Evaluation of CSF and Plasma Biomarkers of Brain Melanocortin Activity in Response to Caloric Restriction in Humans

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Abstract

The melanocortin neuronal system, comprised of hypothalamic proopiomelanocortin (POMC) and agouti-related protein (AgRP) neurons, is a leptin target that regulates energy balance and metabolism, but studies in humans are limited by lack of reliable biomarkers for brain melanocortin activity. The objective of this study was to measure the POMC prohormone and its processed peptide, β-endorphin (β-EP), in cerebrospinal fluid (CSF) and AgRP in CSF and plasma after calorie restriction to validate their utility as biomarkers of brain melanocortin activity. CSF and plasma were obtained from 10 lean and obese subjects after fasting (40h) and refeeding (24h) and from 8 obese subjects before and after 6-weeks of dieting (800 kcal/day) to assess changes in neuropeptide and hormone levels. After fasting, plasma leptin decreased to 35% and AgRP increased to 153% of baseline. During refeeding AgRP declined as leptin increased; CSF β-EP increased but POMC did not change. Relative changes in plasma and CSF leptin were blunted in obese subjects. After dieting, plasma and CSF leptin decreased to 46% and 70% of baseline; CSF POMC and β-EP decreased; plasma AgRP increased. At baseline AgRP correlated negatively with insulin and HOMA-IR and positively with the Matsuda index. Thus following chronic calorie restriction POMC and β-EP declined in CSF while acutely only β-EP changed. Plasma AgRP, however, increased after both acute and chronic restriction. These results support the use of CSF POMC and plasma AgRP as biomarkers of hypothalamic melanocortin activity and provide evidence linking AgRP to insulin sensitivity.
Introduction

The melanocortin neuronal system plays a key role in regulating energy balance and metabolism (11, 35). This system is comprised of hypothalamic proopiomelanocortin (POMC) and agouti-related protein (AgRP) neurons whose peptide products interact with downstream melanocortin receptor (MC-R) expressing neurons (16). The POMC-derived peptide α-MSH inhibits food intake and stimulates energy expenditure while AgRP is an MC-R antagonist that stimulates food intake and inhibits energy expenditure. Defects in POMC synthesis, peptide processing and in MC-R signaling cause obesity in rodents and humans (4, 6, 31). POMC and AgRP neurons are responsive to a variety of metabolic signals that regulate energy and glucose homeostasis, including leptin and insulin (17). The physiology of this system has been extensively studied in rodents but studies in humans are limited by the lack of biomarkers for brain POMC and AgRP. Since levels of the intact POMC prohormone in cerebrospinal fluid (CSF) have been shown to correlate with hypothalamic POMC in rodents, we have focused on similar measurements in human CSF (23). Although it is the POMC-derived peptide α-MSH that engages brain MC-Rs, CSF α-MSH levels are low and difficult to detect. In rodents, CSF POMC, rather then α-MSH, has been show to reflect hypothalamic POMC activity (23). We have previously shown that high levels of POMC are present in human CSF and that concentrations vary as a function of body weight, adiposity and leptin (19). We found no correlation between CSF POMC and plasma POMC which is of pituitary origin. We also measured AgRP in human CSF and plasma. In contrast to POMC, there is evidence that plasma and hypothalamic AgRP levels are correlated in rodents (12) and we have demonstrated a correlation between plasma AgRP and adiposity in humans (19). However, previous CSF and plasma measurements were all performed in the basal
state and the effects of feeding and weight loss on these parameters have not yet been studied in humans.

Food restriction induces a host of hormonal and neuronal responses that serve to maintain energy balance (3). Plasma leptin falls after acute and chronic food restriction and is accompanied by a rise in levels of the soluble leptin receptor (sOB-R) which may impact leptin transport into brain (2, 28). Fasting suppresses POMC and stimulates AgRP in the rodent hypothalamus; these effects can be reversed by leptin (10). Such changes in melanocortin activity stimulate appetite and have been implicated as a cause of recidivism after diet-induced weight loss. Objectives of the current study were to examine hormonal and neuropeptide responses to acute fasting and refeeding (RF) in healthy lean vs obese human subjects as compared to chronic diet-induced weight loss in obese subjects. Accordingly, we measured POMC, AgRP and leptin in CSF and AgRP, leptin and other hormones in plasma in order to validate POMC and AgRP measurements as biomarkers of melanocortin activity after acute and chronic caloric restriction and to examine related changes in plasma and CSF leptin and sOB-R levels. ß-endorphin (ß-EP) was also measured in CSF as both ß-EP and α-MSH are derived together from POMC and their levels in the hypothalamus usually change in parallel (9, 13). Effects on insulin sensitivity and glucose tolerance were studied as the melanocortin system can impact glucose metabolism independently of changes in body weight (35).

