Neuropeptide levels, lifestyle and BMI in postpartum women – a follow-up study

Degree Project in Medicine

By: Ameli Ingemansson

Supervisor: Ulrika Andersson Hall
Co-supervisor: Agneta Holmäng

Programme in Medicine

Institute of Neuroscience and Physiology, Department of Physiology,
Sahlgrenska Academy

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1. Abstract

"The association between neuropeptide levels, lifestyle and BMI in postpartum women – a follow-up study"

Author: Ameli Ingemansson.

Supervisor: Ulrika Andersson Hall. Co-supervisor: Agneta Holmäng

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Background: Women with a pre-pregnancy BMI >25 kg/m² both have a higher risk of pregnancy-related complications such as gestational diabetes, as well as of remaining overweight post-partum, which is a well-known risk factor for many welfare-diseases. Among other factors, lifestyle changes and neuropeptide levels may influence BMI development during pregnancy and post-partum.

Aim: To follow up women 3 – 5 year post-partum and to measure their levels of neuropeptides (leptin, insulin and Agouti-related peptide (AgRP)) in cerebrospinal fluid (CSF) and serum (s), and to study if BMI changes (pre- and post-partum) and fat mass (post-partum) were related to neuropeptide levels and insulin sensitivity. Lifestyle characteristics such as diet and physical activity were also studied.

Methods: Women (n=25) were recruited from a previous pregnancy study conducted in 2010 – 2013. Blood and CSF samples were collected at caesarean and post-partum, body composition were determined, and questionnaires regarding dietary intake, physical activity was distributed. The women were divided into three groups based on their BMI changes from pre-pregnancy to follow-up visits: Loss, Stable and Gain.

Results: The lowest BMI was found in the Stable group (BMI 26.2 ± 4.5 kg/m^2 at follow up), where also s-AgRP levels remained higher post-partum compared with the other groups. The BMI Gain group (BMI 31.7 ± 3.1 kg/m^2 at follow up) had the lowest CSF/S leptin quota, highest increase in CSF insulin levels and were also found to have the highest insulin

resistance at follow up (HOMA-IR = 2.4 ± 0.8). Additionally, BMI correlated to changes in s-AgRP levels post-partum (ρ = -0.511) and to CSF/S leptin (ρ = -0.672). Regarding lifestyle, fat intake correlated (ρ = 0.576) to the increment of CSF AgRP after pregnancy, and physical activity correlated (ρ = -0.507) to lower fat mass.

Conclusions: High s-AgRP levels and a high CSF/S leptin quota, in addition to physical activity might predict lower BMI after pregnancy. Evidence of correlation between fat intake and CSF AgRP levels was found for the first time and would be of interest to study further.

Key-words: Agouti-related peptide, weight-loss, pregnancy, lifestyle, BMI.

2. Introduction

The rising proportion of overweight individuals in our population is a major concern. In Sweden, as many as 38.5% of women are overweight (Body Mass Index >25 kg/m^2) or even obese (BMI >30 kg/m^2) at the start of pregnancy (1). The BMI of pregnant women positively correlates with increased risk of maternal and fetal complications (2), where gestational diabetes mellitus is one of the most common health problems (3). Women with gestational diabetes have a higher risk for developing type II diabetes later in life (4). Pre pregnancy BMI together with excessive gestational weight gain during pregnancy does not only predict short-term morbidity and higher weight retention after pregnancy, but also potential lifelong obesity (5). Gestational weight gain alone explains half of the post-partum weight retention (6), and is therefore the greatest independent predictor (7).

During pregnancy the energy intake increases and thermogenesis is supressed (8, 9), to achieve a favourable condition for foetal development and lactation. This change in energy balance is believed to partly depend on the orexigenic Agouti-related Peptide (AgRP) and the anorexigenic neuropeptides leptin and insulin, and their interactions within the brain.

Concurrently with fat-mass gain and placental growth during pregnancy, serum (S) and cerebrospinal fluid (CSF) levels of leptin are increased (10, 11). They are both strongly correlated with BMI and body fat during pregnancy (12). Even though obese and overweight women have higher levels of CSF leptin, there is a negative correlation between the quota CSF/S and increasing BMI, due to a saturable transport of leptin to the brain at the blood-brain barrier (BBB) (13). The contradictory increase in appetite, despite an elevation of this anorexigenic peptide in pregnant women in general, and obese pregnant women in particular (14, 15), points to a central resistance to leptin with increasing central concentrations.

As neuropeptides also interact and regulate each other's secretion, the suppressive effects that leptin exerts on AgRP (16, 17) may be reversed by leptin resistance (18), which is believed to eventually initialize a compensatory increase in CSF AgRP (19-21). Thus, increased food intake in pregnant women can be seen as a consequence of higher central concentrations of AgRP along with weight gain and foetal and placental growth, which might be necessary to counteract the inhibitory effects of leptin at the brain (22). This would further explain how obese women are able to keep a positive energy balance.

The theory of relative resistance due to saturable transport over the BBB is also applicable to insulin (23). Insulin regulates appetite in a faster manner than leptin, as it is secreted as a direct response to food intake, compared to the continuous release of leptin from adipose tissue. Peripheral insulin resistance develops during pregnancy and serum insulin levels are elevated to ensure normal glucose metabolism and adequate nutrition of the foetus. There is an inverse correlation between insulin sensitivity and fat-mass gained in normal weight pregnant women, and this correlation seems to be even more accentuated along with pregnancy, and in obese women (24). The decreased sensitivity to insulin late in pregnancy is believed to be caused by corticosteroids and placental hormones (25). In most cases, insulin sensitivity will return to normal shortly after delivery as the placenta is removed.

Results from an earlier study from our lab showed for the first time that AgRP was produced in the human placenta, and that s-AgRP levels were elevated in pregnancy (19). This study also showed that all three of the neuropeptides AgRP, insulin and leptin in CSF were higher in obese and overweight women than in normal-weight women during pregnancy. Accordingly, insulin and leptin levels in serum were higher in obese women compared to normal weight women, but their quota in CSF/S was lower. Serum (S)-AgRP, however, was higher in serum in normal weight women. The conclusions drawn from this study were that elevated levels of CSF AgRP throughout pregnancy protected women from the suppressive effects of leptin and insulin on appetite, and that high CSF AgRP promoted a positive energy balance in overweight and obese pregnant women. It was also suggested that high s-AgRP levels were coupled with lower BMI and a favourable metabolic profile. These findings support the theory of resistance at the level of the brain for leptin, and suggest that central AgRP interacts with the effects of leptin to meet the metabolic requirements of pregnancy. However, the actions of AgRP on energy balance are still poorly understood, especially during times of weight gain or weight loss, such as during and after pregnancy. A follow up study was therefore initiated to study how the neuropeptides develop along with BMI changes after pregnancy.

The different determinants for weight development after pregnancy are, of course, not only neuropeptides. For example, it has been shown that physical activity (PA) has many beneficial effects after pregnancy, including physical and mental health and also impacts on weight loss (26). If weight loss is a desirable objective PA should be combined with diet recommendations and reduced energy intake (27). Thus, these parameters are also of interest when studying factors connected to weight changes.

