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Branched chain amino acids, history of gestational diabetes, and breastfeeding: The Bogalusa Heart Study

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Abstract

Background/Aims: To examine the associations between history of gestational diabetes mellitus (GDM) and breastfeeding with branched-chain amino acids (BCAA) and their metabolites in later life.

Methods and Results: 638 women (mean age 48.0 y) who had participated in the Bogalusa Heart Study and substudies of pregnancy history had untargeted, ultrahigh performance liquid chromatography-tandem mass spectroscopy conducted by Metabolon© on serum samples. Metabolites were identified that were BCAA or associated with BCAA metabolic pathways. History of GDM at any pregnancy (self-reported, confirmed with medical records when possible) as well as breastfeeding were examined as predictors of BCAA using linear models, controlling for age, race, BMI, waist circumference, and menopausal status. None of the BCAA differed statistically by history of either GDM or breastfeeding, although absolute levels of each of the BCAA were higher with GDM and lower with breastfeeding. Of the 27 metabolites on the leucine, isoleucine and valine metabolism subpathway, 1-carboxyethylleucine, 1-carboxyethyvaline, and 3-hydroxy-2-ethylpropionate were higher in women with a history of GDM, but lower in women in women with a history of breastfeeding. Similar results were found for alpha-hydroxyisocaproate, 1-carboxyethylisoleucine, and N-acetylleucine.

Conclusions: GDM and breastfeeding are associated in opposite directions with several metabolites on the BCAA metabolic pathway.

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Author Contributions

All authors: writing – reviewing and editing. Emily Harville: conceptualization, methodology, formal analysis, writing – original draft and reviewing and editing. Lydia Bazzano: investigation, supervision, project administration. Jiang He: conceptualization, funding acquisition. Tanika Kelly: methodology, supervision, resources. Lu Qi: conceptualization. Wei Perng: conceptualization, methodology.

Data Availability

Data are available from the BHS executive committee upon request and signing of data use agreements.

Competing Interests: The authors have no competing interests to declare.

Keywords

Metabolomics; diabetes; gestational; lactation; breast feeding; amino acids; branched-chain

Introduction

At least one in four, and likely more, women who develop diabetes during their pregnancies (gestational diabetes mellitus, GDM) will progress to type 2 diabetes (T2DM) in later life [1]. GDM is associated with a 40–60% increased risk of later cardiovascular disease (CVD) [2], and this increased risk appears to be present even among those who do not develop T2DM [3, 4]. Although GDM to T2DM progression shares many risk factors with overall T2DM epidemiology, some factors may differ. For instance, the Diabetes Prevention Program found the effect of metformin to be reduce the progression to diabetes by 40% in women with a history of GDM, while in similar women who did not have a history of GDM metformin did not reduce the progression to diabetes.[5]: Breastfeeding is protective against this progression: a systematic review of 13 cohort studies found that, compared to no lactation, history of lactation was associated with a pooled relative risk of 0.66, 0.58–0.90 for progression to diabetes [6].

Branched chain amino acids (BCAA) are the most abundant of essential amino acids [7]. They have been implicated in the development of insulin resistance, type 2 diabetes, and cardiovascular disease, and have physiologic function in lipid and glucose metabolism and protein synthesis [7]. BCAA and other metabolites, including aromatic amino acids and C3 and C5 acylcarnitines, have been associated with diabetes, insulin resistance, metabolic syndrome, and related complications [8–10].

While the long-term effects of GDM on BCAA have not been studied extensively, a possible role for BCAA in the prognosis of GDM during pregnancy has been investigated in a few cases. A targeted NMR metabolome study indicated that women with GDM had a metabolic profile during pregnancy associated with raised lipids and lipoprotein constituents in VLDL subclasses, greater triacylglycerol enrichment across lipoprotein articles, higher BCAA and aromatic amino acids and different fatty acid, ketone body, adipokine, liver and inflammatory marker profiles [11]. In another study, valine, leucine, and isoleucine were higher in women with GDM than in controls at both early 2nd and early 3rd trimester [11]. These higher BCAA levels in GDM women were also found 11 months postpartum in another study, although BCAA levels did not differ during pregnancy in this sample [12]. In women with a history of GDM, BCAA (isoleucine, leucine, and valine) were associated with an increased chance of progressing to T2DM[13].

In this analysis, we explored how BCAA and BCAA-related metabolites differed in midlife women in the Bogalusa Heart Study based on history of GDM and breastfeeding. We hypothesized that BCAA would be higher in women with a history of GDM and lower in those with a history of breastfeeding. We also hypothesized that breastfeeding would serve as an effect modifier of the association between history of GDM and BCAA.

