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Amino acid profile in women with gestational diabetes mellitus treated with metformin or insulin

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ABSTRACT

Aims: We compared the effects of metformin and insulin treatments of gestational diabetes mellitus (GDM) on amino acid metabolism.
Methods: 217 pregnant women diagnosed with GDM were randomized to receive either metformin or insulin. ^{1}H nuclear magnetic spectroscopy was used to determine serum concentrations of alanine, glutamine, glycine, isoleucine, leucine, valine, histidine, phenylalanine, tyrosine, glucose and lactate at the time of diagnosis and at 36 gestational weeks (gw).
Results: Majority of the amino acid concentrations increased from 30 to 36 gw. The rise in alanine (16% vs. 8%, $p < 0.0001$), isoleucine (11% vs. 5%, $p = 0.035$) and lactate (29% vs. 14% $p = 0.015$) was larger in the metformin group compared to insulin group. Baseline alanine, glycine, isoleucine, leucine, valine and tyrosine were positively related to slightly earlier delivery. Alanine at 36 gw was positively associated with birth weight and glutamine with gestational hypertension or preeclampsia. Lactate at 36 gw was not associated with any adverse outcome.
Conclusions: Compared to insulin metformin caused a greater increase in serum alanine, isoleucine and lactate concentrations. Although the observed differences in the metabolic variables were relatively small and not outright concerning, additional studies and follow-up data are required to ensure the safety of metformin use in pregnancy.
The trial was registered in Clinicaltrials.gov, NCT01240785; http://clinicaltrials.gov/ct2/show/NCT01240785.

1. Introduction

Gestational diabetes mellitus (GDM) is a growing health concern worldwide, affecting both the mother and the fetus. It increases risk for pregnancy and neonatal complications such as macrosomia, preeclampsia, neonatal hypoglycemia and hyperbilirubinemia and need for neonatal intensive care [1,2]. Also, GDM is associated with a significant risk of later type 2 diabetes in the mothers [3]. In the offspring, GDM may lead to obesity and metabolic syndrome, particularly if associated with maternal obesity [4,5].

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Metformin restores normoglycemia without increasing insulin secretion, and is thus unlikely to cause hypoglycemia. It decreases plasma glucose concentration by inhibiting gluconeogenesis in the liver, and according to recent findings, it may also act through several gut-mediated mechanisms [6]. However, the exact mechanism of action is still not completely understood. The ease of oral administration, and its cost compared to insulin, makes metformin an appealing alternative for GDM treatment. While recent evidence is supporting the safety of metformin in the treatment of GDM [7], detailed data on the effect on maternal and fetal metabolism and long term consequences is mostly lacking.

In a recent meta-analysis, metformin reduced gestational weight gain, incidences of neonatal hypoglycaemia, neonatal intensive care and gestational hypertension compared to insulin treatment [7]. Metformin was also associated with slightly lower gestational age at birth, although not with preterm labor [7].

A metabolomic approach aims to identify metabolite profiles and pathways underlining various physiological and pathophysiological conditions, such as GDM. In type 2 diabetes aromatic amino acids (AAA) and branched-chain amino acids (BCAA) have been related to insulin resistance [8], whereas in GDM the relationship seems not to be so straightforward. Serum AAA and BCAA levels were higher in GDM patients in some studies, but the results altogether have been inconsistent [9].

In non-pregnant populations metformin has been shown to affect serum alanine, phenylalanine and several other amino acids, yet there is some discrepancy between the results and study settings [10–19].

In rodents, metformin administration has been demonstrated to reduce gluconeogenesis from lactate and glycerol by altering hepatocellular redox state [20]. Due to reduced gluconeogenesis [21], metformin could provoke accumulation of lactate in blood. However, in a Cochrane Review on metformin treatment of type 2 diabetic subjects, no elevation of blood lactate levels was observed [22]. To our knowledge, there are no previous studies on the effect of metformin on amino acid profile or blood lactate in GDM patients.

Alike GDM, pregnancy alone causes alterations in the metabolic profile. In a Finnish cohort study, there were significant differences in the serum concentrations of most amino acids between pregnant and non-pregnant women [23]. BCAA isoleucine and leucine levels however, didn't differ in any trimester from non-pregnant women. In another study, 160 non-diabetic pregnant women were followed up during pregnancy, and significant differences were detected in serum concentrations of 12 amino acids between trimesters [24].

The main aim of our study was to compare concentrations of maternal amino acids and lactate in women diagnosed with GDM treated with metformin and insulin. The relevance of the possible differences in the amino acid profile was examined by relating them to clinical data. Moreover, we determined at the time of GDM diagnosis possible differences in amino acid metabolism between GDM patients needing medical treatment and those achieving adequate metabolic control with diet alone.

2. Subjects, materials and methods

The trial was conducted between June 2006 and December 2010 in Turku University Hospital, Turku, Finland. It was approved by the Ethics Committee of Southwest Hospital District of Finland, the Finnish National Agency of Medicines, and the European Union Drug Regulatory Agency (EUDRA). The trial was registered in Clinicaltrials.gov, NCT01240785; http://clinicaltrials.gov/ct2/show/NCT01240785. All the participants signed an informed consent.

The study population has been described previously in detail by Tertti et al. [25,26] and Pellonperä et al. [27]. Briefly, 217 women with GDM and singleton pregnancy were randomized in an open-label clinical trial to receive either metformin (n = 110) or insulin (n = 107). Additional serum samples from 126 women who achieved sufficient glycemic control by diet and lifestyle modifications alone were included as a control group regarding the amino acids at baseline. Clinical records and serum samples were available for 109, 107 and 103 women in the metformin, insulin and diet groups, respectively.

