Serum Neuropeptide Y and Leptin Levels compared between Non-pregnant and Pregnant Women in Overall, Non-obese, and Obese Subjects

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ABSTRACT

Objective: The primary objective of this study was to compare serum NPY and leptin levels between non-pregnant and pregnant women in overall, non-obese, and obese subjects. The secondary objective was to compare these peptides between non-obese and obese pregnant women.

Methods: Fasting venous blood was collected from non-pregnant women before open abdominal surgery and from pregnant women when admitted to the delivery room during the latent phase of labor.

Results: There were 12 non-obese and 14 obese subjects in the non-pregnant group and 9 non-obese and 30 obese subjects in the pregnant group. Systolic blood pressure (SBP) was comparable, but heart rate (HR) was higher in pregnant compared to non-pregnant women. Mean±S.E.M serum NPY levels were lower in the pregnant than in the non-pregnant group in overall (0.54±0.02 and 1.34±0.08, respectively), non-obese (0.53±0.05 and 1.23±0.14, respectively), and obese (0.54±0.03 and 1.43±0.09, respectively) subjects ($p<0.01$ for all), but these were comparable between obese and non-obese pregnant subjects. Serum NPY was positively correlated with SBP ($R=0.281$, $p<0.05$), but negatively correlated with HR ($R=-0.324$, $p<0.01$). Serum leptin levels were not different between pregnant and non-pregnant groups, but were significantly higher in obese than non-obese pregnant subjects ($p<0.001$). Serum leptin levels were positively correlated with body weight, BMI, waist and hip circumferences in overall and pregnant subjects ($p<0.001$ all).

Conclusion: In pregnancy, decreased NPY levels might be associated with inhibition of SBP rising as well as increased HR. Leptin levels might not be associated with pregnancy, but associated mainly with obesity.

Keywords: Neuropeptide Y; Leptin; non-pregnant; pregnant women (Siriraj Med J 2018;70: 204-212)

INTRODUCTION

Pregnant state is related with an increase in gestational weight gain (GWG) which is a unique and complex biological phenomenon that supports the functions of growth and development of the fetus.[1] The mean total GWG of normal weight adult women giving birth to term infants, is from maternal, placental, and fetal components, ranging from a low of 10.0 to a high of 16.7 kg.[1] For maternal component, fat appears to be increased in pregnant women, which preferentially deposits over the hips, back, and upper thighs throughout pregnancy.[2] Increased fat deposition leads to increased gene expression and secretion of certain adipokines from adipose tissue which are leptin and neuropeptide Y (NPY).[3,4]

Leptin is a peptide mainly produced and secreted from adipose tissue into the circulation in proportion to fat

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storage.[5-7] Leptin concentrations are positively correlated with indices of obesity including body weight, BMI, and waist and hip circumferences and is markedly increased in obese humans and in various animal models of obesity compared to controls or non-obese population.[8-11] Leptin acts as an afferent satiety signal to inhibit food intake[12] and stimulates energy expenditure.[13] In addition, leptin was found to be involved in blood pressure regulation. It increases sympathetic activity as chronic leptin infusion causes an increase in mean arterial pressure (MAP) and heart rate (HR) in mice.[14,15] On the other hand, acute and chronic leptin administration to rat aortic ring was shown to increase nitric oxide (NO) production and secretion leading to vasodilation.[16,17] In a pathological condition of obesity, chronic elevated serum leptin levels (hyperleptinaemia)[18] caused endothelial dysfunction and impaired NO-mediated vasodilatory effect of leptin[19] which led to increased blood pressure. The human placenta has been shown to be a source of leptin synthesis and secretion during pregnancy.[20] A previous study reported that circulating leptin levels and body fat were found to be higher during the early and late trimesters of pregnancy, than the pre-pregnant period[20] suggesting that the increase of this hormone might be from both placental and adipose production.