3. Aim

The purpose of this study was to follow up women whose neuropeptide levels in the CSF and serum were measured during pregnancy in a study carried out 3 – 5 years earlier (19) to find out how their weights developed after pregnancy, and if weight differences relate to changes in neuropeptide levels, and metabolic health. As diet and PA are believed to play major roles in postpartum weight loss (28), we analyzed PA and food intake. With more knowledge about changes of these parameters, we might find clues to who will normalize their weight, and predict the risk of developing or remaining overweight or obese after pregnancy.

4. Material and methods

4.1 Subjects

All 74 women from the earlier study (19) 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. They were all planned for caesarean section and CSF samples were extracted before the lumbar anaesthesia to minimize the numbers of lumbar puncion. 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 present 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 years after their latest pregnancy.

4.2 Anthropometry and body composition

Body composition of the participants was determined by a whole-body GE Lunar iDXA (Dual-energy x-ray absorptiometry) scan, where fat mass (%) of total body mass was calculated by making transverse scans from head to toe (29). All participants wore light-weight clothes and had their metal jewellery removed. Also android and gynoid distributed fat mass were analysed via Prodigy enCORE software (30). Android fat mass is fat distributed around the abdomen between the ribs and the pelvis, and gynoid fat is the fat distributed around the hips and upper thighs (For visualization see Appendix1). Both were computed in percent of total mass of the area.

4.3 Blood and CSF sampling

After an overnight fast, venous blood and CSF samples were collected. Before spinal anaesthesia lumbar punction was used to extract 12 ml of CSF, and together with blood samples directly transported to the Laboratory for Clinical Chemistry and the Neurochemistry Department and Diagnostics Research Unit at the Sahlgrenska University Hospital in Mölndal.

4.4 Hormone assays

Leptin and AgRP in CSF and serum were analysed by enzyme-linked immunosorbent assay (R&D systems) at the Neurochemistry Department and Research Unit. CSF insulin was analysed with a double antibody radioimmunoassay (Linco Research, St Charles, MO, USA) at the Department of Clinical Science, Lund University. All other biochemical analyses were performed by the accredited clinical chemistry lab (SWEDAC ISO 15189). Due to technical difficulties or subjects declination, CSF was only sampled from 17 of the 25 women.

4.5 Questionnaires regarding lifestyle

The participants reported their dietary intake during the three previous months by completing a food frequency questionnaire. This questionnaire has been validated in the large SOS

(Swedish Obese Subjects) study at Sahlgrenska Academy (31). The participants also completed a short questionnaire regarding PA at work and during spare time where they ranked their levels of activity on a scale from 0/1 – 4 (see Appendix 3). Insulin resistance was estimated by HOMA-IR (Homeostatic Model Assessment – Insulin Resistance) which is a highly sensitive and specific method to assess insulin resistance (32).

All the personal information regarding the participants was transcoded so that no results could be backtracked.

5. Statistical methods

The clinical data and the questionnaire results were collected and entered in Excel databases. Statistical analyses were performed using SPSS.

In the earlier study women were divided into groups based on their BMI before pregnancy; normal weight (NW) with BMI 18.5 – 25 kg/m^2, overweight (OW) with BMI >25 kg/m^2, and obese (OB) with BMI >30 kg/m^2. The women in our study were divided into three groups based on changes in BMI from before pregnancy to after, with a total range of BMI changes from -3.2 – 6.7 kg/m^2. The three groups were divided into quartiles, with a midfield consistent of the second and third quartile. Hence, the 1:st quartile (<-0.6) = BMI Loss, the 2:nd and 3:rd quartile (-0.4 – 2.1) = BMI Stable, and the 4:th quartile (>2.6) = BMI Gain.

The groups were compared for significant differences by medians (because of small sample sizes) using the Kruskal Wallis test, which is a non-parametric analysis of variance that does not assume normal distributed populations. The Whitney U method, with a significance level of <0.05 was used as Post-Hoc test. Because of outliers particularly in serum and CSF-analyses, relations were evaluated by calculation of Spearman's correlation coefficient (ρ).

Even though there were clinical data that deviated >2 SD from the mean, no data were excluded during the analyses. This was because of the restricted numbers of participants, and uncertainty if deviations were due to interpersonal differences or analysing errors.

6. Ethics

This project was undertaken at the Sahlgrenska hospital and was approved by the ethical committee at the University of Gothenburg (dnr 402-08 and dnr 750-15) as a part of the PONCH study. Women from the earlier study (19) were contacted and asked to participate, with information verbally and written. Informed consent was obtained from all participants.

7. Results

7.1 Body composition and background characteristics

7.1.1 Background characteristics

In the earlier study on neuropeptides at caesarean, women were divided into groups based on their pre-pregnancy BMI (Body Mass Index); Normal Weight (NW), Over Weight (OW), and Obese (OB). In the current study, these women were divided into quartiles based on their BMI development from pre-pregnancy to follow up, with the two quartiles in the middle equalling the Stable group. Since the previous study, most women of NW were distributed into the Stable group. OW mostly into Stable and Gain groups, and OB into Loss and Stable groups (Figure 1).

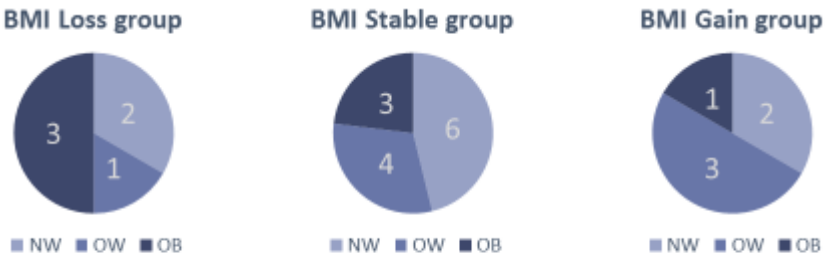

Figure 1. Distribution by numbers of participants from earlier BMI groups; normal weight (NW) over weight (OW) and obese (OB), into new groups based on BMI development from pre-pregnancy to follow-up; Loss, Stable and Gain.

Background characteristics in groups based on BMI development are displayed in Table 1 (for background characteristics based on previous groups, see Appendix 2).

The BMI at follow up was significantly different between the groups, with the Stable group having the lowest BMI. During pregnancy there was no difference in insulin resistance, as measured by the Homeostatic Model Assessment – of Insulin Resistance (HOMA-IR) whereas at follow up the Gain group had higher insulin resistance than the Stable group. No significant differences were found between the groups regarding P-glucose, neither during pregnancy, nor at the follow up (results not shown) and time since last pregnancy.

Table 1. BMI and background characteristics in groups based on BMI development.

