

Postprandial Levels of Branched-chain and Aromatic Amino Acids Associate With Gestational Diabetes Mellitus

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Abstract

Aims

Serum branched-chain amino acids (BCAAs) and aromatic amino acids (AAAs) are associated with obesity, insulin resistance and type 2 diabetes mellitus (T2DM). We want to investigate the levels of these amino acids in women with GDM and subsequently examine their changes in response to an oral glucose tolerance test (OGTT).

Methods

43 GDMs and 67 non-GDMs during their second trimester were recruited in this study. A 75-g OGTT was administered, and fasting, 1-h, and 2-h blood samples were obtained. Serum BCAA and AAA levels were measured by liquid chromatography-tandem mass spectrometry.

Results

The differences of BCAAs and AAAs between women with GDM and controls during their second trimester were not evident during fasting, while became significant after a 75-g glucose load. Glucose ingestion decreased the levels of BCAAs and AAAs in both groups. Notably, GDMs showed a delayed and blunted decrease of these amino acids compared to non-GDMs. The risks of 2-h change of BCAAs and AAAs for GDM were significant.

Conclusions

We identified that the differences of BCAAs and AAAs between women with GDM and controls during their second trimester, which were not evident during fasting, could be provoked by performing OGTT.

Introduction

Pregnancy is associated with remarkable changes in metabolism to support fetal demands and prepare for energy requirements in the postpartum period. In a healthy pregnancy, a decline in insulin sensitivity enhances fetal availability of metabolic substrates[1,2]. However, women in whom insulin secretion does not increase appropriately to compensate for the increased insulin resistance are at high risk for gestational diabetes mellitus (GDM)[3]. GDM has increased dramatically in recent years, which is defined as glucose intolerance observed during pregnancy[4,5]. GDM is associated with an increased risk of various maternal and perinatal complications, such as cesarean delivery, macrosomia, shoulder dystocia, neonatal hypoglycemia, hypocalcemia and subsequent type 2 diabetes mellitus (T2DM)[5,6].

Metabolomics has provided novel approaches to understand diabetes, thus attracting worldwide attention. Recently, a number of studies[7-9] have shown that the fasting levels of branched-chain BCAAs and aromatic amino acids (AAAs), including valine (Val), leucine (Leu), isoleucine (Ile), tyrosine (Tyr), and phenylalanine (Phe) were associated with obesity, insulin resistance, and T2DM in non pregnant populations. In prospective studies, increased concentrations of fasting Val, Leu, Ile and Tyr, Phe were associated with future diabetes[10]. Considering that T2DM and GDM share similar characteristics[11], we hypothesize that these amino acids may be beneficial in studies regarding GDM. Some prior studies investigated the association between these amino acids and GDM, however, the results were relatively contradictory[12-14].

Denise M. Scholtens and coworkers found that fasting Leu/Ile, Phe were higher and Val had a trend to be higher in mothers with high fasting blood glucose (FBG) than in mothers with low fasting glucose at ~28 weeks' gestation[12]. Women with GDM had higher fasting Val ,Leu ,Ile and Tyr, Phe than women in the control group among obese pregnant women in their second trimester of pregnancy[13]. However, Danuta Dudzik et al. reported that there were no changes in fasting BCAAs or AAAs in women with GDM compared to women in the control group in the second trimester of pregnancy[14].

Previous studies analyzing the differences in metabolite profiles between women with GDM and healthy controls mostly focused on fasting state, which failed to discover the metabolic changes and flexibility of the subjects. The oral glucose tolerance test (OGTT) is widely used as a standard method to establish the diagnosis of T2DM and provides a chance to observe the physiological changes during glucose ingestion[15,16]. An OGTT induces a transition from catabolism to anabolism which is accompanied by many changes in metabolite concentrations including BCAAs and AAAs to achieve glucose homeostasis[17]. Studying the challenged metabolic state is important, as people in the modern society live most of the days in a postprandial state and metabolic abnormalities may be masked in the fasting state.

