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Mokkala, Kati

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Increased intestinal permeability, measured by serum zonulin, is associated with metabolic risk markers in overweight pregnant women

Kati Mokkala[a,b,*], Outi Pellonperä[c], Henna Röytiö[a,b], Pirkko Pussinen[d], Tapani Rönnemaa[e], Kirsi Laitinen[a,b]

[a] Institute of Biomedicine, University of Turku, Finland
[b] Functional Foods Forum, University of Turku, Finland
[c] Department of Obstetrics and Gynecology, Turku University Hospital and University of Turku, Finland
[d] Oral and Maxillofacial Diseases, University of Helsinki and University Hospital of Helsinki, Finland
[e] Department of Medicine, Institute of Clinical Medicine, University of Turku and Turku University Hospital, Finland

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ABSTRACT

Background. Increased intestinal permeability with subsequent metabolic endotoxemia, i.e., elevated circulating levels of bacterial lipopolysaccharide, LPS, has been introduced as a novel initiator of obesity related metabolic disturbances in non-pregnant individuals. The objective was to investigate the extent to which intestinal permeability, measured by serum zonulin concentration, is related to metabolic endotoxemia and metabolic risk markers in overweight pregnant women.

Methods. This was a cross-sectional study including 100 pregnant overweight women in early pregnancy. Serum zonulin was analyzed using ELISA, and markers for metabolic endotoxemia (LPS), inflammation (high-sensitive C-reactive protein and glycoprotein acetylation GlyA), glucose metabolism (fasting glucose and insulin), and lipid metabolism were measured.

Results. Higher serum zonulin concentration associated positively with LPS ($P = 0.02$), inflammatory markers ($P < 0.001$), insulin ($P < 0.001$), insulin resistance ($P < 0.001$), and triglycerides ($P = 0.001$), and negatively with insulin sensitivity ($P = 0.001$) (ANOVA with Tukey's corrections or Kruskal–Wallis nonparametric test with Bonferroni correction for zonulin quartiles). All the observed associations were confirmed ($P < 0.015$) in a linear regression model adjusted with potential confounding factors. Both LPS and GlycA showed positive relationship with insulin resistance, serum insulin, triglycerides, total and LDL-cholesterol and negative relationship with insulin sensitivity ($P \leq 0.03$) in the univariate linear regression. Positive relationship was also found between LPS and HDL-cholesterol ($P = 0.03$).

Conclusions. Our findings suggest that increased serum zonulin concentration, i.e., increased intestinal permeability, contributes to metabolic endotoxemia, systemic inflammation, and insulin resistance in overweight pregnant women. By reinforcing

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; GlycA, glycoprotein acetylation; hs-CRP, high-sensitive C-reactive protein; HOMA2-IR, homeostatic model assessment-method; IL-6, interleukin-6; LPS, lipopolysaccharide; PCA, principal component analysis; QUICKI, Quantitative Insulin Sensitivity Check Index; TNF-α, tumor necrosis factor-α.

* *Corresponding author at:* 20014 University of Turku, Finland. Tel.: +358 400822273.
E-mail address: Kati.Mokkala@utu.fi (K. Mokkala).

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intestinal barrier, it may be possible to manipulate maternal metabolism during pregnancy with subsequent health benefits.

1. Introduction

Maternal metabolic and inflammatory profiles during pregnancy are altered to secure the optimal growth and development of the fetus [1]. In obese pregnant women, metabolic changes, including elevation in glucose production, serum lipids, and insulin resistance [2], are exacerbated and subsequently may lead to an increased risk of complications in both mother and child. Indeed, the obesity-related increased risk for maternal adverse outcomes during pregnancy includes gestational diabetes [1], with heightened risk for subsequent type 2 diabetes [3] and cardiovascular disease (CVD) [4]. The consequences for the offspring include an increased risk for insulin resistance and obesity [3]. Because of the importance of pregnancy conditions on health of both the mother and child, a deeper understanding of the underlying mechanisms of obesity-related metabolic disturbances during pregnancy is necessary.

