ACYLATION STIMULATING PROTEIN IS ASSOCIATED WITH PREGNANCY WEIGHT GAIN

Among the proteins secreted by adipocytes, acylation stimulating protein (ASP), which plays a crucial role in energetic balance regulation, merits particular attention. ASP is a protein of the C3 complement system, responsible for glucose and lipids metabolism in an insulin-independent mechanism. ASP’s role during pregnancy and its interactions with pregnancy hormones remains unknown. The lipogenic character of ASP may impose a question as to what extent this hormone participates in pregnant women lipogenesis, and what is the basal and postprandial ASP secretion during the second trimester of pregnancy. The results of the examinations of 26 pregnant women during the second trimester of their first pregnancy were analyzed. Due to the limited data available in the literature, a control group was examined. The group consisted of 8 healthy non-pregnant patients within similar age ranges. Blood samples were collected in order to determine ASP, total cholesterol, HDL, LDL and triglyceride levels. Basal ASP levels present in obese pregnant women (group OBP; 30.20 ± 2.13 ng/mL) were significantly higher than those in the healthy control group (group LnP; 20.49 ± 1.97 ng/mL), P<0.05. Mann-Whitney U test- analysis of these group differences indicated that OBP patients had significantly higher ASP levels than controls at 30 (P<0.01), 60 (P<0.01), and 120 (P<0.01) min after a meal. After a meal, the incremental ASP area under the curve in group OBW patients was significantly higher from that observed in control group LnP (718,9 ± 263,9 ng/mL x 2h vs. 35,1 ± 14,6 ng/mL x 2h, P<0.05). Basal concentration of triglycerides, total cholesterol and LDL cholesterol were significantly higher in all pregnant women compared to the group of non-obese non-pregnant women. It was found that lipid parameters were highly dependent upon body mass gain during pregnancy. Group OBP demonstrated significantly higher basal concentrations of all parameters of lipid metabolism in comparison with the remaining groups of pregnant patients. In conclusion, we found abnormalities of ASP and lipid profiles in lean, overweight,
and obese pregnant women strictly connected with obesity. Acylation stimulating protein correlated with lipid parameters, suggesting increased risk of dyslipidemia in obese pregnant women.

Key words: obesity, pregnancy, acylation stimulating protein (ASP), adipose tissue.

INTRODUCTION

Obesity has become one of the most serious health problems of epidemic proportions affecting the first world. It’s been well established that obesity and fat distribution are strongly related to deranged metabolic functions and are also associated with an elevated incidence of cardiovascular disease, diabetes mellitus, cancer and liver diseases (1-4). Furthermore, obesity in pregnancy has implications to morbidity and mortality rates in both mother and baby (5, 6). Recently, a great deal of attention has focused on circulating adipokines, and studies have been carried out to elucidate the adipokines involvement in the mechanisms of abnormal weight gain or obesity in the mother and their critical influence on the programming of the metabolic pathways of her fetus, as well as its risk for having a phenotype which predisposes one to metabolic syndromes later in life (7). For many years, fatty tissue has been considered as an organ for internal secretions. The most recent studies indicate that the main complications of obesity are provoked by adipokines produced by fatty tissue (8-10). Fat cells produce many cytokines which are involved in the body’s energetic regulation and lipids metabolism (11, 12). Particular attention should be paid to ASP, which is among the proteins secreted by adipocytes, and it plays a crucial role in energetic balance regulation. ASP constitutes of a protein of the C3 complement system and is responsible for glucose and lipids metabolism in an insulin-independent mechanism. During the process of fat cell formation, ASP synthesis gradually increases (13, 14). In vitro chylomicrons stimulate ASP synthesis in the human adipocyte (15). Masłowska et al. (8) have proven that insulin enhances ASP synthesis. Both insulin and ASP suppress fatty tissue lipolysis (16). ASP increases triglyceride synthesis and storage in the fat cell and it also decreases fatty acids secretion from adipocytes (17, 18). In vitro studies revealed that ASP significantly stimulates glucose uptake in adipocyte (15, 19). In humans, an increased ASP secretion was found after a fatty meal, while the ASP concentrations remained unchanged after an oral administration of glucose. Higher ASP values are observed in the case of obesity, in nephrotic syndrome, in metabolic disorders with distinct dyslipidaemia and insulin resistance (20). ASP concentrations decrease with body mass (21). Further evidence of ASP physiological function has been obtained in studies conducted on rodents. ASP administered into the peritoneum of laboratory rodents causes an increase in
triglycerides (22). In rodent-based studies, Ahrén et al. proved that ASP has a
direct impact upon pancreas beta cells, which causes increased secretion of
insulin (21). Both insulin and ASP may share another feature, for instance, tissue
resistance (22). Insulin resistance is accepted as the metabolic syndrome
component (23, 24). The latest studies indicate that tissue immunity to ASP may
also occur (22, 25).