Methods

Study population

The Bogalusa Heart Study is a series of studies of cardiovascular risk, in a semirural, biracial population (65% white and 35% black), founded by Dr. Gerald Berenson in 1973. This analysis combines results from two follow-up studies conducted in 2011–2016: Bogalusa Babies, which examined reproductive outcomes within the BHS, and BiCEPS (Brain, CognitivE and Physical performance Study), which links vascular risk factors across the lifespan with cognitive and physical performance. 1804 women participated in Bogalusa Babies. The most common reason for not participating in both studies was not being available to visit the clinic. In most cases, women completed both studies on the same day, although this was not a requirement. 741 of these women were included in BiCEPS and the metabolomic study (description of study population in Table 1). Compared to other Babies participants, women included in the analysis were more likely to be postmenopausal (58% vs. 44%), somewhat more likely to have smoked (23% vs. 20%, p=0.09), and less likely to have breastfed (35% vs. 42%) and to be black (37% vs. 44%); mean age at first pregnancy was slightly older (22.7 vs. 22.2, p=0.06) as was mean adult BMI (27.6 vs 26.7 kg/m^2, p=0.03). Educational distribution was also somewhat different, with more high school graduates and fewer college+ graduates (p=0.04). There were no differences in self-reported GDM prevalence, parity, or age at interview. Eleven women had diabetes diagnosed before their last pregnancy and therefore were not at risk for GDM for at least one pregnancy; they were excluded from analyses of GDM. This analysis was limited to women with at least one previous pregnancy: n=631 with information on GDM and n=638 with information on breastfeeding.

Reproductive history

All reproductive history variables in this analysis were self-reported, although women were encouraged to consult a baby book, if they had one. During the interview, women were asked whether they had ever been pregnant, the outcome of each pregnancy, and pregnancy complications, including GDM. Reproductive history assessed included number of pregnancies, number of births, and adolescent pregnancy (<16 or <18 years at first pregnancy). Recall has generally been shown to be accurate for reports of GDM (specificity=98%, sensitivity=92%) [14]. In this study, for the subgroup of women for whom we were able to consult medical records (n=381), kappa for agreement between self-report and medical records was between 70 and 85 (depending on how incomplete medical records were treated) when clustering was not accounted for, and 80 to 91 when clustered kappas [15] were calculated. History of GDM was defined as the occurrence at any pregnancy, so if a woman had multiple pregnancies but reported GDM in only one, she was defined as having had a history of the complication. We also examined any history of diabetes during pregnancy, gestational or chronic, as a predictor.

For each pregnancy, the participant was asked if she breastfed and for how long; these were summarized as ever breastfeeding and >6 months total lifetime breastfeeding. Maternal self-report of breastfeeding is generally reliable in the first few years [16, 17], although we are not aware of long-term studies of this.

Metabolite Profiling

Untargeted, ultrahigh performance liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS) was conducted by Metabolon© using BHS fasting serum samples that had been stored at −80°C since the 2013 to 2016 visit [18]. Rigorous quality assurance was conducted during metabolomics profiling which included the use of blanks, blind duplicates (5% of the BHS samples), and standard biochemical compounds which were integrated into every analyzed sample. Untargeted metabolomics profiling resulted in the detection and quantification of 1,466 metabolites. Prior to the statistical analysis, additional quality control and manipulation of the metabolite data was undertaken. Batch effects were assessed using principal components analysis, which revealed no evidence of clustering of metabolite data by run-days. Data filtering included the exclusion of 213 metabolites that were missing or below the detection threshold in more than 80% of samples and 51 metabolites with a reliability coefficient <0.3 based on blind duplicate analysis. Among the 1,202 metabolites passing quality control, 3 were BCAAs (valine, leucine, and isoleucine) and 27 were identified as on the leucine, isoleucine and valine metabolism subpathway according to Metabolon documentation. In addition, levels of isoleucine, valine, and leucine were summed to create a total BCAA index. We also examined the pattern identified by Perng et al. [19–21] as associated with childhood obesity and birthweight for gestational age which incorporates BCAA as well as related metabolites. The factors making up this BCAA pattern were summed, both weighted as listed in their appendix, and unweighted. Metabolites were standardized (mean 0, SD 1) for analysis. BCAA were normally distributed; other metabolites on the pathway with a substantial skew were log-transformed. Metabolites below the limit of detection were imputed as 1/2 the LOD; if more than 10% of the sample met this criterion (n=8), a sensitivity analysis was run deleting those observations (supplementary material).

Reproductive history was examined as a predictor with metabolites as outcomes; linear models were used. Initial analysis controlled only for age; subsequent analysis also controlled for race, BMI and waist circumference at time of metabolite measurement, mean BMI at prior adult visits, and menopausal status. Prior adult BMI was missing for 88 participants (11.8%), so multiple imputation was used to impute values for missing covariates [22]. These analyses were separated due to concerns for their possible role as intermediates, but as results were very similar regardless, only the fully adjusted models are presented. Due to the metabolomic differences that have been found around menopause [23, 24], interactions with menopausal status were examined; none were significant. Statistical analyses were performed in SAS (version 9.4; SAS Institute, Cary, NC). To assess the effect of multiple comparisons, results were examined for significance after correction for false discovery rate (q=0.05). In addition, metabolites were compared for their effect size (beta) and precision (width of confidence interval) as other indicators of strength of association.

