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Metformin and insulin treatment of gestational diabetes: effects on inflammatory markers and IGF-binding protein-1 – secondary analysis of a randomized controlled trial

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Abstract

Background: Gestational diabetes mellitus (GDM) is characterized by disturbed glucose metabolism and activation of low-grade inflammation. We studied whether metformin treatment has favorable or unfavorable effects on inflammatory markers and insulin-like growth factor-binding protein 1 (IGFBP-1) in GDM patients compared with insulin, and whether these markers associate with major maternal or fetal clinical outcomes.

Methods: This is a secondary analysis of a previous randomized controlled trial comparing metformin ($n = 110$) and insulin ($n = 107$) treatment of GDM. Fasting serum samples were collected at the time of diagnosis (baseline, mean 30 gestational weeks [gw]) and at 36 gw. Inflammatory markers serum high-sensitivity CRP (hsCRP), interleukin-6 (IL-6), matrix metalloproteinase-8 (MMP-8) and glycoprotein acetylation (GlycA) as well as three IGFBP-1 phosphoisoform concentrations were determined.

Results: In the metformin and insulin groups combined, hsCRP decreased ($p = 0.01$), whereas IL-6 ($p = 0.002$), GlycA ($p < 0.0001$) and all IGFBP-1 phosphoisoforms ($p < 0.0001$) increased from baseline to 36 gw. GlycA ($p = 0.02$) and non-phosphorylated IGFBP-1 ($p = 0.008$) increased more in patients treated with metformin than those treated with insulin. Inflammatory markers did not clearly associate with pregnancy outcomes but non-phosphorylated IGFBP-1 was inversely associated with gestational weight gain.

Conclusions: Metformin had beneficial effects on maternal serum IGFBP-1 concentrations compared to insulin, as increased IGFBP-1 related to lower total and late pregnancy maternal weight gain. GlycA increased more during metformin treatment compared to insulin. The significance of this observation needs to be more profoundly examined in further studies. There were no evident clinically relevant relations between inflammatory markers and pregnancy outcome measures.

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Trial registration: The trial comparing metformin and insulin treatment was registered in ClinicalTrials.gov (NCT01240785) November 3, 2010. Retrospectively registered.

Keywords: Gestational diabetes, Metformin, Low-grade inflammation, Insulin-like growth factor-binding protein 1, IGFBP-1

Background

Gestational diabetes mellitus (GDM) is a growing health concern. It is associated with obesity and low-grade inflammation and increases the risk for pregnancy complications, such as macrosomia, preeclampsia, neonatal hypoglycemia and hyperbilirubinemia and the need for neonatal intensive care [1, 2]. In the long term GDM causes metabolic perturbations – it increases the risk for obesity and metabolic syndrome in the offspring [3] and the risk for type 2 diabetes (T2DM) in the mother [4]. Metformin treatment of GDM reduces gestational weight gain (GWG), gestational hypertension, the incidence of neonatal hypoglycemia and the need for neonatal intensive care compared to insulin treatment [5]. Although the benefits of metformin treatment during the pregnancy have been well characterized, there are concerns regarding the long term effects specially on the offspring [6]. Furthermore we do not know whether metformin has beneficial effects on low-grade inflammation compared to insulin.

Elevated serum IL-6 and high-sensitivity C-reactive protein (hsCRP) are markers of inflammation and predict the onset of T2DM [7]. Dysregulation of inflammation may be involved also in the pathogenesis of GDM [8]: hsCRP [9, 10], IL-6 [11] and glycoprotein acetylation (GlycA) [12] are related to GDM, and hsCRP also predicts the persistence of glucose intolerance postpartum [13]. Matrix metalloproteinase 8 (MMP-8), a more recent inflammatory marker, is related to intra-amniotic infection [14] and cervical ripening [15], but MMP-8 activity seems also to be increased in patients with GDM [16].

Besides inflammatory markers, a low serum concentration of insulin-like growth factor-binding protein 1 (IGFBP-1) is associated with GDM and an unfavorable metabolic profile [17]. IGFBP-1, in particular, is thought to play a significant role during pregnancy by regulating plasma glucose levels and being related to fetal growth [18]. Phosphorylation of IGFBP-1 increases its affinity to insulin like growth factor 1 (IGF-1). In the normal state, the highly phosphorylated isoform (high-pIGFBP-1) prevails, but during pregnancy, a non-phosphorylated IGFBP-1 (non-pIGFBP-1) is also detected. In cord blood, both phosphoisoforms are decreased in GDM and inversely associated to birth weight [19].

Based on earlier studies, metformin may have anti-inflammatory properties, as demonstrated by suppression of IL-6 (in vitro) [20] and hsCRP [21]. While insulin inhibits IGFBP-1 production [22], metformin appears to increase

IGFBP-1 expression [23]. However, the possible effects of metformin on inflammatory markers in GDM pregnancy have not been studied in sufficiently large patient cohorts to give an unambiguous answer, and its effects on IGFBP-1 in GDM pregnancy have not been studied previously.

