

Cerebrospinal fluid levels of insulin, leptin, and agouti-related protein in relation to BMI in pregnant women

Gustavsson C1, Andersson Hall U1, Pelanis A2, Karlsson OI2, Andersson L1, Svedin P1, Mallard C1, Myntti A1, Andreasson U3, Zetterberg H3,4, Blennow K3, Holmäng A1.

¹ Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy at University of Gothenburg, Box 432, SE-405 30 Gothenburg, Sweden

² Department of Anesthesiology, Sahlgrenska University Hospital, Gothenburg, Sweden

³ Clinical Neurochemistry Lab, Institute of Neuroscience and Physiology, Sahlgrenska Academy at University of Gothenburg, SE-431 80 Mölndal, Sweden

⁴ UCL Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom

Abbreviated Title: Cerebrospinal fluid levels of insulin, leptin and agouti-related protein in pregnant women.

Key terms: cerebrospinal fluid, insulin, leptin, agouti-related protein, pregnancy

Word count:

Number of figures and tables:

Corresponding author and person to whom reprint requests should be addressed:

Carolina Gustavsson, Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy at University of Gothenburg, Box 432, SE-405 30 Gothenburg, Sweden

Disclosure Statement: The authors have nothing to disclose.

Funding sources: Supported by grants from Novo Nordisk Foundation, the Swedish Research Council (No. 12206 and 14002), the Swedish Diabetes Association Research Foundation, The Swedish federal government under the LUA/ALF agreement, IngaBritt and Arne Lundbergs Foundation, Freemasonry Barnhus Board in Gothenburg, Hjärnfonden, the Knut and Alice Wallenberg Foundation and the Torsten Söderberg Foundation. The study sponsors had no involvement in the study design, in the collection, analysis or interpretation of data, in the writing of the manuscript or in the decision to submit the manuscript for publication.

Abstract

OBJECTIVE: During pregnancy, metabolic interactions must be adapted, though neuroendocrine mechanisms for increased food intake are poorly understood. The objective of this study was to characterize differences in insulin, leptin, and agouti-related protein (AgRP) levels in serum and cerebrospinal fluid (CSF) in pregnant women with normal weight (NW) and pregnant women with overweight (OW) or obesity (OB). Placenta as a source for increased peripheral AgRP levels during pregnancy was also investigated.

METHODS: Women were recruited at admission for elective cesarean section. Insulin, AgRP, and leptin were measured in serum and CSF from 30 NW, 25 OW, and 21 OB at term. Serum during pregnancy and placenta at term were collected for further AgRP analysis.

RESULTS: Immunohistology showed placental production of AgRP and serum AgRP levels increased throughout pregnancy. CSF AgRP, leptin, and insulin levels were higher in OW and OB than NW. Serum leptin and insulin levels were higher and AgRP lower in OB than NW.

CONCLUSIONS: High serum AgRP levels might protect from the suppressive effects of leptin during pregnancy. Pregnant women with OB and OW might further be protected from the suppressive effect of leptin by high CSF AgRP levels. Evidence was found, for the first time, of human placental AgRP production mirrored by levels in the circulation.

The rising tide of obesity in women of reproductive age is of particular concern. In the US, the prevalence is 32.4%—an increase of 70% over a 10-year period¹. Among pregnant women in the US, the prevalence of overweight and obesity has almost doubled in the last 20 years, and nearly half of women in the US begin their pregnancies overweight or obese¹. An elevated pre-pregnancy BMI is associated with a linear increase in adverse maternal and fetal outcomes². High pre-gravid BMI and excessive gestational weight gain (GWG) are predictors of short-term postpartum morbidity and higher postpartum weight retention; the latter increases the risk of future pregnancies and of lifelong obesity³.

Pregnancy is characterized by increased metabolism and neuroendocrine changes. For example, food intake is increased and thermogenesis is suppressed^{4,5} to produce a favorable energy balance for the growth and development of fetal and maternal tissues and to increase fat stores in preparation for lactation. However, the mechanisms of the increased food intake are poorly understood. The hypothalamus is the primary brain region regulating energy homeostasis. The arcuate nucleus, a key hypothalamic area involved in food intake and body weight, consists of two neuronal populations with opposite effects on food intake: neurons that co-express agouti-related protein (AgRP) and neuropeptide Y (NPY), which stimulate food intake, and neurons that when activated co-express pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), which inhibit food intake⁶. Both AgRP/NPY and POMC neurons are important target areas for insulin and leptin and act as sensors of peripheral energy stores.

During pregnancy, serum leptin levels are elevated^{7,8}. Leptin is well known as an adipocyte-derived hormone that reduces appetite, but the level of hyperleptinemia observed in pregnant humans and animals suggest that leptin insensitivity is a feature of pregnancy. In humans, leptin seems to be synthesized by the placenta, as the *ob* gene is expressed in syncytiotrophoblasts, trophoblasts, and amnion cells⁹. Consistent with placental production, leptin levels are highest during the second trimester of human pregnancy and fall abruptly postpartum¹⁰. Leptin usually reduces food intake by binding to the long form of the receptor (OB-Rb) in the hypothalamus and modulating the activity of key neurons, including AgRP and POMC neurons, that regulate energy expenditure and appetite. Leptin binds to POMC and the POMC-derived MSH peptides that decrease calorie consumption, and

suppresses release of AgRP. This orexigenic peptide stimulates food intake by counteracting the effects of alpha-MSH at brain melanocortin receptors¹¹. This hypothalamic bioactivity requires transport of leptin into the CNS. Leptin is too large to cross the blood–brain barrier through simple diffusion and is believed to get into the brain via a saturable transport system and carried by the short form of the leptin receptor¹¹. Leptin may also have access to brain areas outside the blood–brain barrier. Despite the hyperleptinemia of pregnancy, calorie consumption and appetite increase during gestation, pointing to the existence of a leptin-resistant state at the level of the hypothalamus that is likely modified to assure that the metabolic needs of the developing fetus are met¹².