	BMI Loss (n)	**BMI Stable** (n)	**BMI Gain** (n)	**P**
Pre-pregnancy BMI (kg/m^2)	29.6 (21.8 – 34.0) (6)	26.2 (18.0 – 33.3) (13)	27.7 (23.3 32.4) (6)	0.345 a. 0.174 b. 0.631 c. 0.380
Gestational weight gain (kg)	11.0 (7.1 – 18.0) (6)	12.0 (-2.0 – 17.0) (13)	16.0 (10.0 – 27.0) (6)	0.100 a. 0.628 b. 0.077 c. 0.053
BMI at follow up (kg/m^2)	27.0 (20.2 – 31.5) (6)	26.2 (18.4 – 33.7) (13)	31.7 (27.6 – 37.0) (6)	**0.024** a. 0.930 **b. 0.016** **c. 0.014**
Weight change post-partum (kg)	-6.2 (-8.8 – -1.8) (6)	1.1 (-1.1 – 5.6) (13)	12.9 (7.1 – 17.3) (6)	**<0.001** **a. 0.001** **b. 0.004** **c. 0.001**
BMI change from pre-pregnancy to follow up (kg/m^2)	-2.2 (-3.2 – -0.7) (6)	0.4 (-0.4 – 2.1) (13)	4.5 (2.7 – 6.7) (6)	**<0.001** **a. 0.001** **b. 0.004** **c. <0.001**
Age (years)	42.1 (36.7 – 45.0) (6)	40.9 (27.4 – 46.7) (13)	36.9 (29.6 – 42.6) (6)	0.163 a. 0.292 b. 0.055 c. 0.237
Parity (n)	3 (2 – 3) (6)	2 (1 – 3) (13)	2 (1 – 2) (6)	**0.035** a. 0.070 **b. 0.018** c. 0.212
Time since last pregnancy (years)	6 (4 – 6) (6)	4 (2 – 6) (13)	6 (4 – 6) (6)	0.468 a. 0.930 b. 1.000 c. 0.329
HOMA-IR during pregnancy	1.1 (0.3 – 4.6) (6)	1.2 (0.1 – 3.4) (13)	1.1 (0.4 – 1.7) (6)	0.866 a. 0.329 b. 0.873 c. 0.539
HOMA-IR at follow up	1.0 (0.8 – 2.8) (5)	1.1 (0.4 – 2.3) (13)	2.4 (0.7 – 2.8) (6)	0.080 a. 0.522

b. 0.273
c. 0.023

Values are medians, with minimum and maximum values within parenthesis. P-values based on Kruskall Wallis. Significance at the <0.05 level.

a. Whitney U comparison between Loss – Stable
b. Whitney U comparison between Loss – Gain
c. Whitney U comparison between Stable – Gain

Parity was significantly higher in the Loss group, with a median of one more child than the other groups. Additionally, we found negative correlations between parity and HOMA-IR, S-leptin, weight change and BMI at follow up. Though these correlations were weak (data not shown), they all pointed in the same direction, with parity in this study being correlated with weight loss and the positive metabolic changes that followed.

7.1.2 BMI development

All groups increased their weight during pregnancy and decreased it at follow up (Figure 2). BMI after pregnancy was significantly different between the groups, with Stable group having the lowest BMI generally. Weight changes significantly differed between the three groups in order Loss – Stable – Gain, and the Loss group not only tended to gain less weight during pregnancy, they also lost significantly more after pregnancy (Table 1).

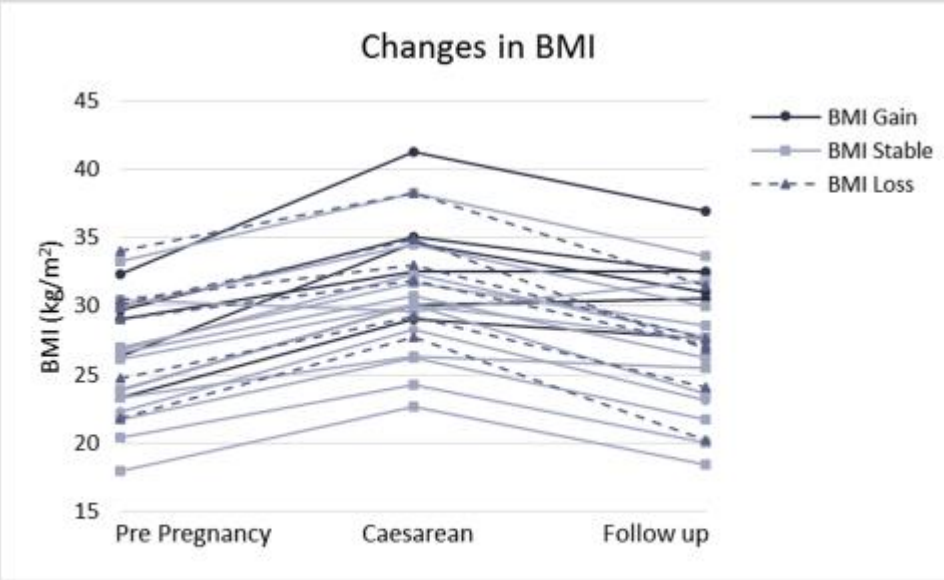

Figure 2. BMI development (kg/m²) from before pregnancy, at caesarean, to follow up, groupwise.

In Table 2 it is shown that BMI at follow up correlated strongly and significantly to serum (s) -leptin, and weaker but significantly to Cerebrospinal Fluid (CSF) leptin. Furthermore it correlated negatively to the ratio CSF/S leptin at follow up, suggesting increased leptin transportation to the brain with loss in BMI. Additionally, weight loss after pregnancy correlated significantly to the change in HOMA-IR which suggests that insulin resistance was reduced by weight loss. BMI at follow up further correlated negatively to the change in s-AgRP levels which may indicate elevated levels of orexigenic s-AgRP among women with lower BMI after pregnancy.

Table 2. Spearman correlations (ρ) to post-partum weight loss and BMI at follow up.

		CSF leptin at follow up	S-leptin at follow up	CSF/S leptin at follow up	S-AgRP change post-partum	HOMA-IR at follow up	HOMA-IR change post-partum
BMI at follow up	ρ	**0.571**	**0.729**	**-0.672**	**-0.511**	**0.500**	**0.455**
	p	0.017	0.000	0.003	0.021	0.013	0.022
	n	17	25	17	20	24	25
Weight loss post-partum	ρ	0.216	**0.439**	-0.459	-0.373	0.373	**0.429**
	p	0.405	0.028	0.064	0.105	0.073	0.033
	n	17	25	17	20	24	25

7.1.3 Body composition

Not surprisingly, fat mass was found to be lower in the BMI Stable group (Table 3) with a tendency of android fat distributed the same between the groups, but gynoid not.

Table 3. Body composition at follow up.

	BMI Loss (n)	**BMI Stable** (n)	**BMI Gain** (n)	**P**
Fat mass (%)	38.5 (29.6 – 45.9) (6)	38.0 (23.3 – 45.5) (13)	43.5 (39.8 – 48.2) (6)	0.077 a. 0.792 b. 0.055 **c. 0.039**
Android fat (%)	45.9 (25.8 – 47.1) (6)	38.6 (17.8 – 53.1) (13)	50.6 (44.2 – 55.1) (6)	**0.039** a. 0.861 **b. 0.025** **c. 0.023**
Gynoid fat (%)	39.6 (36.1 – 50.6) (6)	43.2 (27.7 – 48.9) (13)	46.3 (41.9 – 50.7) (6)	0.096 a. 0.930 b. 0.078 **c. 0.044**

Values are medians, with minimum and maximum values within parenthesis. P-values based on Kruskall Wallis. Significance at the <0.05 level.

a. Whitney U comparison between Loss – Stable
b. Whitney U comparison between Loss – Gain
c. Whitney U comparison between Stable – Gain

Fat mass % positively correlated with s-leptin, CSF leptin, and HOMA-IR at the follow up, which was expected as central and peripheral leptin, as well as insulin resistance, tend to increase with body fat (Table 4.). Furthermore there was negative correlations to CSF/S leptin, s-AgRP and the change of s-AgRP after pregnancy, in accordance with the correlations to BMI values mentioned previously. S-insulin at follow up had a stronger positive correlation to fat with android than gynoid distribution.