The metabolomics analysis project was approved by the Tulane University IRB.

Results

The study population was two-thirds white and one-third black, with the majority post-menopausal (Table 1). Diabetes diagnosis was associated with higher levels of BCAA

(standardized beta for leucine, 0.7160 (SE 0.10), p<0.01; isoleucine, 0.7220 (SE 0.10), p<0.01; valine 0.5516 (SE 0.10), p<0.01), and history of GDM was associated with higher likelihood of diabetes (OR 5.88, 95% CI 3.38–10.24). None of the BCAA was statistically significantly different among women with a history of GDM (Table 2), or a history of breastfeeding. No interactions were found between GDM and breastfeeding (GDM and breastfeeding were not correlated, p for association=0.65). The BCAA pattern identified by Perng et al. [19, 20] was not associated with neither GDM nor breastfeeding, either as a weighted (beta for GDM 0.12, 95% CI −0.30, 0.53; ever breastfed beta −0.15, 95% CI −0.41, 0.11) or unweighted (beta for GDM 0.18, 95% CI −0.51, 0.87; ever breastfed beta −0.24, 95% CI −0.67, 0.18) score.

27 metabolites were quantified as part of the leucine, isoleucine and valine metabolism subpathway (Table 3). In general, metabolites that were higher after GDM or diabetes during pregnancy were lower with a history of breastfeeding. Results were consistent whether the associations were assessed by effect size, precision, or statistical strength. Most strongly associated with GDM were 1-carboxyethylleucine, 1-carboxyethyvaline, 3-hydroxy-2-ethylpropionate, and alpha-hydroxyisocaproate, all of which were higher in women with history of GDM. History of breastfeeding was inversely associated with levels of 1-carboxyethylisoleucine, 1-carboxyethylleucine, 2-hydroxy-3-methylvalerate, alpha-hydroxyisovalerate, N-acetylleucine, and 1-carboxyethylvaline. 3-hydroxy-2-ethylpropionate was positively associated with history of breastfeeding. These associations hel for overall diabetes, with additional positive associations with 1-carboxyethylisoleucine, 3-methyl-2-oxovalerate, 4-methyl-2-oxopentanoate, and with breastfeeding longer than 6 months, with an additional inverse association with alpha-hydroxyisocaproate. However, no associations were statistically strong enough to meet the FDR threshold.

Discussion

GDM is a strong predictor of later-life diabetes and has been rising in recent years [25]. For these reasons, protective factors (such as breastfeeding) and mechanistic indicators (such as metabolites) are of great interest. In this analysis, we explored how long-term metabolism might be altered in women with GDM, whether breastfeeding was associated with related changes, and whether the two interacted, by examining levels of BCAA and related metabolites in a cohort of women an average of 23 years after first pregnancy.

Principal findings

We did not find significantly higher levels of BCAA in women with a history of GDM, nor significantly lower of BCAA with history of breastfeeding, nor was there interaction between the two. It may be that the women in this study were still too young to be at high risk of diabetes. The number of cases of GDM was also relatively small, so power was limited. Several BCAA-related metabolites were examined. One noticeable fact was that metabolites that were significantly higher in those with a history of diabetes in pregnancy were usually lower in women with a history of breastfeeding. For instance, 1-carboxyethylleucine, 1-carboxyethylvaline, and 1-carboxyethylisoleucine were all lower with breastfeeding and higher with history of GDM or diabetes during pregnancy. The

carboxyethyl-modified amino acids are poorly characterized overall, though carboxyethylvaline peptides of β-hemoglobin have been suggested as markers for severity of diabetes [26]. While a lower risk of diabetes with history of breastfeeding has been found in women without a history of GDM [27–29]; studies differ on whether this effect is stronger in women with a history of GDM or not [30, 31]. Our results do not support a stronger effect among women with GDM, ss we found no interaction between the two. BCAA have been shown to be elevated prior to development of diabetes rather than as a consequence of it, so it may be that the lower levels of metabolites after breastfeeding reflect a long-term metabolic shift. Generally, relationships were stronger with those with any history of diabetes and a longer period of breastfeeding; as this includes the women who developed diabetes at the youngest ages and who breastfed the longest, this was expected.

Strengths and weaknesses

Limitations of the study include a relatively small number of women with a history of GDM and lack of clinical confirmation in many cases. We do not have a replication cohort, although this study serves as a replication for some other studies. A single measurement means that up or downregulation of pathways over time cannot be examined. The time frame of the study can be considered a strength or a limitation; while it is long enough to ensure that any metabolic alterations were maintained for an extended period after pregnancy, many women were not yet at the age of peak T2DM diagnosis, which occurs between 45 and 64. Samples had been stored from 1–4 years at −80° before analysis; any degradation due to storage would be non-differential as participants' samples were stored for similar lengths of time and there is no reason to think that variation in the length is correlated with participant characteristics.