Neuropeptide Y (NPY), a 36 amino acid peptide[21], is the most potent orexigenic factor in the hypothalamus which increases body weight and adiposity by increasing food intake.[22] NPY levels in the circulation are also found to be higher in obese than non-obese or normal weight subjects.[4] NPY has another function implicated in cardiovascular regulation by modulating the sympathetic nervous system including vasoconstriction and control of heart rate and coronary blood flow.[23] Elevated NPY concentrations were also reported in hypertension.[24] NPY induces vasoconstriction directly by activation of its Y1 receptor, as well as indirectly by potentiating the effects of ATP and norepinephrine (NE) on adrenergic receptors.[25] Furthermore, plasma NPY concentrations were significantly correlated with BMI, waist circumference, HR, systolic blood pressure (SBP), and diastolic blood pressure (DBP).[26]

In summary, NPY and leptin are implicated in the regulation of food intake, body weight control, and cardiovascular modulation. The primary objective of this study was to compare serum NPY and leptin levels between pregnant and non-pregnant women categorized into non-obese and obese subgroups. As pregnancy is accompanied both by changes of adiposity and cardiovascular events, comparisons of their levels within the same BMI group might help to avoid the confounding effect of obesity. Furthermore, the secondary objective of this study was to compare serum NPY and leptin levels between non-obese and obese pregnant women to investigate the effect of obesity in pregnancy. Comparisons of these hormones between non-pregnant and pregnant women classified by BMI could reveal the changes of these peptides without the confounding effect of obesity. The changes of these peptides in pregnancy could probably explain physiological changes during pregnancy.

MATERIALS AND METHODS

Subjects

The study protocols were approved by the Siriraj Institutional Review Broad (Si 533/2009 for non-pregnant and Si 545/2015 for pregnant subjects) of the Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. Informed consents were obtained from all participants prior to the study. Sample size calculation for the comparison between the mean of 2 independent groups was obtained as follows: $n = 2 (Z_{\alpha/2} + Z_{\beta})^2/((\mu_1-\mu_2)/\sigma)2$, where $\alpha = 0.05$, 2 sided test, $Z_{\alpha/2} = 1.96$, $\beta = 0.2$, $Z_{\beta} = 0.842$, σ = common SD, $\mu_1-\mu_2$ = difference in mean, n = sample size per group. Sample size was obtained from previously published papers as 12 per group for NPY[27] and 2 per group for leptin.[28] In this study, there were 65 subjects who were classified into the pregnant group (n = 39) and the non-pregnant group (n = 26). For the non-pregnant group, recruited subjects were female patients undergoing open abdominal surgery. Phase of menstrual cycle of non-pregnant women could not be controlled because 23 subjects had myoma uteri and 3 subjects had adenomyosis which presented with irregular menstrual cycle. Exclusion criteria included subjects who underwent endocrine therapy (e.g., steroids, hormone replacement therapy, and thyroxine therapy) and had pregnancy, lactation, traumatic operation, malignancy diseases, operations related to endocrine diseases, severe abdominal inflammation, menopause, and/or hypertension. For the pregnant group, subjects who underwent either cesarean or normal labor, had antenatal care, and were in labor at Faculty of Medicine Siriraj Hospital, were healthy, and had age of at least 18 years old, gestational age of at least 34 weeks, and singleton, were recruited into this study. Exclusion criteria included subjects with gestational hypertension, human immunodeficiency virus (HIV) infection, type 1 or type 2 diabetes mellitus, metabolic syndrome, hypertension, polycystic ovarian syndrome, other endocrine disorders, previous history of chronic diseases, smoking habits, malignancies, pre-term membranes rupture, drug use that might affect blood glucose and insulin levels, and drug administration for

pre-term delivery risk and had fetuses with malformations and fetal distress during delivery.

Demographic data of subjects

Age of subjects and body mass index (BMI) of the non-pregnant group (range and median) were obtained from questionnaires and medical records. BMI of the pregnant women was calculated from pre-delivery body weight, which was obtained from medical records when subjects were admitted to the delivery room, subtracted by baby weight, placental weight, and estimated amniotic fluid weight. The estimated amniotic fluid weight was calculated as 6% of the gestational weight gain obtained from medical records as a previous study reported that amniotic fluid accounted for 6% of the gestational weight gain.[29] BMI of both non-pregnant and pregnant women were classified into non-obese (BMI < 25) and obese groups (BMI ≥ 25) according to the classification of BMI for Asian populations.[30] Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and heart rate (HR) were measured by an automated sphygmomanometer. There were 12 non-obese and 14 obese subjects in the non-pregnant group and 9 non-obese and 30 obese subjects in the pregnant group.