GDM diagnosis was determined by Finnish national criteria. The diagnostic cut-off values in 2 h 75 g oral glucose tolerance test (OGTT) were $\geq$ 4.8 mmol/L (fasting), $\geq$10.0 mmol/L (1 h) and $\geq$ 8.7 mmol/L (2 h) until the release of new guidelines in December 2008, and thereafter $\geq$ 5.3, $\geq$10.0 and $\geq$ 8.6 mmol/L, respectively. 228 women were recruited before and 91 women after the change in the diagnostic criteria. Inclusion criteria were newly diagnosed GDM on the basis of at least two pathologic plasma glucose values in OGTT and the need for medical treatment of hyperglycemia (during diet therapy fasting plasma glucose $\geq$ 5.5 mmol/L and/or 1 h postprandial glucose $\geq$ 7.8 mmol/L). Exclusion criteria included cardiac or renal insufficiency, liver disease, metformin use within 3 months preceding pregnancy, or during pregnancy before the OGTT. 99% of the women were Caucasian.

Metformin was started at 500 mg daily and increased up to 2000 mg if needed (median 1500 mg). Additional insulin was given to 23 participants due to unsatisfactory glucose control with metformin only. Insulin treatment was accomplished using NPH insulin and/or rapid-acting insulin lispro or insulin aspart. Medication was initiated at mean 30 gestational weeks (gw).

Overnight fasting serum samples were collected after GDM diagnosis (mean 30 gw) when evaluating the need for medical therapy. Another fasting serum sample was drawn at 36 gw from medically treated patients. Serum concentrations of 9 amino acids (alanine, glutamine, glycine, isoleucine, leucine, valine, histidine, phenylalanine and tyrosine), glucose and lactate were determined using high-throughput proton (^{1}H) nuclear magnetic resonance spectroscopy protocol [28]. All samples were stored at −70 °C before analyses.

Serum C-peptide and HbA1c was measured at baseline using routine laboratory methods. HbA1c was determined also at 36 gw in those mothers receiving antihyperglycemic medication.

We examined the associations between metabolic variables and the following clinical outcomes: gestational weight

gain, preeclampsia or gestational hypertension, gestation length, the incidence of caesarean section, birth weight, neonate admission to neonatal intensive care unit (NICU) and neonatal intravenous glucose given for any indication. Gestational weight gain was defined as last measured weight at maternity clinic minus self-reported weight before pregnancy. Besides absolute value in grams, birth weight was calculated in SD units (deviation from Finnish general population mean adjusted for gestation length). Due to low rates of macrosomia (birth weight > 2.0 SD and/or >4500 g) and small for gestational age (birth weight < −2.0 SD), we calculated prevalence of birth weight >90th and <10th percentile of population adjusted for gestation length as indicators of low and high birth weight, respectively.

2.1. *Statistical analysis*

Due to a few missing serum samples at each time point and rejection of individual measurements in NMR quality control (maximally 3% of samples), there is some variation in the number of samples between various variables. The normality of metabolite data was examined using Shapiro-Wilk test while n < 100 and Kolmogorov-Smirnov test with Lilliefors correction for larger samples sizes. Two-sample *t*-test or Mann-Whitney *U* test was used for comparison of clinical and metabolite data. Alike, pairwise analyses were performed using pairwise *t*-test or Wilcoxon signed-rank test. Categorical clinical data comparison between groups was done with Chi-square or Fisher's exact test.

Associations between metabolic data and C-peptide, HbA1c and clinical outcomes were tested by general regression modelling. All non-binary variables were centered and scaled into SD units, excluding birth weight, which already was calculated as population SD units. With binary outcome variables logistic regression was applied. All regression models were adjusted for clinically relevant confounding factors: prepregnancy BMI and smoking. Comparison between groups (diet vs. medical treatment or insulin vs. metformin) were performed using a regression model assuming interaction between each metabolite and group. To achieve more robust confidence intervals, adjusted bootstrap percentile method was used for regression coefficients.

P-value below 0.05 was considered as statistically significant. For regression coefficients between metabolites and clinical outcomes p-values are represented with and without FDR-adjustment at baseline and 36 gw. All analyses were done on statistical software R (R version 3.3.2).

3. Results

The maternal and neonatal clinical data of the medical treatment group have been published previously [25]. Briefly, there was no difference between the two antihyperglycemic treatment groups with respect to baseline characteristics, glycemic control or clinical outcomes (Table 1). Induction of delivery was applied more often in the insulin than in metformin group (54.2% vs. 37.6%, p = 0.014).

Patients in the diet group were significantly younger (p = 0.037) and their median HbA1c% (p = 0.001), fasting OGTT (p = 0.004), and 1-h OGTT (p = 0.006) were slightly lower compared to those who required medication (Table 1). Also, induction of delivery was applied more often on patients requiring medication (45.8% vs. 30.1%, p = 0.007).

Compared to the diet group glucose was higher (p < 0.0001) and glutamine lower (p = 0.009) in women needing medication (Table 2). Phenylalanine tended to be higher in the group needing medication (p = 0.067).

