Cerebrospinal Fluid and Serum Concentrations of Insulin, Leptin, and Agouti-Related Protein in Relation to Weight Change after Pregnancy- A Follow-up Study

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Abstract

Objective: To examine whether overweight and obesity after pregnancy can be predicted by late pregnancy concentrations of neuropeptides and adipokines in CSF and serum and whether this relates to known risk factors, such as high pre-gravid BMI and excessive gestational weight gain.

Methods: Women (n=25, BMI =27±5 kg/m^2) recruited at admission for elective caesarean section were followed up (5 ± 1yrs) after pregnancy and divided into groups depending on BMI development from the start of pregnancy. Their weight changes after pregnancy, fat mass, and insulin sensitivity (HOMA IR) in relation to CSF and serum AgRP, leptin, soluble LepR, insulin and serum adiponectin concentrations were compared during and after pregnancy

Results: Serum AgRP concentration in late pregnancy and change in serum AgRP concentration after pregnancy predicted both short- and long-term weight changes. A decreased transport of leptin into CSF during pregnancy was shown to be reversed by increased CSF/serum quota after pregnancy. Serum adiponectin increased after pregnancy in women who returned to their pre-pregnancy weight and showed a negative correlation with HOMA-IR.

Conclusions: These results suggest that serum AgRP concentration in late pregnancy may predict weight change after pregnancy. Our findings suggest that circulating AgRP may be physiologically important in long-term regulation of body weight.

Introduction

Global obesity rates have increased over the past three decades—and the epidemic shows no signs of abating. Obesity rates are higher in women than men, ages 20 and over, and high pre-gravid BMI and excessive gestational weight gain predict short-term postpartum morbidity and higher postpartum weight retention (1-3). The latter increases the risk for complications in future pregnancies and for lifelong obesity (4).

Women with gestational weight gain above the pre-pregnancy BMI based recommendations from the Institute of Medicine (IOM) for maternal nutritional needs, retain significantly more weight postpartum than do women with weight gain within or below the recommendations, independent of pre-pregnancy BMI or body fat at baseline.[5] Published data consistently show a decrease in mean body weight within the first year after pregnancy, with an increase in body weight after that period. Even so, 56% of women gain up to 5 kg, and 13% of women gain 5–10 kg, at one year after pregnancy.[6] Excess pregnancy weight retention and failure to lose weight in an appreciable time are risk indicators for overweight and obesity in midlife up to 15 years after pregnancy.[7,8] It has also been suggested that the term 'postpartum weight retention' only should be used within a limited time period (up to 12–18 months postpartum), as other lifestyle associated factors influence the gain in body weight beyond one year after pregnancy.[9]

Much of the present understanding of environmental risk factors for obesity has come from cross-sectional comparisons that provide limited information on causation. Individual variation in pregnancy weight retention and gain is substantial, and it is very difficult to distinguish clinically important predictors that may predict development of obesity in later life. Change in lifestyle factors, such as diet, physical activity, and lactation, might contribute to the development of obesity, but are still not fully understood and can only partly explain the increment in body weight and fat mass [10]. Given the significant increase in obesity rates and the connection of adiposity to morbidity and mortality, we aimed to identify endogenous factors related to pregnancy that may have an impact on the development of obesity and insulin sensitivity in later life.

AgRP neurons are responsive to a variety of metabolic signals that regulate energy and glucose homeostasis, including leptin and insulin [11]. The physiology of this system has been extensively studied in rodents but studies in humans are limited due to the lack of biomarkers for brain AgRP. In a recent study, plasma AgRP was evaluated as a marker of hypothalamic AgRP activity and suggested to be linked to insulin sensitivity [12].

In a newly published study in pregnant women, we found that placental production of AgRP and serum AgRP levels increased throughout pregnancy but with great inter-individual variation. CSF AgRP, leptin, and insulin concentrations were higher in overweight and obese compared with normal weight women, whilst serum AgRP concentration was lower[13]. These findings suggest that both central and peripheral AgRP interacted with effects of leptin and insulin to meet the physiological and metabolic demands of pregnancy.

We now hypothesize that serum AgRP levels in late pregnancy might predict weight change, fat mass and insulin sensitivity five years after pregnancy. The purpose of this study was to follow up these women in a non-pregnant state and compare their weight changes after pregnancy with

pre-and pregnancy values. Fat mass (body composition only at follow-up), and insulin sensitivity (HOMA IR) in relation to CSF and serum AgRP, leptin, insulin and serum adiponectin concentrations were compared during and after pregnancy.