In women, insulin resistance gradually develops during pregnancy to ensure adequate nutrition for the fetus, and insulin secretion increases to maintain normal glucose metabolism. In lean women, the changes in insulin sensitivity during pregnancy are inversely related to the increasing maternal fat mass¹³. Because it is released immediately in response to food intake, insulin is a likely candidate for feedback mechanisms that down-regulate appetite and thereby end food intake. Entry of insulin into the brain is likely facilitated by an insulin receptor-mediated transporter, which functions mainly at physiological levels of plasma insulin¹⁴. Insulin receptors are found in several brain regions but do not regulate glucose metabolism in the CNS. Interestingly, the CSF/serum insulin ratio is significantly associated with whole-body insulin sensitivity, and insulin transport into the CSF is reduced in insulin-resistant subjects¹⁵.

Insulin mainly acts in the arcuate nucleus and binds to its receptors highly expressed in NPY/AgRP and POMC/CART neurons. Because it decreases NPY and stimulates POMC expression, insulin promotes reduced food intake¹⁶. Insulin and leptin both activate POMC neurons, but they seem to differentially regulate AgRP, with insulin stimulating and leptin inhibiting its synthesis¹⁷. AgRP may be important during pregnancy, both for maternal energy expenditure and for fetal development. AgRP expression in the hypothalamus and placenta, and its serum and CSF levels, increase during pregnancy, and the placenta is the main peripheral source of AgRP¹⁸.

The aim of this study was to characterize the differences in insulin, leptin, and AgRP levels in serum and CSF in relation to BMI, energy intake, weight gain, and insulin sensitivity in normal weight, overweight, and obese pregnant women at term.

Methods

Subjects

This prospective study was approved by the local ethics committee at the University of Gothenburg (nr 402–08) and performed at Sahlgrenska University Hospital, Gothenburg, Sweden. Written informed consent was obtained from all subjects before participation. Subjects were 74 healthy women with uncomplicated pregnancies at term (age 33.4 ± 0.5 yr, mean \pm SEM) divided into three groups based on BMI at the first antenatal visit: normal weight (18.5–24.9 kg/m², n = 30), overweight (25.0–29.9 kg/m², n = 21), and obese (≥ 30 kg/m², n = 25). Subjects were screened by medical history before participation. All subjects were nonsmokers in good general health who were on no medications. Subjects were free from past or present major psychiatric disorders, alcoholism, neurological disease, diabetes, and renal and hepatic disease. Subjects were excluded if they had used weight loss supplements or dieted during the 6 months before the study. Subject characteristics are presented in Table 1.

Protocol

All pregnant subjects were undergoing elective cesarean section the morning after an overnight fast. Before surgery, a self-administered dietary questionnaire was used to assess energy intake during the 3 previous months¹⁹. The questionnaire has a semi-quantitative food frequency design and was validated in Swedish men and nonpregnant women against a 4-day food record and 24-h energy expenditure and nitrogen excretion. From these comparisons, valid estimates of energy intake were obtained in normal weight, overweight, and obese subjects¹⁹.

A 10-ml venous blood sample was taken by venipuncture before infusion of 1.0 liter of Ringer-acetate solution. After the infusion and before spinal anesthesia, the patient was placed in a seated or lying position, an introducer needle was inserted into the interspinous ligament at L3-4, and a 25-gauge Whitacre 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. In all groups, the first 0.5 ml of CSF obtained was discarded before collection of the sample for study. All CSF samples were immediately transferred to polyethylene tubes and placed on ice. Samples were then centrifuged,

aliquoted, and stored at -80°C until assays were performed. The serum samples obtained immediately before CSF collection were similarly centrifuged, aliquoted, and stored at -80°C .

Hormone assays

Biochemical analyses were performed by the accredited (SWEDAC ISO 15189) Laboratory for Clinical Chemistry and the Neurochemistry Department and Diagnostics Research Unit at Mölndals Sjukhus, Sahlgrenska University Hospital. Insulin was measured with the Elecsys kit (Roche, Cat. no. 12017547122) on a fully integrated Cobas 6000 analyzer (Roche). ELISA kits were used for agouti-related protein (AgRP, R&D Systems, Cat. no. DAGR00), pro-opiomelanocortin (POMC, MyBioSource, Cat no. MBS2508350), and leptin (R&D Systems, Cat. no. DLP00). All assays were performed as recommended by the manufacturer. The ELISA plates were read on a Vmax plate reader (Molecular Devices), and the concentrations were determined with Softmax software (Molecular Devices). Insulin, AgRP, and POMC were analyzed in undiluted samples. For analysis of leptin, CSF samples were diluted 2-fold and serum samples 100-fold. The quantitative insulin sensitivity check index (QUICKI) was calculated as $1/(\text{Log}(s\text{-insulin})(\text{mU/l}) + \text{Log}(p\text{-glucose})(\text{mmol/l}))^{20}$.

Statistical analyses

The results are given as means \pm SEM. Statistical analyses were done with IBM SPSS Statistics 21 (SPSS, Chicago, IL). Multiple comparisons were evaluated by one-way ANOVA and Turkey's post hoc test. Pair-wise comparisons were evaluated by independent sample *t* test. Correlations were examined by linear regression analysis with Pearson's correlation test. For all comparisons, statistical significance was defined as $P < 0.05$.

