BMC Pregnancy and Childbirth

RESEARCH

Open Access

Association between serum branched chain amino acids, mammalian target of rapamycin levels and the risk of gestational diabetes mellitus: a 1:1 matched case control study

Lingling Cui[1], Zhiqian Li[1], Xinxin Liu[1], Zhonglei Li[2], Jiaxin Li[1], Yingying Guo[1], Huijun Zhou[1], Xiaoli Yang[1], Zhengya Zhang[1], Yuting Gao[1], Lina Ren[1] and Linlin Hua[3*]

Abstract

Background To investigate the association between serum branched chain amino acids (BCAAs), mammalian target of rapamycin (mTOR) levels and the risk of gestational diabetes mellitus (GDM) in pregnant women.

Methods 1:1 matched case–control study was conducted including 66 GDM patients and 66 matched healthy pregnant women (± 3 years) in 2019, in China. Fasting bloods of pregnant women were collected in pregnancy at 24 ~ 28 weeks gestation. And the serum levels of valine (Val), leucine (Leu), isoleucine (Ile) and mTOR were determined. Conditional logistic regressions models were used to estimate the associations of BCAAs and mTOR concentrations with the risk of GDM.

Results Concentrations of serum Val and mTOR in cases were significantly higher than that in controls ($P < 0.05$). After adjusted for the confounded factors, both the second tertile and the third tertile of mTOR increased the risk of GDM (*OR* = 11.771, 95%*CI*: 3.949–35.083; *OR* = 4.869 95%*CI*: 1.742–13.611, respectively) compared to the first tertile of mTOR. However, the second tertile of serum Val (*OR* = 0.377, 95%*CI*:0.149–0.954) and the second tertile of serum Leu (*OR* = 0.322, 95%*CI*: 0.129–0.811) decreased the risk of GDM compared to the first tertile of serum Val and Leu, respectively. The restricted cubic spline indicated a significant nonlinear association between the serum levels of mTOR and the risk of GDM (*P* values for non-linearity = 0.0058).

Conclusion We confirmed the association of higher mTOR with the increased risk of GDM in pregnant women. Pregnant women who were in the certain range level of Val and Leu were at lower risk of GDM. Our findings provided epidemiological evidence for the relation of serum BCAAs and mTOR with risk of GDM.

Keywords Branched chain amino acids, Mammalian target of rapamycin, Gestational diabetes mellitus, Pregnancy, Glucose tolerance

*Correspondence:
Linlin Hua
hualinlin@zzu.edu.cn
Full list of author information is available at the end of the article

Background

Gestational diabetes mellitus (GDM), commonly defined as impaired glucose tolerance with onset or first recognition during pregnancy [1]. The prevalence of GDM was increasing globally and approximately 14.0% of live births worldwide were affected by hyperglycemia during pregnancy in 2021 according to an estimate of International Diabetes Federation [2, 3]. Several serious adverse pregnancy outcomes correlated highly with GDM, including macrosomia, hypoglycemia, fetal hypoxia, and fetal malformation for the infants [4–6]. Moreover, GDM might also be associated with developing type 2 diabetes, metabolic syndrome, and cardiovascular disease later in life for the pregnancy women [7]. As the age and pre-pregnant body mass index (BMI) increased gradually, the prevalence of GDM aroused great attention around the world, and the adverse effect of GDM became one of the important public health problems. Therefore, earlier identification of GDM for pregnant women became particularly crucial.

Branched chain amino acids (BCAAs), including leucine (Leu), isoleucine (Ile), and valine (Val), played critical roles in the regulation of energy homeostasis, nutrition metabolism, gut health, immunity and disease in humans and animals [8]. Recently, many animal experimentations and population-based studies reported that increased circulating BCAAs level owned the predictive values for type 2 diabetes [9–12]. There were also a few studies conducted to address the correction between elevated circulating BCAAs levels and increased risk of GDM, but the relevant conclusions were contradictory. For example, a case–control study found that circulating concentrations of BCAAs were independently and positively associated with GDM through modifying insulin resistance and secretion among the Korean population [13]. However, a nested case–control study reported that the levels of the BCAAs did not differ significantly between GDM and normal glucose tolerance [14]. And another study also showed that the increase in BCAAs occurred postpartum since the BCAAs did not differ during pregnancy, as compared to normoglycemic women [12].

Mammalian target of rapamycin (mTOR), an atypical serine/threonine kinase, was regulated by cellular nutrient and metabolic status [15]. The mTOR signaling pathway was a key component that regulated many major cellular processes and was implicated in various pathological conditions, including cancer, neurodegeneration, and metabolic diseases [16]. Previous studies suggested that placental protein kinase B (Akt)/mTOR signaling was substantially upregulated in GDM patients [17, 18]. And other studies indicated that paeoniflorin [19], olive oil [20] and 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$) [21] could serve as a potential therapeutical strategy for patients with GDM through suppressing mTOR signaling. However, there were few studies to explore the association between circulating mTOR levels and risk of gestational diabetes mellitus (GDM). And our study might be useful for identifying biomarkers and understanding the mechanistic underpinnings of GDM.

Therefore, to investigate the associations between the serum levels of BCAAs and mTOR and risk of GDM, we conducted the 1:1 matched case–control study among 132 pregnant women (including 66 cases and 66 controls).

