha-melanocyte stimulating hormone (alpha-MSH) are correlated with insulin resistance in obese men. *International Journal of Obesity and Related Metabolic Disorders*, **24**, 1260–1264.

Katsuki, A., Sumida, Y., Gabazza, E.C., Murashima, S., Tanaka, T., Furuta, M., Araki-Sasaki, R., Hori, Y., Nakatani, K., Yano, Y. & Adachi, Y. (2001) Plasma levels of agouti-related protein are increased in obese men. *Journal of Clinical Endocrinology and Metabolism*, **86**, 1921–1924.

Li, J.Y., Finniss, S., Yang, Y.K., Zeng, Q., Qu, S.Y., Barsh, G., Dickinson, C. & Gantz, I. (2000) Agouti-related protein-like immunoreactivity: characterization of release from hypothalamic tissue and presence in serum. *Endocrinology*, **141**, 1942–1950.

Nam, S.Y., Kratzsch, J., Wook Kim, K., Rae Kim, K., Lim, S.K. & Marcus, C. (2001) Cerebrospinal fluid and plasma concentrations of leptin, NPY, and alpha-MSH in obese women and their relationship to negative energy balance. *Journal of Clinical Endocrinology and Metabolism*, **86**, 4849–4853.

Pullicino, E., Coward, W.A., Stubbs, R.J. & Elia, M. (1990) Bedside and field methods for assessing body composition: comparison with the deuterium dilution technique. *European Journal of Clinical Nutrition*, **44**, 753–762.

Rajora, N., Boccoli, G., Burns, D., Sharma, S., Catania, A.P. & Lipton, J.M. (1997) alpha-MSH modulates local and circulating tumor necrosis factor-alpha in experimental brain inflammation. *Journal of Neuroscience*, **17**, 2181–2186.

Reizes, O., Lincecum, J., Wang, Z., Goldberger, O., Huang, L., Kaksonen, M., Ahima, R., Hinkes, M.T., Barsh, G.S., Rauvala, H. & Bernfield, M. (2001) Transgenic expression of syndecan-1 uncovers a physiological control of feeding behavior by syndecan-3. *Cell*, **106**, 105–116.

Renz, M., Tomlinson, E., Hultgren, B., Levin, N., Gu, Q.M., Shimkets, R.A., Lewin, D.A. & Stewart, T.A. (2000) Quantitative expression analysis of genes regulated by both obesity and leptin reveals a regulatory loop between leptin and pituitary-derived ACTH. *Journal of Biological Chemistry*, **275**, 10429–10436.

Shen, C.P., Wu, K.K., Shearman, L.P., Camacho, R., Tota, M.R., Fong, T.M. & Van der Ploeg, L.H. (2002) Plasma agouti-related protein level: a possible correlation with fasted and fed states in humans and rats. *Journal of Neuroendocrinology*, **14**, 607–610.

Shimizu, H., Tanaka, Y., Sato, N. & Mori, M. (1995) Alpha-melanocyte-stimulating hormone (MSH) inhibits insulin secretion in HIT-T 15 cells. *Peptides*, **16**, 605–608.

Shutter, J.R., Graham, M., Kinsey, A.C., Scully, S., Luthy, R. & Stark, K.L. (1997) Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. *Genes and Development*, **11**, 593–602.

Vinson, G.P., Whitehouse, B.J., Dell, A., Bateman, A. & McAuley, M.E. (1983) alpha-MSH and zona glomerulosa function in the rat. *Journal of Steroid Biochemistry*, **19**, 537–544.

Wilson, B.D., Bagnol, D., Kaelin, C.B., Ollmann, M.M., Gantz, I., Watson, S.J. & Barsh, G.S. (1999) Physiological and anatomical circuitry between Agouti-related protein and leptin signalling. *Endocrinology*, **140**, 2387–2397.

Yang, Y.K., Thompson, D.A., Dickinson, C.J., Wilken, J., Barsh, G.S., Kent, S.B. & Gantz, I. (1999) Characterization of Agouti-related protein binding to melanocortin receptors. *Molecular Endocrinology*, **13**, 148–155.

Yeo, G.S., Farooqi, I.S., Challis, B.G., Jackson, R.S. & O'Rahilly, S. (2000) The role of melanocortin signalling in the control of body weight: evidence from human and murine genetic models. *Quarterly Journal of Medicine*, **93**, 7–14.

Zimanyi, I.A. & Pelleymounter, M.A. (2003) The role of melanocortin peptides and receptors in regulation of energy balance. *Current Pharmaceutical Design*, **9**, 627–641.