Blood collection

Fasting venous blood samples were collected in the clot activator, silicone coated-tubes (BD Vacutainer®, Becton, Dickinson and Company Franklin Lakes, NJ, USA) during the fasting state from non-pregnant women before open abdominal surgery and from pregnant women when admitted to the delivery room during the latent phase of labor. For all subjects, venous blood had been drawn before intravenous fluid was given. Blood samples were immediately kept in an ice bucket during the transferring process and incubated at 4°C for 1 hour, balanced weight, and separated by centrifuging at 3,000 rpm at 4°C for 15 minutes. Then, serum samples were aliquoted and stored at -70°C, until analysis of serum NPY and leptin.

Serum NPY and leptin measurement

NPY and leptin serum levels were measured by commercial enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) kits (Phoenix Pharmaceuticals, Burlingame, California, USA), respectively. The protocol was done according to the manufacturer's procedures. The range of NPY detection was 0-100 ng/ml and the minimum detectable concentration was 0.11 ng/ml, with 100% and 14.3% cross-reactivity with neuropeptide Y (human, rat, mouse, porcine) and neuropeptide Y (3-36) (human, rat, mouse), respectively. Leptin detection range was 0.312-20.0 ng/ml, with 100% cross-reactivity with human leptin. For NPY, intra-assay variation was 8.84% for pregnant subjects and 10.1% for non-pregnant subjects, and inter-assay variation was 1.62%. For leptin intra-assay variation was 5.73% for pregnant subjects and 8.95% for non-pregnant subjects and inter-assay variation was 1.39%. Samples were run in duplicate and absorbance measured at 450 nm by Synergy HT Multi-Detection Microplate Readers (BioTek Instruments, Inc., Winooski, VT, U.S.).

Statistical analysis

Kolmogorov-Smirnov test was performed to test normality of the data. For normally distributed data, comparisons between the non-pregnant and pregnant groups as well as the non-obese and obese pregnant groups were performed by unpaired T-Test. Comparisons of non-normal distributed data were performed with a non-parametric test. Correlation coefficient was calculated by using the 2-tails Pearson product-moment correlation for normally-distributed data or the Spearman's rank correlation coefficient test for non-normally distributed data. Data were presented as mean±S.E.M. Statistical analysis was performed with PASW statistics 18.0 (SPSS Inc., Chicago, IL, USA). A p-value less than 0.05 was considered as statistical significance.

RESULTS

Demographic and clinical data

Demographic data of the subjects including age and BMI are shown in range and median in Tables 1-4. Clinical data including SBP, DBP, MAP, and HR compared between the non-pregnant and pregnant groups in overall, non-obese, and obese subjects are shown as mean±S.E.M. in Tables 1, 2, and 3, respectively, as well as between non-obese and obese pregnant subjects in Table 4.

DBP was significantly higher in pregnant than non-pregnant women in overall subjects ($p<0.05$) (Table 1) and had a trend to be higher in pregnant than non-pregnant women in non-obese (Table 2) and obese groups (Table 3). HR was significantly higher in pregnant than non-pregnant women in the overall (Table 1), non-obese (Table 2), and obese groups (Table 3) ($p<0.05$ all). SBP and MAP were significantly higher in obese than non-obese pregnant women ($p<0.05$) (Table 4).

Serum levels of NPY and leptin compared between non-pregnant and pregnant women

Serum levels of NPY and leptin compared between

non-pregnant and pregnant women in overall, non-obese, and obese subjects are shown in Fig 1.

Serum NPY levels were significantly lower in the pregnant group than in the non-pregnant group in overall ($p<0.001$), non-obese ($p<0.01$), and obese ($p<0.0001$) subjects (Fig 1). Serum leptin levels were not different between the pregnant and non-pregnant groups in overall, non-obese, and obese subjects (Fig 1).

Serum levels of NPY and leptin in pregnant women compared between non-obese and obese subjects

Serum levels of NPY and leptin in pregnant women compared between non-obese and obese subjects are shown in Fig 2.

Serum NPY levels were comparable between non-obese and obese pregnant women (Fig 2). Serum leptin levels were significantly higher ($p<0.001$) in obese pregnant women compared to non-obese pregnant women (Fig 2).