Metabolite concentrations at baseline and at 36 gw in the medication groups are shown in Table 3. There was no difference between metformin and insulin groups in any of the metabolite values at baseline (Table 3). In medically treated groups combined, the concentration of metabolites increased from baseline to 36 gw except valine and glucose which decreased and tyrosine which was stable. Examining amino acids in the medication groups separately, alanine, glutamine, glycine, isoleucine, leucine and phenylalanine rose significantly in both groups, while valine was unchanged. The upregulation of histidine was significant in the metformin (p = 0.009) but not in the insulin group (p = 0.15). As expected, glucose levels decreased with both medications. Lactate increased in both groups.

The comparison of changes in metabolic variables between the medication groups is shown in Fig. 1. Serum glucose decreased similarly in metformin and insulin groups. There were no differences in the change in AAAs (phenylalanine, tyrosine or histidine), but BCAA isoleucine (median 11% vs. 5%, p = 0.035) and most noticeably alanine (mean 16% vs. 8%, p < 0.0001) increased more in the metformin group. Lactate (mean 29% vs. 14%, p = 0.015) increased more in metformin than insulin group.

Within the metformin group the results concerning amino acid and lactate concentrations and their changes were essentially similar in those who received metformin only and in those needing additional insulin (results not shown).

3.1. *Associations between amino acids, C-peptide and HbA1c*

C-peptide levels at baseline were significantly associated with alanine (57 pM C-peptide/SD alanine, 95% CI: 22; 92), isoleucine (72 pM C-peptide/SD isoleucine, 95% CI: 41; 104), leucine (59 pM C-peptide/SD leucine, 95% CI: 27; 93) and phenylalanine (76 pM C-peptide/SD phenylalanine, 95% CI: 44; 109). Other amino acids measured did not significantly associate with C-peptide levels.

Baseline HbA1c was positively associated with phenylalanine (0.066 HbA1c(%)/SD phenylalanine, 95% CI: 0.028; 0.10). At 36 gw HbA1c was strongly related to alanine in metformin treated women (0.12 HbA1c(%)/SD alanine, 95% CI: 0.046; 0.21) whereas in insulin treated women the association was statistically nonsignificant (−0.020 HbA1c(%)/SD alanine, 95% CI: −0.097; 0.049).

3.2. *Associations between metabolic variables and clinical outcomes*

Associations between clinical outcomes and metabolites at baseline and at 36 gw are shown in Table 4.

Table 1 – Comparison of clinical data.

Variable	Metformin	Insulin	p-value	Any antihyperglycaemic medication	Diet only	p-value
Age (years)	31.9 ± 5.01	32.0 ± 5.47	0.89	31.9 ± 5.23	30.6 ± 5.05	0.037
Smoking	9 (8.6)	17 (16.0)	0.099	26 (12.3)	9 (8.8)	0.36
Primipara	42 (38.5)	49 (45.8)	0.28	91 (42.1)	46 (44.7)	0.67
Pre-pregnancy BMI (kg/m^2)	29.5 ± 5.91	28.9 ± 4.71	0.41	29.2 ± 5.35	28.9 ± 5.41	0.67
HbA1c% at OGTT	5.48 ± 0.34	5.51 ± 0.34	0.49†	5.49 ± 0.34	5.35 ± 0.31	0.001†
HbA1c at OGTT (mmol/mol)	36.3 ± 3.69	36.7 ± 3.72		36.5 ± 3.70	35.0 ± 3.38	
HbA1c% at 36 gw	5.68 ± 0.33	5.69 ± 0.36	0.82			
HbA1c at 36 gw (mmol/mol)	38.5 ± 3.63	38.6 ± 3.89				
OGTT fasting	5.52 ± 0.55	5.57 ± 0.42	0.44	5.54 ± 0.49	5.38 ± 0.43	0.004
OGTT 1 h	11.2 ± 1.49	11.2 ± 1.24	0.61†	11.2 ± 1.37	10.9 ± 1.06	0.006†
OGTT 2 h	8.33 ± 1.76	7.91 ± 1.75	0.076	8.12 ± 1.77	7.81 ± 1.91	0.15
C-peptide at baseline (nmol/L)	1.05 ± 0.33	1.05 ± 0.29	0.90†	1.06 ± 0.31	1.01 ± 0.32	0.10†
Gestational hypertension	2 (1.8)	4 (3.7)	0.44‡	6 (2.8)	4 (3.9)	0.73‡
Preeclampsia	5 (4.6)	10 (9.3)	0.17	15 (6.9)	2 (1.9)	0.063
Assisted vaginal delivery	9 (8.3)	8 (7.5)	0.83	17 (7.9)	7 (8.6)	0.82
Caesarean section	15 (13.8)	18 (16.8)	0.53	33 (15.3)	16 (15.5)	0.95
Induction of delivery	41 (37.6)	58 (54.2)	0.014	99 (45.8)	31 (30.1)	0.007
Gestational weight gain (kg)	7.97 ± 5.24	7.82 ± 5.27	0.83	7.89 ± 5.25	8.82 ± 5.33	0.14
Gw at delivery	39.2 ± 1.40	39.4 ± 1.58	0.43	39.3 ± 1.49	39.3 ± 2.26	0.89
Birth weight (g)	3610 ± 490	3590 ± 450	0.78	3600 ± 470	3560 ± 550	0.54
Birth weight (SD)	0.17 ± 1.05	0.15 ± 0.96	0.91	0.16 ± 1.0	-0.02 ± 1.1	0.15
Macrosomia	5 (4.6)	1 (0.9)	0.21‡	6 (2.8)	5 (4.9)	0.34‡
Birth weight < 10th percentile	12 (11.4)	9 (8.4)	0.46	21 (9.9)	12 (11.7)	0.64
Birth weight > 90th percentile	15 (14.3)	17 (15.9)	0.74	32 (15.1)	12 (11.7)	0.41
Umbilical artery pH	7.28 ± 0.09	7.28 ± 0.08	0.57	7.28 ± 0.08	7.27 ± 0.09	0.16
Apgar-score at 5 min	8.80 ± 1.02	8.85 ± 0.98	0.81†	8.82 ± 1.0	8.85 ± 0.86	0.93†
Admission to NICU	33 (30.1)	39 (36.4)	0.36	72 (33.5)	31 (30.1)	0.54
I.V. Glucose	25 (23.1)	25 (23.6)	0.94	50 (23.4)	25 (24.5)	0.82