Results

Maternal characteristics

Characteristics of the study participants are presented in Table 1. The ages of the participants did not differ; the mean age was about 34 years. GWG and gestational age at birth did not differ significantly between the groups. However, birth weight was significantly higher in the obese group than in the normal weight group. Self-reported total energy intake did not differ between the three groups (Table 1), nor did the intake of carbohydrate, fat, and protein during the last 3 months of pregnancy (data not shown).

Insulin sensitivity

All women were normoglycemic, and their fasting plasma glucose levels did not differ (Table 2). Serum insulin levels were significantly higher, and QUICKI was significantly lower, in obese women than in normal weight women. As expected, QUICKI correlated negatively with BMI ($r = -0.42$, $P < 0.001$), plasma glucose ($r = -0.68$, $P < 0.001$), and serum insulin ($r = -0.87$, $P < 0.001$) in the total study population. QUICKI values also correlated with serum leptin ($r = -0.53$, $P < 0.001$), CSF leptin ($r = -0.36$, $P < 0.01$), CSF/serum leptin ratio ($r = -0.42$, $P < 0.001$), CSF insulin ($r = -0.47$, $P < 0.001$) and birth weight ($r = -0.24$, $P < 0.05$). Interestingly, insulin sensitivity correlated with CSF AgRP ($\beta = -97.29$, $r = -0.27$, $P < 0.05$) (Figure 2A) and the CSF/serum insulin ratio ($r = 0.34$, $P < 0.01$).

Serum and CSF insulin levels

As described in Table 2, serum insulin concentrations were significantly higher in obese women than in normal weight women. Similarly, CSF insulin concentrations were significantly higher in both overweight and obese women than normal weight women (Table 3). Interestingly, the CSF/serum insulin ratio was significantly lower in obese women than normal weight women (Figure 1A). CSF insulin correlated with BMI ($r = 0.24$, $P < 0.05$). By simple linear regression analysis, serum insulin concentration and CSF leptin were positively related ($\beta = 27.50$, $r = 0.28$, $P < 0.05$) (Figure 2B). Serum insulin also correlated with BMI ($r = 0.38$, $P = 0.001$), plasma glucose ($r = 0.36$, $P < 0.01$), CSF leptin ($r = 0.40$, $P < 0.01$), serum leptin ($r = 0.38$, $P < 0.01$). The CSF insulin concentration was significantly associated with serum glucose ($\beta = 0.051$, $r = 0.29$, $P < 0.05$) (Figure 2C), serum leptin (r

= 0.26, $P < 0.05$), and CSF leptin ($r = 0.26$, $P < 0.05$). Serum AgRP correlated negatively ($r = -0.28$, $P < 0.05$), and CSF AgRP correlated positively ($r = 0.28$, $P < 0.05$), with serum insulin. CSF insulin was significantly associated with the CSF/serum leptin ratio ($r = 0.26$, $P < 0.05$) and serum insulin ($r = 0.033$, $P < 0.05$).

Serum and CSF leptin levels

Both serum and CSF leptin levels were significantly higher in overweight and obese women than in normal weight women (Table 3), although the level was much lower in CSF than in serum (Table 3), and both correlated with BMI ($r = 0.49$, $P < 0.001$) ($r = 0.40$, $P < 0.001$). CSF leptin was also associated with GWG ($r = 0.25$, $P < 0.05$). The CSF/serum ratio was significantly lower in the obese women than in normal weight women (Figure 1B). Serum leptin correlated with serum glucose ($r = 0.35$, $P < 0.01$).

Serum and CSF AgRP levels

Serum AgRP levels were significantly lower in obese women than in normal weight women (Table 3). In contrast, CSF AgRP levels were significantly lower in normal weight women than in overweight or obese women (Table 3). Interestingly, simple linear regression analysis of the CSF-AgRP concentration as a function of total energy intake showed a positive relationship between CSF-AgRP and total energy intake ($\beta = 0.005$, $r = 0.39$, $P < 0.05$) (Fig. 2D). The CSF/serum AgRP ratio was significantly higher in obese women than in normal weight women (Figure 1C). Accordingly, simple linear regression analysis of the CSF/serum AgRP ratio as a function of BMI showed a positive relationship between the ratio and BMI in the total population ($\beta = 10.79$, $r = 0.43$, $P < 0.01$) (Fig. 2E). The CSF/serum AgRP ratio correlated negatively with GWG ($r = -0.37$, $P < 0.05$) and positively with total energy intake ($r = 0.36$, $P < 0.05$).

Serum POMC levels

The serum levels of POMC did not differ between the groups (Table 3). However, POMC levels correlated with GWG ($r = 0.37$, $P < 0.01$) and birth weight ($r = 0.26$, $P < 0.05$).

Discussion

This study shows that obese and overweight pregnant women have higher serum and CSF levels of leptin and higher CSF levels of AgRP and insulin than normal weight pregnant women. GWG was similar in all the three groups, but the infants of obese women had higher birth weight than infants of normal weight women. The obese women also had the highest insulin resistance with the lowest QUICKI values which also correlated with birth weights. The CSF/serum leptin and the CSF/serum insulin ratios were lower, and the CSF/serum AgRP ratio was higher, in obese than in normal weight women. Additionally, CSF insulin, CSF/serum insulin ratio, CSF AgRP and serum and CSF leptin levels and the ratio correlated with QUICKI. Total energy intake was positively associated with CSF-AgRP and CSF/serum AgRP ratio, and birth weight correlated with serum POMC in the women.