The primary aim of this study was to compare the effects of metformin and insulin treatment on the inflammatory markers hsCRP, IL-6, MMP-8, GlycA and three IGFBP-1 phosphoisoforms. The secondary aim was to examine whether variation in these variables at baseline (mean 30 gestational weeks, gw) or at late pregnancy (36 gw) are associated with the maternal and the neonatal outcomes. We hypothesized that metformin has beneficial effects on the inflammatory markers and IGFBP-1 compared to insulin.

Methods

Study design

The present study is a secondary analysis of a previous randomized trial [24], in which women with a singleton pregnancy and newly diagnosed GDM were treated either with metformin ($n = 110$) or insulin ($n = 107$) in an open-label randomized design. The original randomized trial was powered to prove non-inferiority of treatment to the primary outcome, which was birth weight. Since this was a secondary analysis, no power-analysis was made to calculate the sample size. However, an additional post-hoc power analysis is included as a supplementary file (Additional file 1). The patients were recruited at the Turku University Hospital on their first visit for management of GDM and they were randomized by the physician using sealed envelopes. GDM diagnosis was made based on the Finnish national guidelines and oral glucose tolerance test (OGTT) thresholds as described before [24]. Metformin treatment was started at a daily dose of 500 mg daily and increased up to 2000 mg if needed (median 1500 mg). Additional insulin was given to 23 participants in the metformin group due to unsatisfactory glucose control with metformin only. For insulin treatment, NPH insulin and/or rapid-acting insulin lispro or insulin aspart were used. The trial was approved by the Ethics Committee of the Southwest Hospital District of Finland, the Finnish National Agency of Medicines, and the European Union Drug Regulatory Agency (EUDRA) and registered retrospectively in ClinicalTrials.gov (NCT01240785, <http://clinicaltrials.gov/ct2/show/NCT01240785>). All participants provided written informed consent. The detailed design

131 and outcomes of the randomized trial have been re-
132 ported elsewhere [24].

133 For the present analysis Clinical data and serum sam-
134 ples from the previous randomized trial were available
135 from 109 and 107 patients of the metformin and insulin
136 groups, respectively. Those patients in the metformin
137 group who received additional insulin are included in
138 the metformin group unless otherwise specified.

139 Biochemical methods and clinical variables

140 Fasting blood samples were drawn at baseline after the
141 GDM diagnosis had been confirmed (mean 30 [20–34]
142 gw) and at 36 gw. Serum concentrations of hsCRP and IL-
143 6 were measured using ELISA [human C-reactive protein
144 (CRP) ELISA kit, R&D Systems, Minneapolis, USA;
145 interleukin-6 (IL-6) ELISA kit, R&D Systems, Minneap-
146 olis, USA]. MMP-8, non-pIGFBP-1, low-pIGFBP-1, high-
147 pIGFBP-1 were determined using ELISA and an immu-
148 noenzymometric assay, as described earlier [15, 25], and
149 GlycA according to a high-throughput proton (¹H)
150 nuclear magnetic resonance spectroscopy protocol [26].

151 Glucose values of the 2 h 75 g OGTT were available at
152 the time of GDM diagnosis. C-peptide, HbA1c, age and
153 pre-pregnancy BMI were assessed as risk factors for GDM
154 and insulin resistance, to examine the relationship with
155 the risk factors, the inflammatory markers and IGFBP-1's.
156 HbA1c was determined using high pressure liquid chro-
157 matography and fasting serum C-peptide by an electro-
158 chemiluminescence immunoassay. Both analytes were
159 measured at baseline and HbA1c also at 36 gw.

160 Associations between inflammatory markers, IGFBP-1
161 phosphoisoforms and the following clinical outcomes
162 were studied, A) maternal outcomes: GWG, preeclampsia
163 or gestational hypertension, gestation length, induction
164 of labor, incidence of cesarean section, and B) fetal out-
165 comes: birth weight, neonate admission to NICU and neo-
166 natal intravenous glucose given for any indication. Total
167 GWG was defined as the last measured weight at the ma-
168 ternity clinic minus self-reported weight before pregnancy,
169 and late GWG as the weight gain from the initiation of
170 antihyperglycemic medication. Birth weight was expressed
171 in grams and in SD units (deviation from the mean value
172 of the Finnish general population adjusted for gestation
173 duration [27]). Birth weight > 90th percentile was used as
174 an additional indicator of large for gestational age and a
175 birth weight below < 10th percentile was used to calculate
176 the incidence of children of small for gestational age.

177 Statistical analyses

178 Categorical clinical data comparison between groups was
179 done with the χ^2 -test and Fisher's exact test. Comparisons
180 of means or medians was done using the Mann-Whitney
181 U or t-test, depending on how the data was distributed.
182 Wilcoxon's test or the t-test was used for testing

metabolite changes from baseline to 36 gw. An ANCOVA 183
analysis was used to adjust for any differences between the 184
compared groups. The normality of distributions was ex- 185
amined using the Shapiro-Wilk test when $n < 100$ and 186
Kolmogorov-Smirnov's test with Lilliefors's correction for 187
larger samples sizes. For correlations, Spearman's rank 188
correlation was used. For linear and logistic regression 189
analyses, continuous variables were first centered and 190
scaled, except for birth weight which already was 191
expressed in terms of SD-units. Regression analyses were 192
run both unadjusted and adjusted for treatment (metfor- 193
min or insulin) and/or pre-pregnancy BMI, which was a 194
priori thought to be the most clinically important con- 195
founding factor. Group-specific regression coefficients are 196
given if the pharmacological treatment interacted signifi- 197
cantly ($p < 0.05$) with the association between the inde- 198
pendent and outcome variable in the regression model. 199
Confidence intervals (CI) for regression coefficients were 200
acquired with the adjusted bootstrap percentile method. 201