Data is shown as mean ± SD or n (%), p-value is given for Mann-Whitney U (†) or *t*-test and for categorical data Chi-square or Fisher's exact test (‡), metformin n = 103–109, insulin n = 101–107, diet only n = 100–103 (except for umbilical artery pH n = 80), medication group (metformin or insulin) n = 204–216. OGTT = oral glucose tolerance test, Gw = gestational weeks, SD = standard deviation, NICU = neonatal intensive care unit, I.V. = intravenous.

Table 2 – Comparison of serum metabolite concentrations at baseline in women treated with diet only and women needing antihyperglycemic medication.

Variable	Any antihyperglycemic medication *mean ± SD or median [IQR] (μmol/L)*	Diet only *mean ± SD or median [IQR] (μmol/L)*	p-value
Amino Acids			
Alanine	394 ± 44	391 ± 43	0.53
Glutamine	371 ± 56	386 ± 48	0.009
Glycine	207 ± 36	205 ± 31	0.70
Branched-Chain AA			
Isoleucine	53 [47–63]	53 [47–60]	0.42†
Leucine	70 [64–79]	71 [63–78]	0.52†
Valine	109 [97–125]	107 [98–123]	0.69†
Aromatic AA			
Phenylalanine	86 [79–94]	84 [78–90]	0.067†
Tyrosine	37 [34–42]	38 [35–42]	0.52†
Histidine	69 ± 9.5	69 ± 8.8	0.95
Glucose Metabolism			
Glucose (mmol/L)	4.1 [3.8–4.4]	3.9 [3.6–4.1]	<0.0001†
Lactate (mmol/L)	1.2 [1.0–1.5]	1.2 [1.0–1.4]	0.68†

p-value is given for Mann-Whitney U (†) or t-test when appropriate,medication group (metformin or insulin) n = 205–208, diet only n = 124–126, SD = standard deviation, IQR = interquartile range

Table 3 – Serum metabolite concentrations at baseline and at 36 gestational weeks in various medication groups.

Variable	Any antihyperglycemic medication			Metformin			Insulin		
	Baseline	36 gw	p-value	Baseline	36 gw	p-value	Baseline	36 gw	p-value
Amino Acids									
Alanine	392 ± 44	438 ± 56	<0.0001	395 ± 45	454 ± 51	<0.0001	389 ± 44	421 ± 57	<0.0001
Glutamine	371 ± 56	384 ± 65	0.009	370 ± 55	381 ± 61	0.044	372 ± 58	387 ± 69	0.028
Glycine	207 ± 36	222 ± 40	<0.0001	201 ± 36	215 ± 36	<0.001	212 ± 36	229 ± 43	<0.0001
Branched-Chain AA									
Isoleucine	53 [47–63]	59 [50–68]	<0.0001†	53 [47–61]	60 [48–70]	<0.0001†	56 ± 12	58 ± 13	0.041
Leucine	70 [64–79]	75 [68–84]	<0.0001†	72 ± 11	78 ± 14	0.0001	73 ± 12	76 ± 14	0.025
Valine	110 [97–124]	106 [96–120]	0.002†	112 ± 18	110 ± 19	0.19	109 ± 21	105 ± 21	0.056
Aromatic AA									
Phenylalanine	86 [78–94]	92 [85–97]	<0.0001†	86 ± 9.9	92 ± 9.6	<0.0001	87 [80–95]	92 [83–102]	0.002†
Tyrosine	37 [34–42]	39 [34–42]	0.48†	38 ± 6.6	38 ± 6.5	0.98	37 [34–42]	39 [35–44]	0.15†
Histidine	69 ± 9.6	71 ± 10	0.004	68 ± 8.8	71 ± 8.9	0.009	69 ± 10	71 ± 11	0.15
Glucose Metabolism									
Glucose	4.1 ± 0.38	3.9 ± 0.43	<0.0001	4.1 ± 0.40	3.8 ± 0.43	<0.0001	4.1 ± 0.38	3.9 ± 0.43	0.003
Lactate	1.2 ± 0.31	1.4 ± 0.40	<0.0001	1.2 ± 0.34	1.5 ± 0.39	<0.0001	1.2 ± 0.28	1.4 ± 0.40	0.011

Variable concentrations are shown as mean ± SD or median [IQR] (μmol/L), glucose and lactate are mmol/L, p-value is given for Wilcoxon signed-rank (†) or t-test when appropriate, combined n = 186–190, metformin (including those who received metformin + insulin) n = 96–99, insulin only n = 90–91, gw = gestational weeks, IQR = interquartile range. At baseline there were no significant differences between two treatment groups (p > 0.05).