Obese women, whose weight gain and total energy intake during the last 3 months of pregnancy were similar to those of normal and overweight women, seem to be protected from the suppressive effect of leptin and insulin by an increased CSF/serum AgRP ratio. This might be a way to secure the metabolic demands of pregnancy also in the obese women.

BMI, GWG, total energy intake, and birth weight

The three groups did not differ in self-reported energy intake or in the intake of carbohydrate, fat, and protein during the last 3 months of pregnancy, and, GWG was similar in all three groups. GWG tended to be lowest in obese women, but the difference was not significant, and their offspring had the highest birth weight. The average GWG in each of the three groups was in line with Swedish Medical Birth Registry for maternal body mass index class. These data also show that mean GWG decreases with increasing maternal BMI²¹.

The infants of the obese women had higher birth weights. Birth weight correlated positively with plasma glucose values at term and negatively with QUICKI values. These correlations reflect the fetal nutrition supply, which increases with reduced insulin sensitivity and adiposity in the women. Maternal glucose levels, even if below those diagnostic of diabetes, are strongly and continuously associated with increased birth weight²².

Interestingly, we also found that serum POMC was positively associated with birth weight, which has to our knowledge, not been reported before. Normal gestation is associated with profound modifications of maternal corticotroph function. Plasma cortisol nearly doubles around mid-gestation and ACTH increases slightly. POMC is not found in nonpregnant women; it becomes measurable in early pregnancy, increases during the second trimester, and remains constant during the remaining gestation²³. POMC plasma levels do not vary diurnally, are higher in multiple pregnancies and not affected by glucocorticoid administration, and return to normal levels within 3 days after birth, consistent with its placental origin²³. However, the physiological function of POMC is unknown. POMC is primarily concentrated at the fetal–maternal interface²⁴, pointing to a potential role in maternal and fetal nutrition and might in that way be connected to fetal growth. Further research is needed to understand its physiological role.

Insulin sensitivity

QUICKI values were significantly lower in obese women, reflecting their reduced insulin sensitivity, than in normal weight women. All women were normoglycemic and did not differ in fasting plasma glucose values; however, the obese women had greater insulin resistance and thus higher insulin levels. The obese women also had higher CSF insulin values and consistently lower CSF/serum insulin ratios. CSF insulin and plasma glucose were strongly associated in all groups.

Insulin is transported from plasma to CSF by a saturable transport system that is very slow. Even after 4 h of superphysiologic levels of plasma insulin, CSF insulin levels are below fasting plasma insulin levels²⁵. Because of its slow transport to the brain via the CSF circulation, insulin an improbable satiety signal. In addition to the correlation between obesity and CSF insulin levels, these findings suggest that insulin in the brain could, like leptin, be a continuing signal reporting on or regulating energy reserves, rather than an immediate satiety signal²⁶. The transit of insulin from plasma to CSF is decreased in several animal models of insulin resistance²⁷⁻²⁹ but has not been studied in human pregnancy.

Evidence of altered transport of insulin across the blood–brain barrier has been found in insulin-resistant women with a high BMI¹⁵. As in our study, these results showed that blood and CSF

concentrations of insulin were correlated, and the ratio was positively associated with whole-body insulin sensitivity, which was also lower in insulin-resistant obese subjects. Insulin levels in this study were on average 92% lower in CSF than in serum¹⁵. In our study, mean insulin levels were 96% lower in CSF than serum; the CSF/serum insulin ratio ranged from 3.9% in the normal weight group to 2.9% in the obese pregnant women. Pregnancy is an insulin-resistant condition per se, which could explain the higher ratio in the women in our study than was reported in nonpregnant women¹⁵. A higher ratio during pregnancy, likely reflecting reduced transport, would counteract the feedback mechanisms of insulin in CNS that downregulate appetite and terminate food intake. This might be a mechanism to ensure that the metabolic needs of the developing fetus are met, in both insulin-resistant normal weight women and obese women.

Leptin sensitivity

We found that serum and CSF levels of leptin were strongly associated with BMI. The highest serum and CSF leptin levels and the lowest ratio were in the obese women. Interestingly, we found a similar pattern for CSF and serum insulin levels. Leptin resistance, like insulin resistance, with increased circulating leptin levels and concurrent hyperphagia, has been reported in both human and animal pregnancy studies^{30,31}. In a recent study, leptin levels in serum and CSF were compared in pregnant and nonpregnant women of normal weight³². The serum levels were higher in the pregnant women, but the CSF leptin levels did not differ between the two groups, which consequently resulted in a lower CSF/serum leptin ratio in the pregnant women (~1% vs. 2.1% in nonpregnant women)³². We did not have a nonpregnant group, but the lowest ratio in the obese group was 1.3%. In another study³³, lower ratios were found in healthy and preeclamptic pregnant women, and CSF and serum leptin levels correlated negatively. In contrast, in our study, CSF and serum leptin correlated positively, and the CSF levels were higher in overweight and the obese women than in normal weight women.