Results are reported with 95% CI; $p < 0.05$ was consid- 202
ered statistically significant. Bonferroni adjustment was 203
applied on the regression analyses. Statistical analyses 204
were run on the R statistics software (version 3.3.2, 205
<http://cran.r-project.org>). This study adheres to CON- 206
SORT guidelines (<http://www.consort-statement.org>) for 207
reporting clinical trials. 208

209 Results

The study population characteristics are given in Table 1. 210
Metformin and insulin groups were similar in terms of 211
OGTT values, HbA1c at both time points, C-peptide, age, 212
pre-pregnancy BMI and GWG. There were no differences 213
in birth weight or proportion of primipara. There were no 214
differences between the metformin and insulin groups re- 215
garding pregnancy outcomes, except for higher labor in- 216
duction rates in the insulin group compared to the 217
metformin group (54.2% vs. 37.6%, $p = 0.014$). 218

219 Inflammatory markers and IGFBP-1's at baseline and 220 change from baseline to 36 gw

Comparing metformin and insulin groups at baseline, 221
there were no differences except for marginally lower low- 222
pIGFBP-1 in the metformin group (21.0 vs. 24.0, $p = 0.04$). 223
Within the metformin group, the inflammatory marker 224
and IGFBP-1 concentrations did not differ when com- 225
pared to those who required additional insulin treatment. 226
Baseline and 36 gw values of the inflammatory markers 227
and IGFBP-1's are provided in detail in Additional file 2. 228

Changes in inflammatory markers and IGFBP-1 phospho- 229
isoforms and comparison of changes are shown in Table 2. 230
In the metformin and insulin groups combined, the hsCRP 231
concentration decreased from baseline to 36 gw, whereas the 232
IL-6, GlycA and IGFBP-1 concentrations increased. GlycA 233
($p = 0.02$) and non-pIGFBP-1 ($p = 0.008$) increased more in 234

T1

T2

t1.1 **Table 1** Clinical characteristics of the study population

t1.2	Variable	Metformin	n	Insulin	n	p-value
t1.3	Patients characteristics					
t1.4	Age (years)	31.9 ± 5.01	109	32.0 ± 5.47	107	0.89
t1.5	Smoking	9 (8.6)	105	17 (16.0)	106	0.099
t1.6	Primipara	42 (38.5)	109	49 (45.8)	107	0.28
t1.7	Pre-pregnancy BMI (kg/m ²)	29.5 ± 5.91	109	28.9 ± 4.71	107	0.41
t1.8	Glucose metabolism					
t1.9	HbA1c% at OGTT	5.48 ± 0.34	109	5.51 ± 0.34	107	0.49†
t1.10	HbA1c at OGTT (mmol/mol)	36.3 ± 3.69		36.7 ± 3.72		
t1.11	HbA1c% at 36 gw	5.68 ± 0.33	101	5.69 ± 0.36	95	0.82
t1.12	HbA1c at 36 gw (mmol/mol)	38.5 ± 3.63		38.6 ± 3.89		
t1.13	OGTT fasting (mmol/L)	5.52 ± 0.55	109	5.57 ± 0.42	107	0.44
t1.14	OGTT 1 h (mmol/L)	11.2 ± 1.49	109	11.2 ± 1.24	107	0.61†
t1.15	OGTT 2 h (mmol/L)	8.33 ± 1.76	108	7.91 ± 1.75	106	0.076
t1.16	C-peptide at baseline (nmol/L)	1.05 ± 0.33	103	1.05 ± 0.29	101	0.90†
t1.17	Pregnancy outcomes					
t1.18	Gestational hypertension	2 (1.8)	109	4 (3.7)	107	0.44‡
t1.19	Preeclampsia	5 (4.6)	109	10 (9.3)	107	0.19‡
t1.20	Assisted vaginal delivery	9 (8.3)	109	8 (7.5)	107	0.83
t1.21	Cesarean section	15 (13.8)	109	18 (16.8)	107	0.53
t1.22	Induction of labor	41 (37.6)	109	58 (54.2)	107	0.014
t1.23	Gestational weight gain (kg)	7.97 ± 5.24	108	7.82 ± 5.27	107	0.83
t1.24	Weight gain in late gestation (kg)	1.79 ± 2.62	109	2.15 ± 2.97	107	0.35
t1.25	Gw at delivery	39.2 ± 1.40	109	39.4 ± 1.58	107	0.43
t1.26	Neonatal outcomes					
t1.27	Birth weight (g)	3610 ± 490	109	3590 ± 450	107	0.78
t1.28	Birth weight (SD)	0.17 ± 1.05	105	0.15 ± 0.96	107	0.91
t1.29	Birth weight (centiles)	54.8 ± 28.9	105	54.3 ± 28.9	107	
t1.30	Macrosomia	5 (4.6)	109	1 (0.9)	107	0.21‡
t1.31	Birth weight < 10th percentile	12 (11.4)	105	9 (8.4)	107	0.46
t1.32	Birth weight > 90th percentile	15 (14.3)	105	17 (15.9)	107	0.74
t1.33	Admission to NICU	33 (30.1)	108	39 (36.4)	107	0.36
t1.34	Newborn I.V. glucose	25 (23.1)	108	25 (23.6)	106	0.94