At *baseline* high concentrations of alanine, glycine, all three BCAAs, isoleucine, leucine and valine, one of the three AAA, tyrosine and glucose were associated with earlier delivery. The associations were significant also after FDR-adjustment in alanine (−0.39 weeks/SD alanine, 95% CI: −0.81; −0.18), tyrosine (−0.39 weeks/SD tyrosine, 95% CI: −0.82; −0.16) and glucose (−0.36 weeks/SD glucose, 95% CI: −0.62; −0.15). Birth weight was after FDR-adjustment positively linked to isoleucine (0.19 SD-units/SD isoleucine, 95% CI: 0.060; 0.31) and glucose (0.25 SD-units/SD glucose, 95% CI: 0.12; 0.37). In logistic regressions only glucose increased risk for birth weight > 90th percentile (OR: 1.6/SD glucose, 95% CI: 1.2; 2.2).

The probability of admission to NICU was associated with higher baseline glucose (OR: 1.4/SD glucose, 95% CI: 1.1; 1.8).

High glutamine at baseline associated with decreased gestational weight gain (−1.3 kg/SD glutamine, 95% CI: −1.9; −0.64) while the effect for valine was the opposite and non-significant after FDR-adjustment (0.58 kg/SD valine, 95% CI: 0.049; 1.1). Lactate did not relate to any of these clinical outcomes at baseline.

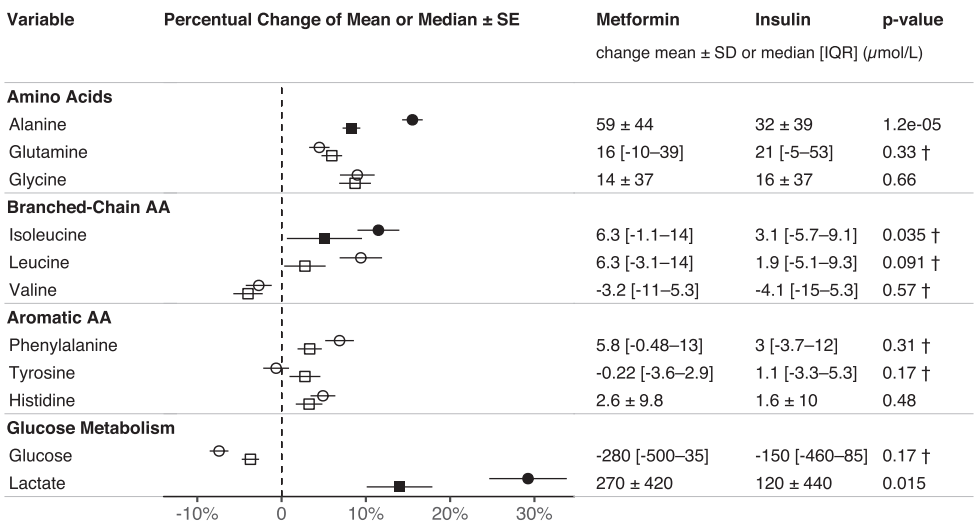

Fig. 1 – Comparison of changes in metabolic profiles from baseline to 36 gestational weeks between metformin and insulin treatment groups circles = metformin group, squares = insulin group, black square or circle denote significant p-value (<0.05). p-value is given for Mann-Whitney U (†) or t-test when appropriate. Metformin (including those who received metformin + insulin) n = 96–99, insulin n = 90–91. AA = amino acids, SE = standard error, IQR = interquartile range.

At 36 gestational weeks of all amino acids measured in medication groups combined (n = 189–193), only alanine was significantly linked to birth weight and the effect was mostly explained by the strong association in those assigned to metformin (metformin group: 0.31 SD-units/SD alanine, 95% CI: 0.11; 0.52, insulin group: 0.051 SD-units/SD alanine, 95% CI: −0.12; 0.28). Low glucose associated to birth weight < 10th percentile (OR: 0.47/SD glucose, 95% CI: 0.25; 0.89).

Combined risk for gestational hypertension or preeclampsia was significantly increased if glutamine concentration was high at 36 gw (OR: 2.3/SD glutamine, 95% CI: 1.3; 4.4).

Lower gestational weight gain was associated with histidine at 36 gw, but only in the metformin group (−1.4 kg/SD histidine, 95% CI: −2.4; −0.38).

Lactate at 36 gw did not relate to any of these clinical outcomes.

4. Discussion

Maternal amino acid profile significantly changes during the last half of pregnancy [23,24]. In our study we have focused on three major questions: first, the reflection of amino acid concentrations on the need of antihyperglycemic medication during GDM, second, the influence of metformin and insulin treatments on the changes in amino acid concentrations after 30 gw, and third, the relation of amino acid and lactate levels to clinical outcome variables.

The need for medication of GDM may result from higher insulin resistance and/or impaired insulin secretion capacity. In our study, shortly after diagnosis of GDM (mean 30 gw) glutamine was lower in patients needing medical treatment, compared to patients who reached sufficient glycemic control with dietary intervention only. This is in accordance with studies in non-pregnant individuals demonstrating an inverse correlation between serum glutamine concentration, insulin resistance and type 2 diabetes risk [8,29].

Phenylalanine, which tended to be higher in patients requiring medical treatment, has been associated with insulin resistance and risk of developing type 2 diabetes in previous studies [8,30]. In accordance with those studies we observed that phenylalanine was strongly correlated with serum C-peptide concentration, which in turn is regarded to be a marker of insulin resistance in pre-diabetic conditions preceding type 2 diabetes. Moreover, serum phenylalanine was at baseline positively associated with HbA1c. A strong relationship was also observed between baseline C-peptide and alanine, isoleucine and leucine, suggesting a link between these amino acids and insulin resistance.