To comprehensively understand the association between amino acids and GDM, we investigated the BCAAs and AAAs during an OGTT across 3 time points in the GDM and healthy controls during the second trimester of pregnancy. This study may shed light on the metabolic dysregulation of BCAAs and AAAs underlying GDM which is one of the most serious health problems today.

Methods

Study population

The study enrolled pregnant women aged 20–41 years at 20–29 weeks' gestation age attending antenatal care at the Maternity and Child Health Center of Suzhou Municipal Hospital between August 1, 2017, and February 28, 2018. All subjects were Han Chinese descent. Women with the following characteristics were excluded in the study: women with pre-existing diabetes mellitus, women with chronic diseases requiring medication during pregnancy except for levothyroxine, women with a history of smoking and women with missing data. Finally, we investigated 43 women with GDM as the case group and 67 women without GDM (non-GDM) as the control group. GDM was diagnosed by performing a 75-g OGTT according to the International Association of Diabetes and Pregnancy Study Groups criteria[15]. All subjects provided written informed consent for inclusion in the study. Ethics approval was obtained from the hospital. This study was approved by the Research Ethics Committees of the Affiliated Suzhou Hospital of Nanjing Medical University and was carried out in according with the principles of the Declaration of Helsinki as revised in 2008.

Characteristics' collection

The anthropometric data (pre-pregnancy weight (pre-weight)), height, gestational age (week) at sample collections, lifestyle factors and history of medication were obtained from clinical medical records through a standard questionnaire. Systolic and diastolic blood pressure (expressed in mmHg) was measured twice (Omron Model HBP-1100, Omron Company, Dalian city, Liaoning Province, China) with the participant in a sitting position, and the mean value was used for further analysis. Pre-BMI was calculated as the subject's pre-weight (kg) divided by their height squared (m^2).

Samples and laboratory measurements

After an overnight fasting of at least 8h, subjects at 20–29 weeks' gestation age underwent a 75-g OGTT where fasting, 1-hour (1-h), and 2-hour (2-h) blood samples were collected. Routine blood biochemical parameters were measured using a fully automatic biochemical analyzer (Hitachi 7000, Tokyo, Japan). Subsequently, the serum samples were stored at -80°C prior to the subsequent detection of BCAAs and AAAs. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to quantify serum BCAAs and AAAs as we previously reported[18]. Chromatographic separation was carried out on a Synchronis HILIC column (150 mm \times 2.1 mm, 5 μm , column temperature 35°C ; Shimadzu, Kyoto, Japan) with a mobile phase (acetonitrile: ammonium acetate = 89:11, v/v, 0.6 mL/min). The retention times for L-Val, L-Leu, L-Ileu, L-Phe and L-Tyr were 8.45 min, 5.33 min, 5.96 min, 4.56 min and 8.87 min, respectively. The MS analysis was performed using a QTrap 5500 mass spectrometer (AB Sciex, Concord, Ontario, Canada). Amino acids concentrations were determined using multiple reaction monitoring (MRM) with different transitions, matrix effect of 98.7%-107.3%, and recovery of 92.7%-102.3% [18].

Statistical analyses

Continuous variables with a normal distribution or approximate normal distribution were reported as mean \pm standard deviation; Comparisons between the non-GDM and the GDM group were performed using student's t test. Within group, we used paired t test to compare amino acids values after OGTT with fasting values. Percent changes of the amino acids at 1-h/2-h were calculated as follows: percent changes=(Concentration at 1-h/2-h – Concentration at fasting)/Concentration at fasting \times 100%. In order to analyse the change patterns between the two groups, $P < 0.05/3 = 0.017$ was considered statistically significant after Bonferroni correction (across 3 time points). logistic regression models were used to quantify the association of BCAAs and AAAs with GDM using group category as the response variable and fasting and 2-h change of amino acid concentrations as the independent variable. Three models were used: (1) unadjusted, (2) adjusted for age, gestational age, (3) adjusted for age, gestational age and pre-BMI. Statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 software, with $P < 0.05$ (except for specially stated) considered as significant. GraphPad Prism 5 was used to create the figures.