Table 4. Spearman correlations (ρ) to body composition at follow up.

		S-insulin at follow up	**CSF leptin at follow up**	**S-leptin at follow up**	**CSF/S leptin at follow up**	**S-AgRP at follow up**	**S-AgRP change post-partum**	**HOMA-IR at follow up**
Fat mass by DXA (%)	ρ	**0.527**	**0.498**	**0.832**	**-0.790**	**-0.445**	**-0.539**	**0.535**
	p	0.007	0.042	0.000	0.000	0.026	0.014	0.007
	n	25	17	25	17	25	20	24
Android fat (%)	ρ	**0.616**	**0.534**	**0.758**	**-0.776**	-0.361	**-0.510**	**0.520**
	p	0.001	0.027	0.000	0.000	0.076	0.022	0.009
	n	25	17	25	17	25	20	24
Gynoid fat (%)	ρ	**0.423**	**0.522**	**0.833**	**-0.685**	-0.325	**-0.522**	**0.410**
	p	0.035	0.032	0.000	0.002	0.113	0.018	0.047
	n	25	17	25	17	25	20	24

7.2 Neuropeptides

7.2.1 Agouti-related peptide (AgRP)

We found an interesting narrowed span of s-AgRP levels at follow up (Figure 3). The Stable group were the only group that remained at high s-AgRP levels after pregnancy, while the other two decreased (Table 5).

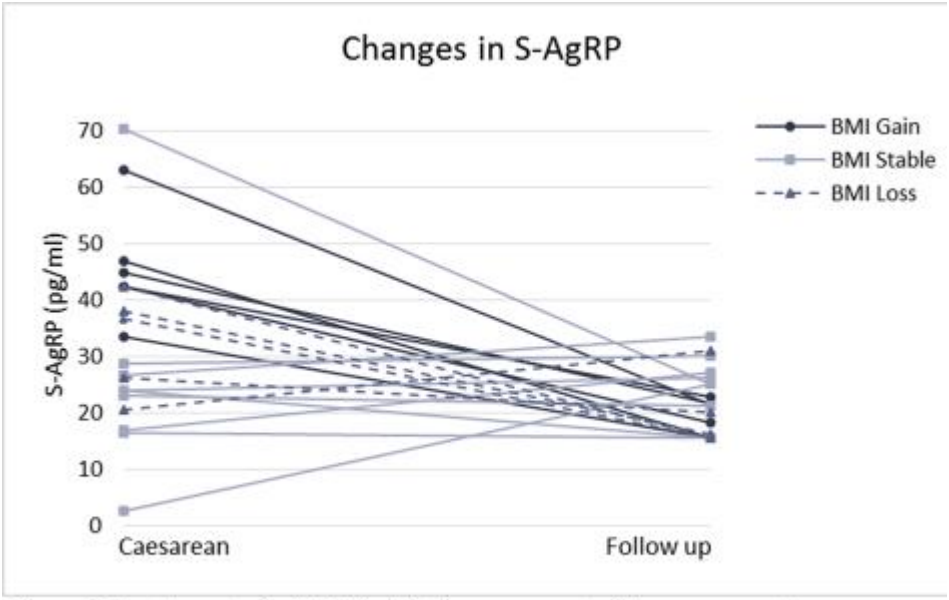

Figure 3. Development of s-AgRP (pg/ml) from caesarean to follow up, groupwise.

Table 5. S-AgRP at follow up (FU) and the difference from pregnancy (Δ).

		BMI Loss (n)	**BMI Stable** (n)	**BMI Gain** (n)	**P**
S-AgRP (pg/ml)	FU	16 (16 – 31) (6)	25 (16 – 33) (13)	20 (16 – 23) (6)	0,471 a. 0.373 b. 0.624 c. 0.285
	Δ	-21 (-27 – 10) (5)	1 (-45 – 22) (9)	-24 (-42 – 18) (6)	**0,035** a. 0.205 b. 0.201 **c. 0.013**
CSF AgRP (pg/ml)	FU	34 (19 – 47) (4)	41 (30 – 63) (19)	38 (35 – 39) (3)	0,432 a. 0.287 b. 0.593 c. 0.351
	Δ	8 (4 – 20) (4)	25 (11 – 43) (8)	12 (-20 – 27) (3)	0,092 **a. 0.026** b. 0.724 c. 0.306

Values are medians, with minimum and maximum values within parenthesis. P-values based on Kruskall Wallis. Significance at the <0.05 level.

a. Whitney U comparison between Loss – Stable
b. Whitney U comparison between Loss – Gain
c. Whitney U comparison between Stable – Gain

Nearly all women increased their CSF AgRP at follow up (Figure 4). The Stable group tended to have a greater increase in CSF AgRP than the other groups (Table 5). Interestingly, changes in CSF AgRP correlated weakly but significantly to changes in fat intake post-partum (See diet correlations in Figure 8). It also negatively correlated to years from last pregnancy (r = -0.608, p = 0.016), in other words; the longer time elapsed since the last pregnancy, the more similar CSF AgRP levels were to those during pregnancy.

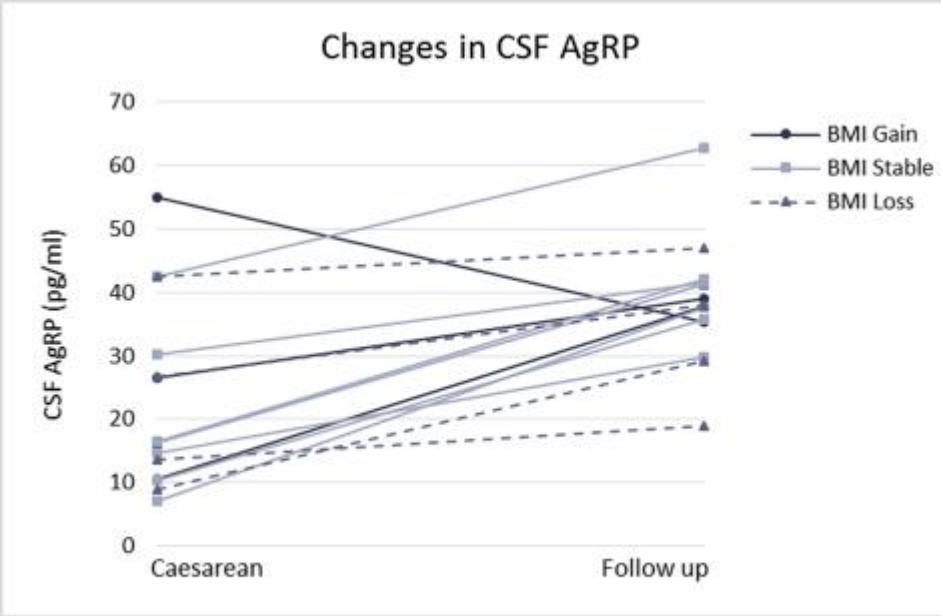

Figure 4. Development of CSF AgRP (pg/ml) from caesarean to follow up, groupwise.