TABLE 1. Clinical parameters of overall subjects compared between non-pregnant and pregnant women: number (n) of subjects (%), age and BMI (range and median), SBP, DBP, MAP, and HR (mean±S.E.M). *p<0.05, ***p<0.001

Parameters	Overall subjects (n = 65)		P-value
	Non-pregnancy	Pregnancy	
n (percentage)	26 (40%)	39 (60%)	-
Age (range), year	28-53	20-41	-
Age (median), year	45.0	28.0	-
BMI (range), kg/m^2	17.5-44.0	18.8-38.8	-
BMI (median), kg/m^2	26.3	27.5	-
SBP (mmHg)	118.1±2.0	118.2±1.0	0.956
DBP (mmHg)	72.8±1.7	77.1±0.8	0.026*
MAP (mmHg)	87.9±1.6	90.8±0.7	0.113
HR (bpm)	76.9±2.0	86.3±1.4	<0.0001***

TABLE 2. Clinical parameters of non-obese subjects compared between non-pregnant and pregnant women: number (n) of subjects (%), age and BMI (range and median), SBP, DBP, MAP, and HR (mean±S.E.M). *p<0.05

Parameters	Non-obese subjects (n = 21)		P-value
	Non-pregnancy	Pregnancy	
n (percentage)	12 (57.14%)	9 (42.86%)	-
Age (range), year	28-51	20-39	-
Age (median), year	43.5	27.0	-
BMI (range), kg/m^2	17.5-22.8	18.8-22.3	-
BMI (median), kg/m^2	20.6	21.7	-
SBP (mmHg)	117.3±3.9	114.4±2.4	0.812
DBP (mmHg)	70.6±2.2	74.4±1.8	0.239
MAP (mmHg)	85.0±1.8	87.8±1.7	0.935
HR (bpm)	81.1 ± 2.0	87.4 ± 1.8	0.034*

Correlations between 2 factors

Correlations between serum NPY and leptin with other factors in overall, non-pregnant, and pregnant subjects are shown in Table 5.

Serum NPY levels were significantly positively correlated with SBP (R=0.281, p<0.05), but significantly negatively correlated with HR (R=-0.324, p<0.01) in overall subjects. Serum leptin levels were significantly positively correlated to body weight, BMI, and waist and hip circumferences in overall and pregnant subjects (R=0.470-0.628, p<0.001 all). Serum leptin levels had a significant positive correlation with hip circumference in non-pregnant subjects (R=0.480, p<0.05).

TABLE 3. Clinical parameters of obese subjects compared between non-pregnant and pregnant women: number (n) of subjects (%), age and BMI (range and median), SBP, DBP, MAP, and HR (mean±S.E.M). ***p<0.001

Parameters	Obese subjects (n = 44)		P-value
	Non-pregnancy	Pregnancy	
n (percentage)	14 (31.82%)	30 (68.18%)	-
Age (range), year	28-51	21-41	-
Age (median), year	43.5	29.0	-
BMI (range), kg/m^2	25.2-44.0	25.1-38.8	-
BMI (median), kg/m^2	29.1	28.1	-
SBP (mmHg)	121.8±3.2	119.3±1.1	0.482
DBP (mmHg)	74.6±2.3	77.9±0.9	0.208
MAP (mmHg)	90.4±2.5	91.7±0.7	0.610
HR (bpm)	72.1±2.6	87.5±1.4	<0.0001***

TABLE 4. Clinical parameters of pregnant subjects compared between non-obese and obese pregnant women: number (n) of subjects (%), age and BMI (range and median), SBP, DBP, MAP, and HR (mean±S.E.M). *p<0.05

Parameters	Pregnancy (n = 39)		P-value
	Non-obese	Obese	
n (percentage)	9 (23.08%)	30 (76.92%)	-
Age (range), year	20-39	21-41	-
Age (median), year	27.0	29.0	-
BMI (range), kg/m^2	18.8-22.3	25.1-38.8	-
BMI (median), kg/m^2	21.7	28.1	-
SBP (mmHg)	114.4±2.4	119.3±1.1	0.044*
DBP (mmHg)	74.4±1.8	77.9±0.9	0.076
MAP (mmHg)	87.8±1.7	91.7±0.7	0.016*
HR (bpm)	87.4 ± 1.8	87.5±1.4	0.137