Results

Characteristics of the study population

The clinical characteristics of the 110 pregnant women at 20–29 weeks' gestation were presented in Table 1. A total of 43 women with GDM (age, 30.98 ± 4.63 years) and 67 women without GDM (age, 29.10 ± 3.63 years) were included. Women with GDM were older and had higher pre-BMI than the non-GDMs. Subjects in the GDM group had higher glucose levels in all three time points during OGTT. There were also significant differences in SBP and DBP between the two groups.

Table 1
Characteristics of the study population

	GDM (n=43)	Non-GDM (n=67)	P
Age (years)	30.98±4.63	29.10±3.63	0.02
Pre-weight (kg)	70.93±11.70	55.25±8.04	<0.001
Pre-BMI (kg/m ²)	27.69±4.76	21.33±3.01	<0.001
Gestational age (days)	180.86±13.67	171.78±6.69	<0.001
SBP (mmHg)	120.40±14.90	106±11.95	<0.001
DBP (mmHg)	75.4±9.89	67.75±8.48	<0.001
HbA1c (%)	5.37±0.55	4.77±0.36	<0.001
Fasting glucose (mmol/L)	5.53±1.00	4.07±0.40	<0.001
1-h glucose (mmol/L)	11.64±1.91	7.37±1.31	<0.001
2-h glucose (mmol/L)	9.43±1.78	6.06±1.08	<0.001

Continuous variables with a normal distribution or approximate normal distribution were represented as mean±standard deviation; Comparisons between the non-gestational diabetes mellitus (non-GDM) and the GDM groups were performed using student's t-test. The statistically significant difference was defined as P < 0.05.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; OGTT, oral glucose tolerance test; 1-h glucose, one-hour glucose at OGTT; 2-h glucose, two-hour glucose at OGTT.

Comparison of serum amino acid concentrations during an oral glucose tolerance test between women with gestational diabetes mellitus (GDM) and women without GDM (non-GDM)

We examined the amino acid concentrations in each group (Table 2). There was no significant difference between the GDM group and non-GDM group in fasting status. After a 75-g OGTT, amino acids levels differed between the GDM group and non-GDM group. All of the 1-h amino acids in the GDM group, including Val, Leu, Ile and Tyr, Phe, were higher than those in the non-GDM group. Moreover, except for Phe, amino acids at 2-h showed similar results as those at 1-h.

Table 2
Comparisons of serum amino acid concentrations in the non-gestational diabetes mellitus (non-GDM) and GDM during an oral glucose tolerance test (OGTT)

Amino acids ($\mu\text{mol/L}$)	GDM (n=43)	Non-GDM (n=67)	P
0-Val	199.62 \pm 33.15	201.8 \pm 24.69	0.694
1-h Val	200.32 \pm 32.33	170.14 \pm 22.74	<0.001
2-h Val	166.65 \pm 27.86	151.31 \pm 20.92	0.001
0-Leu	119.91 \pm 20.64	120.70 \pm 14.02	0.810
1-h Leu	118.30 \pm 22.33	92.52 \pm 12.12	<0.001
2-h Leu	89.56 \pm 16.21	77.91 \pm 11.76	<0.001
0-Ile	60.93 \pm 9.91	59.04 \pm 7.81	0.266
1-h Ile	62.04 \pm 14.53	42.37 \pm 6.85	<0.001
2-h Ile	45.04 \pm 10.64	33.10 \pm 6.25	<0.001
0-Tyr	42.16 \pm 7.40	44.22 \pm 6.42	0.125
1-h Tyr	46.73 \pm 8.16	35.85 \pm 5.89	<0.001
2-h Tyr	38.52 \pm 7.60	29.42 \pm 5.34	<0.001
0-Phe	91.39 \pm 19.29	91.17 \pm 12.96	0.947
1-h Phe	87.72 \pm 14.20	80.30 \pm 12.20	0.004
2-h Phe	78.53 \pm 14.16	74.53 \pm 11.37	0.105

Values were expressed as mean \pm standard deviation. Comparisons between the non-gestational diabetes mellitus (non-GDM) and GDM groups were performed using student's t-test. The statistically significant difference was defined as $P < 0.05$.