We also found that CSF leptin and CSF/serum leptin correlated strongly with QUICKI values, although not as strongly as CSF insulin. The serum glucose values also correlated strongly with CSF insulin but not at all with CSF leptin. Both insulin and leptin act in the hypothalamus to enhance

peripheral insulin sensitivity and thereby decrease plasma glucose³⁴. Similarly, in rats, leptin sensitizes the hypothalamus to insulin to regulate plasma glucose³⁵. In that study, leptin infused directly into the CNS reduced hepatic glucose production in the liver and thereby lowered circulating glucose levels. Our findings might also mirror an indirect effect of leptin in the hypothalamus required to increase hypothalamic sensitivity to insulin to adjust plasma glucose. If this is an important interaction, leptin resistance during pregnancy might contribute to the insulin resistance that develops during pregnancy. Glucose is the most important fuel for fetal and placental tissues and is also important for maternal metabolism. Thus, the supply of glucose gets a metabolic priority for the pregnant woman, requiring adaptations to ensure that the glucose is continuously available in case of variations in maternal nutrition and insulin sensitivity.

AgRP in serum and CSF

The serum AgRP level was significantly lower in obese women than normal weight women, but the CSF AgRP level and the CSF/serum AgRP ratio were higher. In a recent study, AgRP was measured in CSF for the first time in human pregnancy and was found to be higher in normal weight women at term than in nonpregnant women³². Although both pregnant and nonpregnant subjects had comparable CSF leptin levels, absolute CSF AgRP levels were significantly higher in pregnant women, suggesting that leptin suppresses AgRP less effectively during pregnancy³². Increases in AgRP mRNA have also been consistently reported in the hypothalamus of rodents during pregnancy^{36,37}. In rats, serum AgRP increase during pregnancy and do not decrease before delivery, implying that the placenta is the main source of AgRP in maternal serum¹⁸. These data also indicated that AgRP is a major factor for fetal development and for maternal energy expenditure during pregnancy. AgRP is usually present as a trimer and passes the blood–brain barrier very slowly³⁸. Thus, peripheral AgRP probably does not directly regulate the secretion of different peptides in the brain but has long-lasting appetite-stimulating properties. Indeed, food intake increases after a single centrally administered dose of AgRP, and the effect lasts for up to a week; in contrast, the response to NPY lasts for hours rather than days³⁹.

We found that CSF levels of AgRP and the CSF/serum AgRP ratio correlated strongly and positively with maternal BMI and were positively associated with total energy intake, pointing to the importance of AgRP in regulating energy status and food intake during pregnancy. Moreover, we found positive association with AgRP ratio and serum insulin and also with CSF/serum leptin ratio. These results are very interesting since cross-talk between the leptin and insulin signaling pathways in the hypothalamus appears to be important in the regulation of AgRP^{40,41}. The forkhead transcription factor FOXO1, an important mediator of insulin signaling in peripheral tissues, is inactivated in the hypothalamus by both insulin and leptin^{40,41}. Activation of hypothalamic FOXO1 blocks the action of both leptin and insulin and stimulates AgRP expression, resulting in increased food intake^{40,41}. Compared with insulin and leptin, of which the high serum concentrations also resulted in higher CSF level, serum levels of AgRP were lower in obese subjects but resulted in higher CSF levels of AgRP. The only positive correlations that we found between energy intake and the other peptides measured were with CSF AgRP and the CSF/serum AgRP ratio. This finding suggesting the importance of this peptide in regulating energy expenditure and food intake during pregnancy and confirms findings in rodents^{18,42}. Interestingly, serum AgRP levels correlated with GWG only in the obese group. Since GWG decreases with maternal BMI, and obese women tended to have the lowest GWG, AgRP might counteract the inhibitory effects of insulin and leptin and thereby ensure that the energy demands of obese pregnant women are met.

The mechanisms of increased food intake during pregnancy are complicated and poorly understood, and few studies have been done in human pregnancies. Our study covers only a small portion of the complex interactions that drive the neuroendocrine adaptation to pregnancy; the brain is the site of action of many hormones originating from the ovary and placenta. Gonadal steroid hormones influence feeding behavior, and hyperphagia starts very early in pregnancy⁴³, an important period that we have not studied. Nor have the short-term signals, including neural signals from the stomach and gut that help regulate meal size been studied. More research is therefore needed to confirm our results and to gain deeper insight in the neuroendocrine adaptations to pregnancy

Conclusion

During pregnancy, metabolic interactions must be adapted to supply fetal nutritional demand and to store energy in preparation for the metabolic demands of lactation. These adaptations were important for human survival and prioritized in our evolutionary biologic history. In today's "obesogenic" environment, with unlimited access to energy-rich foods, these adaptive processes may not be compatible with a healthy outcome of pregnancy. Obesity is now the most common clinical risk factor in obstetric practice. Our data suggest, for the first time in human pregnancy, that obese women, whose weight gain and total energy intake during the last 3 months of pregnancy were similar to those of normal and overweight women, are protected from the suppressive effect of leptin and insulin by an increased CSF/serum AgRP ratio. This might be a way to secure the metabolic demands of pregnancy in obese women.