ASP’s role during pregnancy and its interactions with pregnancy hormones still
remains unknown. The studies of Saleh et al. have shown a greater influence of
ASP upon the fatty tissues of adipocytes in women when compared with male
adipocytes, as well as a close relationship between ASP and subcutaneous fatty
tissue lipogenesis in women. The authors suggest that hormone-related differences
may modulate ASP function (22). Furthermore, it has been demonstrated that ASP
correlates well with fatty tissue mass, resistance, dislipidaemia, and a higher
frequency of heart and vascular system diseases (26, 27).

The lipogenic characteristic of ASP may impose a question as to what extent
this hormone participates in pregnant women lipogenesis and what is the basal
and postprandial ASP secretion during the second trimester of pregnancy.
Although there is an unquestionable evidence of the association of ASP with
obesity, such information currently is contradictory to pregnancy. Therefore, the
aim of this study was to examine the classical and novel risk of adipogenic factors
in lean, overweight and obese women in the second trimester of pregnancy.

MATERIALS AND METHODS

Subjects

The study included 26 women with singleton pregnancies in the 2nd trimester of gestation.
Gestational age was confirmed by ultrasound scan. The exclusion criteria included pregnant women
with confirmed multiple pregnancies, cardiovascular diseases, diabetes mellitus, hypertension
arterialis, chronic inflammatory diseases and acute infections which may affect acute phase protein.
Due to little data available in the literature, a second control group was studied, which was
composed of 8 healthy and non-pregnant female patients within a similar age range. The patients
were recruited from the Obstetrics and Gynaecology Clinic of the Medical University of Silesia in
Ruda Śląska. The experimental protocol was approved by the Ethical Committee for Human
Experimentation of the Medical University of Silesia. All patients filled in a questionnaire which
consisted of questions concerning marital status, employment, previous operations, illnesses,
obstetric history, contraceptive use, smoking, alcohol consumption and paternal characteristics.

All the patients were thoroughly examined, and anthropometric examinations were performed.
After the evaluation of body mass and height, the body mass index (BMI) was determined using the
following formula: BMI = body mass (kg) / [height(m)]². Accordingly, the examined women were
divided into 4 groups: group CP - control group of non-pregnant lean women, group LnP – group
of lean pregnant women, in whom body mass index was determined within the ranges of 18.9 –
24.9, group OWP – group of overweight pregnant women, in whom body mass index was
determined within the ranges of 25.0 – 29.9, group OBP – group of obese pregnant women, in
whom body mass index was determined > 30.0.
Experimental design

All subjects were examined on two separate occasions. On the day of examination, subjects were admitted to the physiology testing unit after a two-day isocaloric (2000 kcal) diet. The meals contained 55% carbohydrates, 25% protein, and 20% fat. On the day of the examination, the subjects were after asked to fast from 20.00 h of the previous night. The heights and weights were recorded. Blood samples were drawn at baseline (mentioned as time 0 in the figure) between 08.00 and 09.00 h and 30, 60 and 120 min after the end of the standard mixed-meal as described previously.

Analyses

Acetylation stimulating protein (ASP) - was determined by the immunoenzymatic method, using ELISA (Linco Research, Missouri, USA). The method sensitivity was 0.12 ng/ml. Insulin was determined by the radioimmunological method using RIA (Linco Research, Missouri, USA), the method’s sensitivity being 2.0 mIU/l. Glucose and lipids were determined using an enzymatic kit, with the use of a commercial diagnostic set. Insulin sensitivity was estimated by the homeostatic model assessment [HOMA-IR index], which is the product of plasma glucose and insulin, divided by 22.5.