Material and methods

Subjects
The study was approved by the ethical committee at the University of Gothenburg (dnr 402-08 and dnr 750-15). Informed consent was obtained from all participants after receiving verbal and written information. All 74 women from the earlier study (ref Gustavsson) were contacted by phone, and 25 women agreed to participate in this follow-up. Inclusion criteria in the first study were uncomplicated pregnancies and healthy subjects, screened by medical history. All subjects were normoglycemic, non-smokers, and did not have a risk consumption of alcohol at the entry of the study. Dieting and use of weight-loss supplements within 6 months before pregnancy were excluding factors, and no dietary recommendations were given to the women in the study. Exclusion criteria for the follow up study were pregnancy during the last 12 months or history of diabetes, neurological, hepatic-, renal- or major psychiatric disease. Four of the participants had been pregnant during the time period between these two studies, and the average time of follow up was 5±1 years after their latest pregnancy.

Eight healthy normal weight women were recruited at local maternity wards for measurement of AgRP during pregnancy. Venous blood samples were taken after an overnight fast three times during pregnancy and at 6 months postpartum.

Protocol
After an overnight fast, venous blood and CSF samples were collected. For CSF, an introducer needle was inserted into the interspinous ligament at L3-4, and a 25-gauge Whitacre needle or a 25-gauge Pajunk Pencil Point Spinal Needle was inserted through the introducer into the subarachnoid space. Ten milliliters of CSF were removed with a 10-ml syringe. Hemorrhagic samples were excluded. The first 0.5 ml of CSF was discarded. Study samples were centrifuged, aliquoted, and stored (–80°C). Due to technical difficulties or subjects declination, CSF was sampled from 17 out of the 25 women. Body composition was determined using whole-body GE Lunar iDXA (Dual-energy x-ray absorptiometry) scan, where whole body, android and gynoid fat mass were analysed via Prodigy enCORE software.

Biochemical assays
Agouti-related protein (AgRP), Leptin, Leptin receptor (LepR), and Adiponectin were measured in the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital Mölndal. ELISA kits were used for AgRP, leptin, LepR, and adiponectin (R&D Systems). All assays were performed as recommended by the manufacturer. ELISA plates were read on a Vmax plate reader and concentrations determined with Softmax software (Molecular Devices). Insulin and AgRP concentrations were measured in undiluted, LepR in 5-fold diluted, and adiponectin in 100-fold diluted samples. For leptin analysis, CSF samples were diluted 2-fold and serum samples 100-fold. Serum insulin and plasma glucose concentrations were measured in the Clinical Chemistry Laboratory at Sahlgrenska University Hospital Mölndal using Elecsys kits (Roche) on a Cobas

6000 analyzer (Roche). CSF insulin was analysed with a double antibody radioimmunoassay (Linco Research, St Charles, MO, USA) at the Department of Clinical Science, Lund University. HOMA-IR was calculated as fasting insulin (mU/l) x fasting glucose (mmol/l)/22.5.

Statistical methods

The women were divided into groups based on changes in BMI from before pregnancy to follow-up, where women gaining more than $2kg/m^2$ were named BMI Gain and the rest BMI Stable. Results are presented as mean ± SD. BMI Gain was compared to BMI Stable using independent student t-test or in the case of CSF measurements the non-parametric independent-samples Mann Whitney U test due to low numbers. For longitudinal comparisons, pregnancy to follow-up, paired t-test was used apart for CSF measurements where Wilcoxon singed ranked test was used for BMI Gain. Correlations were analysed for the whole population using Pearson correlations. Logistical regression was used for all pregnancy variables individually to predict group belonging at follow-up. Only one variable (AgRP) showed significant regression, and no further multivariate models were found to fit the data. For determination of Michaelis-Menten type kinetics for saturable transport of leptin, all measurements from our previous pregnancy study were included (n=58, ref Gustavsson), and a non-linear regression analysis was performed using the model $[Leptin]_{CSF} = Vmax[Leptin]_{serum} /([Leptin]_{serum} + K_m)$. *P*<0.05 was considered significant.