Materials and Methods

Study participants and protocols
Study participants were healthy men and women (age 22-45 yrs) who were non-smokers and were not taking medications. Women were studied in the early follicular phase of the menstrual cycle. Subjects with a history of eating disorders, recent weight change ± 5%, or use of weight loss products or dieting within 6 months of starting the study were excluded. This study was approved by the Columbia University Institutional Review Board and written informed consent was obtained from all subjects.

**Study 1: Fasting-refeeding protocol**

Ten subjects (7 M, 3 F) were studied: 6 lean (BMI 23.1± 0.9 kg/m²); 4 obese (BMI 33.0 ± 2.2). Subjects were admitted to the clinical research center at 1000h (Day1), after fasting since dinner at 1800h the previous day, and continued to fast for a total of 40h. They had free access to water and received intravenous hydration with 1L of normal saline. Lumbar puncture (LP) was performed at the conclusion of the 40h fast (1000h; Day2). Subjects were refed 200% of calculated (Harris-Benedict equation) caloric requirements over the next 24h. Meals (55% carbohydrate, 15% protein, 30% fat) were provided by the Bionutrition Research Core: breakfast (1000h), lunch (1300h), dinner (1900h), snacks (1600/2200h); breakfast the following day (0800h). 20% of calories was provided at each meal and 10% at each snack. A second LP was performed after refeeding (1000h; Day 3). 10 ml of CSF were collected at each LP. Blood was obtained during fasting (F) at 1000h-F16, 1800h-F24 (Day1), 1000h-F40 (Day 2) and refeeding (RF) before lunch-RF3 and dinner-RF8 and before 0800h-RF22 and after breakfast 0900h-RF23, 1000h-RF2 (Day 3). Subjects consumed an average of 190 ± 8.6% of their caloric requirement.
One subject developed a mild headache after the first LP so did not have a second LP but was re-fed and had blood drawn.

Study 2: Low Calorie diet protocol

Nine obese (BMI 33.3 ± 1.6kg/m²; range 30 to 41) female subjects were recruited. Eight subjects were studied before and after 6-wks on an 800 kcal/day liquid diet (Optifast™). CSF (10 ml) was collected by LP after an overnight fast at baseline and after 6-wks of diet. Blood was obtained concomitantly. A 2h oral glucose (75g) tolerance test (OGTT) was performed on a separate day before and at the end of the diet in 7 subjects. Hunger and satiety were assessed by visual analog scale (VAS) before each OGGT. One subject was withdrawn after developing a mild headache after the first LP.

Assays

Leptin and sOB-R were measured in plasma and CSF by ELISA (R&D Systems, Minneapolis, MN) (19). POMC was measured by two-site ELISA (19, 27); no crossreactivity with ACTH, α-MSH or β-EP. β-EP was measured by RIA as previously described; 3% crossreactivity with POMC (25). β-EP was also measured with a newly developed 2-site ELISA that is specific for β-EP and does not crossreact with POMC. This assay employs the same antibody used in the RIA for capture and a monoclonal antibody (MAB5276, Millipore, Temecula, CA) to met-enkephalin (N-terminal of β-EP) that was biotinylated for detection; sensitivity is 2 pg/ml.
AgRP was measured by ELISA and RIA with relative specificities for full-length AgRP and AgRP \textsuperscript{83-132} respectively (19, 34). The ELISA (R&D Systems) uses full-length human AgRP standard; 17% cross-reactivity with AgRP \textsuperscript{83-132}. The RIA uses an antibody provided by Dr. Barsh and human AgRP \textsuperscript{83-132} standard (Phoenix Pharmaceuticals); 20% crossreactivity with full-length AgRP.

Insulin was measured by Immulite1000 (Siemens Healthcare Diagnostics). Glucose was measured by the hexokinase method. Total ghrelin was measured by ELISA (Millipore, Billerica, MA).