Val, valine; Leu, leucine; Ile, isoleucine; Tyr, tyrosine; Phe, phenylalanine; 0, fasting; 1-h, one- hour at OGTT; 2-h, two-hour at OGTT

The Amino Acid Changes during the oral glucose tolerance test (OGTT)

The amino acid changes during the OGTT in the GDM group and non-GDM group were illustrated in Figures 1–3. Compared with the baseline levels, all the amino acids at 2-h significantly reduced after the OGTT in both groups(Fig. 1A,B). We also described the percent changes and significance of changes in both groups(Fig. 2A,B) and specific results were available in Table S1. In the GDMs, compared to baseline, Val, Leu, Ile and Phe at 1-h didn't show any difference and reduced significantly at 2-h ranging from 12.75%-25.30%. Tyr primarily increased by 12.56% at 1-h and lowed by 7.59% at 2-h. In the non-GDMs, compared to baseline, all the amino acids initially decreased small during 1-h and became more pronounced during 2-h. On the other hand, our results revealed that the percent change in Ile was 43.90% in the non-GDM group which was largest among the amino acids. Similarly, the largest percent change was also Ile in the GDM group. Furthermore, we compared the percent changes in the two groups (Fig. 3) and specific results were seen in Table S2. Pronounced differences were observed after

glucose ingestion between the two groups. Val, Leu, Ile and Phe showed a delayed decrease in the GDMs compared to the non-GDMs; within the first hour there was no significant decrease in Val, Leu, Ile and Phe in the GDMs. At 2-h, the GDMs displayed smaller decrease in all five amino acids compared with the non-GDMs.

The odds ratio (OR) for the association of amino acids with gestational diabetes mellitus (GDM)

Logistic regression model was used to analyze the risks of amino acids levels (fasting and 2-h change of amino acids) for GDM (Table 3). In the crude model, the risks of fasting BCAAs (Val, Leu, and Ile) and fasting AAAs (Tyr and Phe) for GDM were insignificant. We further discovered that 2-h change of BCAAs and AAAs for GDM were evidently significant. These significant associations remained the same or became slightly attenuated after adjustment for age and gestational age. By contrast, the associations were moderately attenuated after further adjusting for pre-BMI. The OR of 2-h change of Val, Ile, Tyr for GDM still existed while 2-h response of Leu, Phe disappeared.

Table 3
The crude and adjusted logistic regression model of amino acids with gestational diabetes mellitus

	Fasting			2h-change		
	Beta(95% C.I.)	Beta(95% C.I.)	Beta(95% C.I.)	Beta(95% C.I.)	Beta(95% C.I.)	Beta(95% C.I.)
	Model 1	Model 2	Model 3	Model 1	Model 2	Model.3
Val	0.997 (0.984,1.011)	1.001 (0.985,1.017)	0.991 (0.971,1.010)	1.061 (1.031,1.092) ^{***}	1.060 (1.027,1.093) ^{***}	1.035 (1.001,1.070) [*]
Leu	0.997 (0.975,1.020)	1.002 (0.974,1.030)	0.985 (0.952,1.018)	1.070 (1.034,1.106) ^{***}	1.071 (1.029,1.115) ^{**}	1.040 (0.996,1.085)
Ile	1.026 (0.981,1.073)	1.019 (0.966,1.075)	0.970 (0.906,1.038)	1.149 (1.084,1.218) ^{***}	1.157 (1.082,1.238) ^{***}	1.108 (1.028,1.194) ^{**}
Tyr	0.955 (0.901,1.013)	0.952 (0.891,1.018)	0.942 (0.867,1.024)	1.445 (1.255,1.665) ^{***}	1.423 (1.227,1.650) ^{***}	1.302 (1.118,1.516) ^{**}
Phe	1.001 (0.977,1.026)	1.000 (0.972,1.028)	0.990 (0.957,1.024)	1.044 (1.001,1.089) ^{**}	1.044 (0.996,1.095)	1.017 (0.964,1.073)

Results are based on analyses of the study population (43 women with gestational diabetes mellitus (GDM) and 67 women without GDM(non-GDM)).

