Potential Biomarkers of Metabolic Syndrome in a Group of Small for Gestational Age Neonates

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Objective - Small for gestational age (SGA) infants have an increased risk of developing metabolic syndrome (MS) later in life. The aim of our study was to analyze some of the potential MS biomarkers in SGA neonates and to find the most indicative neonatal biomarker associated with MS in SGA. Materials and Methods - Serum adiponectin, fasting glucose, insulin, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and triglycerides were evaluated in 43 small (SGA) and 25 appropriate for gestational age (AGA) neonates in the first month of life. Glucose/insulin (G/I) ratio and the homeostasis model assessment of insulin resistance index (HOMA-IR) were calculated. Results - Mean serum adiponectin in SGA was significantly lower than in AGA neonates (36.6 vs. 42.6 mg/L, P=0.021). In SGA infants a statistically insignificant higher fasting insulin levels (9.8 vs. 6.4 mIU/L), lower G/I ratio (1.9 vs. 3.0 mol/IU) and higher HOMA-IR (2.6 vs. 1.4) was observed. The mean serum triglyceride level in SGA neonates was significantly higher than in AGA neonates (1.7 vs. 1.4 mmol/L, P=0.031). Conclusion - SGA neonates demonstrated significantly reduced serum adiponectin and significantly increased serum triglyceride levels in comparison to AGA infants in the first month of life. Triglycerides appeared to be the most promising biomarker reflecting metabolic tendencies in SGA newborns and could possibly be used in predicting the future development of MS.

Introduction

Many epidemiological studies demonstrated that low birth weight for gestational age was associated with increased incidence for cardiovascular and metabolic diseases later in life (1). Accordingly, the fetal origins or the thrifty phenotype hypothesis was formed which states that the unfavourable intrauterine environment leads to the reprogramming of the endocrine and metabolic pathways in the fetus, and so enables the fetus short term survival (2). However, in the long term, this reprogramming might be detrimental and develop into the metabolic syndrome (MS), also known as the syndrome X or the insulin resistance syndrome (3), which encompasses central obesity, dyslipidemia, arterial hypertension and glucose intolerance or diabetes type 2 both indicating insulin resistance (4). The interplay between glucose metabolism, insulin resistance, endothelial dysfunction, systemic low-grade inflammatory and prothrombotic state may have a crucial role in the development and progression of MS (5).

Studies of pathophysiological mechanisms of MS revealed potential biomarkers
of MS, but so far it has not yet been clearly determined when in postnatal life pathological changes become evident and what impact do they have on the progression of diseases related to MS. Adiponectin, which enhances insulin sensitivity and can therefore protect the vasculature from the detrimental effects of insulin resistance and diabetes, alone or in combination with other biomarkers was studied in early postnatal life (6-8), in the first two years of life (9), in prepubertal children (10-13) and young adults (14). In all studies, adiponectin was demonstrated to be lower in small for gestational age (SGA) in comparison to appropriate for gestational age (AGA) infants (15).

Insulin resistance of peripheral tissues is proposed to be additional mechanism by which sufficient glucose in growth restricted infants is provided to the brain (16, 17). Glucose, insulin, insulin-like growth factors and lipids were studied in umbilical cord plasma of SGA and AGA newborns (18-20) and in the first days postnatally (21, 22). There are conflicting reports on the levels of glucose, insulin and lipids in SGA neonates, but all of the studies demonstrated the level of triglycerides to be higher in SGA neonates.

The purpose of our study was to analyse biomarkers of MS: serum adiponectin, insulin resistance and lipid profile in a group of SGA and AGA neonates and to find the most indicative neonatal biomarker of MS while being small for gestational age.

**Patients and Methods**

**Patients**

A total of 43 SGA neonates hospitalized at the Department of Neonatology, University Children’s Hospital Ljubljana, whose parents agreed to participate in the study, were enrolled in the study. The inclusion criteria were birth weight and/or birth length under the 10th percentile for the newborn’s gestational age and gender according to Slovenian percentile curves. Only neonates from single pregnancies were included. Neonates with chromosomal anomalies, organ malformations, intracerebral haemorrhage or congenital infection were excluded. In the control group 25 AGA newborns were recruited, with birth weight and birth length between the 10th and 90th percentile for gestational age and gender. All newborns in both groups were breast or bottle fed, with similar frequencies ranging around 3 hours. At recruitment, data of newborns (gender, gestational age, birth weight, birth length and head circumference) were retrieved from clinical records. The characteristics of both groups are presented in Table 1.