Statistical analyses

All values are expressed as means ± SEM. A Comparison of baseline concentrations and the magnitude between baseline and the peak were performed using ANOVA followed by post hoc analysis using Bonferroni. The incremental area under the curve (AUC) for each hormone profile was calculated using the trapezoid method after subtracting the basal AUC from the calculated AUC. The significance of the differences between the individual groups was assessed using Mann-Whitney U test. The relationship between the variables was assessed using the Pearsons coefficient of correlation. P-values less than 0.05 were considered significant.

RESULTS

Demographic, clinical and biochemical characteristics of each subject group as well as baseline ASP levels and HOMA – IR indexes are given in Table 1.

There were no differences between the four groups in respect to age, gestational age and HDLC values. Moreover, there were no differences between LnP and LP groups in initial body weight, BMI, delta weight gain and TC. Weight gain was more than 2-fold higher in OWP and 4-fold higher in OBP as compared with the lean controls (LnP and LP). Although total cholesterol TC and HDLC concentrations were not significantly different between both the lean groups, fasting plasma TG concentrations and HOMA-IR values were significantly higher and HDLC concentrations lower in the overweight and obese pregnant women. Baseline ASP concentrations were significantly different among the four groups (P<0.001), with concentrations OWP and OBP being higher than concentrations in the LnP and LP groups.

ASP responses to the mixed meal

As shown in Fig. 1, ASP concentrations rose significantly in the OWP and OBP groups after the meal, reaching a momentum after 60 min (the maximal
percent increase in ASP 56% OWP, P<0.01 and 77% OBP, P<0.01, compared with baseline) followed by a subsequent rise back to baseline, though in both these groups it was higher by the end of the study period (120 min), whereas ASP concentration variations seemed to be higher in the LnP group after the meal, there were no differences between the peak values and the basal concentrations (Fig. 2).

The incremental AUC of plasma ASP in OBW patients (718.9 ± 263.9 x 2h) was significantly higher than that observed in the control group LnP (35.1 ± 14.6 x 2h), P<0.01, demonstrating an increase of ASP secretion in obese pregnant women (Fig. 3).

Basal concentration of triglycerides, total cholesterol and LDL cholesterol levels were significantly higher in all pregnant women compared to the group of non-obese non-pregnant women. It was found that lipid parameters were highly dependent upon body mass gain during pregnancy. Group OBP demonstrated significantly higher initial concentrations of all parameters of lipid metabolism in comparison with the remaining groups of pregnant patients (Table 1).

### Table 1. Demographic, clinical and biochemical characteristics of the studied subjects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LnP n=8</th>
<th>LP n=8</th>
<th>OWP n=11</th>
<th>OBP n=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.2 ± 3.1</td>
<td>22.2 ± 2.1</td>
<td>23.4 ± 2.5</td>
<td>23.6 ± 2.4</td>
</tr>
<tr>
<td>Height [cm]</td>
<td>164.4 ± 4.7</td>
<td>162.8 ± 6.6</td>
<td>163.9 ± 5.1</td>
<td>164.8 ± 8.2</td>
</tr>
<tr>
<td>Prepregnancy Weight (kg)</td>
<td>57.4 ± 8.1</td>
<td>56.4 ± 5.6</td>
<td>61.1 ± 2.2**</td>
<td>86.0 ± 11.2***#</td>
</tr>
<tr>
<td>Prepregnancy BMI (kg/m²)</td>
<td>21.2±2.0</td>
<td>22.1 ± 1.8</td>
<td>26.1 ± 1.3**</td>
<td>35.1 ± 1.2**#</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>-</td>
<td>21.2 ± 3.1</td>
<td>21.5 ± 2.5</td>
<td>21.3 ± 1.8</td>
</tr>
<tr>
<td>Delta Weight gain (kg)</td>
<td>+5.9±0.7</td>
<td>+6.5 ± 0.8</td>
<td>+14.7 ± 1.7**</td>
<td>+22.3 ± 2.4**#</td>
</tr>
<tr>
<td>TC (ng/dL)</td>
<td>171.6 ± 12.6</td>
<td>182.3 ± 19.5</td>
<td>192.7 ± 20.6*</td>
<td>251.3 ± 39.3***#</td>
</tr>
<tr>
<td>HDLC (ng/dL)</td>
<td>63.1 ± 5.8</td>
<td>62.3 ± 8.3</td>
<td>57.8 ± 6.3</td>
<td>59.0 ± 8.5</td>
</tr>
<tr>
<td>LDLc (ng/dL)</td>
<td>105.1 ± 10.9</td>
<td>121.6 ± 10.1</td>
<td>121.2 ± 18.8</td>
<td>167.0 ± 20.1**</td>
</tr>
<tr>
<td>TG (ng/dL)</td>
<td>108.9 ± 15.2</td>
<td>219.8 ± 25.6*</td>
<td>228.8 ± 26.2*</td>
<td>258.1 ± 30.2*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.9 ± 0.51</td>
<td>2.91 ± 0.42*</td>
<td>3.4 ± 1.26*</td>
<td>5.23 ± 0.99**#</td>
</tr>
<tr>
<td>Fasting ASP (ng/mL)</td>
<td>20.49 ± 1.97</td>
<td>25.50 ± 2.59*</td>
<td>28.67 ± 1.61**</td>
<td>30.20 ± 2.13***#</td>
</tr>
</tbody>
</table>