t1.35 Data is shown as mean ± SD or n (%). The p-value is given for the t-test or the Mann-Whitney U (indicated with †) and for categorical data for the χ^2 -test or
t1.36 Fisher's exact test (indicated with ‡). The number of mothers with clinical variables varied slightly due to missing data for some variables. OGTT = oral glucose
t1.37 tolerance test, gw = gestational weeks, SD = standard deviation, NICU = neonatal intensive care unit, I.V. = intravenous. Birth weight in SD and centiles were
t1.38 adjusted for Finnish population growth charts. Macrosomia was defined as birth weight > 4500 g or > 2 SD

235 patients treated with metformin than with insulin but other-
236 wise there were no statistically significant differences in these
237 changes between the groups.

238 **Correlations between inflammatory markers, age, pre-**
239 **pregnancy BMI and measures of glucose metabolism**
240 Spearman's correlations for inflammatory markers, IGFBP-
241 1's, age and variables related to pre-pregnancy BMI and glu-
242 cose metabolism among the metformin and insulin treated
F1 243 patients are represented in Fig. 1. At baseline, hsCRP and IL-
244 6 correlated positively and IGFBP-1 phosphoisoforms

inversely with pre-pregnancy BMI and C-peptide. GlycA cor-
related at baseline with HbA1c and C-peptide but not with
pre-pregnancy BMI. MMP-8 measured at baseline correlated
only weakly with pre-pregnancy BMI.

Regression analyses between inflammatory markers,
IGFBP-1's and clinical outcomes in metformin and insulin
treated patients

Baseline
Non-pIGFBP-1 at baseline was associated with lesser
total and late GWG (Table 3 and Additional file 3). After

Table 2 Change in concentrations of inflammatory markers and IGFBP-1 phosphoisoforms from baseline to 36 gestational weeks

Variable	Metformin and insulin combined		Metformin		Insulin		p-value for comparison of changes (metf vs ins)
n	179		94		85		
	median/mean (95% CI)	p-value	median/mean (95% CI)	p-value	median/mean (95% CI)	p-value	
Inflammation							
hsCRP (mg/L)	-0.47 [-1.3; -0.014]	0.011	-0.45 [-1.7; 0.16]	0.028	-0.47 [-1.8; 0.093]	0.18	0.72
IL-6 (ng/L)	0.70 [0.20; 1.40]	0.002	0.85 [0.50; 1.8]	0.002	0.62 [-0.19; 1.4]	0.13‡	0.31
MMP-8 (µg/L)	0.0 [-2.0; 0.80]	0.50	-0.70 [-2.0; 1.0]	0.76	0.70 [-2.0; 2.6]	0.20	0.28
GlycA (mmol/L)	0.11 [0.089; 0.13]	< 0.0001	0.15 [0.11; 0.18]	< 0.0001‡	0.091 [0.064; 0.12]	< 0.0001‡	0.020
IGFBP-1							
Non-phosphorylated (µg/L)	17.0 [13.0; 20.5]	< 0.0001	21.0 [14.0; 26.0]	< 0.0001	13.4 [7.9; 18.9]	< 0.0001‡	0.008
Low-phosphorylated (µg/L)	6.0 [4.0; 7.9]	< 0.0001	6.0 [3.6; 7.5]	< 0.0001	4.0 [-2.0; 4.0]	0.021	0.081
High-phosphorylated (µg/L)	300 [190; 410]	< 0.0001‡	260 [110; 420]	0.001‡	340 [180; 500]	< 0.0001‡	0.48‡

Median/mean change from baseline to 36 gestational weeks [95% confidence interval (CI)]. Positive values indicate increase and negative values decrease. p-values are given for the one-sample t-test (indicated with ‡) or Wilcoxon's signed rank test (comparisons not indicated by ‡). For comparison of changes between metformin and insulin groups, Mann-Whitney's U-test or the t-test (indicated with †) was used. hsCRP = high sensitivity CRP, IL-6 = interleukin 6, MMP-8 = matrix metalloproteinase 8, GlycA = glycoprotein acetylation, IGFBP-1 = insulin-like growth factor-binding protein 1. n-values for GlycA are 190, 99 and 91 for combined, metformin and insulin groups, respectively