Results

Women were divided into groups according to BMI development from the start of pregnancy to the follow-up five years (5 ± 1 yrs) after pregnancy, with characteristics displayed in Table 1. Although there was no difference in pre-pregnancy BMI, there was significantly higher gestational weight gain and insulin resistance (HOMA-IR) during pregnancy in the BMI Gain group. After pregnancy, the BMI Gain group had higher BMI and body fat whereas there was no difference in insulin resistance. There were no differences between the groups regarding age, parity or time since pregnancy at the follow-up. There was also no difference between groups in terms of breastfeeding with an average of 4.2 ± 3.7 months of exclusive breastfeeding and 8.0 ± 6.5 months of total breastfeeding.

CSF concentrations of AgRP, leptin and insulin, and serum concentrations of AgRP, leptin, insulin, adiponectin and leptin receptor were measured during pregnancy and at follow-up after pregnancy (Table 2). Women who returned to their pre-pregnancy weight had lower s-AgRP levels during pregnancy compared to both other groups. Using logistic regression, s-AgRP was the only parameter during pregnancy that predicted which group the women would belong to (p=0.045, OR=1.24). S-AgRP increased in BMI Stable after pregnancy whereas it decreased in BMI Gain (Table 2, and changes in Fig 1), where both groups showed similar levels at follow up. When analysing s-AgRP for the total population, there was no significant difference between pregnancy and follow-up. CSF-AgRP did not differ between groups and did not change from pregnancy to follow-up.

S-leptin decreased after pregnancy in BMI Stable and the whole population, where BMI Gain stayed at a higher level at follow-up. CSF-leptin was similar in all groups and did not change after pregnancy. The ratio of CSF:serum leptin as a measure of transport into CNS was increased

significantly after pregnancy in the BMI Stable group and the whole population. Changes in s-AgRP correlated with changes in CSF:serum leptin for the total population (r=0.679, p=0.015).

S-Insulin decreased after pregnancy in the total population with no differences between groups, whereas CSF insulin decreased only in the BMI stable group (Table 2).

S-Adiponectin increased after pregnancy in BMI Stable, and in the population as whole, but not in BMI Gain (Table 2 and Fig 1b). At follow-up, adiponectin concentration correlated inversely with HOMA-IR (R=-0.453, P=0.026) and positively with the leptin CSF:serum ratio (R=0.559, P=0.020). Adiponectin and AgRP concentrations developed similarly after pregnancy with changes in AgRP and changes in adiponectin from pregnancy to follow-up correlating (R=0.597, P=0.005), a correlation that remained after controlling for BMI at follow up.

The adipokines adiponectin and leptin are often expressed as a ratio. This ratio showed increases for BMI Stable after pregnancy, but not for BMI Gain with a significantly lower ratio than Stable at follow-up (Table 2and Fig 1c). It also correlated negatively to HOMA-IR at follow-up (R=-0.503, P=0.012).

Leptin receptor (LepR) was analysed in a limited number of samples during pregnancy (n=9). For the women analysed, LepR was significantly reduced after pregnancy (Table 2). BMI Gain had lower LepR concentrations than BMI Stable at follow up. There were also strong negative correlations at follow-up between LepR with both CSF-leptin and s-leptin (r=-0.648, p=0.005 and r=-0.579, p=0.002 respectively), and a positive correlation between LepR and CSF:serum leptin ratio (r=0.528, p=0.029).

The relationship between hormones and body composition is displayed in Table 3. The strongest correlations with all body fat parameters are found with leptin in both CSF, serum and the ratio CSF:serum. Serum insulin also correlates strongly with all body fat measurements, whereas CSF insulin only correlated with android fat and the ratio android:gynoid fat. Serum AgRP and adiponectin showed similar correlations, with negative correlations only to android fat and the ratio adroid:gynoid fat. LepR show strong negative correlations with whole body, android and gynoid fat.

After showing an increase of plasma AgRP during pregnancy in normal weight women in our previous study (ref), one could expect a decrease in AgRP after delivery. This is not seen at follow up for the whole population of women in this study (Table 1), where only BMI Gain decreased AgRP levels. We therefore also investigated a population of normal weight women that were followed during pregnancy in our previous study (ref), and measured AgRP 6 months after pregnancy (Fig 2). Interestingly, plasma AgRP concentrations increased during pregnancy and were even higher at 6 months postpartum (Fig 2A). Though not all women increased their serum levels of AgRP, and the change in AgRP from trimester 3 to 6 months postpartum correlated with the body weight at this time point (Fig 2B).