**Statistical analysis**

Data are expressed as mean ± SEM. CSF hormone and neuropeptide levels in the fasted and re-fed states and before and after dieting were analyzed by paired-t-test or paired Wilcoxon signed rank test. Plasma levels of leptin and AgRP measured over time were analyzed by repeated measures ANOVA. Areas under the hormone response curves (AUC) during the OGTT were calculated by trapezoid analysis and compared by paired-t-test. Correlations were determined by linear regression analysis using Pearson’s correlation. Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR) (15). Insulin sensitivity during the OGTT was calculated by the Matsuda index (MI) (14).

**Results**
Study 1: Fasting and refeeding (RF)

Changes in leptin and sOB-R in plasma and CSF

Plasma leptin changed over time during fasting and RF (p<0.001) (Fig. 1). Levels decreased from baseline (upon admission) of 15.4 ± 5.1 to 6.1 ± 2.3 ng/ml after fasting (p=0.002) and then increased to 20 ± 6.2 ng/ml during RF (p=0.02 vs baseline). Plasma leptin suppressed to 35.1 ± 3.4% of baseline in the entire group but the degree of suppression was more profound in lean vs. obese subjects (28.7 ± 3.1 vs 44.8 ± 3.4% of baseline; p=0.009) (Fig. 1). The peak percent increase from baseline during RF was 173 ± 19 vs 130 ±17% in lean vs obese (p=0.16). CSF leptin was 139 ± 41 pg/ml after fasting and increased to 205 ± 55 pg/ml after RF (p=0.004). The percent increase in CSF leptin was greater in lean vs obese subjects (275± 56 vs 131 ± 5.7%; p=0.016) (Fig. 1). Thus relative changes in plasma and CSF leptin were more pronounced in lean subjects after fasting and RF.

Plasma sOB-R increased over time during fasting and RF (p=0.004). Baseline sOB-R was 22.4 ± 2.0 ng/ml vs 23.3 ± 2.3 after fasting; levels continued to increase during RF to 25.7 ± 3.0 ng/ml (p<0.05). The ratio of CSF to plasma leptin expressed as a percentage was 3.3 ± 0.48 % after fasting and decreased to 1.9 ± 0.33 % after RF (p=0.008). Thus higher plasma sOB-R was associated with a lower CSF to plasma leptin ratio. CSF sOB-R did not change significantly after fasting vs RF (0.233 ±0.05 vs 0.256 ± 0.06 ng/ml; p=0.14).

Changes in insulin and ghrelin
Serum insulin decreased from 9.9 ± 2.5 to 5.4 ± 1.3 µIU/ml after 40h fasting (p=0.005). Plasma ghrelin was 658 ± 91 and 657 ± 99 pg/ml after 16h and 24h fasting and tended to decrease after 40h (539 ± 72) fasting (p=0.09). Ghrelin then decreased after 3h and 8h of RF (63 and 51% of baseline; p<0.001) and returned to 90% of baseline the following morning.

Changes in POMC and β-EP in CSF and AgRP in CSF and plasma

The concentration of POMC in CSF was not different after fasting vs RF (p=0.49). In contrast the concentration of β-EP in CSF after fasting was 77.5% of the concentration after RF (p=0.04); (Fig. 2). The POMC to β-EP ratio tended to be higher during fasting (p=0.11). CSF levels of AgRP were not different during fasting vs. RF. However plasma AgRP changed over time during fasting and RF (p<0.001) (Fig. 2). Plasma AgRP increased from 68 ± 7.8 to 103 ± 16 pg/ml after 40h of fasting (p=0.02); levels then decreased towards baseline at the end of RF. Plasma AgRP was higher in lean vs obese subjects at baseline (80 ±10 vs 50 ±4.8 pg/ml; p=0.04) and after fasting (131 ± 19 vs 62 ± 4.5 pg/ml; p=0.02); AgRP correlated negatively with BMI at baseline (r=-0.719; p=0.02) and after fasting (r= -0.741; p=0.01). Plasma AgRP correlated negatively with plasma leptin (r= -0.642), CSF leptin (r= -0.652) (p<0.05) and insulin (r= -0.600; p=0.07). The relationship between plasma leptin and AgRP throughout the fasting-RF protocol is shown in Fig.2. The decrease in leptin after fasting is paralleled by an increase in plasma AgRP (153% of baseline) and with RF the increase in leptin is paralleled by a decrease in AgRP. CSF AgRP was measured by ELISA and RIA with relative specificities for the full-length (FL) and C-terminal (CT) peptides respectively. Although no difference was noted after...
fasting vs RF with either assay, the calculated ratio of CT to FL AgRP was 2.24 ± 0.37 after fasting vs 1.58 ± 0.22 after RF (p=0.02).