Comparison to other studies

We are unaware of other studies that have tackled this precise question; most related studies have addressed metabolites during or shortly after pregnancy. A previous study found no association between GDM and BCAA in either maternal or cord blood during pregnancy [32]. Some other variables showed no association with either GDM or breastfeeding, even though one might be expected. We found no associations with isobutyrylcarnitine, which has been found to be higher in pregnant women with GDM [33], nor with tiglylcarnitine, which has been associated with metabolic syndrome [34]. Also, the BCAA-related profile found to be associated with GDM and childhood obesity was not associated with the outcomes studied here [20]. Other metabolites have been associated with diabetes and related phenotypes: alpha-hydroxyisocaproate was lower in those who had breastfed and higher in those with a history of diabetes in pregnancy; this metabolite can be detected in low quantities in the urine of diabetics [35]. Higher alpha-hydroxyisovalerate was lower with breastfeeding in this analysis and has been associated with visceral fat mass [36] and isolated post-challenge diabetes [37]. 4-methyl-2-oxopentanoate has been shown to affect insulin gene expression and is higher in those with a history of diabetes [38].

Meaning of the study

Generally, metabolites earlier in the metabolic pathways were more strongly associated with the outcomes than those further down the pathways (for instance, the first three metabolites

in the leucine precursor-product order are N-acetylleucine, 4-methyl-2-oxopentanoate, and alpha-hydroxyisocaproate; 3-methyl-2-oxovalerate and alpha-hydroxyisovalerate, steps 2 and 3 in the isoleucine pathway, were also significant; N-acetylisoleucine did not reach the p-value threshold but was similar in magnitude to the effect seen for N-acetylleucine). This is consistent with the women with a history of GDM but not active diabetes having milder disturbances, rather than the whole pathway being disturbed, as would be seen with T2DM. Other metabolites identified as associated with history of diabetes during pregnancy or breastfeeding include 3-methyl-2-oxovalerate, which was higher in those with a history of diabetes during pregnancy, consistent with previous research indicating that it is a predictive biomarker of impaired fasting glucose [39]. It is produced in the initial step in the oxidative process of isoleucine, and is often one of the more responsive members of the pathway. 3-hydroxy-2-ethylpropionate was also higher in those with a history of diabetes in pregnancy, but was unusual in also being higher in those who had breastfed. This metabolite is part of the R-pathway of isoleucine metabolism; levels reflects shunting of BCAAs away from mitochondrial metabolism and can be co-incident with diabetes.[40] Although it did not meet the FDR threshold, N-acetylleucine was also higher in those with a history of diabetes during pregnancy and lower in those with a history of breastfeeding. However, given the lack of a replication cohort and confirmation in an independent cohort, the results remain exploratory, and no clinical meaning can be assigned to them.

Unanswered questions and future research

In conclusion, we did not find that BCAA levels were higher in those with a history of GDM or lower in those with a history of breastfeeding, but did find this pattern with some metabolites, particularly the 1-carboxyethyl metabolites of the BCAAs. Such findings increase support for the idea that breastfeeding is beneficial for long-term metabolic health, and suggest one possible mechanism. Future studies should attempt to replicate these results in an independent sample, and, if replicated, explore whether BCAA or its metabolites can be used as clinically useful individual or combined predictors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BCAA	Branched chain amino acids
BHS	Bogalusa Heart Study
BiCEPS	Brain, CognitivE and Physical Performance Study

GDM	Gestational diabetes mellitus
UPLC-MS/MS	ultrahigh performance liquid chromatography-tandem mass spectroscopy

References

[1]. Kim C, Newton KM, Knopp RH (2002) Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes Care 25(10): 1862–1868 [PubMed: 12351492]

[2]. Shostrom DCV, Sun Y, Oleson JJ, Snetselaar LG, Bao W (2017) History of Gestational Diabetes Mellitus in Relation to Cardiovascular Disease and Cardiovascular Risk Factors in US Women. Front Endocrinol (Lausanne) 8: 144 10.3389/fendo.2017.00144 [PubMed: 28694789]

[3]. Retnakaran R, Shah BR (2017) Role of Type 2 Diabetes in Determining Retinal, Renal, and Cardiovascular Outcomes in Women With Previous Gestational Diabetes Mellitus. Diabetes Care 40(1): 101–108. 10.2337/dc16-1400 [PubMed: 27821407]

[4]. Fadl H, Magnuson A, Ostlund I, Montgomery S, Hanson U, Schwarcz E (2014) Gestational diabetes mellitus and later cardiovascular disease: a Swedish population based case-control study. BJOG 121(12): 1530–1536. 10.1111/1471-0528.12754 [PubMed: 24762194]

[5]. Aroda VR, Christophi CA, Edelstein SL, et al. (2015) The effect of lifestyle intervention and metformin on preventing or delaying diabetes among women with and without gestational diabetes: the Diabetes Prevention Program outcomes study 10-year follow-up. J Clin Endocrinol Metab 100(4): 1646–1653. 10.1210/jc.2014-3761 [PubMed: 25706240]