Fig.3 displays CSF levels as a function of serum levels for leptin (Fig. 3a) and AgRP (Fig. 3b) during pregnancy and at follow up. Leptin measurements indicate a saturable transport mechanism during both pregnancy and follow-up, however with a threefold higher K_m value during pregnancy. For AgRP there seems to be no correlation between CSF and serum levels.

Discussion

In this study, we re-examined women four to six years after pregnancy and divided them into two groups depending on their BMI development from the start of pregnancy to the follow-up. We also followed the weight development in another population of pregnant women to 6 months postpartum. In both populations, serum AgRP levels in late pregnancy and changes in serum AgRP after pregnancy seem to predict weight changes both short-term and long-term which is a novel observation. Earlier suggested findings of a decreased transport of leptin into CSF during pregnancy has been confirmed in this study by increased CSF/serum ratio after pregnancy, and reduced K_m values, most clearly seen in the women that returned to their pre-pregnancy weight. Interestingly, the leptin ratio correlates with soluble leptin receptor levels, indicating influence on leptin transport across the blood brain barrier (BBB). Serum adiponectin increased after pregnancy in the women that were weight stable or decreased their weights and showed a negative correlation with QUICKI after controlling for BMI.

AgRP predicts weight changes after pregnancy

Women who returned to their pre-pregnancy weight at follow-up (BMI Stable) had lower serum AgRP in late pregnancy compared with those who remained at an increased weight. Logistic regression showed that this was the only parameter during pregnancy predicting weight development afterwards. A negative correlation was also found to android fat at follow-up. S-AgRP increased in the BMI Stable women after pregnancy whereas it decreased in those women who did not returned to their pre-pregnancy weight. Both groups showed similar levels at follow up. CSF-AgRP did not differ between groups at follow-up and did not change from pregnancy values. After showing an increase of circulating AgRP levels during pregnancy in our previous study (13) probably due to placental release, and reported higher circulating AgRP in pregnant compared to non-pregnant women (Page-Wilson), we expected to find a decrease post-partum in all women. Instead, we found an increase in AgRP at the five year follow-up in the weight stable women. We also observed, in another population of pregnant women, that the circulating AgRP increased overall at 6 months postpartum but that the change was highly individual and correlated to the maternal weight change and body weight from late pregnancy to 6 months after.

Most of the actions of AgRP are believed to take place in the hypothalamic melanocortin system and injection of AgRP into the cerebral ventricles induces hyperphagia and obesity in rodents (14–16). However, increasing evidence indicates not only that hypothalamic AgRP contributes to obesity but also that peripheral AgRP plays a significant role in energy balance and obesity, with fasted humans and animals showing increased circulating levels of AgRP(17-19). In a recent cross-sectional study with lean and overweight/obese men and women, plasma (but not CSF) AgRP correlated inversely with BMI, leptin, insulin, HOMA and fat mass (20).
AgRP has been shown to cross the BBB, from the blood to the brain, much slower than the saturably transported leptin, and slower than almost all other non–saturably transported neurotrophins or endogenous peptides (21). This passage has been shown not to be self-inhibited by excess AgRP given by intravenous injection, indicating the absence of a saturable transport system, and not cross-inhibited by leptin or α-MSH. HPLC measurements also showed that the small amount of AgRP crossing the BBB did so in intact form to the brain parenchyma rather than binding to capillary endothelial cells (21). This is in agreement with our results showing no relationship between CSF and serum AgRP in pregnant or non-pregnant states.

This could also clarify why intracerebroventricular injections of AgRP are more effective than short exposure to peripheral AgRP for reduction of energy expenditure and stimulation of food intake (22). Even though single bolus peripheral AgRP may not exert a potent acute effect on food intake and energy expenditure compared to centrally administered AgRP (14-16), it could still play important regulatory roles in situations such as fasting. It has been shown by radioactive labelled tracer after intravenous bolus injection, that AgRP enters peripheral organs at different influx rates, all of which were higher than into spinal cord and brain and was fastest in the liver and adrenal gland (22). Thus, the differential uptake of AgRP by peripheral organs could be a peripheral regulated process that is modulated by food deprivation. Although we did not find significant correlations after an overnight fast between serum AgRP and leptin, insulin, and HOMA-IR, which might be due to a low number of subjects in our analysis, this has been shown in another study (12).