**Study 2: Low Calorie Diet (LCD)**

**Changes in leptin and sOB-R in plasma and CSF**

Mean weight loss after 6 weeks of dieting was 8.6% (Fig. 3). Plasma leptin decreased to 46% (p=0.009) and CSF leptin to 70% of baseline (p= 0.004) (Fig. 3). Plasma sOB-R increased to 114% of baseline (p=0.04). CSF sOB-R did not change. The ratio of CSF to plasma leptin expressed as percent was 1.32 ±0.19% at baseline vs 1.76 ± 0.18 % after weight loss (p=0.08).

**Changes in insulin, glucose metabolism and ghrelin**

Fasting serum insulin decreased from 15.0 ± 3.6 to 5.1 ± 0.7 µIU/ml, fasting glucose decreased from 91.3 ± 4.1 to 84.9 mg/dl ± 4.1 (p=0.04) and HOMA-IR decreased from 3.4 ± 0.9 to 1.0 ±0.2 (p=0.02) (Figs 3&5). The AUC for insulin during the OGTT decreased (p=0.03) but the AUC for glucose was not different. The MI calculated during the OGGT increased from 5.1 ± 2.1 to 9.2 ± 2.5 (p=0.02) (Fig. 5). Fasting plasma ghrelin increased after weight loss (Fig. 3) and the AUC for ghrelin during the OGGT increased by 136% (p=0.04). Fasting plasma ghrelin before weight loss correlated negatively with serum insulin (r= -0.837; p= -0.009) and with percent weight loss after diet (r= -0.766; p=0.02). Ghrelin did not correlate with VAS scores.
Changes in POMC and β-EP in CSF and AgRP in CSF and plasma

The concentrations of POMC and β-EP in CSF decreased significantly following weight loss. Mean and individual changes are shown in Fig. 4. CSF POMC decreased to 86% of baseline (p=0.003). CSF β-EP, measured by RIA and highly specific ELISA, decreased to 87% and 71% of baseline respectively (p<0.05). There was no change in the POMC to β-EP ratio. CSF AgRP did not change after weight loss when measured by either ELISA (20.6 ±2.1 vs 19.0 ± 1.5 pg/ml) or RIA (39.4 ± 6.8 vs 43.8 ± 6.0 pg/ml). However plasma AgRP increased significantly from 61.7 ± 9.7 to 72.0 ± 11 pg/ml (p=0.03) (Fig. 4). The CSF POMC to plasma AgRP ratio decreased to 74% of baseline (p=0.005); the CSF POMC to CSF AgRP (RIA) ratio decreased to 75% of baseline (p=0.01), indicating decreased melanocortin activity after weight loss.

At baseline, plasma AgRP correlated negatively with serum insulin (r= -0.807; p=0.008) and HOMA (r= - 0.633; p=0.13) (Fig. 5). There was a positive correlation between plasma AgRP and the MI calculated during the baseline OGTT (r = 0.777; p=0.04) (Fig. 5). These correlations were no longer evident after weight loss. Subjects tended to report less hunger before the OGTT done after weight loss (2.27±0.9) than before weight loss (4.5±1.0; p=0.05). There were no significant correlations between ratings of hunger and satiety with POMC or AgRP at baseline or after weight loss. However the percent change in plasma AgRP after weight loss correlated positively with VAS hunger scores (r=0.733; p=0.06) and the percent change in the ratio of POMC to plasma AgRP correlated negatively with hunger scores (r=- 0.836; p=0.02), suggesting that relative changes in melanocortin activity may contribute to changes in appetite after weight
loss. The percent change in plasma AgRP also tended to correlate with the percent change in ghrelin \( (r=0.656; p=0.08) \).