References

1. Huda S, Brodie L, Sattar N. Obesity in pregnancy: prevalence and metabolic consequences. *Semin Fetal Neonatal Med.* 2010;15(2):70-76.
2. Linné Y. Effects of obesity on women's reproduction and complications during pregnancy. *Obes Rev.* 2004;5(3):137-143. Review.
3. Gunderson E, Abrams B, Selvin S. Does the pattern of postpartum weight change differ according to pregravid body size? *Int J Obes Relat Metab Disord.* 2001;25(6):853-862.
4. Forsum E, Löf M. Energy metabolism during human pregnancy. *Annu Rev Nutr.* 2007;27:277-292.
5. Abelenda M, Puerta M. Inhibition of diet-induced thermogenesis during pregnancy in the rat. *Pflügers Arch.* 1987;409:314-317.
6. Varela L, Horvath T. Leptin and insulin pathways in POMC and AgRP neurons that modulate energy balance and glucose homeostasis. *EMBO Rep.* 2012;13(12):1079-1086.
7. Butte N, Hopkinson J, Nicolson M. Leptin in human reproduction: serum leptin levels in pregnant and lactating women. *J Clin Endocrinol Metab.* 1997;82:585-589.
8. Lewandowski K, Horn R, O'Callaghan C, et al. Free leptin, bound leptin, and soluble leptin receptor in normal and diabetic pregnancies. *J Clin Endocrinol Metab.* 1999;84:300-306.
9. Masuzaki H, Ogawa Y, Sagawa N, et al. Nonadipose tissue production of leptin: leptin as a novel placenta derived hormone in humans. *Nat Med* 1997;3(1029-1033).
10. Lee M, Wardlaw S. The central melanocortin system and the regulation of energy balance. *Front Biosci.* 2007;12:3394-4010.
11. Banks W, Kastin A, Huang W, Jaspan J, Maness L. Leptin enters the brain by a saturable system independent of insulin. *Peptides.* 1996;17:305-311.
12. Ladyman S, Augustine R, Grattan D. Hormone interactions regulating energy balance during pregnancy. *J Neuroendocrinol.* 2010;22:805-817.
13. Catalano P, Roman-Drago N, Amini S, Sims E. Longitudinal changes in body composition and energy balance in lean women with normal and abnormal glucose tolerance during pregnancy. *Am J Obstet Gynecol.* 1998;179:156-165.
14. Banks W, Jaspan J, Kastin A. Selective, physiological transport of insulin across the blood-brain barrier: novel demonstration by species-specific radioimmunoassays. *Peptides.* 1997;18(8):1257-1262.
15. Heni M, Schöpfer P, Peter A, et al. Evidence for altered transport of insulin across the blood-brain barrier in insulin-resistant humans. *Acta Diabetol.* 2014;51(4):679-681.
16. Porte DJ, Baskin D, Schwartz M. Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from *C. elegans* to humans. *Diabetes* 2005;54(5):1264-1276.
17. Xu A, Kaelin C, Takeda K, Akira S, Schwartz M, Barsh G. PI3K integrates the action of insulin and leptin on hypothalamic neurons. *J Clin Invest* 2005;115(4):951-958.
18. Szczepankiewicz D, Pruszyńska-Oszmałek E, Kaczmarek P, et al. Changes of agouti-related protein in hypothalamus, placenta, and serum during pregnancy in the rat. *J Endocrinol.* 2009;202(1):35-41.
19. Lindroos A, Lissner L, Sjostrom L. Validity and reproducibility of a self-administered dietary questionnaire in obese and non-obese subjects. *Eur J Clin Nutr.* 1993;47(7):461-481.
20. Katz A, Nambi S, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab.* 2000;85(7):402-410.
21. Cedergren M. Effects of gestational weight gain and body mass index on obstetric outcome in Sweden. *Int J Gynaecol Obstet.* 2006;93(3):269-274.
22. HAPO S. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. Associations With Neonatal Anthropometrics. *Diabetes.* 2009;58(2):453-459.
23. Raffin-Sanson M, Keyzer Yd, Bertagna X. Proopiomelanocortin, a polypeptide precursor with multiple functions: from physiology to pathological conditions. Review. *Eur J Endocrinol.* 2003;149(2):79-90.