Leptin transport across BBB increased after pregnancy and leptin resistance decreased

Our data shows a relationship between serum and CSF leptin indicating a saturable transport into CSF. This has been found in earlier studies, in both humans and mice, which have shown saturation at similar serum levels to ours indicating that the most effective transport into BBB is at low circulating levels (44). During pregnancy, increased food intake and increased fat deposits in the presence of hyperleptinemia suggests that gestation is a state of physiological resistance to leptin, which is useful to allow a positive energy balance and gain of energy stores needed for reproductive success. It has been shown in rats that central resistance to leptin during pregnancy results from two processes, with an alteration in the hypothalamic intracellular cascade mechanisms of leptin and a reduction in the transport of leptin through the BBB (24). A lower CSF/plasma leptin percentage has been shown in pregnant compared with non-pregnant women consistent with reduced leptin transport into the brain (30). This putative transitory condition reducing the suppressive effects on food intake of leptin during pregnancy, to our knowledge, has not been shown before in the same women in pregnant and non-pregnant state. Our serum and CSF data show a threefold higher K_m value during pregnancy, consistent with a lower saturation concentration and a reduced transport across the BBB. CSF leptin did not change after pregnancy suggesting that leptin may also be less effective in suppressing the orexigenic effect of AgRP due to changes in leptin signaling within the hypothalamus. No differences in CSF leptin between pregnant and non-pregnant women were also found in another study (30).

Circulating leptin decreased after pregnancy, most pronounced in those women who were weight stable, whereas those who gained weight had a small non-significant leptin reduction compared with the pre-pregnancy values. The CSF/serum leptin ratio increased significantly only in the BMI Stable group at follow-up. A reduction in the transport of leptin through the BBB has been shown in obese humans (25, 26), indicating that decreased passage of leptin into the CNS is a general mechanism of leptin resistance. Leptin is a 16-kDa protein and too large to cross this barrier by simple diffusion, but an active translocation across the BBB has been proposed (27). Little is known about leptin entry into CNS but it has been suggested that the soluble short form of the leptin receptor, LepR, might be involved in the transport of leptin through the BBB (28, 29).

LepR was significantly reduced after pregnancy with lowest follow-up concentrations in women who gained weight compared with those who were weight stable. There were also strong negative correlations between LepR with both CSF-leptin and s-leptin, and a positive correlation with

leptin ratio at follow-up in agreement with a recent study (45). LepR also showed strong negative correlations with whole body, android and gynoid fat depots.

Soluble LepR concentrations have been shown to increase during pregnancy in women, in parallel with the rise in circulating leptin levels (30-32) and a decline in CSF leptin (30), supporting the hypothesis that this binding protein prevents leptin transport into CSF mediating leptin resistance during pregnancy. This is in accordance with studies showing that LepR might antagonize the central transport of leptin both in vitro and in vivo in animal models (33, 34), limiting the access of leptin to the brain and hypothalamus also in humans. Interestingly, in a recent study, it was demonstrated that leptin binding to and signaling through LepR is not required for efficient transport across human endothelial monolayers (35). The multiligand receptor, low-density lipoprotein receptor-related protein-2 (LRP-2) has recently been suggested as a candidate for BBB transporter for leptin (35). We hope that a deeper understanding of leptin BBB transport will help to clarify the exact causes for leptin resistance seen in pregnancy and obesity in future studies.

Adiponectin increased after pregnancy and correlated to insulin sensitivity

Serum adiponectin increased after pregnancy in all women and significantly in those who were weight stable compared with their pre-pregnancy BMI. At follow-up, adiponectin concentration correlated inversely with HOMA-IR and with both android and the ratio android/gynoid fat. Circulating adiponectin and AgRP levels evolved similarly and correlated strongly from pregnancy to follow-up, even after controlling for BMI.

Pregnancy is characterized by increased insulin resistance (36), in association with the release of the major regulatory cytokines adiponectin and leptin from adipose tissue. Circulating adiponectin levels are decreased during the third trimester in parallel with reduction of maternal insulin sensitivity compared with the first and second trimesters (37, 38). Adiponectin gene expression during pregnancy has been shown to mainly be derived from adipose tissue, and the levels were lower in obese compared to lean women (39). Plasma adiponectin was also negatively correlated with insulin sensitivity and maternal BMI in agreement with our findings although they did not measure body fat mass or fat depots.