**Discussion**

Although previous studies suggest that concentrations of POMC in CSF and of AgRP in plasma may be useful biomarkers of hypothalamic POMC and AgRP activity, the effects of caloric restriction on these parameters and their relationship to leptin and insulin have never been studied in humans. This study demonstrates changes in melanocortin peptides after fasting and RF and after diet-induced weight loss that correspond to the known changes in POMC and AgRP in the hypothalamus. Changes in CSF leptin are also demonstrated for the first time as related to plasma leptin and sOB-R. Importantly more evidence is provided that supports the use of plasma AgRP as a marker of hypothalamic AgRP activity and links plasma AgRP to insulin sensitivity.

Plasma leptin fell to 35% of baseline after 40h of fasting and then rapidly rebounded after 24h of RF. Relative changes in both plasma and CSF leptin were greater in lean vs the obese subjects, suggesting that in lean individuals, the brain receives a more robust signal indicating energy deficit and surplus. Changes in the CSF to plasma leptin ratio were compared after fasting and RF. We hypothesized that the ratio would be lower during fasting due to increased sOB-R that can inhibit leptin transport into brain \((20, 28)\). However the ratio was actually higher after
fasting vs RF which may be due to the fact that sOB-R levels continued to increase during RF.

By comparison, after dieting and achieving 8.6% weight loss, plasma and CSF leptin decreased to 46% and 70% of baseline respectively but the CSF to plasma leptin ratio did not change despite an increase in sOB-R levels. Weight loss during the fast was minimal compared to the diet, but there was a comparable fall in leptin (3, 33).

The concentration of POMC in CSF did not change after 40h of fasting but decreased after dieting. However CSF β-EP declined in both cases. Thus acute caloric deprivation affects release of processed POMC peptides, while chronic restriction leads to a decrease in the POMC prohormone and the processed peptides. This is consistent with an initial effect on peptide release and a more delayed effect on POMC synthesis but could also reflect changes in POMC processing. Changes in POMC processing enzymes have been reported in the hypothalamus during fasting (22). In contrast, plasma AgRP increased after both acute and chronic caloric restriction. This is consistent with studies showing more rapid changes in hypothalamic AgRP expression compared to POMC during fasting (10, 21). Unfortunately α-MSH could not be reliably measured in CSF possibly due to degradation or inactivation by prolylcarboxypeptidase (29). Our α-MSH assay is specific for the amidated peptide and does not detect the inactivated peptide. However CSF β-EP may serve as a marker of both hypothalamic β-EP and α-MSH given that levels of both peptides typically change in parallel (13, 32). As with POMC, CSF β-EP is of brain origin (25). Thus it is likely that the decline in CSF β-EP during fasting is a reflection of a decline in hypothalamic β-EP and α-MSH.
AgRP was measured in CSF and plasma but only plasma AgRP showed consistent changes in both studies. The increases in plasma AgRP during fasting and dieting mirror the expected changes in hypothalamic AgRP under those conditions. The reciprocal changes in plasma leptin and AgRP during fasting and RF are consistent with the known inhibitory effect of leptin on AgRP in the hypothalamus. Higher levels of plasma AgRP have been reported in rats during fasting (12, 26) and in humans before vs after breakfast (26). We have previously demonstrated negative correlations between plasma AgRP and BMI and leptin in lean and obese subjects (19). Similar negative correlations are again demonstrated. These results suggest that plasma AgRP is of hypothalamic origin but how brain AgRP gains access to the circulation remains unclear. Heavy AgRP fiber staining is found in the median eminence that could be a source of secretion into the blood (7). Although the adrenals may also be a source for circulating AgRP (18) it is notable that plasma AgRP did not change in rats after adrenalectomy (12). In contrast to plasma AgRP, CSF AgRP did not increase significantly after fasting or dieting. The explanation for this is unclear but may relate to anatomical differences in AgRP fiber tracks that gain access to CSF and blood respectively (1, 7). However in CSF there was relatively more AgRP measured by RIA vs ELISA after fasting. This is consistent with changes in AgRP processing resulting in relatively more AgRP 83-132 which has more biological activity than the full-length peptide (5). We have confirmed by HPLC that both forms of AgRP are present in CSF (19, 34).