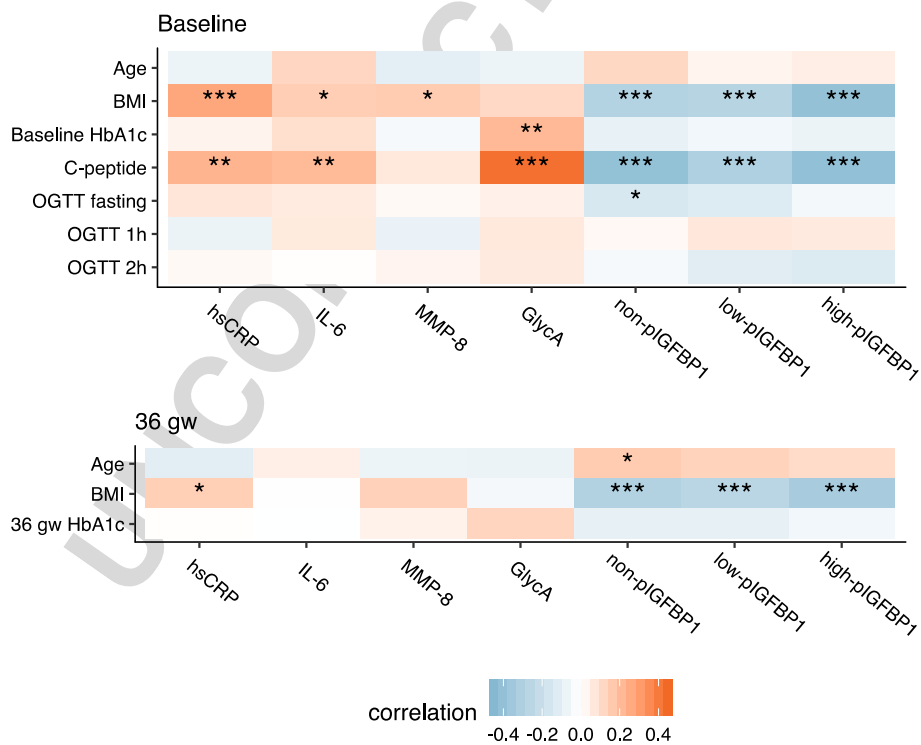


Fig. 1 Heatmap representation of Spearman's correlations between age, pre-pregnancy BMI and glucose metabolism with inflammatory markers and IGFBP-1 phosphoisoforms at baseline (n = 196–208) and at 36 gestational weeks (n = 181–198). BMI = body mass index, OGTT = oral glucose tolerance test, gw = gestational weeks, hsCRP = high sensitivity CRP, IL-6 = interleukin 6, MMP-8 = matrix metalloproteinase 8, GlycA = glycoprotein acetylation, non/low/high-pIGFBP-1 = non/low/high-phosphorylated insulin-like growth factor-binding protein 1. *p < 0.05, **p < 0.01, ***p < 0.001. This figure was created using ggplot2 in R

Table 3 Regression models with significant ($p < 0.05$) association of inflammatory markers and IGFBP-1 concentrations with maternal and neonatal outcomes

Independent variable	Outcome	β -estimate [95% CI] (p -value)	n total
Baseline			
non-pIGFBP-1	total GWG (kg/SD)	-1.2 [-2; -0.64] (< 0.001)*	201
MMP-8	late GWG (kg/SD)	0.41 [0.022; 0.77] (0.035)	202
non-pIGFBP-1	late GWG (kg/SD)	0.45 [-0.87; -0.13] (0.021)	202
hsCRP	length of gestation (weeks/SD)	0.2 [0.028; 0.36] (0.044)	202
high-pIGFBP-1	induction of labor (OR/SD)†	0.67 [0.48; 0.92] (0.0094)	202
non-pIGFBP-1	birth weight (SD/SD)	-0.15 [-0.32; -0.052] (0.027)	198
36 gestational weeks			
non-pIGFBP-1	total GWG (kg/SD)	-1.1 [-1.8; -0.52] (0.0027)*	188
non-pIGFBP-1	late GWG (kg/SD)	-0.55 [-0.96; -0.21] (0.0069)	189
non-pIGFBP-1	cesarean section (OR/SD)‡	0.49 [0.24; 0.84] (0.043)	189
MMP-8	birth weight (SD/SD)	-0.17 [-0.34; -0.037] (0.022)	185

Both metformin and insulin treated patients were included. Induction of labor was performed in 92 and cesarean section in 26 women. Data is given as regression β -estimates or odds ratios (OR) in respect to one SD change of the predictor [95% confidence interval, CI] (p -value). The reference groups for binary outcomes were no induction of labor (†) and vaginal delivery (‡). SD = standard deviation, GWG = (maternal) gestational weight gain, pIGFBP-1 = phosphorylated insulin-like growth factor-binding protein 1, MMP-8 = matrix metalloproteinase 8, hsCRP = high sensitivity CRP. * $p < 0.0045$ (Bonferroni)

adjustment for pre-pregnancy BMI, both non-pIGFBP-1 (-1.5 kg/SD, $p < 0.0001$) and low-pIGFBP-1 (-0.99 kg/SD, $p = 0.0037$) were inversely associated with total GWG and non-pIGFBP-1 (-0.47 kg/SD, $p = 0.019$) with late GWG (see Additional file 4 for adjusted regression results). Irrespective of these adjustments, MMP-8 was associated with late, but not total GWG. Only after adjustment for pre-pregnancy BMI, was hsCRP associated with total GWG (0.72 kg/SD, $p = 0.05$). HsCRP was positively associated with the gestation length and was not affected by adjustment for pre-pregnancy BMI (0.20 weeks/SD, $p = 0.048$). Non-pIGFBP-1 was associated with lower birth weight before (-0.15 SD-units/SD, $p = 0.027$) and after (-0.14 SD-units/SD, $p = 0.049$) adjustment for pre-pregnancy BMI.