7.2.1. Insulin

Most of the participants had a small increment of their CSF insulin after pregnancy, as can be seen in Figure 5. Three participants decreased their CSF insulin and

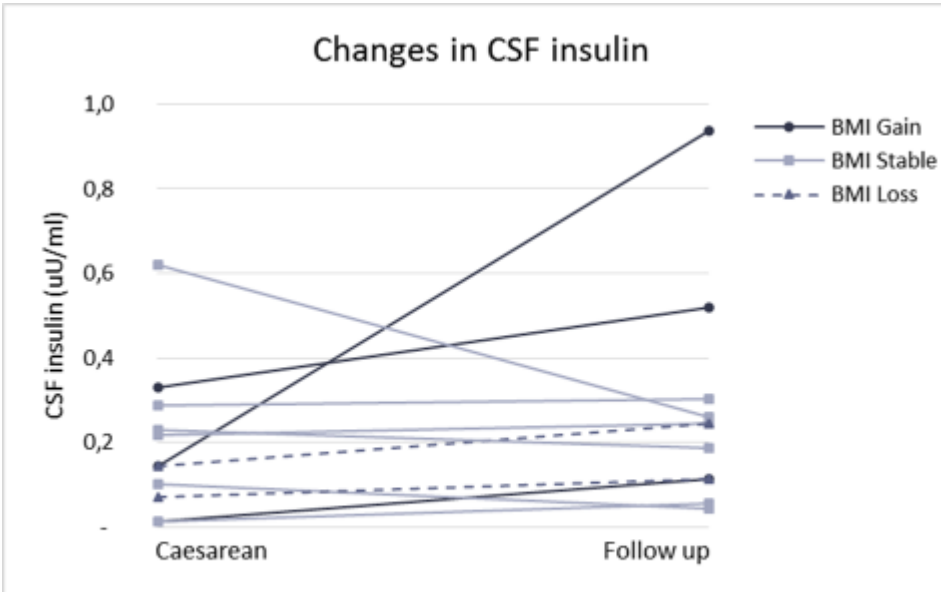

Figure 5. Development of CSF insulin (uU/ml) from caesarean to follow up, groupwise.

they were all from the BMI Stable group. The two women who had the highest increase in CSF insulin were from the BMI Gain group, and this group also increased their CSF insulin significantly more than the other groups. No differences regarding serum insulin were found between the groups (results not shown).

Table 6. CSF insulin at follow up (FU) and the difference from pregnancy (Δ).

		BMI Loss (n)	**BMI Stable** (n)	**BMI Gain** (n)	**P**
CSF Insulin (uU/ml)	FU	0,22 (0,12 – 0,39) (4)	0,06 (0,04 – 0,30) (7)	0,52 (0,12 – 0,94) (3)	0,179 a. 0.296 b. 0.372 c. 0.086
	Δ	0,04 (-0,04 – 0,10) (3)	0,01 (-0,36 – 0,04) (5)	0,19 (0,10 – 079) (3)	**0,036** a. 0.180 b. 0.077 **c. 0.025**

Values are medians, with minimum and maximum values within parenthesis. P-values based on Kruskall Wallis. Significance at the <0.05 level.

a. Whitney U comparison between Loss – Stable
b. Whitney U comparison between Loss – Gain
c. Whitney U comparison between Stable – Gain

7.2.2 Leptin

CSF/S leptin levels in the BMI Gain group was lower than the Stable group at follow up (Figure 6 and Table 7). The saturable transport over the BBB developing during pregnancy did thus not in general seem to be reversed post-partum as many of the participants actually increased their CSF/S leptin quota post-partum.

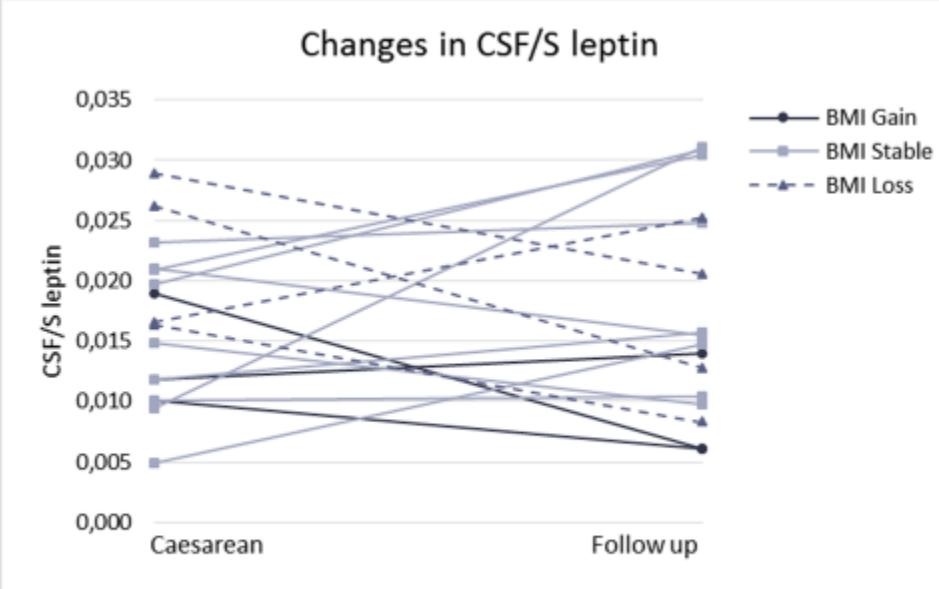

Figure 6. Development of leptin transportation at the blood-brain barrier from caesarean to follow up, groupwise.

Table 7. Serum and CSF/S leptin at follow up (FU) and the difference from pregnancy (Δ).

		BMI Loss (n)	**BMI Stable** (n)	**BMI Gain** (n)	**P**
CSF/S leptin	FU	0,017 (0008 – 0,025) (4)	0,020 (0,010 – 0,031) (10)	0,006 (0,006 – 0,14) (3)	0,073 a. 0.396 b. 0.154 **c. 0.028**
	Δ	-0,008 (-0,013 – 0,009) (3)	0,003 (-0,006 – 0,022) (8)	-0,004 (-0,013 – 0,002) (3)	0,240 a. 0.153 b. 0.827 c. 0.221

Values are medians, with minimum and maximum values within parenthesis. P-values based on Kruskall Wallis. Significance at the <0.05 level.

a. Whitney U comparison between Loss – Stable
b. Whitney U comparison between Loss – Gain
c. Whitney U comparison between Stable – Gain

7.3 Lifestyle

In Figure 7 it is shown that in the BMI Stable group we found that all of the participants except for one person decreased their energy intake. In the other groups the change in energy intake was more diverse.

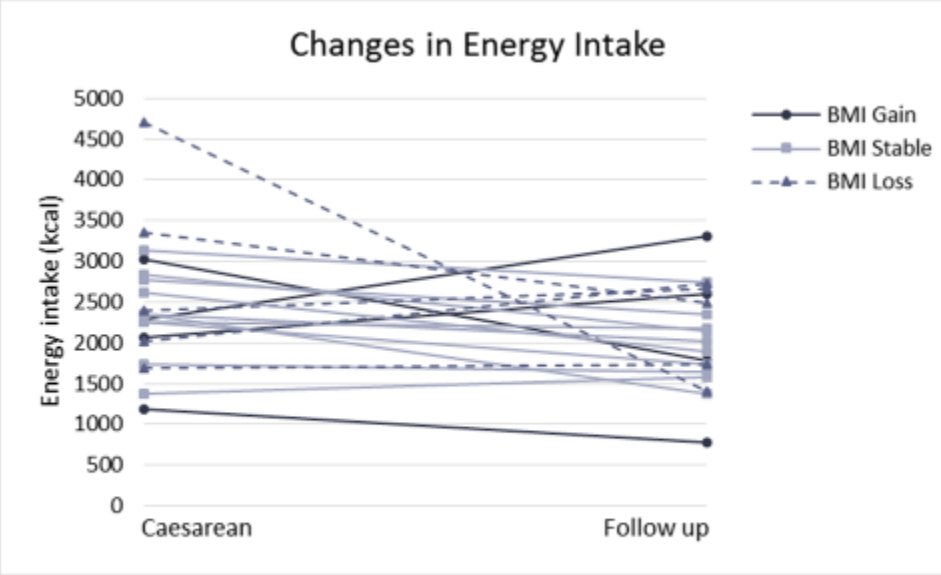

Figure 7. Development of energy intake (kcal) from caesarean to follow up, groupwise.