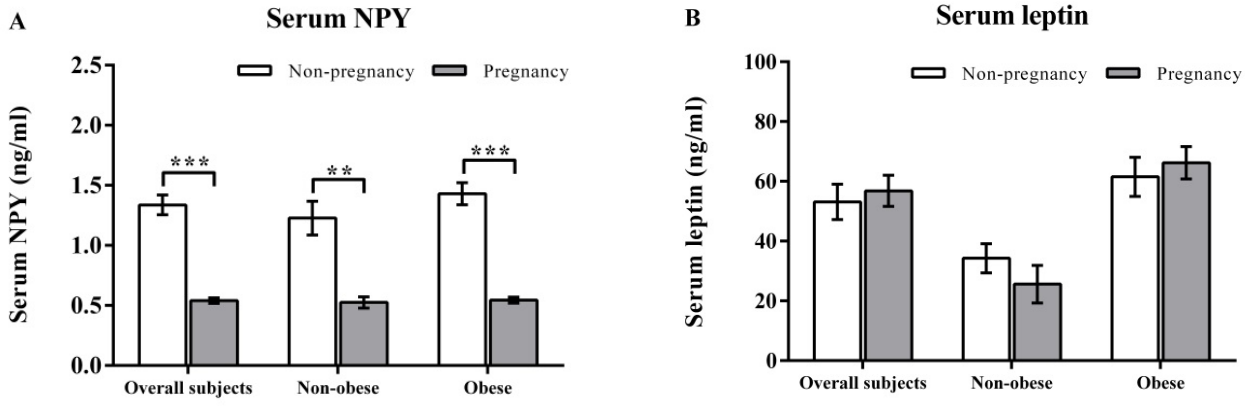

Fig 1. Serum levels of NPY and leptin compared between non-pregnant and pregnant women in overall, non-obese, and obese subjects. Panels A and B show serum NPY and leptin, respectively. Values are expressed as mean±S.E.M. **p<0.01, ***p<0.001 compared between non-pregnant and pregnant women.

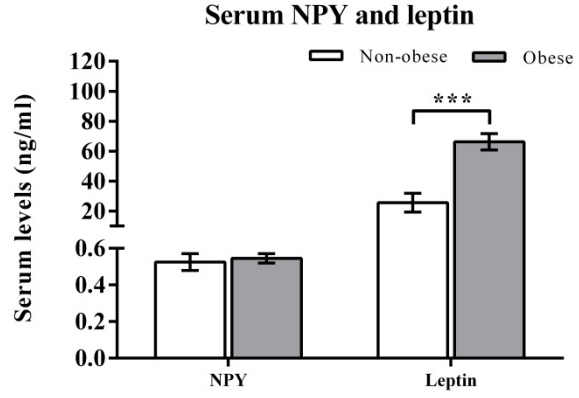

Fig 2. Serum NPY and leptin levels in non-obese and obese pregnant women. Values are expressed as mean±S.E.M. ***p<0.001 compared between non-obese pregnant and obese pregnant women.

TABLE 5. Correlations between serum NPY and leptin with other factors in overall, non-pregnant, and pregnant subjects.

R = correlation coefficient, SBP = systolic blood pressure, HR = diastolic blood pressure, BMI = body mass index, *p<0.05, **p<0.01, ***p<0.001

Factors	Overall subjects (n = 65)		Non-pregnant subjects (n = 26)		Pregnant subjects (n = 39)	
	R	P-value	R	P-value	R	P-value
Serum NPY						
- SBP	0.281	0.012*	0.035	0.850	0.131	0.379
- HR	-0.324	0.004**	-0.149	0.415	-0.066	0.665
Serum leptin						
- Body weight	0.545	<0.0001***	0.296	0.218	0.590	<0.0001***
- BMI	0.583	<0.0001***	0.366	0.124	0.645	<0.0001***
- Waist circumference	0.470	<0.0001***	0.211	0.386	0.618	<0.0001***
- Hip circumference	0.628	<0.0001***	0.480	0.038*	0.663	<0.0001***

DISCUSSION

This study focused on the comparisons of serum NPY and leptin levels between pregnant and non-pregnant women. During pregnancy, increased maternal adiposity might lead to changes of NPY and leptin levels, therefore, subgroup analysis by BMI classification was performed in this study to decrease confounding effects of obesity from pregnancy.