Gestational week 36

Similarly to baseline, non-pIGFBP-1 measured at 36 gw was associated with lesser total and late GWG, and after adjustment for pre-pregnancy BMI also low-pIGFBP-1 was associated with total GWG. In the metformin group, MMP-8 was related to higher late GWG (0.74 kg/SD, $p = 0.35$) and hsCRP with longer gestation (0.40 weeks/SD, $p = 0.046$), and these associations were unaffected by adjustment for pre-pregnancy BMI. A high non-pIGFBP-1 concentration was related to a lower incidence for cesarean section (OR: 0.49, $p = 0.043$), but this association was no longer significant after adjustment for pre-pregnancy BMI. A high MMP-8 was associated with lower birth weight (-0.17 SD-units/SD, $p = 0.022$), and this association was not affected by pre-pregnancy BMI.

When the regression p -values at each time point were adjusted using Bonferroni method, the associations between non-pIGFBP-1 and GWG remained significant at

both time points in models irrespective of adjustment for pre-pregnancy BMI. In addition the association between low-pIGFBP-1 at baseline and total GWG was significant in the regression adjusted for pre-pregnancy BMI. Regression results for metformin and insulin groups separately are shown in Additional file 5, for those models in which there was a significant interaction ($p < 0.05$) in the association between the independent and outcome variable. None of the p -values for metformin and insulin groups separately reached Bonferroni adjusted threshold of $p < 0.0045$.

Discussion

Seven biomarkers at the time of GDM diagnosis and at 36 gestational weeks were analyzed and the effects of metformin and insulin treatment on the biomarker concentrations and their relation to clinical outcomes were compared. In addition to the traditional markers hsCRP and IL-6, also MMP-8 and GlycA were included in the analyses, since both of these markers are promising markers of cardiovascular risk outside pregnancy [28, 29].

In both treatment groups hsCRP decreased from baseline to 36 gw, as demonstrated previously in non-diabetic obese and normal-weight pregnant women [30]. To our knowledge, this is the largest sample comparing the effect of metformin and insulin on hsCRP in GDM. In another large trial comparing metformin and insulin treatment in GDM (the MiG trial), CRP remained unchanged from GDM diagnosis to 36 gw [31]. Notwithstanding the different quantification method, this difference may be explained by lower baseline hsCRP in the MiG study [31]. Conversely, hsCRP has been related to BMI [32], which was higher in MiG than in our cohort; this emphasizes the

319 possible effects of ethnicity and the need for absolutely
320 identical diagnostic criteria for GDM.

321 In line with previous reports in non-diabetic subjects,
322 IL-6 increased during the last trimester of pregnancy
323 [30]. IL-6 is secreted to a large extent by adipocytes and
324 correspondingly higher serum concentrations are associ-
325 ated with higher BMI [30]. However, IL-6 has also anti-
326 inflammatory effects [33] and considering the lack of as-
327 sociations with any adverse outcomes in our data, the
328 complexity of IL-6 signaling in pregnancy remains in-
329 completely understood. Still, we have demonstrated that
330 compared with insulin metformin treatment of GDM
331 does not appear to affect serum IL-6.

332 Previously it has been shown that, in the presence of
333 premature rupture of membranes, maternal serum IL-6
334 predicts preterm delivery at 72 h before delivery [34]. In
335 our data there was an inverse, albeit statistically non-
336 significant association between IL-6 at 36 gw and gesta-
337 tion length.

338 Serum GlycA increased in both treatment groups but
339 more in response to metformin treatment. This is in
340 contrast to a previous study in non-diabetic individuals
341 where metformin did not affect serum GlycA [35]. How-
342 ever, the serum concentrations of some glycoproteins,
343 such as α -1-acid glycoprotein and α -1-antitrypsin,
344 change in normal pregnancy [36], and this confuses the
345 interpretation of GlycA. In general, pregnancy is associ-
346 ated with activation of the innate immune system and
347 with an increase in the concentration of acute phase
348 proteins in the serum. An overall increase of GlycA dur-
349 ing pregnancy has been reported previously in a popula-
350 tion cohort study [37] and this probably reflects changes
351 in the immune system [38]. High GlycA predicts T2DM
352 [39] and cardiovascular [29] risk in non-pregnant
353 women. Similarly, in pregnancy it has been associated
354 with insulin resistance, a poor lipid profile [40] and
355 GDM in obese women [12]. In agreement with this,
356 GlycA correlated with HbA1c and C-peptide at baseline
357 but not with HbA1c at 36 gw. These results suggest that
358 GlycA may not be a reliable marker of inflammation
359 near term, possibly due to changes in glycoprotein com-
360 position [36].