Values represent regression coefficients from the logistic regression analyses and their 95% confidence intervals (C.I.) for the risk of GDM

The statistically significant difference was defined as $P < 0.05$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

2-h changes which were calculated as Concentration at 2-h - Concentration at fasting.

Model 1: unadjusted. Model 2: adjusted for age and gestational age. Model 3: adjusted for age, gestational age and pre-BMI.

Discussion

In this study, we profiled three time points of BCAAs and AAAs during a 75-g OGTT in 43 women with GDM and 67 healthy controls during their second trimester using a targeted metabolomics approach. Overall, the levels of the BCAAs and AAAs did not differ significantly between GDM and non-GDM groups at the fasting status. After glucose load, our data on the 2-h levels of BCAAs and AAAs decreased apparently compared to the fasting status in both groups supporting the known action of insulin in suppression of proteolysis. Further, we found different patterns of change in BCAAs and AAAs after glucose load between the two groups with a delayed and blunted decrease in GDMs. We also observed the negative relationships between 2-h change of BCAAs and AAAs with GDM.

According to the previous studies, the increasing levels of fasting Val, Leu, Ile, Tyr, and Phe are the amino acids that are most consistently associated with obesity and T2DM outside pregnancy[7–9]. However, the associations between these amino acids and GDM are inconsistent. Fasting Leu/Ile, Phe were higher and Val had a trend to be higher in Northern European mothers with high fasting blood glucose (FBG) (>90th percentile) compared to mothers with low FBG (<10th percentile) between 24 and 32 weeks' gestation[12]. Similarly, Sara L. White performed a metabolic study among obese pregnant women in their second trimester of pregnancy found that women with GDM had higher fasting Val, Leu, Ile and Tyr, Phe than women in the control group[13]. In contrast to the former two researches, our study didn't find any differences in fasting BCAAs and AAAs between GDMs and non-GDMs. This finding was in agreement with Danuta Dudzik et al. who compared the metabolic profiles between women with GDM and women in the control group during their second trimester using targeted metabolomics[14]. The discrepancy could be explained by the differences in races, ages, maternal BMI, methods to assess amino acids levels and small sample sizes. One of recent studies including 83 pregnant women with gestational age ≥ 25 weeks proved that significantly elevated levels of Val, Ile, Tyr and Phe in T2DM pregnant women, but not among GDM mothers[19]. Another study from America investigating the effects of GDM on intermediary metabolism in late pregnancy observed that women with GDM whose fasting glucose of 5.83mmol/l or greater had elevated levels of fasting Val, Leu, Ile compared with controls[20]. Meanwhile, they did not observe any differences between subjects with GDM whose fasting glucose less than 5.83mmol/l and controls. Combining the latter two studies, we speculated that different glucose metabolism disorders meant varying degrees of islet dysfunction might be other reason for the discrepancy.

After glucose ingestion, an earlier report showed a gradual decrease of Val, Leu, Ile and Tyr, Phe in nonpregnant populations with normal glucose tolerance, which was in agreement with healthy pregnant women in this study[21]. Notably, we found marked differences between healthy controls and GDMs in glucose-provoked alterations in these amino acids. Val, Leu, Ile and Phe unchanged and Tyr increased during the first one hour of the OGTT in the GDM group, after which they blunted decreased. A previous study observed Val, Leu, Ile and Tyr increased during the first 30 minute of the OGTT and then blunted decreased in obese individuals[22]. The patterns of decrease (delayed and blunted) in these amino acids in GDMs similar to obese individuals reflected a dysregulation of proteolysis which might partly be associated with impaired insulin sensitivity. Furthermore, we noticed that percent changes of the amino acids at 2-h in healthy pregnant women were smaller in comparison to nonpregnant healthy populations found by Qin Wang[21]. But percent changes were similar in extent to insulin resistant individuals and prediabetes. Also, we observed percent changes at 2-h in GDMs generally decreased less than healthy controls even less than T2DM from Qin Wang's study[21]. We speculated that insulin resistance in GDM during the second trimester of pregnancy seemed to be more severe than T2DM.