Data are presented as mean (SEM). Groups: lean non-pregnant (LnP), lean pregnant (LP), overweight pregnant (OWP), obese pregnant (OBP); TC, total cholesterol; HDLC, high-density lipoprotein; LDLc, low-density lipoprotein cholesterol; TG, triglycerides; HOMA-IR, homeostatic model assessment insulin resistance index; * p<0.05 vs. LnP group; ** p<0.05 vs. LnP&LP groups; * p<0.05 vs. OWP group; Conversion from metric to SI Units: TC, nanograms/deciliter x 0.0259 = millimoles/liter; HDLC, nanograms/deciliter x 0.0136=millimoles/liter; LDLc, nanograms/deciliter x 0.000 = millimoles/liter; TG, nanograms/deciliter x 0.0114= millimoles/liter.
The HOMA index in obese pregnant women (OBP; $5.23 \pm 0.99$) was substantially higher than in lean pregnant women (LP; $2.91 \pm 0.42$), $P < 0.001$ and in the group of non-obese non-pregnant (LnP: $1.9 \pm 0.51$), $P < 0.001$ (Table 1).
Statistically, in obese pregnant women (group OBP), the basic ASP concentrations were significantly higher than basal ASP concentrations revealed by the group of non-obese non-pregnant women (group LnP, \( P<0.001 \)), and they were similar to those of overweight pregnant women (group OWP). The ASP concentrations in the group of non-obese pregnant participants (group LP) and in the group of overweight pregnant women (group OWP) were, higher in comparison with the obtained levels in the group of non-obese non-pregnant women (group LnP), and this was noted to be statistically significant. Statistically significant changes were also observed in the ASP concentrations in the group of non-obese pregnant women (group LnP) and overweight pregnant women (group OWP; \( P<0.05 \)) (Table 4, Fig. 3).

Both in the group of non-obese pregnant women (group LnP) and in the group of lean pregnant women (group LP), a difference which was noted to be statistically significant (\( P<0.05 \)) was observed in the decrease of the ASP concentration after 30, 60 and 120 minutes was registered. In the groups of overweight pregnant subjects (group OWP) and obese pregnant women (group OBP), a significant statistical increase (\( P<0.05, P<0.01 \)) of values in the 30th and 60th minute was observed, followed by another concentration drop in the 120th minute, which values obtained for the group OWP were similar to the basic ones, while in group OBP, significantly higher values were recorded (\( P<0.05 \))( Fig. 3).

Statistical analyses indicated significantly lower postprandial ASP decrease (\( P<0.001 \)) in obese pregnant women (group OBP) when compared with lean pregnant (group LP), overweight pregnant participants (group OBP) and non-

Fig. 3. AUC ASP of the studied group. Data are presented as mean (SEM). Groups: lean non-pregnant (LnP), lean pregnant (LP), overweight pregnant (OWP), obese pregnant (OBP).
obese non-pregnant (group LnP) women. Also, the group of obese pregnant women demonstrated a statistically significant after-meal decrease in the ASP concentration compared to lean pregnant women (group LP) and non-obese non-pregnant women (group LnP) (Fig. 4).