361 Serum MMP-8 was rather constant during the last tri-
362 mester of pregnancy, and to our knowledge this is the
363 first longitudinal study characterizing MMP-8 in GDM.
364 Outside GDM, MMP-8 is associated with chorioamnio-
365 nitis [14] and preterm delivery [41]. Although we did
366 not observe an association between maternal serum
367 MMP-8 and gestation length, MMP-8 was associated
368 with a slightly reduced birth weight. Serum MMP-8 may
369 indicate subclinical inflammation of the placenta or the
370 chorion, which would affect birth weight.

371 In normal pregnancy, serum IGFBP-1 increases during
372 the first trimester and then decreases slightly before

another peak just before delivery [42]. In our data, 373
IGFBP-1 phosphoisoform concentrations increased from 374
baseline to 36 gw in both treatment groups. Non- 375
pIGFBP-1 concentrations increased significantly more in 376
women treated with metformin, and there was a trend 377
towards a higher concentration of low-pIGFBP-1. In line 378
with this, metformin causes a marked increase in 379
IGFBP-1 in non-pregnant women with the polycystic 380
ovary syndrome [43]. Metformin increases insulin sensi- 381
tivity and this might decrease insulin levels. There is a 382
negative feedback loop from insulin to the production of 383
IGFBP-1 [22], and this might explain the difference in 384
serum IGFBP-1 levels between the treatment groups. 385
Another possibility is that the increase in IGFBP-1's in 386
the metformin group is a consequence of dietary 387
changes in response to gastrointestinal symptoms often 388
occurring during metformin use. Previously metformin 389
treatment has been related to lower GWG when com- 390
pared to either insulin [44] or placebo [45]. And al- 391
though in our data there were no differences in GWG 392
between the treatment groups, non-pIGFBP-1 and low- 393
pIGFBP-1 were inversely associated with GWG. 394

Neither at baseline nor at 36 gw was there any appar- 395
ent association between inflammatory markers, IGFBP- 396
1's and clinical outcomes, with the exception of the in- 397
verse association between non-pIGFBP-1, low-pIGFBP-1 398
and GWG. 399

IGFBP-1 phosphoisoform concentrations were associ- 400
ated with healthier metabolic profiles, as expected, but 401
high non-pIGFBP-1 and low-pIGFBP-1 were also related 402
to lesser GWG. High pre-pregnancy BMI and high 403
GWG are two major risk factors of excessive fetal 404
growth. In spite of that, IGFBP-1's in our data were not 405
clearly associated with any birth weight variables. This is 406
in contrast with previous results from a population co- 407
hort where low IGFBP-1 throughout pregnancy was re- 408
lated with a higher birth weight [46]. The discrepancy 409
may at least in part be explained by the fact that our 410
study population, having GDM and being therefore at 411
risk for fetal macrosomia, were given intensive dietary 412
and lifestyle counselling after the GDM diagnosis to pre- 413
vent excessive weight gain. 414

Metformin has been found to reduce the risk of gesta- 415
tional hypertension in comparison to insulin [5] and the 416
risk of preeclampsia when compared to placebo [45]. 417
This effect however was unlikely mediated by reduction 418
of insulin resistance in obese patients [47]. In line with 419
these findings, neither IGFBP-1's nor the inflammatory 420
markers were associated with the risk of hypertensive 421
disorders in our data. 422

Baseline high-pIGFBP-1 in all patients requiring met- 423
formin or insulin and low-pIGFBP-1 in metformin- 424
treated patients was associated with a lower risk for in- 425
duction of labor. This may reflect a better overall 426

427 metabolic health of patients with high serum IGFBP-1
428 while having a lower overall risk for pregnancy compli-
429 cations (of which induction of labor was the most fre-
430 quent). The induction rate of labor was marginally
431 higher in patients treated with insulin. This might reflect
432 the physicians' perception that GDM treated with insulin
433 is more severe than GDM without insulin treatment.

434 In our study, at baseline the inflammatory markers
435 hsCRP, IL-6 and GlycA, and IGFBP-1 phosphoisoforms
436 correlated stronger with fasting C-peptide and pre-
437 pregnancy BMI than with fasting or postprandial glucose.
438 Hence, inflammatory markers and IGFBP-1 phosphoiso-
439 forms seem to indicate obesity related insulin resistance.

440 We have demonstrated that metformin affects serum
441 GlycA and non-pIGFBP-1 in GDM, and that the associa-
442 tions between these markers and clinical outcomes are
443 similar irrespective of the antihyperglycemic treatment
444 used. Based on this data it is unlikely that metformin, at
445 least when started this late in pregnancy, has any signifi-
446 cant impact on the systemic low-grade inflammation
447 that is present in GDM [9–12] or reflects morbidity later
448 in life [13]. Follow-up studies are needed to assess the
449 long term safety of metformin treatment of GDM on
450 children. Further on, it needs to be studied whether pos-
451 sible long term consequences are associated with the
452 changes in serum inflammatory markers or IGFBPs.

453 Strengths and limitations of the study

454 We have included two relatively novel inflammatory
455 markers, MMP-8 and GlycA, and provide longitudinal
456 data of their changes during the last trimester of preg-
457 nancy. The study design was a randomized controlled
458 trial – a setting that improves the reliability of results.
459 Even so, there are some limitations to our study.