In non-pregnant populations, obesity contributes to multiple lifestyle-associated chronic disorders. Obesity has been associated with low grade inflammation, a condition characterized as increased concentrations of inflammatory markers including C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) [5]. Recent studies suggest low grade inflammation as an underlying mechanism for a range of metabolic disturbances like metabolic syndrome, type 2 diabetes, dyslipidemia, and cardiovascular disease [6–8]. Whether obese women during pregnancy exhibit the same low grade inflammatory status is less well known; it has been suggested that they have higher serum CRP and IL-6 concentrations compared to normal weight pregnant women [9–11].

Low grade inflammation is considered to originate from the adipose tissue, explaining the strong link between obesity and low grade inflammation. In pregnancy, another source for low grade inflammation is the placenta, which by producing cytokines may have an active role in mediating the inflammation in obese women and those with GDM [1]. The latest research indicates that the gut may be an additional source for low grade inflammation [12–15]. This may arise from an increased permeability of the intestine that allows the passage of gram-negative bacterial endotoxin, lipopolysaccharide (LPS) into circulation. The increased circulatory LPS, i.e., metabolic endotoxemia, may induce inflammatory responses [16,17] through the activation of toll-like receptor 4 [5]. We have shown that there is considerable variation in intestinal permeability among overweight and obese pregnant women [18].

Currently, the role of increased intestinal permeability during pregnancy as a source of low grade inflammation and subsequent metabolic disturbances is poorly understood. Therefore, we studied the impact of intestinal permeability on metabolic endotoxemia, inflammation, and glucose and lipid metabolism by using serum zonulin as its biomarker. Further, we evaluated the interrelations of these risk markers in overweight pregnant women.

2. Methods

2.1. Study Subjects and Design

This cross-sectional study included 100 overweight women in early pregnancy (median: 13.0; IQR: 11.0–15.0 weeks of gestation) living in Southwest Finland. The data were collected from healthy overweight women participating in a mother–infant dietary intervention trial (ClinicalTrials.gov, NCT01922791). The inclusion criteria of this study were overweight (prepregnancy BMI ≥ 25) and early pregnancy (<17 weeks of gestation). The exclusion criteria were gestational diabetes diagnosed in the current pregnancy, multifetal pregnancy, presence of metabolic or inflammatory disease including type 1 and type 2 diabetes, celiac disease and inflammatory bowel disease. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of the Hospital District of Southwest Finland (permission number 115/180/2012). Written informed consent was obtained from all subjects. The study measurements and samples were obtained at the first study visit, which was the baseline of the intervention trial. The mean age of the women was 29.4 ± 4.9 years, and 50% (50/100) were highly educated with college or university degrees. The median pre-pregnancy body mass index (BMI) was 29.8 kg/m^2; IQR 26.9–32.8 kg/m^2. Fifty-two percent of the participants were overweight (BMI 25–30 kg/m^2), and 48% were obese (BMI > 30 kg/m^2). The primary outcome variables were serum markers for metabolic endotoxemia (LPS) and inflammation. The secondary outcome variables were glucose and lipid metabolism.

2.2. Blood Sample Analysis

Serum markers used in this study were as follows: serum zonulin for intestinal permeability and hs-CRP and glycoprotein acetylation (GlycA) for inflammatory status. GlycA is a novel inflammatory biomarker that is composed of a complex of heterogeneous nuclear magnetic resonance signal containing *N*-acetyl sugar groups originating from multiple acute phase circulating glycoproteins; α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin, and transferrin [19]. LPS was used as a marker for metabolic endotoxemia; insulin, glucose, and insulin resistance, estimated by the homeostatic model assessment-method (HOMA2-IR) and insulin sensitivity by the Quantitative Insulin Sensitivity Check Index (QUICKI) for glucose metabolism; serum triglycerides and total, LDL-, and HDL-cholesterol for lipid metabolism. On the morning of study visit, a fasting blood sample was drawn from the antecubital vein of mothers, the serum was separated and analyzed for insulin, glucose, and hs-CRP, and the rest of the samples were frozen in aliquots at −70 °C until analyzed for zonulin, markers for lipid metabolism, and