[6]. Feng L, Xu Q, Hu Z, Pan H (2018) Lactation and progression to type 2 diabetes in patients with gestational diabetes mellitus: A systematic review and meta-analysis of cohort studies. J Diabetes Investig 9(6): 1360–1369. 10.1111/jdi.12838

[7]. Nie C, He T, Zhang W, Zhang G, Ma X (2018) Branched Chain Amino Acids: Beyond Nutrition Metabolism. Int J Mol Sci 19(4). 10.3390/ijms19040954

[8]. Zhao X, Han Q, Liu Y, Sun C, Gang X, Wang G (2016) The Relationship between Branched-Chain Amino Acid Related Metabolomic Signature and Insulin Resistance: A Systematic Review. J Diabetes Res 2016: 2794591 10.1155/2016/2794591 [PubMed: 27642608]

[9]. Wang S, Yu X, Zhang W, et al. (2018) Association of serum metabolites with impaired fasting glucose/diabetes and traditional risk factors for metabolic disease in Chinese adults. Clin Chim Acta 487: 60–65. 10.1016/j.cca.2018.09.028 [PubMed: 30237081]

[10]. Tobias DK, Lawler PR, Harada PH, et al. (2018) Circulating Branched-Chain Amino Acids and Incident Cardiovascular Disease in a Prospective Cohort of US Women. Circ Genom Precis Med 11(4): e002157 10.1161/circgen.118.002157 [PubMed: 29572205]

[11]. White SL, Pasupathy D, Sattar N, et al. (2017) Metabolic profiling of gestational diabetes in obese women during pregnancy. Diabetologia 60(10): 1903–1912. 10.1007/s00125-017-4380-6 [PubMed: 28766127]

[12]. Chorell E, Hall UA, Gustavsson C, et al. (2017) Pregnancy to postpartum transition of serum metabolites in women with gestational diabetes. Metabolism 72: 27–36. 10.1016/j.metabol.2016.12.018 [PubMed: 28641781]

[13]. Tobias DK, Clish C, Mora S, et al. (2018) Dietary Intakes and Circulating Concentrations of Branched-Chain Amino Acids in Relation to Incident Type 2 Diabetes Risk Among High-Risk Women with a History of Gestational Diabetes Mellitus. Clin Chem 64(8): 1203–1210. 10.1373/clinchem.2017.285841 [PubMed: 29945965]

[14]. Carter EB, Stuart JJ, Farland LV, et al. (2015) Pregnancy Complications as Markers for Subsequent Maternal Cardiovascular Disease: Validation of a Maternal Recall Questionnaire. J Womens Health (Larchmt) 24(9): 702–712. 10.1089/jwh.2014.4953 [PubMed: 26061196]

[15]. Yang Z, Zhou M (2014) Kappa statistic for clustered matched-pair data. Stat Med 33(15): 2612–2633. 10.1002/sim.6113 [PubMed: 24532251]

[16]. Dietz P, Bombard J, Mulready-Ward C, et al. (2014) Validation of self-reported maternal and infant health indicators in the Pregnancy Risk Assessment Monitoring System. Matern Child Health J 18(10): 2489–2498. 10.1007/s10995-014-1487-y [PubMed: 24770954]

[17]. Ahluwalia IB, Helms K, Morrow B (2013) Assessing the validity and reliability of three indicators self-reported on the pregnancy risk assessment monitoring system survey. Public Health Rep 128(6): 527–536. 10.1177/003335491312800612 [PubMed: 24179264]

[18]. Evans AM, DeHaven CD, Barrett T, Mitchell M, Milgram E (2009) Integrated, nontargeted ultrahigh performance liquid chromatography/electrospray ionization tandem mass spectrometry platform for the identification and relative quantification of the small-molecule complement of biological systems. Anal Chem 81(16): 6656–6667. 10.1021/ac901536h [PubMed: 19624122]

[19]. Perng W, Gillman MW, Fleisch AF, et al. (2014) Metabolomic profiles and childhood obesity. Obesity (Silver Spring) 22(12): 2570–2578. 10.1002/oby.20901 [PubMed: 25251340]

[20]. Perng W, Rifas-Shiman SL, McCulloch S, et al. (2017) Associations of cord blood metabolites with perinatal characteristics, newborn anthropometry, and cord blood hormones in project viva. Metabolism 76: 11–22. 10.1016/j.metabol.2017.07.001 [PubMed: 28987236]

[21]. Perng W, Tang L, Song PXK, Tellez-Rojo MM, Cantoral A, Peterson KE (2019) Metabolomic profiles and development of metabolic risk during the pubertal transition: a prospective study in the ELEMENT Project. Pediatr Res 85(3): 262–268. 10.1038/s41390-018-0195-5 [PubMed: 30297880]

[22]. (2013) The MIANALYZE Procedure In: SAS/STAT 131 User's Guide. SAS Institute, inc., Cary, NC, pp 5172–5231