The role of BCAAs in human metabolism remains controversial. On the one hand elevated levels are associated with risk of developing type 2 diabetes [8]. On the other hand, co-administration of leucine with metformin has been shown to improve insulin sensitivity on obese mice [31]. In a large Mendelian randomization analysis in humans, risk of type 2 diabetes was related to impaired BCAA metabolism rather than high BCAA alone [32].

4.1. *Changes in metabolite concentrations from baseline to 36 gestational weeks*

Independent of medication group, pregnancy itself had marked influences on 10 of the 11 metabolites analyzed. Most metabolite concentrations, including lactate, increased by approximately 5–25%, while the differences in the changes of metabolites between metformin and insulin groups were in most cases relatively small. However, although modest, the rise in lactate was higher in patients treated with metformin. Another exception was over 80% greater rise in serum

Table 4 – Associations of metabolite concentrations at baseline and 36 gestational weeks with clinical outcomes.

	Gestational Weight gain	Preeclampsia or gestational hypertension	Length of Gestation	Caesarean Section	Birth Weight	Birth Weight < 10th Percentile	Birth Weight > 90th Percentile	NICU Admission	I.V. Glucose Administration
Baseline:									
n +/-		*23 / 275–280*		*43 / 255–260*		*30–31 / 264–269*	*43–44 / 252–256*	*95–96 / 202–206*	*69–70 / 226–230*
total n:	*297–302*		*298–303*		*295–300*				
units	*kg / SD*	*OR / SD*	*weeks / SD*	*OR / SD*	*SD / SD*	*OR / SD*	*OR / SD*	*OR / SD*	*OR / SD*
Amino Acids									
Alanine	−0.3 [−0.97; 0.17]	1.3 [0.82; 1.9]	−0.39 [−0.81; −0.18]**##	0.94 [0.67; 1.4]	0.079 [−0.042; 0.18]	1.1 [0.76; 1.5]	1.0 [0.72; 1.4]	1.1 [0.82; 1.4]	1.3 [0.98; 1.6]
Glutamine	−1.3 [−1.9; −0.64]**##	1.1 [0.68; 1.7]	−0.12 [−0.29; 0.078]	1.1 [0.83; 1.7]	−0.078 [−0.19; 0.056]	1.4 [0.93; 2.5]	0.92 [0.66; 1.3]	1.1 [0.82; 1.3]	1.2 [0.91; 1.6]
Glycine	0.011 [−0.49; 0.59]	1.1 [0.72; 1.8]	−0.2 [−0.37; −0.049]*	0.89 [0.64; 1.3]	−0.034 [−0.15; 0.093]	1.3 [1.0; 1.9]	0.88 [0.59; 1.3]	0.95 [0.77; 1.3]	0.99 [0.74; 1.3]
Branched−Chain AA									
Isoleucine	0.16 [−0.45; 0.81]	1.3 [0.85; 1.9]	−0.23 [−0.44; −0.054]*	0.9 [0.61; 1.3]	0.19 [0.06; 0.31]**#	0.82 [0.55; 1.3]	1.3 [0.92; 1.8]	1.1 [0.83; 1.5]	1.2 [0.87; 1.7]
Leucine	0.44 [−0.19; 1.1]	1.2 [0.8; 1.8]	−0.28 [−0.51; −0.086]**	0.86 [0.59; 1.3]	0.11 [−0.017; 0.24]	1.1 [0.72; 1.7]	1.1 [0.81; 1.6]	1.1 [0.84; 1.4]	1.1 [0.84; 1.5]
Valine	0.58 [0.049; 1.1]*	1.0 [0.62; 1.6]	−0.22 [−0.42; −0.017]*	1.1 [0.77; 1.5]	0.0016 [−0.11; 0.13]	1.4 [1.0; 2.2]	1.1 [0.79; 1.5]	1.0 [0.75; 1.3]	1.1 [0.84; 1.5]
Aromatic AA									
Phenylalanine	0.28 [−0.3; 0.83]	1.4 [0.94; 2.4]	−0.13 [−0.36; 0.062]	0.8 [0.48; 1.2]	−0.034 [−0.15; 0.11]	1.3 [0.84; 1.9]	0.86 [0.6; 1.2]	1.1 [0.86; 1.4]	1.1 [0.82; 1.5]
Tyrosine	0.3 [−0.28; 0.93]	0.98 [0.69; 1.5]	−0.39 [−0.82; −0.16]**##	0.85 [0.5; 1.2]	0.02 [−0.09; 0.14]	1.1 [0.7; 1.7]	1.1 [0.76; 1.6]	1.1 [0.85; 1.5]	1.2 [0.93; 1.6]