There were no significant differences regarding diet or physical activity (PA) between the groups (results not shown). Neither was there any correlation between BMI at follow up and diet. However, the change in CSF AgRP correlated significantly to changes in fat intake post-partum, with no correlation to any other macronutrient (Figure 8).

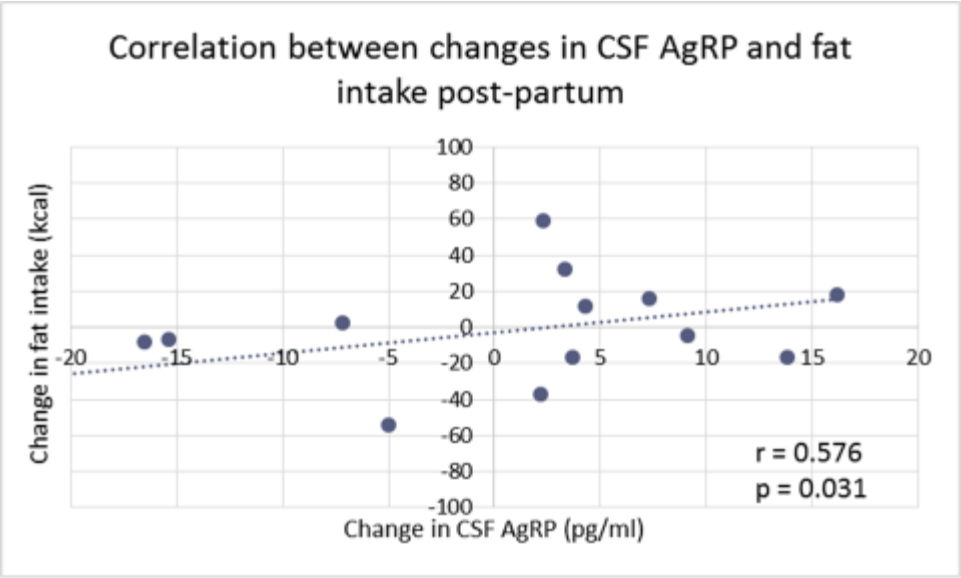

Figure 8. Correlation between changes in fat intake and CSF AgRP post-partum.

Table 8. Spearman correlations (ρ) to physical activity at follow up.

		S-leptin at follow up	S-leptin change post-partum	Fat mass at follow up	Gynoid fat at follow up	Android fat at follow up
Physical activity - leisure	ρ	**-0.482**	**-0.462**	**-0.507**	**-0.547**	**-0.359**
	p	0.015	0.023	0.010	0.005	0.078
	n	25	24	25	25	25
Physical activity - total	ρ	-0.288	-0.393	-0.272	**-0.397**	**-0.126**
	p	0.162	0.058	0.188	0.050	0.547
	n	25	24	25	25	25

There were significant negative correlations between PA at spare time and fat mass. However, android fat did not correlate, but gynoid fat did (Table 8). No significant differences were found between the groups (results not shown).

8. Discussion

Our major findings were that the Stable group had the lowest BMI, and also remained at higher serum Agouti-related peptide (s-AgRP) levels post-partum than the other groups. The BMI Gain group increased their levels of insulin in CSF, and had higher peripheral insulin resistance, measured by HOMA-IR, at the follow up. Low BMI correlated to physical activity (PA), to the increment of s-AgRP, to lower insulin resistance, and to higher CSF/S leptin quota. Evidence of correlation between fat intake and levels of CSF AgRP was found for the first time in human.

Characteristic for the women in the Gain group was that they retained more weight after pregnancy. Furthermore they decreased their levels of peripheral AgRP more than the other groups, and low s-AgRP levels are, as mentioned previously, suggested to be connected to higher BMI (19). They also increased their CSF insulin levels more than the other groups and had higher peripheral insulin resistance, measured by HOMA-IR at follow up.

Women with decreased weight compared with before pregnancy tended to gain less weight during pregnancy, and lost significantly more after pregnancy compared to the other groups, which may depend on lifestyle changes such as more physical activity, since this correlated to fat mass after pregnancy.

The weight-stable women in our study remained at higher s-AgRP after pregnancy. The overall reduction of s-AgRP levels at follow up may be due to the removal of the placenta, which during pregnancy contributes to the peripheral synthesis that makes s-AgRP levels higher and probably also to larger variability during this period. Other peripheral sources of AgRP synthesis are adrenal glands, kidneys, and lungs (33). AgRP is released diurnally from the hypothalamus along with fasting (34) and may be elevated in serum from the adrenal glands in response to exercise (35). The increase in CSF AgRP might be connected to PA and

low BMI together with higher levels of peripheral AgRP found in this study. Women that kept a stable BMI from before pregnancy had lower BMI, fat mass, insulin resistance and CSF insulin. The low and stable levels of s-AgRP further confirms earlier findings which suggests that lower BMI is connected to higher s-AgRP levels in serum (12, 18, 19).

Findings of a correlation between changes in CSF AgRP and changes in fat intake are in accordance with studies on rodents. These studies showed that higher fat intake promotes leptin resistance (36) via an inflammatory process at the AgRP neurons in the hypothalamus (37, 38), with a subsequent increase in CSF AgRP due to the lowered potency of leptin's suppressive effect on these neurons (21, 39). This implicates that fat intake could have an orexigenic effect through altered levels of CSF AgRP, which remains to be proven in humans. Wei W et al. further describe that mice fed a high fat diet, even for a short period, have elevated CSF AgRP levels, but do not develop leptin resistance (21). In other words, fat intake may have a greater impact on energy balance than previously believed, and as described by Milanski et al., even fat quality may impact on the inflammatory response at the AgRP neurons (40). This result is interesting and needs to be studied further, especially with a larger sample size included, and also with determining of fat quality in the diet.

BMI and fat mass at follow up additionally correlated to parameters that have been concluded to be coupled to BMI in earlier studies, such as serum and CSF leptin, HOMA-IR and negatively to CSF/S leptin (19, 41, 42). The lower CSF/S leptin during pregnancy correlated to BMI, and was not in general reversed at follow up. Thus this satiety in transportation to the brain would rather be linked to BMI and fat mass per se.

The negative correlation between parity and HOMA-IR at pregnancy and to HOMA-IR change after pregnancy indicates development of increased insulin resistance with each

pregnancy, but also a greater reversion post-partum, which are both well-known phenomena (43).

One of the strengths of this study is the even distribution between normal weight, over weight and obese participants from the first study. Additionally we were able to look at a longitudinal prospective development of the same women's BMI and neuropeptides, instead of comparing pregnant women to a non-pregnant group. Another advantage is that the time span since their last pregnancy was long enough that their weight and neuropeptide levels should have stabilized.