[23]. Auro K, Joensuu A, Fischer K, et al. (2014) A metabolic view on menopause and ageing. Nature communications 5: 4708 10.1038/ncomms5708

[24]. Ke C, Hou Y, Zhang H, et al. (2015) Plasma Metabolic Profiles in Women are Menopause Dependent. PLoS One 10(11): e0141743 10.1371/journal.pone.0141743 [PubMed: 26580805]

[25]. Bardenheier BH, Imperatore G, Gilboa SM, et al. (2015) Trends in Gestational Diabetes Among Hospital Deliveries in 19 U.S. States, 2000–2010. Am J Prev Med 49(1): 12–19. 10.1016/j.amepre.2015.01.026 [PubMed: 26094225]

[26]. Jagadeeshaprasad MG, Batkulwar KB, Meshram NN, et al. (2016) Targeted quantification of N-1-(carboxymethyl) valine and N-1-(carboxyethyl) valine peptides of beta-hemoglobin for better diagnostics in diabetes. Clin Proteomics 13: 7 10.1186/s12014-016-9108-y [PubMed: 27030792]

[27]. Stuebe AM, Rich-Edwards JW, Willett WC, Manson JE, Michels KB (2005) Duration of lactation and incidence of type 2 diabetes. JAMA 294(20): 2601–2610. 10.1001/jama.294.20.2601 [PubMed: 16304074]

[28]. Villegas R, Gao YT, Yang G, et al. (2008) Duration of breast-feeding and the incidence of type 2 diabetes mellitus in the Shanghai Women's Health Study. Diabetologia 51(2): 258–266. 10.1007/s00125-007-0885-8 [PubMed: 18040660]

[29]. Schwarz EB, Ray RM, Stuebe AM, et al. (2009) Duration of lactation and risk factors for maternal cardiovascular disease. Obstet Gynecol 113(5): 974–982. 10.1097/01.AOG.0000346884.67796.ca [PubMed: 19384111]

[30]. Martens PJ, Shafer LA, Dean HJ, et al. (2016) Breastfeeding Initiation Associated With Reduced Incidence of Diabetes in Mothers and Offspring. Obstet Gynecol 128(5): 1095–1104. 10.1097/aog.0000000000001689 [PubMed: 27741196]

[31]. Gunderson EP, Jacobs DR Jr., Chiang V, et al. (2010) Duration of lactation and incidence of the metabolic syndrome in women of reproductive age according to gestational diabetes mellitus status: a 20-Year prospective study in CARDIA (Coronary Artery Risk Development in Young Adults). Diabetes 59(2): 495–504. 10.2337/db09-1197 [PubMed: 19959762]

[32]. Shokry E, Marchioro L, Uhl O, et al. (2019) Impact of maternal BMI and gestational diabetes mellitus on maternal and cord blood metabolome: results from the PREOBE cohort study. Acta Diabetol 56(4): 421–430. 10.1007/s00592-019-01291-z [PubMed: 30725264]

[33]. Roy C, Tremblay PY, Anassour-Laouan-Sidi E, et al. (2018) Risk of gestational diabetes mellitus in relation to plasma concentrations of amino acids and acylcarnitines: A nested case-control study. Diabetes Res Clin Pract 140: 183–190. 10.1016/j.diabres.2018.03.058 [PubMed: 29626588]

[34]. Yu ZR, Ning Y, Yu H, Tang NJ (2014) A HPLC-Q-TOF-MS-based urinary metabolomic approach to identification of potential biomarkers of metabolic syndrome. Journal of Huazhong

University of Science and Technology Medical sciences = Hua zhong ke ji da xue xue bao Yi xue Ying De wen ban = Huazhong keji daxue xuebao Yixue Yingdewen ban 34(2): 276–283. 10.1007/s11596-014-1271-7

[35]. Liebich HM (1986) Gas chromatographic profiling of ketone bodies and organic acids in diabetes. Journal of Chromatography B: Biomedical Sciences and Applications 379: 347–366. 10.1016/S0378-4347(00)80689-1

[36]. Pallister T, Jackson MA, Martin TC, et al. (2017) Untangling the relationship between diet and visceral fat mass through blood metabolomics and gut microbiome profiling. Int J Obes (Lond) 41(7): 1106–1113. 10.1038/ijo.2017.70 [PubMed: 28293020]

[37]. Chou J, Liu R, Yu J, et al. (2018) Fasting serum α−hydroxybutyrate and pyroglutamic acid as important metabolites for detecting isolated post-challenge diabetes based on organic acid profiles. Journal of Chromatography B 1100–1101: 6–16. 10.1016/j.jchromb.2018.09.004

[38]. Goodison S, Kenna S, Ashcroft SJ (1992) Control of insulin gene expression by glucose. Biochem J 285 (Pt 2): 563–568. 10.1042/bj2850563 [PubMed: 1322137]