LPS. Serum zonulin, a protein responsible for regulating paracellular transport in the intestine [20] was measured using the Zonulin ELISA kit (Immundiagnostik, Bernsheim, Germany). Serum zonulin is a commonly used as a marker for intestinal permeability [12,13,20–22] and it correlates with lactulose/mannitol permeability test results [20,23]. Inter-assay variation in zonulin assay was 10.6% which is below the values reported by the manufacturer and is in line with previous studies using the same kit [12,13]. We used zonulin quartiles for classification of the study subjects into four study groups (Q1-Q4) according to different degree of intestinal permeability, with the highest quartile reflecting the highest intestinal permeability. Glucose, insulin, and hs-CRP concentrations were measured in an accredited Turku University Hospital Laboratory according to the quality control system. Glucose concentration was measured using an enzymatic method utilizing hexokinase (Cobas 8000 automatic c702-analyzer, Roche Diagnostics, Mannheim, Germany). Insulin concentrations were determined with an immunoelectrochemiluminometric assay (a modular E170 automatic analyzer, Roche Diagnostics, Mannheim, Germany). HOMA2-IR was calculated from fasting plasma glucose and fasting insulin using HOMA calculator (http://www.dtu.ox.ac.uk/) [24]. QUICKI was calculated as =1/(log(FastingInsulin) + log(FastingGlu)) [25]. Hs-CRP levels were determined using an automated colorimetric immunoassay on the Dade Behring Dimension RXL autoanalyzer (Siemens Healthcare, Camberly, Surrey, UK). The lower limit of detection was 0.1 mg/L.

Serum lipids and GlycA were quantified from serum samples using a commercial high-throughput proton NMR metabolomics platform (Brainshake, Helsinki, Finland). Details of the experimentation and applications of the NMR metabolomics platform have been described previously [26]. LPS was analyzed using a Limulus amebocyte lysate assay coupled with a chromogenic substrate (HyCult Biochemistry, Uden, the Netherlands). The interassay coefficient of variation was 5.9%.

2.3. Statistics

Sample size was calculated on the expected difference (40%) in LPS values between the highest and lowest serum zonulin quartiles, based on data from previous studies [27] that demonstrated 50% difference in serum LPS between the normal weight and obese women. The sample size was calculated with 90% power and at 5% significance level. The required sample size was 19 subjects/group, but we chose to include 100 subjects, allowing 25 subjects in each quartile.

All statistical analyses were performed using SPSS version 23.0 (IBM, Chicago, IL), with $P < 0.05$ considered as significant. The normality was analyzed by performing Kolmogorov–Smirnov normality test and by visual inspection of histograms. To compare the outcome variables according to serum zonulin quartiles, a Kruskal–Wallis test with Bonferroni corrections was used to analyze the variables that were not normally distributed and one-way ANOVA with Tukey's corrections for normally distributed variables. To particularly investigate the subjects with low grade inflammation having an increased risk for metabolic disturbances, a group of women with a cut-off for hs-CRP > 3 mg/L [28,29] was formed. For this, subjects with hs-CRP < 3 mg/L were excluded (n = 25), resulting in a group of 75 subjects. Subsequently, a linear and an adjusted linear regression analysis with possible confounding factors, was performed for the evaluation of the association between zonulin and serum markers for metabolic endotoxemia, inflammation, glucose, and lipid metabolism. For this, maternal prepregnancy BMI and gestational weeks at sampling were used as covariates. Variables that were not normally distributed were natural log-transformed. To further test whether specific metabolic patterns could be detected, a principal component analysis (PCA) of serum metabolites was performed, with dichotomized zonulin (Q1 and Q2 combined vs. Q3 and Q4 combined) acting as a categorizer. Further, to evaluate relationships between metabolic endotoxemia (LPS) and inflammation (GlycA) among other metabolic markers, inflammation, and glucose and lipid metabolism, Spearman's correlation and univariate linear regression analyses were conducted.