In our study, the risks of 2-h change of BCAAs and AAAs for GDM were significant, while fasting BCAAs and AAAs weren't significant. According to a previous study, linear regression models revealed 2-h change of leucine/isoleucine, valine were associated with fasting insulin in individuals with impaired glucose tolerance[23]. Furthermore, another study from Sweden including 21 healthy individuals discovered that the postprandial responses of the three diabetes associated amino acids (DMAAs, Ile, Tyr, and Phe) were more strongly associated with fasting glucose than the fasting concentrations of the DMAAs[24]. D.O. Mook-Kanamori et al. also found more associations amongst the postprandial amino acid concentrations than in the fasting state with T2DM[25]. We showed that the physiological challenges increased interindividual variation, revealing metabolotypes that were not evident at baseline. The link between 2-h change of BCAAs and AAAs and GDM remained the same or became slightly attenuated after adjustment for age and gestational age, while moderately attenuated after further adjustment for pre-BMI. This decrease suggested pre-BMI accounted in part for the risks of BCAAs and AAAs for GDM.

Investigation of BCAAs and AAAs in response to an oral glucose challenge gave us a unique opportunity to study the changes from catabolism to anabolism. After the OGTT, the differences remained coherent whether the metabolic changes were assessed via relative or absolute concentration changes. Our study revealed that postprandial dysfunction of BCAAs and AAAs might be a novel component of GDMs that is hitherto poorly recognized as a potential interventional target. However, our study still has limitations. Due to its small sample size, confirmation studies should include larger groups. We only investigated the concentrations of BCAAs and AAAs at one timepoint during second trimester. Since metabolism changes substantially in the maternal body in different trimesters of pregnancy, it is important to explore longitudinal metabolomic profiles across gestation in the future.

In conclusion, our results suggested that the differences of BCAAs and AAAs between women with GDM and controls during their second trimester, which were not evident during fasting, could be determined by performing OGTT. The different patterns of decrease in BCAAs and AAAs in GDMs and controls provided deeper understanding of the GDM phenotype. It might therefore be beneficial if we would better understand postprandial dysfunction in GDM beyond fasting. We maintained that this was particularly important because of people mostly living in a non-fasting state and might provide new therapeutic opportunities.

Declarations

Data availability

The datasets generated during and analysed in the current study are available from the corresponding author on reasonable request.

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Author Contributions

BG, QS, and LC participated in the design of the study. QZ, BG, YW, and MC collected the samples. YW and BG performed the statistical analysis. LC helped in interpreting the results. All authors drafted, read, and approved the final manuscript.