24. Raffin-Sanson M, Ferre F, Coste J, Oliver C, Cabrol D, Bertagna X. Pro-opiomelanocortin in human pregnancy: evolution of maternal plasma levels, concentrations in cord blood, amniotic fluid and at the feto–maternal interface. *European Journal of Endocrinology*. 2000;142:53-59.
25. Wallum B, Taborsky GJ, Porte DJ, et al. Cerebrospinal fluid insulin levels increase during intravenous insulin infusions in man. *J Clin Endocrinol Metab*. 1987;64:190-194.
26. Baskin D, Lattemann DF, Seeley R, Woods S, Porte DJ, Schwartz M. Insulin and leptin: dual adiposity signals to the brain for the regulation of food intake and body weight. *Brain Res*. 1999;848:114-123.
27. Begg D, Mul J, Liu M, et al. Reversal of diet-induced obesity increases insulin transport into cerebrospinal fluid and restores sensitivity to the anorexic action of central insulin in male rats. *Endocrinology*. 2013;154:1047-1054.
28. Baura G, Foster D, Kaiyala K, Porte DJ, Kahn S, Schwartz M. Insulin transport from plasma into the central nervous system is inhibited by dexamethasone in dogs. *Diabetes*. 1996;45:86-90.
29. Kaiyala K, Prigeon R, Kahn S, Woods S, Schwartz M. Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. *Diabetes*. 2000;49(1525-1533).
30. Ladyman S. Leptin resistance during pregnancy in the rat. *J Neuroendocrinol*. 2008;20:269-277.
31. Grattan D, Ladyman S, Augustine R. Hormonal induction of leptin resistance during pregnancy. *Physiol Behav*. 2007;91:366-374.
32. Page-Wilson G, Reitman-Ivashkov E, Meece K, et al. Cerebrospinal fluid levels of leptin, proopiomelanocortin, and agouti-related protein in human pregnancy: evidence for leptin resistance. *J Clin Endocrinol Metab*. 2013;98(1):264-271.
33. Celik O, Hascalik S, Ozerol E, Hascalik M, Yologlu S. Cerebrospinal fluid leptin levels in preeclampsia: relation to maternal serum leptin levels. *Acta Obstet Gynecol Scand*. 2004;83(6):519-523.
34. Pocai A, Morgan K, Buettner C, Gutierrez-Juarez R, Obici S, Rossetti L. Central leptin acutely reverses diet-induced hepatic insulin resistance. *Diabetes*. 2005;54:3182-3189.
35. Koch C, Augustine R, Steger J, et al. Leptin rapidly improves glucose homeostasis in obese mice by increasing hypothalamic insulin sensitivity. *J Neurosci*. 2010;30(48):16180-16187.
36. Rocha M, Bing C, Williams G, Puerta M. Pregnancy-induced hyperphagia is associated with increased gene expression of hypothalamic agouti-related peptide in rats. *Regul Pept*. 2003;114:159-165.
37. Trujillo M, Spuch C, Carro E, Senarís R. Hyperphagia and central mechanisms for leptin resistance during pregnancy. *Endocrinology*. 2011;152:1355-1365.
38. Kastin A, Akerstrom V, Hackler L. Agouti-related protein(83–132) aggregates and crosses the blood–brain barrier slowly. *Metabolism*. 2000;49:1444-1448.
39. Hagan M, Rushing P, Pritchard L, et al. Long-term orexigenic effects of AgRP-(83–132) involve mechanisms other than melanocortin receptor blockade. *Am J Physiol Regul Integr Comp Physiol*. 2000;279:R47-R52.
40. Kim M, Pak Y, Jang P, et al. Role of hypothalamic Foxo1 in the regulation of food intake and energy homeostasis. *Nat Neurosci*. 2006;9:901-906.
41. Kitamura T, Feng Y, Kitamura Y, et al. Forkhead protein FoxO1 mediates AgRP-dependent effects of leptin on food intake. *Nat Med*. 2006;12(534-540).
42. Szczepankiewicz D, Wojciechowicz T, Kaczmarek P, Nowak K. Leptin and its receptors in the course of pregnancy in the rat. *Int J Mol Med*. 2006;17(1).
43. Augustine R, Ladyman S, Grattan D. From feeding one to feeding many: hormone-induced changes in bodyweight homeostasis during pregnancy. *J Physiol*. 2008;586:387-397.

Figure Legends

Figure 1. CSF/serum ratios of insulin (A), leptin (B), and AgRP (C) concentrations in normal weight (NW, n = 26–30), overweight (OW, n = 19–20), and obese (OB, n = 14–22) pregnant women. Values are mean \pm SEM. *p < 0.05, **p < 0.01 vs. normal weight women (independent sample t test).

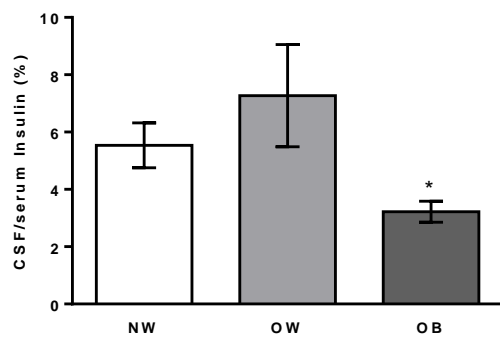
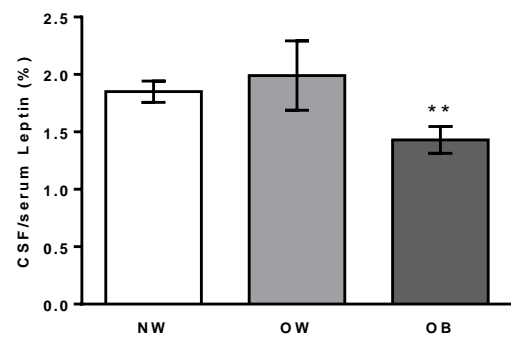
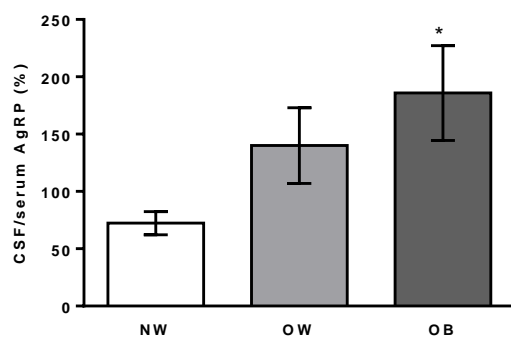
A.**B.****C.**

Figure 2. Correlation between CSF AgRP and QUICKI (A) serum insulin and CSF leptin (B), CSF insulin and plasma glucose (C), total energy intake and CSF AgRP (D), and CSF/serum AgRP ratio and BMI (E) for the entire study population with no controlled variable.