3. Results

3.1. Association of Serum Zonulin with Metabolic Endotoxemia, Markers of Inflammation, and Glucose and Lipid Metabolism

The mean serum zonulin concentration was 46.6 ± 11.1 ng/ml (n = 100). LPS differed according to zonulin quartiles ($P < 0.001$), LPS being highest in Q4 (Table 1). Inflammatory markers hs-CRP and GlycA also differed according to serum zonulin concentrations. When comparing the zonulin quartiles, hs-CRP was significantly lower in the lowest zonulin quartiles (Q1, Q2, Q3) than in the highest quartile (Q4), and GlycA was significantly lower in Q1 and Q2 than in Q3 and Q4 (Table 1). Further, serum zonulin concentration was associated with all markers of glucose metabolism, except with fasting glucose concentration. Insulin concentration and insulin resistance, as evaluated by HOMA2-IR, increased according to serum zonulin concentrations, while insulin sensitivity, evaluated by QUICKI, decreased. Particularly, statistically significant differences between the zonulin quartiles were found as follows: for insulin (Q1 vs. Q3 and Q4; Q2 vs. Q4), HOMA2-IR (Q1 vs. Q3 and Q4; Q2 vs. Q3 and Q4), and QUICKI (Q1 vs. Q3 and Q4; Q2 vs. Q3 and Q4) (Table 1). For lipid concentrations, only serum triglycerides were found to differ according to zonulin quartiles (Q1 vs. Q3 and Q4) (Table 1).

Linear positive relationship was detected between zonulin and LPS, hs-CRP, GlycA, insulin, HOMA2-IR, triglycerides, total and LDL-cholesterol and negative between zonulin and QUICKI in subjects with low grade inflammation (hs-CRP > 3 mg/ml, n = 75) (Table 2). As prepregnancy BMI and weeks of gestation were associated with many of the metabolic markers (Table 3), the relationship between serum zonulin and serum variables in subjects with low grade inflammation was inspected while considering the confounding factors. Again, serum zonulin was found to associate positively with LPS, hs-CRP, GlycA, insulin, HOMA2-IR, triglycerides, and total cholesterol, and inversely with QUICKI in the adjusted linear regression analysis (Table 3).

To clarify whether subjects with higher (combined Q3 and Q4) or lower (combined Q1 and Q2) serum zonulin concentration share similar metabolic patterns, we conducted PCA. Two metabolic patterns were recognized (Supplemental Fig. 1a): subjects with higher serum zonulin were associated with higher concentration of many metabolic risk markers, while subjects with lower serum zonulin concentration were shown

Table 1 – Median [IQR] and mean values (SD) for serum metabolic markers according to zonulin quartiles. n = 100.

Serum variables	Q1	Q2	Q3	Q4	P-value
Zonulin ng/ml (mean (range))	33.9 (25.3–38.2)	42.3 (38.7–46.3)	49.0 (46.5–52.6)	61.2 (53.2–94.7)	
Inflammatory markers					
hsCRP mg/l [a]	4.0 [2.0–6.3][Q4]	3.6 [2.5–6.6][Q4]	4.7 [2.7–6.6][Q4]	8.7 [6.0–13.7]	<0.001
GlycA mmol/l [a]	1.4 [1.3–1.5][Q3, Q4]	1.4 [1.3–1.5][Q3, Q4]	1.6 [1.5–1.6]	1.6 [1.5–1.7]	<0.001
Metabolic endotoxemia					
LPS EU/ml [b]	0.36 (0.06)	0.35 (0.06) [Q4]	0.37 (0.007)	0.41 (0.074)	0.023
Glucose metabolism					
Insulin mU/l [a]	7.0 [5.5–11.5][Q3, Q4]	9.0 [7.0–11.0][Q4]	11.0 [9–13.5]	14.0 [9.5–18.5]	<0.001
Glucose mmol/l [b]	4.74 (0.35)	4.74 (0.27)	4.80 (0.032)	4.83 (0.71)	0.706
HOMA2-IR [a]	0.91 [0.70–1.48][Q3,Q4]	1.15 [0.91–1.42][Q3, Q4]	1.43 [1.18–1.74]	1.8 [1.2–2.3]	<0.001
QUICKI [a]	0.36 [0.33–0.38][Q3,Q4]	0.35 [0.34–0.36][Q3, Q4]	0.33 [0.32–0.34]	0.32 [0.31–0.35]	0.001
Lipid metabolism					
TG mmol/l [a]	0.96 [0.88–1.09][Q3,Q4]	1.00 [0.76–1.44]	1.29 [1.17–1.45]	1.32 [0.95–1.82]	0.001
Total-C mmol/l [a]	4.5 [4.0–4.8]	4.5 [4.1–5.7]	4.9 [4.1–5.7]	4.9 [4.3–5.8]	0.087
LDL-C mmol/l [b]	1.46 (0.38)	1.63 (0.73)	1.74 (0.48)	1.73 (0.55)	0.235
HDL-C mmol/l [b]	1.81 (0.29)	1.77 (0.30)	1.79 (0.33)	1.99 (0.37)	0.062

Statistical significant differences between the quartiles are shown as superscripts.
[a] Kruskal–Wallis nonparametric test with Bonferroni corrections.
[b] One-way ANOVA with Tukey's corrections.

to associate with higher QUICKI (Supplemental Fig. 1a and 1b). In PCA correlation blot, higher serum zonulin was positively associated with metabolic markers except QUICKI and HDL-C, the strongest association being with LPS, triglycerides, and GlycA (Supplemental Fig. 2).