Competing Interests

The authors declare no competing interests

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Figures

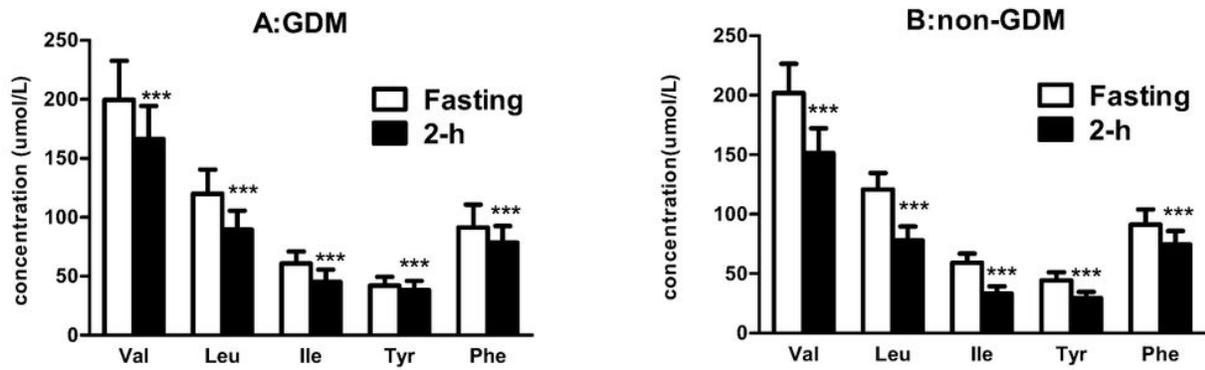


Figure 1

Concentrations of BCAAs and AAAs (Mean \pm SD) between fasting and 2-h after glucose ingestion. A women with gestational diabetes mellitus(GDMs). B non-GDMs. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared amino acids values at 2-h after glucose ingestion with fasting using the paired t test.

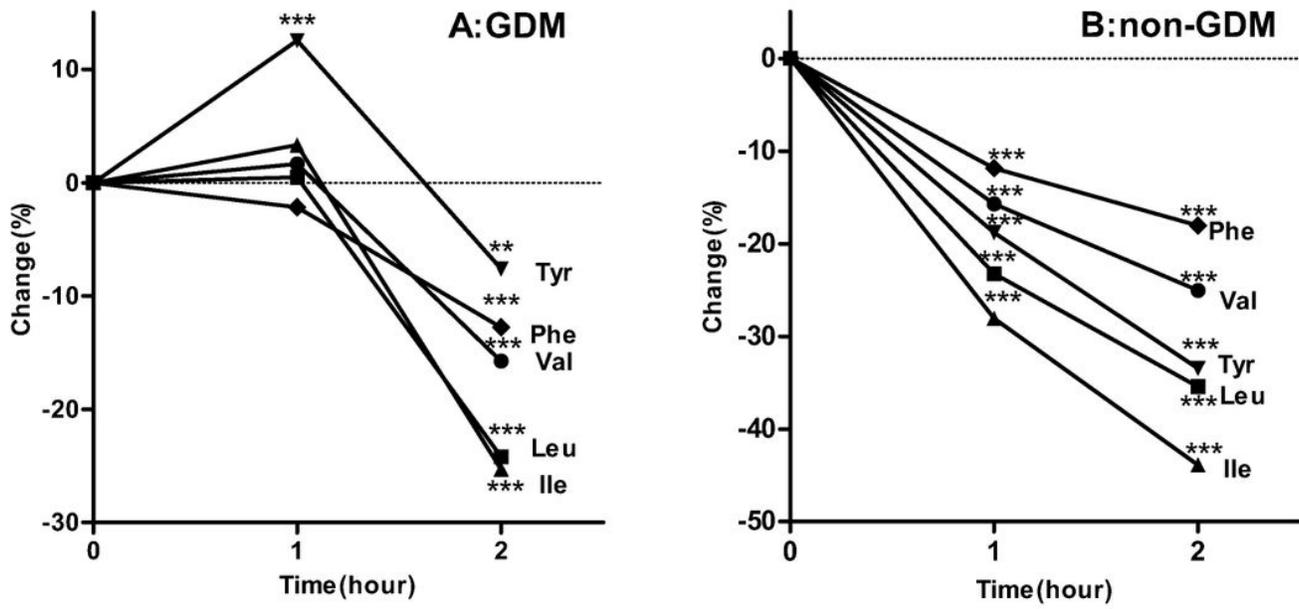


Figure 2

Amino acid changes in response to an oral glucose tolerance test. The dots denoted mean percent change. Percent change was defined as the absolute change in relative to baseline. A women with gestational diabetes mellitus(GDMs). B non-GDMs. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared amino acids values at corresponding time point after glucose ingestion with fasting using the paired t test.