The ratio of adiponectin to leptin showed large and significant increases for the women who had a stable BMI, but not for the group that gained BMI at follow-up. A strong correlation was also found between the ratio and body fat, as well as android and gynoid fat depots.

Decreased adiponectin and increased leptin concentrations resulting in a reduced adiponectin/leptin ratio are associated with increased insulin resistance in for example obesity and type 2 diabetes mellitus (40, 41). The utility of the adiponectin/leptin ratio as a potential noninvasive biomarker of insulin resistance has also been shown in pregnancy where an inverse correlation between insulin resistance index and adiponectin/leptin ratio was found and the ratio during pregnancy was inversely correlated with BMI before pregnancy (42), all in agreement with our study.

In a recent study, most of the studied adipokines including adiponectin, AgRP, leptin and LepR were correlated with anthropometric parameters of adiposity in obese women and men (43). This study employed the calculation of multiple indices of adipokine interactions in order to evaluate possible adipokine synergism and found that particularly AgRP and LepR in combination with

other adipokines such as adiponectin may better reflect functional effects, especially with respect to influence on adipose tissue metabolism and prediction of body composition in specific groups of patients (43).

Conclusion
The prevalence of overweight and obesity in fertile women is rising. High pre-gravid BMI and excessive gestational weight gain increase the risk for overweight and obesity after pregnancy. We compared the same women in a pregnant and non-pregnant state with regards to their weight changes after pregnancy in relation to their pre-pregnancy values of neuropeptides and adipokines. These results show that serum AgRP levels in late pregnancy may predict weight change after pregnancy both within the first 6 months and after several years, and correlates to fat depots after pregnancy. Our findings point to that circulating AgRP might be physiologically important in long-term regulation of body weight. Additional studies with a larger number of women need to confirm these results and also to establish mechanisms behind these findings.

Table 1 Maternal characteristics of the BMI groups Stable and Gain based on BMI development from the start of pregnancy to follow up 5 ± 1 years later.

|	BMI Stable (n=18)	BMI Gain (n=7)	Total (n=25)
Pre-pregnancy BMI (kg/m^2)	26.5 ± 4.5	26.8 ± 3.5	26.6 ± 4.2
Gestational weight gain (kg)	12 ± 4	16 ± 7*	13 ± 6
HOMA IR, pregnancy	1.3 ± 0.7	2.3 ± 1.6*	1.6 ± 1.1
BMI change, pre-pregnancy to follow up (kg/m^2)	-0.4 ± 1.5	4.1 ± 1.5*	0.9 ± 2.5
BMI at follow up (kg/m^2)	26.1 ± 4.3	31.0 ± 3.7*	27.5 ± 4.7
Body Fat (kg)	25 ± 8	36 ± 8*	28 ± 10
Android Fat (%)	38 ± 12	49 ± 5*	41 ± 12
Gynoid Fat (%)	40 ± 7	47 ± 3*	42 ± 7
HOMA IR	1.5 ± 1.0	1.9 ± 1.1	1.6 ± 1.0
Age (years)	40.0 ± 4.9	37.4 ± 5.0	39 ± 5
Parity (n)	2.3 ± 0.6	2.0 ± 0.6	2.2 ± 0.6
Time since last pregnancy (yrs)	4.8 ± 1.3	5.4 ± 1.0	5.0 ± 1.2

Values are presented as mean ± SD. *Significant difference compared to BMI Stable, $p<0.05$ using independent t-test.

Table 2 Levels of AgRP, leptin, insulin, adiponectin, and leptin receptor in serum and CSF during pregnancy and at follow-up.