3.2. *Correlations Among LPS, Inflammation, and Glucose and Lipid Metabolism*

To evaluate the role of metabolic endotoxemia (LPS) as a mediator of increased intestinal permeability, we conducted correlations of LPS with markers of inflammation, glucose, and lipid metabolism. LPS was positively correlated with GlycA (Spearman's correlation coefficients, $\rho = 0.40$, $P < 0.001$), but not with hs-CRP ($\rho = 0.023$, $P = 0.842$). Positive correlation was also detected between LPS and serum triglycerides, LDL-, HDL-, and total cholesterol ($\rho = 0.38$, $P = 0.001$; $\rho = 0.31$, $P = 0.006$; $\rho = 0.29$, $P = 0.011$; and $\rho = 0.40$, $P < 0.001$, respectively).

Low grade inflammation was found to contribute to alterations in serum variables, as GlycA correlated with glucose and lipid metabolism. GlycA was positively correlated with insulin, HOMA2-IR ($\rho = 0.517$, $P < 0.001$ and $\rho = 0.50$, $P < 0.001$, respectively) and

Table 2 – Association of zonulin, LPS and GlycA with markers of metabolic endotoxemia, inflammation and glucose and lipid metabolism within subjects with hs-CRP > 3 mg/l (n = 75) in a linear regression model. [a]

	β (95% CI) LPS EU/ml	R^2	P-value	β (95% CI) hsCRP mg/l[a]	R^2	P-value	β (95% CI) GlycA mmol/l[a]	R^2	P-value
zonulin	0.002 (0.001, 0.004)	0.14	0.001	0.006 (0.002, 0.011)	0.11	0.004	0.005 (0.003, 0.006)	0.25	<0.001
LPS				0.018 (−1.734, 1.771)	0.000	0.983	0.590 (0.260, 0.920)	0.15	0.001
GlycA	0.269 (0.153, 0.386)	0.18	<0.001	2.150 (1.122, 3.178)	0.19	<0.001			
	Insulin mU/l[a]			**HOMA2-IR[a]**			**QUICKI[a]**		
zonulin	0.015 (0.007, 0.023)	0.15	0.001	0.015 (0.007, 0.023)	0.14	0.001	−0.002 (−0.004, 0.001)	0.13	0.001
LPS	1.740 (0.274, 3.206)	0.07	0.02	1.655 (0.188, 3.121)	0.07	0.02	−0.249 (−0.485, −0.014)	0.06	0.03
GlycA	2.221 (1.374, 3. 67)	0.27	<0.001	2.180 (1.332, 3.029)	0.26	<0.001	−0.339 (−0.477, −0.202)	0.25	<0.001
	Triglycerides mmol/l[a]			**total cholesterol mmol/l[a]**			**LDL-cholesterol mmol/l**		
zonulin	0.011 (0.006, 0.017)	0.18	<0.001	0.006 (0.002, 0.010)	0.11	0.004	0.013 (0.002, 0.024)	0.07	0.021
LPS	1.9901 (0.923, 2.879)	0.06	<0.001	1.105 (0.467, 1.742)	0.14	0.001	2.255 (0.398, 4.123)	0.07	0.01
GlycA	2.338 (1.898, 2.778)	0.61	<0.001	0.626 (0.201, 1.050)	0.11	0.004	1.453 (0.223, 2.673)	0.07	0.02
HDL-cholesterol mmol/l									
zonulin		0.006 (−0.001, 0.012)			0.03		0.111		
LPS		1.221 (0.077, 2.365)			0.058		0.03		
GlycA		0.024 (−0.745, 0.793)			0.000		0.951		

The regression coefficient (β) represents the one-unit change in zonulin (ng/ml), LPS (EU/ml) or log-transformed serum GlycA (mmol/l) associated with the change of respective serum marker. R^2 = R-square, 95% CI: 95% confidence interval for β.
[a] Values are natural log-transformed for linear regression analysis i.e. the regression coefficient (β) represents the one-unit increase in serum marker in the natural log-scale associated with the change of zonulin, LPS or GlycA in natural log-scale.