Histidine	−0.15 [−0.75; 0.49]	0.76 [0.52; 1.2]	−0.17 [−0.34; 0.016]	1.3 [0.89; 1.8]	0.083 [−0.033; 0.2]	0.8 [0.5; 1.3]	1.2 [0.85; 1.6]	1.2 [0.93; 1.6]	1.0 [0.73; 1.4]
Glucose Metabolism									
Glucose	−0.31 [−0.85; 0.26]	0.9 [0.54; 1.4]	−0.36 [−0.62; −0.15]**##	1.9 [1.3; 2.8]**##	0.25 [0.12; 0.37]**##	0.79 [0.5; 1.2]	1.6 [1.2; 2.2]**	1.4 [1.1; 1.8]*	1.2 [0.91; 1.6]
Lactate	0.32 [−0.32; 0.85]	1.0 [0.73; 1.5]	−0.049 [−0.23; 0.13]	0.8 [0.55; 1.2]	0.075 [−0.04; 0.21]	1.2 [0.81; 1.7]	1.1 [0.79; 1.6]	1.0 [0.84; 1.4]	1.2 [0.99; 1.6]
36 gw:									
n +/−:		*20 / 172–173*		*29 / 163–164*		*19–20 / 170*	*29 / 160–161*	*59 / 132–133*	*40 / 150–151*
total n:	*191–192*		*192–193*		*189–190*				
Amino acids									
Alanine	0.54 [−0.24; 1.2]	1.4 [0.76; 2.3]	−0.24 [−0.41; 0.016]*	1.3 [0.86; 2]	0.15 [0.029; 0.3]*	1.1 [0.61; 1.7]	1.2 [0.8; 2.0]	0.98 [0.68; 1.4]	1.4 [0.87; 1.9]
Glutamine	−0.33 [−1.1; 0.38]	2.3 [1.3; 4.4]*	−0.12 [−0.31; 0.099]	1.2 [0.84; 1.8]	0.0078 [−0.18; 0.14]	1.2 [0.61; 2.3]	0.98 [0.67; 1.4]	1.2 [0.87; 1.8]	1.3 [0.83; 2]
Glycine	−0.16 [−1.0; 0.56]	1.4 [0.83; 2.5]	−0.15 [−0.31; 0.063]	1.2 [0.81; 1.9]	0.067 [−0.082; 0.2]	1.3 [0.83; 2.2]	1.2 [0.73; 1.9]	0.81 [0.59; 1.1]	0.93 [0.66; 1.4]
Branched−Chain AA									
Isoleucine	−0.15 [−0.68; 0.48]	1.3 [0.7; 2.0]	0.037 [−0.2; 0.26]	1.1 [0.6; 1.7]	0.04 [−0.087; 0.18]	1.1 [0.64; 1.8]	0.87 [0.58; 1.4]	0.95 [0.65; 1.4]	1.0 [0.73; 1.6]
Leucine	−0.018 [−0.58; 0.63]	1.3 [0.78; 1.8]	−0.0063 [−0.25; 0.25]	1.2 [0.61; 1.8]	−7.9e−4 [−0.15; 0.14]	1.2 [0.81; 2.2]	0.9 [0.58; 1.4]	0.94 [0.61; 1.3]	1.0 [0.7; 1.6]
Valine	0.62 [−0.12; 1.2]	1.2 [0.68; 1.9]	−0.091 [−0.31; 0.12]	1.2 [0.79; 1.7]	−0.032 [−0.18; 0.12]	1.3 [0.85; 1.8]	0.93 [0.55; 1.5]	1.0 [0.73; 1.5]	1.2 [0.79; 1.7]
Aromatic AA									
Phenylalanine	0.11 [−0.71; 0.84]	1.2 [0.77; 1.8]	−0.13 [−0.35; 0.1]	1.3 [0.82; 2.1]	−0.036 [−0.17; 0.098]	1.3 [0.84; 1.8]	1.0 [0.62; 1.6]	1.1 [0.76; 1.5]	1.1 [0.73; 1.7]
Tyrosine	0.37 [−0.23; 0.98]	1.1 [0.66; 2.0]	−0.093 [−0.29; 0.12]	1.3 [0.78; 1.8]	0.065 [−0.056; 0.18]	1.1 [0.72; 1.5]	1.5 [0.97; 2.2]*	1.2 [0.83; 1.6]	1.2 [0.79; 1.7]
Histidine	−0.28 [−1.0; 0.36]	1.1 [0.62; 2.1]	−0.095 [−0.32; 0.16]	1.2 [0.71; 1.9]	0.11 [−0.083; 0.23]	0.75 [0.39; 1.2]	1.2 [0.79; 1.9]	1.0 [0.71; 1.5]	0.96 [0.6; 1.6]
Glucose Metabolism									
Glucose	−0.079 [−0.89; 0.64]	0.6 [0.33; 0.92]	−0.029 [−0.22; 0.19]	1.2 [0.75; 1.8]	0.13 [0.0072; 0.27]	0.47 [0.25; 0.89]*	1.2 [0.77; 1.8]	1.1 [0.76; 1.5]	0.91 [0.62; 1.4]
Lactate	−0.13 [−0.89; 0.58]	0.8 [0.45; 1.3]	−0.07 [−0.27; 0.18]	1.1 [0.77; 1.6]	−0.021 [−0.16; 0.13]	1.1 [0.68; 1.8]	0.88 [0.51; 1.3]	0.86 [0.63; 1.2]	0.96 [0.65; 1.3]

Measures are expressed as odds ratios (OR) or regression β−estimates with 95% confidence intervals. At baseline associations were estimated for whole study population (n = 295–303), diet and medical treatment groups. At 36 gw associations were estimated for medical intervention group (n = 189–193). Adjustments were done for BMI at the beginning of pregnancy and smoking. Birth weight was measured in population SD units.

* $p < 0.05$.

** $p < 0.01$.

\# FDR adjusted $p < 0.05$.