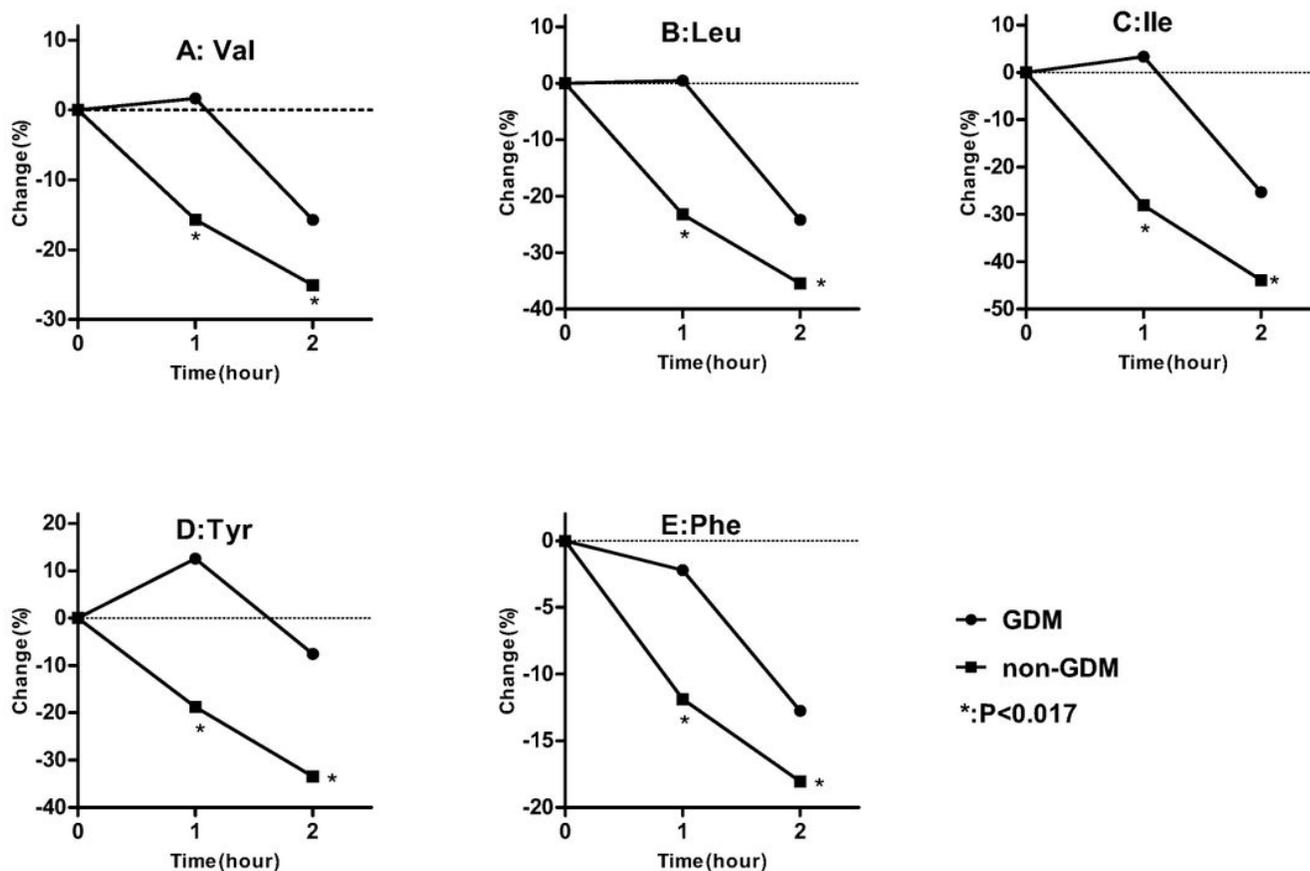


Figure 3

Percent changes of amino acid between the gestational diabetes mellitus (GDM) and non-GDM groups. The dots denoted mean percent change. Percent change was defined as the absolute change in relative to baseline. A Val. B Leu. C Ile. D Tyr. E Phe. * $p < 0.017$, compared percent changes of amino acid at corresponding time point between GDM and non-GDM using student's t-test.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTables.xlsx](#)