Table 3 – Association among zonulin and markers of metabolic endotoxemia, inflammation and glucose and lipid metabolism within subjects with hs-CRP > 3 mg/L (n = 75) in a multiple linear regression model adjusted for log-transformed BMI and gestational weeks.

	β (95% CI) LPS EU/ml	R^2/P^1-value	P^2-value	β (95% CI) hs-CRP mg/l [a]	R^2/P^1-value	P^2-value	β (95% CI) GlycA mmol/l [a]	R^2/P^1-value	P^2-value
zonulin	0.002 (0.001, 0.003)	0.21/0.001	0.002	0.013 (0.003, 0.023)	0.17/0.004	0.015	0.004 (0.002, 0.006)	0.38/<0.001	<0.001
BMI	−0.088 (−0.213, −0.037)		0.164	1.087 (0.122, 2.052)		0.028	0.322 (0.152, 0.492)		<0.001
weeks	0.061 (−0.010, 0.132)		0.090	0.144 (−0.403, 0.691)		0.601	0.073 (−0.023, 0.169)		0.135
	insulin mU/l [a]			HOMA2-IR [a]			QUICKI [a]		
zonulin	0.015 (0.007, 0.022)	0.38/<0.001	<0.001	0.015 (0.007, 0.022)	0.38/<0.001	<0.001	−0.002 (−0.003,−0.001)	0.36/<0.001	<0.001
BMI	1.407 (0.684,2.129)		<0.001	1.394 (0.674, 2.114)		<0.001	−0.211 (−0.329, −0.094)		0.001
weeks	−0.492 (−0.901, −0.082)		0.019	−0.511 (−0.919, −0.103)		0.015	0.087 (0.021, 0.154)		0.011
	Triglycerides mmol/l [a]			Total cholesterol mmol/l [a]			LDL-cholesterol mmol/l		
zonulin	0.009 (0.003, 0.015)	0.28/<0.001	0.003	0.004 (0.000, 0.007)	0.32/<0.001	0.032	0.008 (−0.003, 0.019)	0.21/0.001	0.135
BMI	0.572 (0.022, 1.122)		0.022	0.076 (−0.268, 0.419)		0.662	0.528 (−0.513, 1.569)		0.315
weeks	0.450 (0.139, 0.762)		0.139	0.447 (0.253, 0.642)		<0.001	1.044 (0.454, 1.634)		0.001

The regression coefficient (β) represents the one-unit (ng/ml) increase in zonulin, one-unit (kg/m^2) increase in BMI, or one week increase in gestational weeks associated with the change of serum marker. R^2 = R-square, 95% CI: 95% confidence interval for β. P^1-value = P-value for adjusted multiple linear regression. P^2-value = P-values for each predictor in adjusted multiple linear regression.
[a] Values are natural log-transformed for linear regression analysis, i.e., the regression coefficient (β) represents the one-unit (ng/ml) increase in zonulin, one-unit (kg/m^2) increase in BMI, or one week increase in gestational weeks associated with the change in serum marker in the natural log-scale.

triglycerides, LDL-, and total cholesterol (ρ = 0.79, P < 0.001; ρ = 0.35, P = 0.002; and ρ =0.34, P = 0.003, respectively) and inversely with QUICKI (ρ = −0.50, P < 0.001). Further analysis in univariate linear regression confirmed the results. In addition, LPS was found to associate positively with insulin and HOMA2-IR and negatively with QUICKI (Table 2).

4. Discussion

In this study, we showed that intestinal permeability, evaluated by serum zonulin concentration, is related to metabolic endotoxemia, markers of inflammation, and glucose and lipid metabolism in pregnant overweight women. The results suggest an important role for intestinal permeability in inducing adverse metabolic reactions and thus may have a potential effect on the health of both the mother and the child.

The finding of a positive association between serum zonulin and inflammatory markers indicates that increased intestinal permeability contributes to elevated inflammatory status. It is noteworthy that this association was independent of the degree of mother's obesity. These results are in line with previous studies conducted in non-pregnant populations [12,13,15]. The mechanism for the increase in inflammation through increased intestinal permeability is likely to arise from metabolic endotoxemia as LPS correlated with GlycA. However, this needs to be confirmed, as LPS did not correlate with the typically used marker for low grade inflammation, CRP.