\## FDR adjusted $p < 0.01$. SD = standard deviation, NICU = neonatal intensive care unit, I.V. = intravenous.

alanine in metformin treated women compared to those treated with insulin (59 vs. 32 μmol/L, $p < 0.0001$). Alanine is a fundamental glycogenic amino acid abundant in circulation. During fasting it is released from muscles to be utilized in liver gluconeogenesis. A similar rise in alanine levels in response to metformin therapy as in our study has been described earlier in various study settings [11,19,33], but to our knowledge this effect has not been demonstrated in pregnant population. Higher concentration of serum alanine in metformin vs. insulin treated GDM women may possibly be a consequence of metformin's mechanism of action, i.e. ability to depress gluconeogenesis in the liver [21]. This may also lead to accumulation of other glucogenic substrates such as lactate, as observed in the present study.

There were no differences in the change of AAAs between metformin and insulin groups during the last half of pregnancy. Out of three BCAAs isoleucine increased more ($p = 0.035$) and leucine showed a trend towards higher increase ($p = 0.091$) in patients assigned to metformin. In a study comparing metformin and placebo in coronary disease patients, isoleucine and leucine seemed to rise but there was no significant difference between groups during 18 month treatment [11]. Instead AAAs phenylalanine and tyrosine decreased and histidine increased. Alike, three-month metformin treatment was associated with decreased phenylalanine [15], and tyrosine when metformin was combined with pioglitazone [12]. Isoleucine and leucine concentrations were found to increase significantly during a two-day treatment with metformin in insulin resistant but not in insulin sensitive type 2 diabetes patients [13]. In a study on the Diabetes Prevention Program [14], relative rise of BCAAs isoleucine, leucine and valine, and AAAs phenylalanine and tyrosine at 2 years follow-up did not differ significantly between placebo, lifestyle intervention and metformin treated groups. Yet the increase in the concentration of these metabolites tended to be marginally higher in metformin treated patients [14].

4.2. *Metabolites and clinical outcomes*

Baseline high glucose was related to most of the adverse outcomes, shorter duration of gestation, increased caesarean section rate, higher birth weight and increased NICU admission rate. Measured at 36 gw, glucose level associated only with birth weight. This suggests that at this stage of pregnancy in insulin or metformin treated patients, glucose does not predict other adverse outcomes. However, the lack of other associations may also be explained by the fact that all women were well controlled and thus the variation in glucose levels was very small. In any case, our findings suggest that intervention to prevent and treat hyperglycemia should be initiated early in pregnancy to achieve even better results in the treatment of GDM. The concentration of serum lactate measured at baseline or 36 gw did not associate with any adverse outcomes.

Previous data on amino acid metabolism during pregnancy in relation to pregnancy outcomes are scarce. In a study by Scholtens et al. on the HAPO study population fasting lysine associated inversely with birth weight [34]. In a type 1 diabetic population, serine, threonine, lysine, proline, ornithine and arginine correlated with birth weight [35]. In the present study, isoleucine at baseline and alanine at 36 gw related to higher birth weight. However, the association between birth weight and alanine at 36 gw was no longer significant after applying FDR correction. Even though metformin treatment increased serum alanine, the birth weights between the medical treatment groups were similar, thus leaving an open question whether elevated isoleucine and especially alanine per se promote fetal growth, or rather reflect increased insulin resistance. In placenta mammalian target of rapamycin (mTOR) pathway has been proposed to be an important regulator of nutrient transport, including amino acids [36]. Besides regulation by maternal hormones such as insulin, metformin suppresses mTOR activity via AMP-activated protein kinase (AMPK) dependent [37] and independent [38] manner.

High concentrations of most amino acids at baseline predicted earlier delivery. To our knowledge there are no previous data reporting associations between maternal amino acid profile and gestation length. In agreement with previous data [39], the higher maternal glucose was at baseline, the earlier children were delivered.

At baseline higher glutamine associated with lesser gestational weight gain. On the other hand, higher glutamine at 36 gw predicted increased risk for gestational hypertension or preeclampsia. A previous study has shown that preeclampsia is associated with a lower, rather than a higher glutamine level leaving open the question on the relation between glutamine and hypertensive disorders [40].

There are some limitations in our study. This was a secondary analysis of GDM patients needing medication, utilizing previously collected samples at baseline and at 36 gw. Unfortunately, the study lacks serum samples from the diet group at 36 gw and therefore a comparison of the changes in metabolites during the last months of pregnancy between diet and medication treated mothers was not possible. Also, vast majority of the study population was Caucasian and therefore the results do not necessarily apply to other ethnic groups. Moreover, our patients were in excellent glycemic control and the role of amino acids could be different in less well controlled mothers.

In summary, compared to insulin, metformin treatment of GDM caused a greater increase in serum alanine, isoleucine and lactate concentrations. Independent of treatment group serum alanine measured at 36 gw was associated with higher birth weight, and glutamine with higher incidence of pregnancy induced hypertensive disorders. Although the observed differences were relatively small and not outright concerning, additional studies and follow-up data are required to ensure the safety of metformin use in GDM pregnancy.

Author Contributions

M.H. analyzed the data and wrote the first draft of the manuscript. K.T. provided clinical data of the metformin and insulin treated patients and serum samples of all patients from a previous study, designed the present study and edited and reviewed the manuscript. O.P. provided clinical data of the diet treated patients and reviewed the manuscript. T.R. designed the study and reviewed and edited the manuscript. M.H., K.T. and T.R are the guarantors of this work and, as

such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflicts of Interest

The authors do not have any conflicts of interest.

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