In the neonatal period, a 2 ml blood sample was obtained from newborns. Samples were withdrawn immediately before feeding, in fasting conditions. Blood was immediately centrifugated, and the serum was stored at – 20 °C until processing. Concomitantly, blood lipid concentrations were determined.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SGA (n=43)</th>
<th>AGA (n=25)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male:female, %)</td>
<td>23:20 (53:47)</td>
<td>14:11 (56:44)</td>
<td>0.841</td>
</tr>
<tr>
<td>Gestational age (week)</td>
<td>37.5 ± 2.8</td>
<td>38.6 ± 1.8</td>
<td>0.126</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2236 ± 505</td>
<td>3451 ± 445</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>46.1 ± 3.9</td>
<td>51.3 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>31.6 ± 2.6</td>
<td>34.5 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Small for gestational age; †Appropriate for gestational age.
Laboratory Procedures

Laboratory measurements of adiponectin, insulin and plasma lipids were performed in the laboratory of the Institute of Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana. Laboratory measurements of fasting glucose were performed in the laboratory of the Department of Clinical Biochemistry, University Children’s Hospital, University Medical Centre Ljubljana.

Adiponectin in sera of neonates was measured by radioimmunoassay using 125 I-labeled antigen (Linco Research, Missouri, USA). Serum insulin was determined using automated chemiluminescent immunoassays (analyzer Liaison, Diasorin, Salluggia, Italy). Plasma lipids: total cholesterol, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, triglycerides were measured using routine clinical laboratory methods. Briefly, total cholesterol and triglyceride (TG) concentrations were measured by enzymatic-colorimetric methods; HDL-cholesterol was measured by a direct method and LDL-cholesterol was determined by the Friedewald formula. Blood glucose concentration was determined using a commercial glucometer.

Assessment of Insulin Sensitivity

The ratio between serum glucose and insulin (G/I) was calculated dividing both values. The G/I ratio was taken as an insulin sensitivity index. The homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated as follows: HOMA-IR=fasting I concentration (mIU/L) x fasting G level (mmol/L)/22.5. Lower HOMA-IR values indicate greater insulin sensitivity, whereas higher HOMA-IR values indicate lower insulin sensitivity, in other words insulin resistance. In the absence of cutoff points for insulin levels and HOMA-IR index in the newborn, values in the adult population were considered as the cutoff points (23).

Ethics Statement

The study was approved by the National Medical Ethics Committee (number 33/08/07) and a written informed consent was obtained from one of the parents of each subject prior to the enrolment in the study.

Statistical Analysis

The basic data and measured biomarkers of AGA and SGA newborns were expressed as a mean ± SD or as percentage distribution for gender. Spearman’s rho was used to calculate the correlation coefficients between birth measures and biomarkers for SGA newborns. For the comparison of biomarkers between the AGA and SGA groups of newborns, we performed the Mann-Whitney U test and χ²-test. Biomarkers resulting in univariate significance were separated into two groups according to median value and included in multivariate logistic regression. The χ², influence factor, 95% confidence interval and P value were calculated. Statistical analysis was performed with the SPSS 15.0 software (SPSS, Chicago, IL). P <0.05 was marked as statistically significant.

Results

The mean±SD values of the analyzed parameters in SGA and AGA group of Caucasian infants are represented in Table 2.

The mean serum adiponectin in SGA was 14% lower than in AGA neonates. There was also no significant difference in fasting glucose levels between SGA and AGA neonates. In SGA infants a statistically insignificant higher fasting insulin levels, lower G/I ratio and higher HOMA-IR was observed. In fact, 84% of AGA and only 56 % of SGA infants were insulin sensitive according to their HOMA score (<2). SGA neonates also had higher total cholesterol and LDL levels com-
pared to AGA neonates, but the differences were not statistically significant. The mean serum triglyceride level was significantly higher in SGA compared to AGA neonates. LDL was the only biomarker that was proved to be significantly different between genders, as the mean LDL concentrations were significantly higher in SGA girls (mean±SD 1.6±0.6 mmol/L) than in SGA boys (mean ±SD 1.3±0.5 mmol/L; Z=-2.0; P=0.044).

**Birth Weight, Height and Head Circumference in Correlation with the Measured Biomarkers**

We found a positive correlation between birth measures (weight, height, head circumference) and total cholesterol and LDL levels in SGA neonates (Table 3), while there was no correlation between birth measures and other measured biomarkers. When correlating between birth measures and biomarkers in all the subjects from the study and control group, adiponectin correlated positively with birth weight (r=0.251, P=0.042).