AgRP neurons are known to play a role in responding to insulin signaling and regulating glucose metabolism independent of changes in body weight (8, 24). Plasma AgRP correlated negatively with fasting insulin and HOMA in a cohort of lean and obese subjects and the correlation persisted when adjusted for BMI (19). This is again seen in the present studies. The negative correlation observed at baseline in the diet study is notable given that it involved a more
homogeneous group of overweight/obese women. Furthermore, a strong positive correlation was
noted with plasma AgRP and the MI calculated during the baseline OGTT, providing more
evidence for the use of plasma AgRP as a marker of insulin sensitivity while weight stable.
However, these correlations were no longer evident during weight loss which is associated with
numerous hormonal and metabolic changes and an overall decrease in brain melanocortin
activity.

There were no correlations between ratings of hunger and satiety with POMC or AgRP at
baseline or after weight loss. However the percent change in plasma AgRP after weight loss
correlated positively with hunger and the percent change in the ratio of POMC to plasma AgRP
correlated negatively with hunger, suggesting that changes in melanocortin activity may
contribute to changes in appetite after weight loss. Plasma AgRP did not correlate with ghrelin
but the change in plasma AgRP tended to correlate with the change in ghrelin, consistent with
the known stimulatory effect of ghrelin on AgRP neurons (30).

We have previously shown that AgRP (in plasma and CSF) is positively correlated with CSF
POMC in lean and obese subjects (19). This initially appeared paradoxical given the opposite
roles that POMC and AgRP play in regulating energy balance. However we now show that
under conditions of caloric restriction POMC levels fall and AgRP increases as would be
predicted from animal studies. The explanation for this may be that the activities of both POMC
and AgRP neurons and the entire brain melanocortin circuit are increased in lean vs obese
subjects under basal conditions, but in the setting of a caloric deficit, POMC neuronal activity decreases and AgRP increases to maintain energy balance.

In summary, plasma and CSF leptin decreased substantially after fasting and dieting. The relative changes in both CSF and plasma leptin after fasting and RF were blunted in obese subjects. A significant fall in CSF POMC was only seen after the diet, although CSF β-EP changed in both settings. Plasma AgRP levels increased in both settings and at baseline correlated with insulin, HOMA and MI. This study provides further support for the use of CSF POMC and plasma AgRP measurements as biomarkers of hypothalamic melanocortin activity and provides additional evidence linking plasma AgRP to insulin sensitivity.

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References


**Legends to Figures**

**Figure 1.** Mean (± SEM) plasma leptin over time during the entire period of fasting and refeeding (upper panels). Leptin levels in the entire group are shown on the left, solid circles; leptin levels in the lean subjects are shown in the middle, solid triangles; leptin levels in the obese subjects are shown on the right, open squares. The degree of leptin suppression during fasting was greater in the lean vs obese subjects. Mean plasma and CSF leptin at the time of the 2 LPs after 40h of fasting (solid bars) and 24h of refeeding (hatched bars) (lower panel). The percent increase in CSF leptin after refeeding was higher in lean vs obese subjects (lower right panel). (* p<0.01).

**Figure 2.** Mean (± SEM) CSF POMC, β-EP and AgRP (upper panel) and plasma AgRP (left lower panel) after 40h of fasting (solid bars) and 24h of refeeding (hatched bars). Mean concentrations of plasma AgRP (solid circles) and plasma leptin (open squares) are depicted over the entire time period (lower right panel). (* p<0.05).

**Figure 3.** Percent weight loss for the eight subjects in the diet study (left upper panel). Mean (± SEM) plasma and CSF leptin (upper panel) and plasma sOB-R, serum insulin and plasma ghrelin (lower panel) at baseline before the diet (solid bars) and after 6 weeks of dieting (hatched bars). (*p<0.05).
Figure 4. Mean (± SEM) CSF POMC and β-EP at baseline before the diet (solid bars) and after 6 weeks of dieting (hatched bars) (upper left panel); graphs of individual CSF POMC and β-EP concentrations (upper right panels). CSF and plasma AgRP at baseline before the diet (solid bars) and after the diet (hatched bars) (lower left panel). The ratios of CSF POMC to plasma AgRP and to CSF AgRP before and after the diet (lower right panels). (*p<0.05).

Figure 5. Mean (± SEM) insulin levels over time during the OGTT at baseline (solid circles) and after weight loss (open squares) (upper left panel). Mean calculated HOMA and MI at baseline (solid bars) and after weight loss (hatched bars) (middle panels). Correlation of plasma AgRP with fasting insulin (upper right panel) and the Matsuda index calculated during the first OGTT (lower right panel). (*p<0.05).