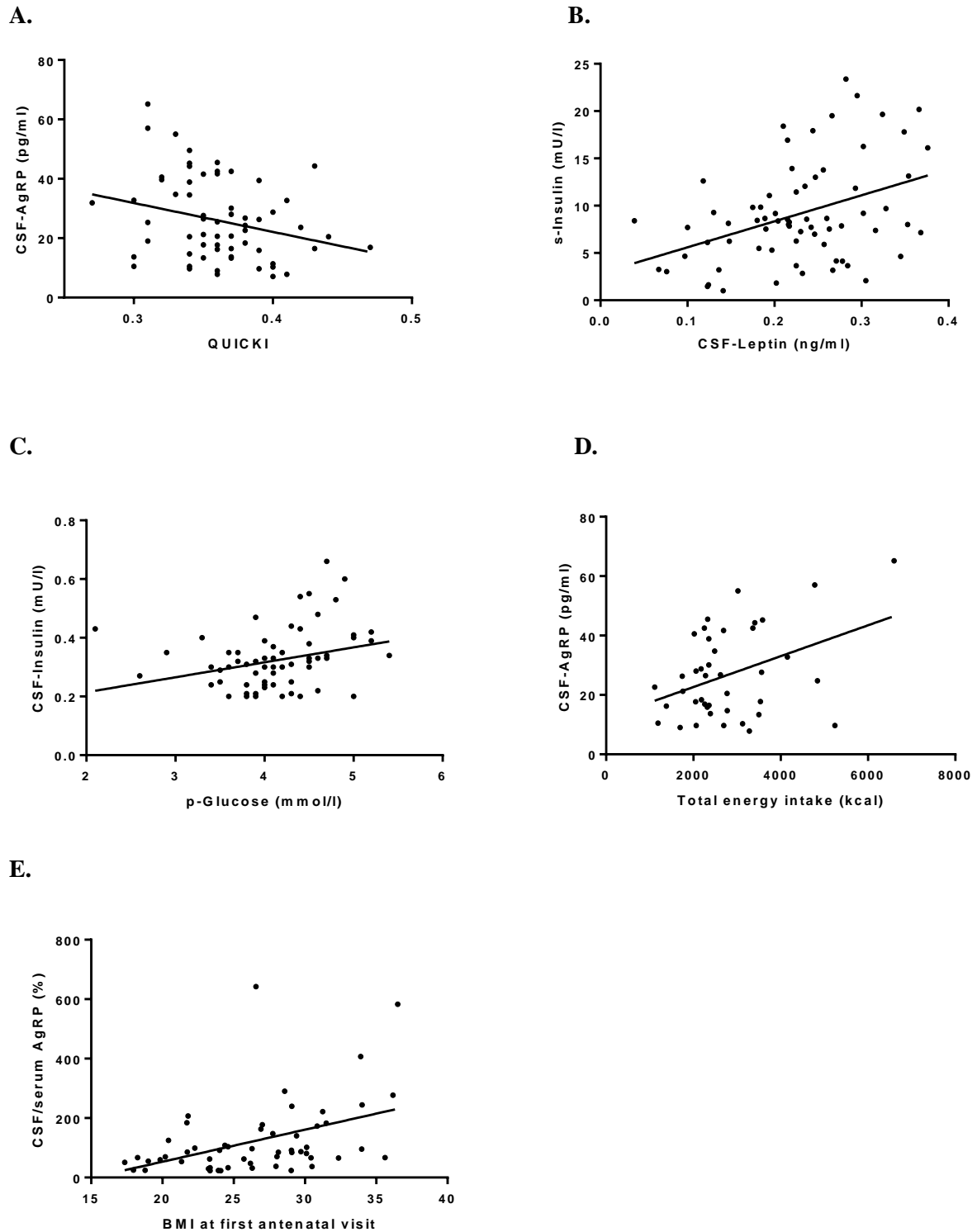


TABLE 1. Maternal characteristics.

Characteristics	Group		
	Normal weight (n = 23–30)	Overweight (n = 11–21)	Obese group (n = 23)
Age at Caesarean (years)	33 ± 0.8	34 ± 1.0	33 ± 0.9
BMI at first antenatal visit (kg/m ²)	22 ± 0.4	28 ± 0.3	33 ± 0.7
Weight gain during pregnancy (kg)	15 ± 0.6	13 ± 1.1	12 ± 2.0
Gestational age at birth (days)	254 ± 6	266 ± 5	254 ± 14
Birth weight, offspring (g)	3551 ± 47	3609 ± 111	3779 ± 98*
Total energy intake (kcal)	2637 ± 197	2691 ± 268	3265 ± 483

Values are mean ± SEM.

* $P < 0.05$ vs. normal weight women (independent sample t test).

TABLE 2. Insulin sensitivity

Characteristics	Group		
	Normal weight (n = 30)	Overweight (n = 21)	Obese (n = 17-23)
Plasma glucose (mmol/l)	4.1 ± 0.1	4.0 ± 0.2	4.2 ± 0.1
Serum insulin (mU/l)	8.1 ± 0.8	10.0 ± 1.4	14.4 ± 1.6**
QUICKI	0.37 ± 0.0	0.36 ± 0.0	0.34 ± 0.0***

Values are mean ± SEM.

* $P < 0.05$, ** $P < 0.01$ vs. normal weight women (independent sample t test).

TABLE 3. CSF concentrations of AgRP, insulin and leptin, and serum concentrations of AgRP, leptin and POMC.

Characteristics	Group		
	Normal weight (n = 26–39)	Overweight (n = 18–21)	Obese (n = 19–23)
Serum			
AgRP (pg/ml)	33.16 ± 2.2	33.08 ± 3.7	23.61 ± 2.6**
Leptin (ng/ml)	11.53 ± 0.9	16.48 ± 1.9*	19.54 ± 1.4***
POMC (ng/ml)	1.81 ± 0.2	1.72 ± 0.4	1.56 ± 0.2
CSF			
AgRP (pg/ml)	20.29 ± 2.0	30.43 ± 3.3*	29.66 ± 3.4*
Insulin (mU/l)	0.29 ± 0.0	0.36 ± 0.0*	0.35 ± 0.0*
Leptin (ng/ml)	0.19 ± 0.0	0.25 ± 0.0*	0.26 ± 0.0**

Values are mean ± SEM.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. normal weight women (independent sample t test).