**Logistic Regression Analysis**

Multivariate logistic regression analysis showed that the concentration of triglycerides was a significant independent predictor of being SGA (the factor of influence 3.8, 95% CI 1.2-12.3) (Table 4). SGA neonates had lower adiponectin levels in comparison

### Table 2. Laboratory Values from SGA* and AGA† Newborns.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>SGA* (n=43)</th>
<th>AGA† (n=25)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (mg/L)</td>
<td>36.6 ± 13.2</td>
<td>42.6 ± 11.3</td>
<td>0.021†</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.8 ± 1.5</td>
<td>4.6 ± 0.6</td>
<td>0.595</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>9.8 ± 10.6</td>
<td>6.4 ± 8.7</td>
<td>0.282</td>
</tr>
<tr>
<td>HOMAIR</td>
<td>2.6 ± 3.2</td>
<td>1.4 ± 2.0</td>
<td>0.300</td>
</tr>
<tr>
<td>Glucose/Insulin</td>
<td>1.9 ± 3.1</td>
<td>3.0 ± 5.0</td>
<td>0.218</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>2.9 ± 0.7</td>
<td>2.9 ± 0.7</td>
<td>0.978</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.5 ± 0.6</td>
<td>1.4 ± 0.6</td>
<td>0.833</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>1.6 ± 1.2</td>
<td>1.6 ± 1.0</td>
<td>0.913</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.7 ± 0.8</td>
<td>1.4 ± 0.6</td>
<td>0.031†</td>
</tr>
</tbody>
</table>

*Small for gestational age; †Appropriate for gestational age; ‡P<0.05; §High density lipoprotein; ‖Low density lipoprotein; ¶Triglyceride.

### Table 3. Correlations Between Birth Measures and Biomarkers Measured for SGA* Newborns.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Birth weight</th>
<th>Birth length</th>
<th>Head circumference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td>r=0.050, P=0.752</td>
<td>r=0.009, P=0.953</td>
<td>r=0.065, P=0.682</td>
</tr>
<tr>
<td>Glucose</td>
<td>r=0.055, P=0.731</td>
<td>r=0.022, P=0.889</td>
<td>r=0.139, P=0.379</td>
</tr>
<tr>
<td>Insulin</td>
<td>r=0.137, P=0.387</td>
<td>r=0.019, P=0.904</td>
<td>r=0.280, P=0.073</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>r=0.390, P=0.012†</td>
<td>r=0.318, P=0.043†</td>
<td>r=0.329, P=0.035†</td>
</tr>
<tr>
<td>HDL</td>
<td>r=0.157, P=0.327</td>
<td>r=0.146, P=0.363</td>
<td>r=0.136, P=0.396</td>
</tr>
<tr>
<td>LDL</td>
<td>r=0.489, P=0.001†</td>
<td>r=0.469, P=0.002†</td>
<td>r=0.401, P=0.0099</td>
</tr>
<tr>
<td>TG</td>
<td>r=0.036, P=0.823</td>
<td>r=0.117, P=0.467</td>
<td>r=0.016, P=0.921</td>
</tr>
</tbody>
</table>

*Small for gestational age; †High density lipoprotein; ‡Low density lipoprotein; ¶Triglyceride; ††P<0.05, ††P<0.01.
to the levels in AGA neonates but in multivariate logistic regression analysis adiponectin did not reach a statistically significant impact (IF 0.4, 95% CI 0.1-1.3). Fig. 1 presents the distribution of SGA and AGA newborns according to adiponectin and triglyceride levels.

Discussion

In SGA neonates, we simultaneously established significantly lower mean serum adiponectin and significantly higher mean serum triglyceride levels in comparison to AGA neonates. There are many reports on lower adiponectin levels in SGA neonates (6-8). It was also reported previously (7, 24, 25), that serum adiponectin levels correlate positively with birth weight, length and head circumference and that there is no gender difference in adiponectin levels, though Ibanez and co-workers (19) reported higher levels of adiponectin in girls who were born SGA. In the study of Cianfarani and co-workers (26) it was showed that the levels of adiponectin were also reduced in SGA children at 8 years of age, and the level was even lower in those with adequate postnatal catch-up growth. Similarly, Jaquet and co-workers (14) demonstrated significantly reduced serum adiponectin levels in young adults born SGA. A fall in serum adiponectin levels during the first two years of life was related to increasing age and greater weight gain in SGA infants (9), but on the other side this could also reflect insulin resistance.
To the best of our knowledge, this is the first report on concomitant establishment of significantly lower serum adiponectin and significantly higher triglyceride levels in newborn period. Recently, the combination of lowered adiponectin and higher triglyceride levels were found in short SGA prepubertal children (27). As mentioned, we demonstrated higher mean triglycerides in SGA newborns and similar result was shown also by the study of Kelishadi and co-workers (18) when assessing cord blood lipid profile of neonates. According to the results of multivariate logistic regression analysis the concentration of triglycerides proved to be the most promising biomarker in our SGA newborn population: the concentration of triglycerides 1.4 mmol/liter and above independently predicted the risk of being SGA. Since adiponectin is known to be reducing plasma concentrations of triglycerides our results are in accordance with this observation.