DISCUSSION

We have demonstrated for the first time that adipogenic proteins such as ASP may be relevant to within physiological range weight gain in pregnancy, because of their different postprandial characteristic pattern release, when compared to their lean nonpregnant counterparts.

The study has proven that there is a significant increase in the fasting plasma ASP levels in obese pregnant women. Significant positive correlations between the fasting ASP levels and TG, LDL-C, HDL-C, total cholesterol levels, and insulin resistance in obese pregnant women were observed.

Excessive body mass gain during pregnancy correlates well with the increased ASP concentrations when compared with the group of non-obese and non-pregnant women. Metabolic resistance is an important feature that is shared between insulin and ASP (25). Insulin resistance has long been recognized during late pregnancy (25, 28). In vitro studies demonstrate that ASP tissue resistance may also occur (25). Currently, ASP’s role during pregnancy and its correlated pregnancy hormones remains beyond the scientific know-how. In vivo studies have shown that estrogen as an anti-fat factor favors the occurrence of hyperlipidemia (29). In rodents after ovariectomy, a decreased fat mass and decreased triglycerides storage in adipocyte have been observed (30, 31). As far as progesterone is concerned, it provokes an excessive amount of blood insulin, which is probably due to its direct impact on pancreas islets and it also stimulates fatty tissue synthesis (26). However, estrogen and progesterone levels vary during pregnancy. During late pregnancy, the estrogen concentration increases as well as the estrogen to progesterone ratio, this favours the decrease of the fatty tissue amount. Further evidence is required before a conclusion can be reached as to whether pregnancy hormones have a direct impact on the ASP metabolism (27). However, the same studies indicate that pregnancy hormones may modify the ASP function, this suggests that the increase in the ASP concentrations during pregnancy is a counterbalance to the estrogen which decreases the number of adipocytes (27). A strong correlation between ASP and BMI in the examined pregnant women indicates the important role of ASP as the factor influencing lipids metabolism and body mass gain during pregnancy. Further studies would be necessary to account for the influence of hormones on the ASP function during the different trimesters of pregnancy.

In our study, we found increased insulin and insulin resistance in the obese pregnant women, and these trends were more pronounced by the higher BMI. We
believe that insulin resistance and adipocyte hormones may influence each other in obese pregnant women, with altered adipocyte hormones aggravating the level of insulin resistance. The increased ASP, LDL-C, HDL-C and total cholesterol in obese pregnant women that was shown in our study may have deleterious effects. As reported in several studies in mice and adipocytes, ASP enhances energy storage through increasing postprandial triglyceride clearance and adipose tissue fatty acid esterification and decreasing hormone-sensitive lipase-mediated lipolysis (32, 33). However, in human studies, increased plasma ASP is associated with obesity, diabetes, and cardiovascular disease (2, 4). Furthermore, increased fasting plasma ASP is associated with a delay in postprandial triglyceride clearance in human beings (34, 35). Whether increased ASP is a compensatory mechanism or an indication of resistance remains unknown.

In conclusion, we found abnormalities of ASP and lipid profiles in lean, overweight, and obese pregnant women which were dependent on obesity. Acylation-stimulating protein correlated with lipid parameters, suggested an increased risk of dyslipidemia in obese pregnant women. We found that ASP as a lipogenic factor contributing to the lipogenesis process during pregnancy. Therefore we assume that an abnormalities of the ASP contributes to the increase of the fatty mass during pregnancy. Further studies will be necessary to determinate the mechanism of ASP and the interaction between gestational hormones.

Conflict of interest statement: None declared.

REFERENCES

2. Reaven G. The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. Endocrinol Matab Clin North Am 2004; 33: 283-303;

29. Höckerstedt A, Jauhiainen M, Tikkanen MJ. Estradiol fatty acid esterification is increased in high density lipoprotein subclass 3 isolated from hypertriglyceridemic subjects. *Atherosclerosis* 2006; 185(2): 264-270.


Received: March 15th, 2008
Accepted: August 1st, 2008

Author’s address: Krystyna Zwirska-Korczala MD, PhD; Department of Physiology, Medical University of Silesia, 41-800 Zabrze, ul. Jordana 19, Poland; e-mail: kkorcz@slam.katowice.pl