In this study, serum NPY levels were significantly lower in pregnant than non-pregnant women. DBP and HR were higher in pregnant than non-pregnant women while SBP and MAP were comparable among groups. Furthermore, NPY had a positive correlation with SBP but a negative correlation with HR. These results suggest that NPY levels in pregnancy were related to increased SBP, but decreased HR. A decrease in NPY levels in pregnancy might be associated with inhibition of SBP rising as well as increased HR. During pregnancy, there is a decrease in total peripheral vascular resistance[31,32] due to vasodilation of the renal and systemic circulation[33], although MAP is maintained[34] because there is a rise in blood volume and cardiac output by 30-50%.[31,32] As NPY has an effect to induce vasoconstriction, decreased NPY levels in pregnant women might lead to vasodilation in pregnancy to maintain uteroplacental circulatory blood flow in order to meet fetal metabolic demands.[35] The progressive increase in HR throughout pregnancy peaking in the late third trimester, likely counterbalances the vasodilation to maintain adequate cardiac output at a functional level until delivery. However, our results were inconsistent with previous studies showing higher NPY levels in pregnant compared to non-pregnant women. The inconsistency of the results might be due to BMI mismatch, different number of subjects, sampling population, and techniques used for analysis of NPY levels. For BMI mismatch, subjects of this study were classified into obese and non-obese as NPY levels are changed according to obesity status[4], while previous studies did not categorize subjects into obese and non-obese subgroups.[36,37] This might lead to unreliable results of the previous studies. Furthermore, to eliminate the confounding effect of obesity, the current body weight of pregnant subjects was calculated from subtraction of maternal body weight by baby weight, placental weight, and amniotic fluid weight. For number of subjects, our study had 26 non-pregnant and 39 pregnant subjects while previous studies had 15 non-pregnant and 33 pregnant subjects[36] and 8 non-pregnant and 8 pregnant subjects[37] which were lower than this study. For sampling population, non-pregnant subjects of our study were recruited from female patients undergoing open abdominal surgery. Twenty three subjects had myoma uteri and 3 subjects had adenomyosis presented with irregular menstrual cycle. On the other hand, other studies recruited healthy non-pregnant subjects without declaration of menstrual cycle. For the analytical methods of peptide concentrations, NPY levels were determined in serum by EIA technique in this study while in plasma by radioimmunoassay (RIA) in previous studies.[36,37] Furthermore, previous studies found that an increase in NPY levels from stress[38] as well as preeclampsia[37] was observed in platelet rich plasma (PRP), but not platelet poor plasma (PPP), suggestive of accumulation of the peptide in platelets.[39] As NPY levels were determined in serum in our study, NPY in platelets could not be measured probably leading to lower NPY levels than plasma.[39]

Serum NPY levels were comparable between non-obese and obese pregnant women and there was no correlation between NPY levels and obesity parameters including body weight, BMI, and waist and hip circumferences in this study. These results suggest that during pregnancy, NPY levels might be associated with hemodynamic regulation rather than obesity.

Serum leptin levels were not different between pregnant and non-pregnant women but were higher in obese compared to non-obese pregnant women. Furthermore, serum leptin levels had positive correlations with body weight, BMI, waist and hip circumferences. These results suggest that leptin levels were obviously associated with obesity rather than pregnant status. Our results were inconsistent with previous studies showing increased leptin levels in pregnant women than non-pregnant women.[20,40] In a previous study, leptin levels of pregnant women were reported without BMI matching at the time of blood sampling to non-pregnant women. As circulating leptin in pregnancy parallels the process of fat accumulation and mobilization[40], the increase in adiposity in pregnant women could lead to increased leptin levels in pregnancy. Our data supported the evidence showing that adipose tissue, not placenta, is a major source of circulating leptin production.[41]

In summary, this study revealed lower levels of serum neuropeptide Y in pregnancy compared to non-pregnancy in both non-obese and obese subjects. Thus, we postulated that, decreased neuropeptide Y levels during pregnancy might contribute to a decline in vasoconstriction leading to vasodilation. Serum leptin levels were higher in obese pregnant than non-obese pregnant women with comparable levels between BMI-matched non-pregnant and pregnant women. Therefore, serum leptin levels were associated with fat accumulation rather than a pregnant state.

One limitation of our study was that the exact body weight of pregnant women during the time of blood collection could not be obtained. However, the estimated body weight of pregnant group is a better representation of current BMI than pre-pregnant BMI or BMI at early gestational age. Estimated amniotic fluid weight used in this study was calculated from GWG instead of ultrasonography and operative record. This is because, in normal practice, the amniotic fluid measurement was reported as low, normal, and high amniotic fluid volume, not estimated into weight of amniotic fluid. As a result, we could not obtain amniotic fluid weight from this technique. Furthermore, this study recruited both normal and cesarean delivery, so the measurement of amniotic fluid during delivery was not applicable to all subjects. Therefore, estimated amniotic fluid weight from GWG might be the most convenient method in normal clinical practice setting. Another study limitation was that blood samples were collected before open abdominal surgery or before delivery which might lead to increased stress levels in the subjects. Another limitation of this study was that non-pregnant subjects were not healthy and had gynecological conditions including myoma uteri and adenomyosis.