Even though adiponectin is known to be reducing hepatic gluconeogenesis, in our study there was no significant difference in fasting glucose levels between SGA and AGA neonates. Adiponectin is also known as insulin sensitizing protein and this could explain our observation of SGA infants having a trend towards higher fasting insulin levels, lower G/I ratio and higher HOMA-IR, but the results proved statistically insignificant. Equal, but statistically significant observations were noted among prepubertal children and adults, who were born SGA (28, 29). Bazaes and co-workers (21) and Iñiguez and co-workers (30) reported lower glucose and insulin levels and higher glucose/insulin ratios in SGA compared to AGA infants at 48 hours of age. They concluded that in early postnatal life, SGA infants display an increased insulin sensitivity with respect to glucose disposal (21). At 3 years, respectively, fasting insulin was significantly higher in SGA infants with catch-up growth in weight, compared to those who did not catch up, and AGA infants (30). In short prepubertal children born SGA, Arends and co-workers observed significantly reduced mean insulin sensitivity level and also significantly higher mean acute insulin response compared to short AGA controls (31).

Insulin is thought to play a key role in the pathogenesis of MS (31). Intrauterine growth restriction has been linked both to increased resistance of peripheral tissues to insulin in order to provide sufficient glucose to the brain (16, 19) and also to diabetes type 2 (32). Insulin hypersecretion leads to increased fatty acid synthesis in the liver and adipose tissue. A compensatory increase in glucose oxidation and diversion away from beta-oxidation lead to compensatory increases in long-chain CoA and triglyceride synthesis in the liver. Triglycerides in the blood are a marker of intracellular hepatic long-chain CoA accumulation and increased VLDL synthesis. Oxidation of glucose is decreased and in the islets apoptosis of beta-cells leads to decreased insulin secretion and type 2 diabetes (32). In the study of Soto and co-workers it was demonstrated that SGA infants had a clear tendency to higher triglycerides at 1 year of age, and this is by some authors thought to be a relevant marker of insulin resistance (33). Though a total of 79 relevant studies in metaanalysis suggest that impaired fetal growth does not have effects on blood cholesterol levels that would have a material impact on vascular disease risk (34), there are studies in which significantly lower mean total cholesterol, HDL and LDL concentrations were found in umbilical venous blood at term age (18). Two studies of prepubertal children born SGA matched to AGA controls demonstrated normal range of mean fasting serum levels of total cholesterol, HDL, LDL and triglycerides, and no difference in fasting serum lipid concentrations (31), but the study of Tenhola and co-
workers demonstrated that nearly half of the SGA children were in the highest quartile for serum total cholesterol of the AGA children at the age of 12 years (35). According to this data intrauterine growth restriction may already influence the level of serum total cholesterol before the teens. SGA children with poor catch-up growth in height may be at the highest risk for hypercholesterolemia.

Conclusion

In summary, already in the neonatal period, we again confirmed a significant reduction in serum adiponectin levels in SGA infants in comparison to AGA infants. Concomitantly, we measured significantly increased serum triglyceride levels in SGA newborns. Lower levels of adiponectin, being a protein with insulin sensitising properties, could lead to insulin resistance which according to our results (higher HOMA-IR in SGA) could be present already in newborn period. From the biomarkers tested, glucose, insulin, total cholesterol, HDL, and LDL cholesterol failed to reflect the presumed endocrine and metabolic differences among SGA and AGA neonates. Considering our results, triglycerides appeared to be the most promising biomarker reflecting metabolic tendencies in SGA newborns and could possibly be used in predicting the future development of MS. Though larger prospective studies are required to further understand the clinical value of triglycerides and adiponectin measurement in SGA children, we intend to follow up the children from our group in order to further analyse the predictive value of both biomarkers.

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Authors’ contributions: Conception and design: PF, DPP; Acquisition, analysis and interpretation of data: PF, DPP, MZ, AJ, MS; Drafting the article: PF, DPP; Revising the article critically for intellectual content: PF, DPP; Approved final version of the manuscript: PF, DPP, MZ, AJ, MS.

Conflict of Interest: The authors declare that they have no conflict of interest.

References