460 Our sample size was designed to prove non-inferiority
461 of metformin or insulin in birth weight in the previously
462 published primary randomized trial (24). Thus, although
463 the study population is fairly large, it was underpowered
464 to reveal or exclude all studied associations between in-
465 flammation markers and IGFBP-1s and outcome vari-
466 ables. There may also be confounding factors that
467 slightly affect both maternal and neonatal outcomes, but
468 the statistical power of multiple adjusted regression
469 models to examine each outcome closely is limited. The
470 serum samples in late pregnancy were taken at mean 36
471 gw of the patients. Since the women delivered at mean
472 39 gw, additional samples taken nearer delivery could
473 have provided important additional information on the
474 effect of metformin and insulin. Our population is repre-
475 sentative of mostly Caucasian patients in excellent gly-
476 cemic control, and these results may not necessarily be
477 generalizable to populations of other ethnicities or with
478 inferior glycemic control. Furthermore the indications
479 for induction, cesarean section and NICU admissions

vary between countries making comparisons of these 480
outcomes between various studies difficult. The trial was 481
registered at ClinicalTrials.gov retrospectively. 482

483 Conclusions

484 Metformin had beneficial effects on maternal serum
485 IGFBP-1 concentrations compared to insulin, possibly
486 due to its favorable effect on insulin resistance. IGFBP-1,
487 the non-phosphorylated isoform in particular, related to
488 lower total and late pregnancy maternal weight gain.
489 Otherwise there were no evident clinically relevant rela-
490 tions between inflammatory markers and pregnancy out-
491 come measures. Compared to insulin metformin caused
492 a similar decrease in serum hsCRP and a similar increase
493 in IL-6 but a slightly greater rise in GlycA. The signifi-
494 cance of GlycA, and of IL-6-CRP-signalling in GDM will
495 need to be more profoundly examined in further studies.

496 Supplementary information

497 **Supplementary information** accompanies this paper at <https://doi.org/10.1186/s12884-020-03077-6>.
498

Additional file 1. Post-hoc power analysis. 500

Additional file 2: Table S1. Comparison of inflammatory markers and 501
IGFBP-1's at baseline and at 36 gestational weeks. 502

Additional file 3: Table S2. Associations of inflammatory markers and 503
IGFBP-1 concentrations with clinical outcomes. 504

Additional file 4: Table S3. Associations of inflammatory markers and 505
IGFBP-1 concentrations at baseline and 36 gestational weeks with clinical 506
outcomes adjusted for pre-pregnancy BMI in metformin and insulin 507
treated patients combined. 508

Additional file 5: Table S4. Regression models with significant ($p <$ 509
0.05) interaction between treatment group (metformin or insulin) and the 510
association between outcome and the independent variable. 511
512

514 Abbreviations

515 BMI: Body mass index; CI: Confidence interval; CRP: C-reactive protein;
516 hsCRP: High-sensitivity C-reactive protein; ELISA: Enzyme-linked
517 immunosorbent assay; FDR: False discovery rate; GDM: Gestational diabetes
518 mellitus; GlycA: Glycoprotein acetylation; Gw: Gestational weeks;
519 GWG: Gestational weight gain; HbA1c: glycated hemoglobin; IGF-1: Insulin-
520 like growth factor 1; IGFBP-1: (non-pIGFBP-1, low-pIGFBP-1, high-pIGFBP-1)
521 non-phosphorylated / low-phosphorylated / high-phosphorylated insulin-like
522 growth factor-binding protein 1; IL-6: Interleukin-6; LDL: Low-density
523 lipoprotein; MMP-8: Matrix metalloproteinase-8; NICU: Neonatal intensive
524 care unit; OGTT: Oral glucose tolerance test; SD: Standard deviation;
525 T2DM: Type 2 diabetes mellitus

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531 Authors' contributions

532 M.H. analyzed the data and wrote the first draft of the manuscript. K.T.
533 provided clinical data on the metformin and insulin treated patients and
534 serum samples of all patients from a previous study, designed the present
535 study and edited and reviewed the manuscript. J.J. carried out the analyses
536 of hsCRP, IL-6, MMP-8 and IGFBP1's and reviewed and edited the manuscript,
537 T.S. participated in the analysis of MMP-8 and reviewed and edited the
538 manuscript, T.R. designed the study and reviewed and edited the manu-
539 script. All authors have approved the final version of the manuscript.

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547 Availability of data and materials

548 The datasets used and/or analysed during the current study are available
549 from the corresponding author on reasonable request.

550 Ethics approval and consent to participate

551 The trial was approved by the Ethics Committee of the Southwest Hospital
552 District of Finland, the Finnish National Agency of Medicines, and the
553 European Union Drug Regulatory Agency (EUDRA). All participants provided
554 written informed consent.

555 Consent for publication

556 Not applicable.

557 Competing interests

558 T.S. and J.J. are inventors of a diagnostic patent for serum MMP-8 (FI 127 416
559 B / 31.5.2018). M.H., K.T. and T.R. do not have any conflicts of interest.

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