A factor to take into consideration was that as the samples were extracted at different times for caesarean and follow up, the neuropeptides were not analysed at the same time in parallel. Hence, there could be systematic errors regarding especially the AgRP and leptin components of the results in thus study. S-insulin was measured by a certified clinical chemistry lab, which should give reproducible results. Both sets of CSF insulin samples were measured simultaneously, and plans to do the same for AgRP and leptin have been made although they were not possible to carry out within the scope of this project. However, looking both at absolute values and changes from one time-point to another reduces the impact of systematic errors.

We should furthermore take into account that planned caesarean section is not randomly performed on pregnant women, but inclusion and exclusion criteria in this study should minimize the risk that our women should differ in terms of progression of the pregnancy. There is also difficulty to recruit women for this kind of study, since testing may give rise to pain, and there was also a small risk for transient health complications from CSF sampling (post-spinal headache). It is also quite time-consuming to fill in extensive questionnaires, so it is possible that women who chose to participate in the follow up study are more ambitious and caring about a healthy lifestyle than women in the general population. This suggests a

possibility of bias in the recruited group, and might lead to selection towards a specific social class and tends to exclude others.

As these results are based on a small group of individuals, they are mostly indicators of what could be of interest for further studies. Special care should be taken when interpreting results on diet and physical activity since there could be difficulties in questionnaire reliability (31). Especially for diet there is a known phenomenon that self-reported diet tends not to be totally reliable, particularly not for obese subjects (44), and on basis of a small number of participants. Hence, no significant differences between the groups were found regarding PA and diet. Though we decided to only use the interpersonal results from the dietary questionnaire, so that at least the changes in diet would be rather reliable since these results are based on reports from the same person, only at different times. Also, using correlations in addition to group analysis allowed us to use the full material as one group with a bigger sample size.

9. Conclusions

For the first time, it was possible to show that CSF AgRP in humans was correlated to fat intake. Both fat intake and fat quality would be interesting to study further, as this study suggests that diet composition could contribute to increased levels of CSF AgRP, and hence energy-homeostasis.

S-AgRP was higher in participants with lower BMI, and BMI correlated negatively to changes in s-AgRP which further confirms earlier findings. Women with lower BMI were also more weight stable than the other groups which has not been shown before. Thus, high levels of s-AgRP and CSF/S leptin after pregnancy, in addition to physical activity might predict who will keep a low and stable BMI.

It is difficult to interpret whether body composition and habits determine neuropeptide levels, or if the reverse case is true. More longitudinal studies would therefore be of interest.

10. Populärvetenskaplig sammanfattning på Svenska

”Neuropeptider, livsstil och BMI efter graviditeten - en uppföljande studie”

Av: Ameli Ingemansson.
Handledare: Ulrika Andersson Hall och Agneta Holmäng
Examensarbete, läkarprogrammet, HT 2016.
Institutet för neurovetenskap och fysiologi, Sahlgrenska akademien, Göteborgs universitet.

Övervikt och fetma är som bekant en av de farligaste riskfaktorerna för flertalet välfärdssjukdomar. Under graviditeten ökar risken för komplikationer hos både mor och barn om kvinnan är överviktig eller fet (har ett ”Body mass index” (BMI) över 25 kg/m^2). Sjukdomar som graviditets-diabetes och blodproppar ökar med stigande BMI, och risken att förbli överviktig/fet ökar ju mer man lagt på sig under graviditeten. Vid en normal graviditet ökar kvinnan i vikt trots att koncentrationen av centralt verkande hungerdämpande hormoner som insulin och leptin ökar. Leptin frigörs av fettmassa och från moderkakan, varför dessa nivåer stiger under graviditeten och vid ökande BMI. Insulin ökar också med stigande BMI, men frigörs snarare vid måltider än kontinuerligt, såsom leptin. Dessa båda koncentrationer stiger således även hos överviktiga/feta kvinnor under graviditeten. Trots att nivåerna av dessa båda hormoner stiger i både serum och i centrala nervsystemet (CNS) under graviditeten och vid ökande BMI, så verkar det föreligga någon form av mättnad både för transporten till hjärnan men också en sorts resistens i hjärnan, ju högre nivåer man utvecklar. Det är på så sätt man förklarar hur överviktiga kvinnor under graviditeten ändå kan tillgodogöra sig energi och fettmassa på liknande sätt som normalviktiga dito, trots höga nivåer av dessa hungerdämpande hormoner.

I en tidigare studie från forskargruppen fastslog man bland annat att moderkakan inte bara producerar hungerdämpande hormoner utan även ett hormon som verkar hungerstimulerande i CNS (AgRP). Detta hormonen har dock en annan effekt i blodet än i hjärnan, och man kopplade samma höga nivåer av AgRP i blod med lågt BMI. Man föreslog också en kompensatorisk ökning av det hungerstimulerande AgRP i CNS som svar på höga nivåer av insulin och leptin, men ville följa upp dessa resultat för att få en mer mångfacetterad bild av kvinnornas hormonnivåer och BMI-utveckling efter genomgången graviditet.

Den här uppföljande studien syftade således till att söka utröna vilka kvinnor som återgick till samma vikt som före graviditeten, vilka som ökade och vilka som minskade i vikt efter graviditeten och vad som var karaktäristiskt för de olika utfallen. Förhoppningen var att resultaten framöver skulle kunna bidra till förutse vilka kvinnor som skulle få vilket utfall. Föreslagna metoder för viktnedgång brukar vara kostrestriktioner och fysisk aktivitet, varför vi även tagit hänsyn till detta i denna studie. Vår primära frågeställning var dock vilka nivåer deras hungerstimulerande- och hungerdämpande hormoner låg på nu, hur de utvecklats sedan kvinnorna deltog i den föregående studien vid deras kejsarsnitt, samt hur detta hängde samman med deras viktutveckling och kroppsammansättning efter graviditeten.

Det togs därför blod och ryggmärgsvätska, och därefter vägdes, mättes och genomfördes en kroppssammansättningsmätning på kvinnorna. Sedan indelades de i tre grupper utifrån hur deras BMI utvecklats över graviditeten och i efterförloppet. Det blev en Uppgångs-, en Stabil- och en Nedgångsgrupp.

Våra primära resultat avseende dessa grupper var att den Stabila gruppen var den som hade lägst BMI, vilket var överraskande. Denna grupp låg också kvar på höga nivåer av AgRP i blodet. Lågt BMI korrelerade med ökade nivåer av s-AgRP och ökad leptintransport till hjärnan. Uppgångsgruppen visade sig ha sänkt sina s-AgRP-nivåer mest. Dessutom hade de

ökat koncentrationen av insulin i CNS mest, och hade även högst perifer insulinresistens av grupperna, vilka båda är bidragande faktorer till det metabola syndromet som sammanhänger med högre BMI. För första gången på människor, hittade vi också samband mellan fettintaget och AgRP i CNS, vilket föranleder misstankar om att kostsammansättningen kan påverka aptiten i högre grad än vad som tidigare trotts. Man kunde inte se någon reversering efter genomgången graviditet avseende den mättade leptintransporten till hjärnan, vilket talar för att denna transport snarare beror på BMI än graviditet.