The strengths of this study were that subgroup analysis of subjects into obese and non-obese was made and the current body weight of pregnant women was subtracted by baby weight, placental weight, and amniotic fluid weight to reduce the confounding effect of adiposity and GWG. The results of this study could be applied in a clinical context as an abnormal increase in NPY levels in pregnancy might lead to increased blood pressure resulting in pregnancy-induced hypertensive diseases including preeclampsia. Further studies will be needed to reveal the effect of decreased NPY induced vasodilation in normal pregnancy.

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REFERENCES

1. Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM Pregnancy Weight Guidelines; Rasmussen KM YA, editors. Weight Gain During Pregnancy: Reexamining the Guidelines. Washington (DC): National Academies Press (US); 2009.
2. Taggart NR, Holliday RM, Billewicz WZ, Hytten FE, Thomson AM. Changes in skinfolds during pregnancy. Br J Nutr. 1967; 21(2):439-51.
3. Trayhurn P, Bing C. Appetite and energy balance signals from adipocytes. Philosophical Transactions of the Royal Society B: Biological Sciences. 2006;361(1471):1237-49.
4. Sitticharoon C, Chatree S, Churintaraphan M. Expressions of neuropeptide Y and Y1 receptor in subcutaneous and visceral fat tissues in normal weight and obese humans and their correlations with clinical parameters and peripheral metabolic factors. Regul Pept. 2013a;185:65-72.
5. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994;372(6505):425-32.
6. Kokot F, Adamczak M, Wiecek A, Cieplok J. Does leptin play a role in the pathogenesis of essential hypertension? Kidney Blood Press Res. 1999;22(3):154-60.
7. Wauters M, Considine RV, Van Gaal LF. Human leptin: from an adipocyte hormone to an endocrine mediator. Eur J Endocrinol. 2000;143(3):293-311.
8. Lonnqvist F, Nordfors L, Jansson M, Thorne A, Schalling M, Arner P. Leptin secretion from adipose tissue in women. Relationship to plasma levels and gene expression. J Clin Invest. 1997;99(10):2398-404.
9. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 1996;334(5): 292-5.
10. Lin S, Thomas TC, Storlien LH, Huang XF. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. Int J Obes Relat Metab Disord. 2000;24(5):639-46.
11. Anukulkitch C, Rao A, Pereira A, McEwan J, Clarke IJ. Expression of genes for appetite-regulating peptides in the hypothalamus of genetically selected lean and fat sheep. Neuroendocrinology. 2010;91(3):223-38.
12. Wilding JP. Neuropeptides and appetite control. Diabet Med. 2002;19(8):619-27.
13. Jequier E. Leptin signaling, adiposity, and energy balance. Ann N Y Acad Sci. 2002;967:379-88.
14. Shek EW, Brands MW, Hall JE. Chronic leptin infusion increases arterial pressure. Hypertension. 1998;31(1 Pt 2):409-14.
15. Kuo JJ, Jones OB, Hall JE. Inhibition of NO synthesis enhances chronic cardiovascular and renal actions of leptin. Hypertension. 2001;37(2 Pt 2):670-6.
16. Vecchione C, Maffei A, Colella S, Aretini A, Poulet R, Frati G, et al. Leptin effect on endothelial nitric oxide is mediated through Akt-endothelial nitric oxide synthase phosphorylation pathway. Diabetes. 2002;51(1):168-73.