		BMI Stable $n_{serum} = 18$ $n_{CSF} = 13\text{-}17$	**BMI Gain** $n_{serum} = 7$ $n_{CSF} = 4\text{-}7$	**Total** $n_{serum} = 25$ $n_{CSF} = 17\text{-}24$
S-AgRP (pg/ml)	Pregnancy	33 ± 13	$63 \pm 16^*$	43 ± 21
	Follow-up	$45 \pm 10^\dagger$	$46 \pm 8^\dagger$	45 ± 9
CSF-AgRP (pg/ml)	Pregnancy	34 ± 14	39 ± 23	36 ± 17
	Follow-up	32 ± 8	33 ± 4	33 ± 7
S-Leptin (ng/ml)	Pregnancy	23 ± 11	37 ± 21	27 ± 15
	Follow-up	$15 \pm 13^\dagger$	$26 \pm 9^*$	$18 \pm 13^\dagger$
CSF-Leptin (ng/ml)	Pregnancy	0.17 ± 0.07	0.21 ± 0.06	0.18 ± 0.07
	Follow-up	0.15 ± 0.07	0.19 ± 0.01	0.16 ± 0.07
CSF:S Leptin %	Pregnancy	0.99 ± 0.76	0.67 ± 0.27	0.89 ± 0.67
	Follow-up	$1.42 \pm 0.50^\dagger$	0.84 ± 0.30	$1.28 \pm 0.52^\dagger$
S-Insulin (mU/l)	Pregnancy	7.8 ± 3.9	12.0 ± 7.7	9.0 ± 5.4
	Follow-up	6.3 ± 3.8	7.8 ± 4.3	$6.7 \pm 3.9^\dagger$
CSF Insulin (mU/l)	Pregnancy	1.6 ± 1.1	1.9 ± 1.7	1.7 ± 1.3
	Follow-up	$1.0 \pm 0.9^\dagger$	2.8 ± 2.8	1.5 ± 1.7
CSF:S Insulin %	Pregnancy	23 ± 18	19 ± 25	22 ± 20
	Follow-up	18 ± 14	28 ± 24	20 ± 16
S-Adiponectin (ug/ml)	Pregnancy	7.5 ± 3.4	$8,7 \pm 5.2$	7.9 ± 3.9
	Follow-up	$10.9 \pm 4.6^\dagger$	7.9 ± 3.2	$10.0 \pm 4.4^\dagger$
S-Adiponectin:Leptin	Pregnancy	457 ± 414	329 ± 267	421 ± 378
	Follow-up	$1410 \pm 1240^\dagger$	$331 \pm 163^*$	$1108 \pm 1157^\dagger$
S-LepR (ng/ml)	Pregnancy	59 ± 9 (n=5)	57 ± 39 (n=4)	58 ± 25 (n=9)
	Follow-up	41 ± 10	$32 \pm 6^*$	$38 \pm 10^\dagger$

Values are presented as mean ± SD. *Significant difference compared to BMI Stable using independent t-test for serum measurements and Whitney U test for CSF measurements (p<0.05). †Significant difference between pregnancy and follow-up using paired t-test (p<0.05).

Figure 1. Comparisons between BMI groups for changes from pregnancy to follow-up. a) serum AgRP b) serum adiponectin and c) serum adiponectin:leptin ratio. Individual values plotted with marker displaying median value. * Significant difference between groups (P>0.05 using independent t-test).
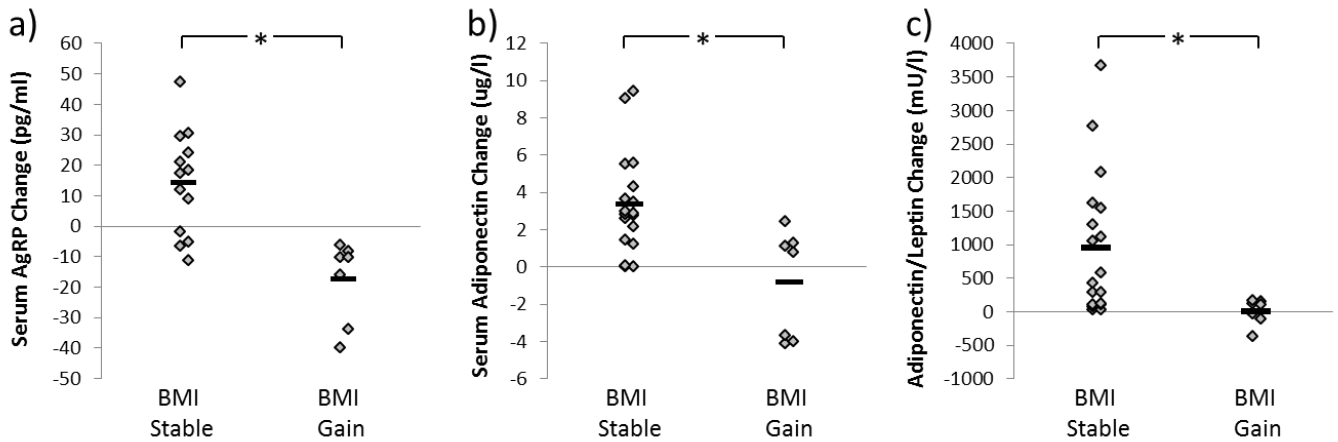
Table 3 Pearson correlations between DXA measurements and concentrations of Insulin, Leptin, AgRP, Adiponectin and Leptin receptor at follow-up in all women; n=25 for serum and n=17 for CSF.