Den enda livsstilskomponenten som kunde kopplas till BMI var fysisk aktivitet på fritiden. Signifikant för ett stabilt och lågt BMI verkade således generellt vara höga nivåer av hungerstimulerande hormonet AgRP i serum och en hög leptintransport till hjärnan, i kombination med träning.

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13. Appendeces

Appendix 1. Gynoid and android fat distribution

Appendix 2. BMI and background characteristics in earlier BMI groups; normal weight (NW), over weight (OW) and obese (OB)

	NW (n)	**OW** (n)	**OB** (n)	**P**

Pre-pregnancy BMI (kg/m^2)	22.8 (18.0 – 24.8) (10)	26.9 (26.3 – 29.7) (8)	30.4 (30.0 – 34.0) (7)	**<0.001** **a. <0.001** **b. 0.001** **c. 0.001**
Gestational weight gain (kg)	14.5 (8.0 – 18.0) (10)	12.0 (9.0 – 24.0) (8)	11.1 (-2.0 – 27.0) (7)	0.412 a. 0.326 b. 0.239 c. 0.642
BMI at follow up (kg/m^2)	23.3 (18.4 – 30.6) (10)	28.2 (26.2 – 32.6) (8)	31.5 (26.9 – 37.0) (7)	**0.002** **a. 0.004** **b. 0.003** c. 0.298
Weight change post-partum (kg)	0.5 (-4.9 – 17.3) (10)	4.1 (-6.0 – 13.6) (8)	-0.1 (-8.8 – 13.9) (7)	0.327 a. 0.424 b. 0.354 c. 0.165
BMI change from pre-pregnancy to follow up (kg/m^2)	0.2 (-1.6 – 6.7) (10)	1.5 (-1.9 – 4.7) (8)	0.0 (-3.2 – 4.6) (7)	0.309 a. 0.450 b. 0.379 c. 0.132
Age (years)	40.6 (29.6 – 46.7) (10)	40.0 (31.6 – 45.0) (8)	41.3 (27.4 – 43.2) (7)	0.881 a. 0.593 b. 0.884 c. 0.817
Parity (n)	2 (2 – 3) (10)	2 (2 – 2) (8)	2 (1 – 3) (7)	0.297 a. 0.593 b. 0.592 c. 0.579
Time since last pregnancy (years)	6 (4 – 6) (10)	5 (2 – 6) 8	4 (3 – 6) (7)	0.102 a. 0.163 **b. 0.033** c. 0.527
HOMA-IR during pregnancy	0.9 (0.2 – 3.4) (10)	1.3 (0.1 – 1.7) (8)	1.1 (0.4 – 4.6) (7)	0.834 a. 0.722 b. 0.558 c. 0.817
HOMA-IR at follow up	1.1 (0.4 – 2.3) (9)	1.0 (0.4 – 2.8) (8)	1.7 (0.8 – 2.8) (7)	0.217 a. 0.847 b. 0.101 c. 0.165

Values are medians, with minimum and maximum values within parenthesis. P-values based on Kruskall Wallis. Significance at the <0.005 level.

a. Whitney U comparison between NW – OW
b. Whitney U comparison between NW – OB
c. Whitney U comparison between OW – OB

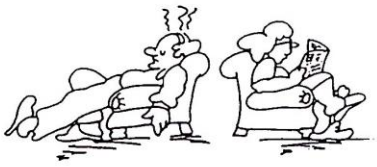
Appendix 3.

FRÅGOR OM FYSISK AKTIVITET

386. HUR MYCKET RÖR OCH ANSTRÄNGER DU DIG KROPPSLIGT UNDER DIN FRITID?

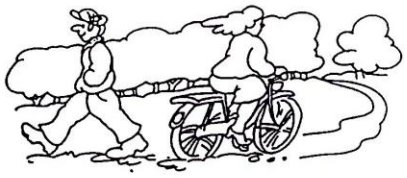
Om Din aktivitet varierar mycket mellan t.ex. sommar och vinter, så försök att ta ett genomsnitt. Frågan gäller det senaste året.

Välj den grupp som bäst motsvarar din egen fysiska aktivitet.



Grupp 1 Stillasittande fritid ☐

Du ägnar dig mestadels åt läsning, handarbete, TV, bio eller annan stillasittande sysselsättning på fritiden.

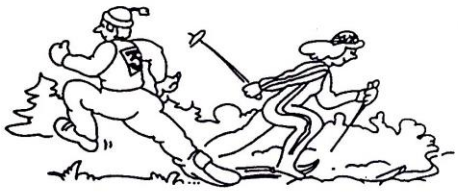


Grupp 2 **Måttlig motion på fritiden** ☐

Du promenerar, cyklar eller rör dig på annat sätt under minst 4 timmar i veckan. I detta inräknas också gång eller cykling till och från arbetet samt söndagspromenader, ordinärt trädgårdsarbete, fiske, bordtennis, bowling.

Grupp 3 **Regelbunden motion och träning** ☐

Du ägnar dig åt t.ex. löpning, simning, tennis, badminton, motionsgymnastik eller liknande, som motionssport. Tyngre trädgårdsarbete och liknande räknas till denna grupp. För att kryssa i grupp 3 skall Du ägna dig åt dessa aktiviteter minst 3 timmar i veckan.



Grupp 4 **Hård träning eller tävlingsidrott** ☐

Du ägnar dig åt hård träning och tävling i löpning, orientering, skidåkning, simning, fotboll, handboll etc regelbundet och minst 4 gånger i veckan.

ett kryss

387. HUR MYCKET RÖR OCH ANSTRÄNGER DU DIG KROPPSLIGT I DITT ARBETE?

Vi vill veta hur kroppsligt ansträngande ditt arbete är, hur mycket Du går, springer, klättrar och lyfter. De yrken, som finns upptagna i nedanstående grupper är bara exempel. Frågan gäller det senaste året. Välj den grupp som bäst motsvarar ditt arbete vad gäller fysisk ansträngning.

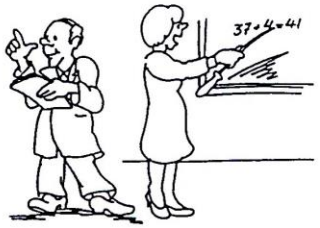
Grupp 0 Ej yrkesarbetande ☐

Pensionärer eller långtidssjukskrivna fyller i grupp 0 eftersom frågan gäller fysisk ansträngning i yrkeslivet.

Grupp 1 Stillasittande arbete ☐

Du har övervägande stillasittande arbete och går inte mycket under arbetstiden. Exempel på sådana arbeten är skrivbordsarbete, urmakeri och montering av lättare delar.

Grupp 2 Lätt men något rörligt arbete ☐



Du har ett arbete där Du går ganska mycket men ej bär eller lyfter tyngre saker. Exempel på sådana arbeten är rörligt expeditionsarbete, lätt industriarbete, förmanssysslor, sådan undervisning där man går mycket, arbete som affärsbiträde.

Grupp 3 Måttligt tungt arbete ☐

Du går mycket och lyfter dessutom ganska mycket eller går mycket uppför trappor eller backar. Exempel är brevbärning, arbete vid tyngre industri, byggnadssnickeri, rörläggningsarbete.

Grupp 4 Tungt arbete ☐

Du har ett tungt kroppsarbete, lyfter tunga föremål och anstränger dig mycket kroppsligt. Exempel är skogsarbete, tungt lantbruksarbete, fiske med tunga redskap, byggnadsgrovarbete.

ett kryss