[39]. Menni C, Fauman E, Erte I, et al. (2013) Biomarkers for type 2 diabetes and impaired fasting glucose using a nontargeted metabolomics approach. Diabetes 62(12): 4270–4276. 10.2337/db13-0570 [PubMed: 23884885]

[40]. Lotta LA, Scott RA, Sharp SJ, et al. (2016) Genetic Predisposition to an Impaired Metabolism of the Branched-Chain Amino Acids and Risk of Type 2 Diabetes: A Mendelian Randomisation Analysis. PLoS medicine 13(11): e1002179 10.1371/journal.pmed.1002179 [PubMed: 27898682]

Highlights

- Branched chain amino acids (BCAA; leucine, isoleucine, and valine) and their metabolites have been associated with diabetes.
- At least one in four women who develop gestational diabetes will progress to type 2 diabetes; breastfeeding is protective against this progression.
- In midlife, BCAA levels were not significantly different in women with a history of gestational diabetes or breastfeeding.
- Gestational diabetes and breastfeeding were associated in opposite directions with levels of several metabolites on the BCAA metabolic pathway.

Table 1.

Bogalusa Heart Study participants with metabolomics and pregnancy data

	N	%
race		
black	232	36.9
white	397	63.1
menopausal status		
premenopausal	249	39.7
postmenopausal	378	60.3
ever smoked		
yes	362	57.5
no	268	42.5
total gravidity		
1	89	14.2
2	256	40.8
3+	282	45.0
total parity		
1	123	19.6
2	289	46.1
3+	215	34.3
any fertility issues	56	8.9
history of low birthweight	114	18.2
history of preterm birth	93	14.7
history of GDM	64	10.2
ever breastfed	210	34.5

	mean	median	SD	min	max
age at interview	48.1	48.7	5.1	35.0	56.7
age at first pregnancy	22.7	21.4	5.2	14.0	45.8
BMI	31.7	31.1	7.7	17.9	66.6

Table 2.

Branched-chain amino acids, history of gestational diabetes, and breastfeeding history

	leucine		isoleucine		valine		sum BCAAs	
	beta	**95% CI**	**beta**	**95% CI**	**beta**	**95% CI**	**beta**	**95% CI**
history of GDM (those with pre-existing diabetes omitted)	0.189	−0.056, 0.433	0.197	−0.047, 0.442	0.159	−0.084, 0.402	0.545	−0.139, 1.229
any diabetes during pregnancy	0.153	−0.076, 0.382	0.164	−0.065, 0.393	0.132	−0.096, 0.360	0.449	−0.192, 1.090
ever breastfed	−0.097	−0.250, 0.056	−0.133	−0.286, 0.019	−0.075	−0.227, 0.077	−0.305	−0.732, 0.122
total breastfeeding more than 6 months	−0.063	−0.269, 0.143	−0.176	−0.382, 0.030	−0.064	−0.269, 0.141	−0.303	−0.880, 0.274
p for interaction between GDM and breastfeeding		0.43		0.39		0.42		0.38

GDM, gestational diabetes mellitus; CI, confidence interval; BCAA, branched-chain amino acids

Table 3.

Branched-chain amino acid metabolites, history of gestational diabetes, and breastfeeding