17. Korda M, Kubant R, Patton S, Malinski T. Leptin-induced endothelial dysfunction in obesity. Am J Physiol Heart Circ Physiol. 2008;295(4):H1514-21.
18. Beltowski J. Leptin and the regulation of endothelial function in physiological and pathological conditions. Clin Exp Pharmacol Physiol. 2012;39(2):168-78.
19. Beltowski J. Role of leptin in blood pressure regulation and arterial hypertension. J Hypertens. 2006;24(5):789-801.
20. Hauguel-de Mouzon S, Lepercq J, Catalano P. The known and unknown of leptin in pregnancy. Am J Obstet Gynecol. 2006;194(6):1537-45.
21. Arora S, Anubhuti. Role of neuropeptides in appetite regulation and obesity – A review. Neuropeptides. 2006;40(6):375-401.
22. Kalra SP, Kalra PS. NPY and cohorts in regulating appetite, obesity and metabolic syndrome: beneficial effects of gene therapy. Neuropeptides. 2004;38(4):201-11.
23. Zhang K, Rao F, Pablo Miramontes-Gonzalez J, Hightower CM, Vaught B, Chen Y, et al. Neuropeptide Y (NPY): Genetic Variation in the Human Promoter Alters Glucocorticoid Signaling, Yielding Increased NPY Secretion and Stress Responses. J Am Coll Cardiol. 2012;60(17):1678-89.
24. Lambert E, Straznicky N, Schlaich M, Esler M, Dawood T, Hotchkin E, et al. Differing pattern of sympathoexcitation in normal-weight and obesity-related hypertension. Hypertension. 2007;50(5):862-8.
25. Brain SD, Cox HM. Neuropeptides and their receptors: innovative science providing novel therapeutic targets. Br J Pharmacol. 2006;147 Suppl 1:S202-11.
26. Baltazi M, Katsiki N, Savopoulos C, Iliadis F, Koliakos G, Hatzitolios AI. Plasma neuropeptide Y (NPY) and alpha-melanocyte stimulating hormone (a-MSH) levels in patients with or without hypertension and/or obesity: a pilot study. Am J Cardiovasc Dis. 2011;1(1):48-59.
27. Petraglia F, Coukos G, Battaglia C, Bartolotti A, Volpe A, Nappi C, et al. Plasma and Amniotic Fluid Immunoreactive Neuropeptide-Y Level Changes during Pregnancy, Labor, and at Parturition1989. 324-8 p.
28. Gyamfi EAA. Maternal serum levels of adiponectin and leptin in non-pregnant, normal pregnant and preeclamptic women 2015.
29. Hytten FE. Weight gain in pregnancy. Clinical Physiology in Obstetrics. 1980:193–233
30. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004;363(9403):157-63.
31. Longo LD. Maternal blood volume and cardiac output during pregnancy: a hypothesis of endocrinologic control. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 1983;245(5):R720.
32. Thornburg KL, Jacobson SL, Giraud GD, Morton MJ. Hemodynamic changes in pregnancy. Semin Perinatol. 2000;24(1):11-4.
33. Thornburg KL, Louey S. Uteroplacental circulation and fetal vascular function and development. Curr Vasc Pharmacol. 2013;11(5):748-57.
34. Meah VL, Cockcroft JR, Backx K, Shave R, Stohr EJ. Cardiac output and related haemodynamics during pregnancy: a series of meta-analyses. Heart. 2016;102(7):518-26.
35. Thaler I, Manor D, Itskovitz J, Rottem S, Levit N, Timor-Tritsch I, et al. Changes in uterine blood flow during human pregnancy. Am J Obstet Gynecol. 1990;162(1):121-5.
36. Petraglia F, Coukos G, Battaglia C, Bartolotti A, Volpe A, Nappi C, et al. Plasma and amniotic fluid immunoreactive neuropeptide-Y level changes during pregnancy, labor, and at parturition. J Clin Endocrinol Metab. 1989;69(2):324-8.
37. Khatun S, Kanayama N, Belayet HM, Bhuiyan AB, Jahan S, Begum A, et al. Increased concentrations of plasma neuropeptide Y in patients with eclampsia and preeclampsia. Am J Obstet Gynecol. 2000;182(4):896-900.
38. Zukowska-Grojec Z. Neuropeptide Y. A novel sympathetic stress hormone and more. Ann N Y Acad Sci. 1995;771:219-33.
39. Paiva SP, Veloso CA, Campos FF, Carneiro MM, Tilan JU, Wang H, et al. Elevated levels of neuropeptide Y in preeclampsia: A pilot study implicating a role for stress in pathogenesis of the disease. Neuropeptides. 2016;55:127-35.
40. Sattar N, Greer IA, Pirwani I, Gibson J, Wallace AM. Leptin levels in pregnancy: marker for fat accumulation and mobilization? Acta Obstet Gynecol Scand. 1998;77(3):278-83.
41. Kawai M, Yamaguchi M, Murakami T, Shima K, Murata Y, Kishi K. The Placenta Is Not the Main Source of Leptin Production in Pregnant Rat: Gestational Profile of Leptin in Plasma and Adipose Tissues 1997.p.798-802.