		CSF Insulin	Serum Insulin	CSF/Serum Insulin	CSF Leptin	Serum Leptin	CSF/Serum Leptin	Serum AgRP	Serum Adiponectin	Serum LepR	Adiponectin :Leptin
Body Fat (%)	R	0.446	**0.512**	0.321	**0.661**	**0.720**	**-0.853**	-0.380	-0.379	**-0.549**	**-0.791**
	P	0.073	**0.009**	0.209	**0.004**	**0.000**	**0.000**	0.061	0.061	**0.004**	**0.000**
Android Fat (%)	R	**0.552**	**0.580**	0.398	**0.670**	**0.683**	**-0.864**	**-0.399**	**-0.436**	**-0.550**	**-0.791**
	P	**0.022**	**0.002**	0.114	**0.003**	**0.000**	**0.000**	**0.048**	**0.029**	**0.004**	**0.000**
Gynoid Fat (%)	R	0.340	**0.420**	0.246	**0.682**	**0.715**	**-0.777**	-0.232	-0.261	**-0.510**	**-0.752**
	P	0.182	**0.037**	0.341	**0.003**	**0.000**	**0.000**	0.264	0.208	**0.009**	**0.000**
And:Gyn ratio	R	**0.612**	**0.571**	0.449	**0.559**	**0.491**	**-0.781**	**-0.452**	**-0.481**	**-0.441**	**-0.670**
	P	**0.009**	**0.003**	0.071	**0.020**	**0.013**	**0.000**	**0.023**	**0.015**	**0.027**	**0.000**

Figure 2 Plasma AgRP in normal weight prospective cohort. A) AgRP plasma concentration through pregnancy and 6 months post-partum. B) Correlation between postpartum change in plasma AgRP (T3 to PP06) and postpartum body weight. N=8
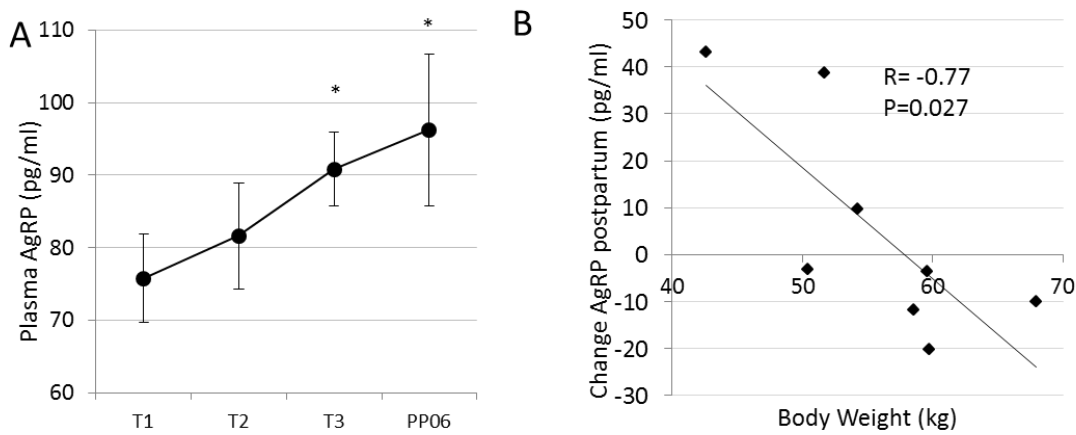
Figure 3 Relation of CSF to serum concentration of a) leptin and b) AgRP during pregnancy and 5±1 years after. N=58 during pregnancy and N=17 at follow up.