In addition to association with serum LPS and inflammatory markers, serum zonulin concentration was directly associated with markers of glucose and lipid metabolism, again indicating a role for an increased intestinal permeability in the unfavorable alterations of these metabolic risk markers. This finding is in line with previous studies conducted in non-pregnant subjects [12–15,22]. Low grade inflammation is a known factor associated with insulin resistance [7,8], as proinflammatory mediators like cytokines are able to impair insulin action [30]. This may be a mechanism explaining our findings, since we found an association between inflammation marker GlycA and HOMA2-IR and QUICKI. Serum LPS was found to associate positively with insulin and HOMA2-IR and negatively with QUICKI, suggesting an interplay with LPS, low grade inflammation and glucose metabolism. Regarding other metabolic markers, triglycerides, total, and HDL-cholesterol were positively correlated with serum zonulin. The positive correlation of zonulin with triglycerides and total cholesterol has been previously detected in non-pregnant subjects, but an inverse correlation has been demonstrated for HDL-cholesterol [13,14]. This discrepancy between the two studies regarding HDL-cholesterol may be explained by the fact that pregnancy itself induces changes in maternal lipids [31]. As also reported before [32], we found relationships between higher serum LPS and serum lipids. The mechanism of how LPS impacts lipid metabolism is not clear but may include induction of lipogenesis in adipocytes [33] or crosstalk between endocannabinoid system and LPS, a mechanism suggested to contribute to adipogenesis in obesity [34]. We also found association between GlycA and lipid metabolism, proposing interplay between inflammation and lipid metabolism in our study subjects.

The clinical significance of our findings may be manifested as a contribution to increased risk for pregnancy complications,

like gestational diabetes, as well as long-term health disturbances after pregnancy. Hs-CRP and GlycA are both related to metabolic disorders; the maternal first trimester CRP has been shown to be associated with the development of gestational diabetes in some studies [35–37] but not all [38–41], and GlycA has been related to chronic inflammation [42], type 2 diabetes, and CVD in non-pregnant subjects [43–45]. Previously, only levels of individual glycoproteins have been investigated in women with normal pregnancy and with gestational diabetes (e.g., [38,46–50]). The novel finding of association of GlycA complex with metabolic risk markers in our study suggests that elevated levels may predict the risk for metabolic disorders, such as insulin resistance in pregnancy as well. The possible role of elevated GlycA in the onset of gestational diabetes remains to be examined in future studies.

The strength of this study is that it was a carefully conducted clinical study, and all the samples were collected at a very concise time frame during early pregnancy. At the same time, since it is unknown whether intestinal permeability is further altered as pregnancy proceeds, a longitudinal study would enable the translation of these findings to clinical implications. Also, this study included only overweight or obese women; thus, whether the relationship between serum zonulin and serum risk markers is the same in normal weight pregnant women needs to be studied, alongside with the potential impact of predisposition to altered glucose metabolism due to family history of diabetes. However, we consider weight not to be a factor affecting interpretations of the study results, since BMI had no impact on the association of serum zonulin and risk markers. The findings in our study suggest strong evidence for the relationship between serum zonulin and risk markers in subjects with low grade inflammation. These relationships were further confirmed in PCA, showing similar association of higher serum zonulin concentration with higher metabolic risk markers.

The detected relationships of increased serum zonulin with markers of glucose and lipid metabolism (i.e., factors that are known to increase the risk for various metabolic disorders) highlight the importance of the contribution that the gut has on metabolic health. The mechanism is likely to originate through inflammatory properties of LPS and possibly some other gut-originated components that cross to the circulation because of increased intestinal epithelial permeability.

Declaration of Interest

The authors have no conflicts of interest to disclose.

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Author Contribution

KM and KL designed the study, KL and HR organized the data collection, KM performed the serum zonulin analyses, PP analyzed the serum LPS, KM and KL analyzed the data and wrote the paper, and OP and TR contributed to the interpretation of the results. All authors read, commented, and approved the final version of the paper. KL has the primary responsibility for final content.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.metabol.2016.12.015.

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