	history of gestational diabetes		any diabetes during pregnancy		ever breastfed		>6 months breastfeeding	
	beta	95% CI	beta	95% CI	beta	95% CI	beta	95% CI
1-carboxyethylisoleucine[a, b]	**0.1937**	**−0.0161, 0.4036**	**0.2950**	**0.0801, 0.5098**	**−0.1729**	**−0.3160, −0.0297**	**−0.2722**	**−0.4648, −0.0795**
1-carboxyethylleucine[a, b]	**0.2909**	**0.0732, 0.5085**	**0.3253**	**0.1111, 0.5395**	**−0.1425**	**−0.2858, 0.0008**	**−0.2636**	**−0.4562, −0.0710**
2,3-dihydroxy-2-methylbutyrate[a,b]	0.0452	−0.2094, 0.2997	0.0521	−0.1866, 0.2908	−0.0872	−0.2463, 0.0718	−0.1522	−0.3664, 0.0621
2-hydroxy-3-methylvalerate[a]	0.0469	−0.1711, 0.2648	0.0414	−0.1626, 0.2453	**−0.1713**	**−0.3067, −0.0360**	**−0.2189**	**−0.4014, −0.0364**
3-hydroxyisobutyrate[a]	0.1672	−0.0872, 0.4215	0.1363	−0.1035, 0.3761	0.1532	−0.0063, 0.3126	−0.0305	−0.2460, 0.1851
3-methyl-2-oxovalerate	**0.2312**	**−0.0050, 0.4675**	**0.2691**	**0.0454, 0.4927**	−0.0337	−0.1834, 0.1160	−0.1005	−0.3022, 0.1013
3-methylglutaconate[a]	**0.2794**	**0.0159, 0.5429**	0.1902	−0.0565, 0.4368	0.0047	−0.1599, 0.1694	0.0110	−0.2110, 0.2392
3-methylglutarylcarnitine (2)[a]	0.0952	−0.1403, 0.3307	0.0157	−0.2052, 0.2366	−0.0164	−0.1638, 0.1309	0.0523	−0.1462, 0.2507
4-methyl-2-oxopentanoate	0.1955	−0.0442, 0.4351	**0.2406**	**0.0139, 0.4674**	0.0300	−0.1217, 0.1816	0.0298	−0.1747,0. 2342
alpha-hydroxyisovalerate[a]	0.0807	−0.1667, 0.3282	0.0575	−0.1738, 0.2887	**−0.1645**	**−0.3182, −0.0108**	−0.1948	−0.4021, 0.0124
beta-hydroxyisovalerate[a]	−0.0643	−0.3187, 0.1902	−0.0544	−0.2924, 0.1836	−0.1169	−0.2754, 0.0416	−0.1132	−0.3271, 0.1007
ethylmalonate[a]	−0.0326	−0.2881, 0.2229	−0.0162	−0.2574, 0.2249	0.1317	−0.0289, 0.2921	0.0659	−0.1516, 0.28214
isobutyrylcarnitine (C4)[a]	−0.0278	−0.2966, 0.2410	−0.0586	−0.3099, 0.1927	−0.0820	−0.2495, 0.0856	−0.1097	−0.3354, 0.1160
isobutyrylglycine[a]	0.0219	−0.2406, 0.2843	0.0138	−0.2323, 0.2598	−0.0775	−0.2415, 0.0864	−0.0040	−0.2251, 0.2170
isovalerate (i5:0)	0.0815	−0.1816, 0.3447	0.0868	−0.1596, 0.3332	0.0466	−0.1175, 0.2107	0.0125	−0.2088, 0.2338
isovalerylglycine[a, b]	0.1434	−0.1237, 0.4104	0.2068	−0.0427, 0.4562	−0.0613	−0.2279, 0.1054	−0.1619	−0.3862, 0.0624
methylsuccinate[a]	0.0228	−0.2347, 0.2830	0.0503	−0.1952, 0.2959	0.0964	−0.0670, 0.2599	−0.0272	−0.2476, 0.1933
N-acetylisoleucine[a]	0.1762	−0.0768, 0.4292	0.1582	−0.0784, 0.3948	−0.1330	−0.2906, 0.0247	−0.1398	−0.3505, 0.0746
N-acetylleucine[a]	**0.2466**	**−0.0061, 0.4992**	**0.2020**	**−0.0341, 0.4382**	**−0.1779**	**−0.3350, −0.0207**	−0.1534	−0.3658, 0.0590
tiglylcarnitine (C5:1-DC)	0.1173	−0.1314, 0.3660	0.0534	−0.1803, 0.2871	0.0989	−0.0568, 0.2546	0.1268	−0.0832, 0.3368
3-methyl-2-oxobutyrate	0.0598	−0.1805, 0.3002	0.1019	−0.1242, 0.3279	0.0283	−0.1225, 0.1791	−0.0524	−0.2557, 0.1509
isovalerylcarnitine (C5)	−0.0795	−0.3414, 0.1822	−0.1049	−0.3497, 0.1399	0.0375	−0.1275, 0.2009	−0.0673	−0.2877, 0.1529
2-methylbutyrylcarnitine (C5)	−0.0272	−0.2692, 0.2148	−0.0316	−0.2623, 0.1900	0.0033	−0.1475, 0.1541	−0.0899	−0.2931, 0.1132

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	history of gestational diabetes		any diabetes during pregnancy		ever breastfed		>6 months breastfeeding	
	beta	**95% CI**	**beta**	**95% CI**	**beta**	**95% CI**	**beta**	**95% CI**
1-carboxyethylvaline[a, b]	**0.2827**	**0.0733, 0.4931**	**0.3512**	**0.1401, 0.5624**	**−0.1753**	**−0.3165, −0.0340**	**−0.2792**	**−0.4692, −0.0892**
3-hydroxy-2-ethylpropionate	**0.2767**	**0.0394, 0.5140**	**0.3308**	**0.1080, 0.5536**	**0.1779**	**0.0291, 0.3266**	0.0936	−0.1079, 0.2951
alpha-hydroxyisocaproate[a,b]	**0.2343**	**0.0027, 0.4660**	**0.2146**	**−0.0054, 0.4347**	−0.1242	−0.2710, 0.0226	**−0.3044**	**−0.5012, −0.1075**
N-carbamoylvaline[a, b]	0.0254	−0.2278, 0.2786	0.0629	−0.1751, 0.3008	0.0757	−0.0829, 0.2344	0.0631	−0.1508, 0.2769

All results adjusted for age, BMI and waist circumference at time of metabolite measurement, average prior adult BMI, race, and menopausal status.

[a]log-transformed to address skewness

[b]>10% below the limit of detection; imputed as ½ LOD; results in supplementary material with values below LOD omitted