Methods

Study design and population

A 1:1 age- (±3 years)-matched case–control study was conducted at the Third Affiliated Hospital of Zhengzhou University. Pregnant women who aged from 18 to 45 years, carried a singleton fetus, and decided to deliver in the study hospital were recruited at their first prenatal visit. The study protocol had been approved by the Ethics Committee of the Third Affiliated Hospital of Zhengzhou University. All the participants have signed the informed consent forms before enrollment. The study had been registered with on the Chinese Clinical Trial Registry (ChiCTR2000028811), and the Registration Date was January 4, 2020.

All participants had been visited from January 2019 to October 2019. Individuals were excluded if they met the following criteria: (a) had the history of smoking and drinking, (b) had pregnant complication or miscarried, (c) had the family history of thyroid disorders, (d) took the medicine influencing hormone secretion and glycol-metabolism, (e) with diabetes mellitus, hypertension, disease of heart, or renal disease in pre-pregnancy. Finally, 66 GDM cases were identified according to the criteria of the International Association of Diabetes and Pregnancy Study Groups (IADPSG) in 2011. For each case, one control was selected randomly from the remaining participants who were free of GDM, and matched by pregnant women's age (±3 years). Thus, a total of 66 cases and 66 controls were included in the study.

Measurements

Anthropometric measurement

Information of sociodemographic characteristics (e.g.age, residential area, and education) and other detailed information including parity, history of previous miscarriages, miscarriage times was obtained by a standard questionnaire. Professional trained researchers instructed pregnant women face to face to fill in the questionnaires, and then timely checked and corrected completed questionnaires. The weight and height of participants were measured by the InBody J30 (Biospace, Seoul, South

Korea) and a stadiometer in the hospital without shoes and wearing light clothing, respectively. Pre-pregnancy BMI was calculated by weight (kg)/height^2 (m^2). Blood pressure was measured twice after 5 min of rest using an appropriate cuff according to arm size. The average of two blood pressure measurements was recorded as the final value. And waist-hip ratio was calculated as the waist (m) divided by the hip (m).

Biochemical measurement

Fasting bloods of pregnant women were collected in pregnancy at 24 ~ 28 weeks gestation. Blood samples were collected and serum was obtained by centrifugation at 3000 × rpm for 10 min at 4 °C. Separated serum samples were put into the liquid nitrogen container, and then stored in − 80 °C refrigerator until analysis.

Concentrations of BCAAs in serum were measured using high-performance liquid chromatography (HPLC) by L-8900 High speed amino acid analyzer (Hitachi High-Tech Science Corporation, Japan). Serum fasting insulin (Wuhan Elabcience Company) and mTOR (Wuhan CUSABIO Company) levels were both determined by enzyme-linked immunosorbent assay (ELISA) following the manufacture's protocol. The levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) in serum were determined using the GPO-PAP method by biochemical analyzer (Shanghai Kehua Bioengineering Co, LTD).

Definitions

Plasma glucose of pregnant women was measured during a 75-g oral glucose tolerance test (OGTT) between 24 and 28 weeks gestation as a routine prenatal examination. The diagnosis of GDM was based on the recommended criterion by IADPSG of exceeded glucose values of 5.1, 10.0, and 8.5 mmol/L at fasting, 1 h, and 2 h.

Education level was divided into three categories: low (junior school or below), middle (high school, technical school, or technical secondary school), and high (junior college, college or above). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR = fasting plasma insulin (μIU/L) × fasting plasma glucose (FPG) (mmol/L)/22.5.

Table 1 Baseline characteristics of case and control group

Characteristic	Case ($n=66$)	Control ($n=66$)	t/χ^2	*P* value
Age (y)	30.9 ± 3.8	30.5 ± 4.5	1.364[a]	0.177
Pre-pregnancy BMI (kg/m2)	22.6 ± 3.2	21.8 ± 3.2	1.343[a]	0.184
Pregnancy times, n (%)			0.545[b]	0.460
1	24(36.4)	20(30.3)		
≥ 2	42(63.6)	46(69.7)		
Parity, n (%)			0.183[b]	0.669
Nulliparous	25 (37.9)	27(40.9)		
Multiparous	41(62.1)	39(59.1)		
Numbers of previous miscarriages, n (%)			1.566[b]	0.211
0	44(66.7)	37(56.1)		
≥ 1	22(33.3)	29(43.9)		
Residential area, n (%)			9.900[b]	0.002
Urban	45(68.2)	27(40.9)		
Rural	21(31.8)	39(59.1)		
Education attainment, n (%)			2.104[b]	0.349
Junior middle school or lower	2(3.0)	5(7.6)		
Senior high school	7(10.6)	4(6.1)		
College or higher	57(86.4)	57(86.3)		
History of previous poor pregnancy outcome, n (%)			-	0.119
No	66(100)	62(93.9)		
Yes	0 (0)	4 (6.1)		
SBP (mmHg)	101 ± 10	112 ± 11	-1.531[a]	0.131
DBP (mmHg)	65 ± 18	64 ± 8	-0.750[a]	0.456
Waist-hip ratio	0.88 ± 0.05	0.85 ± 0.06	3.151[a]	0.002

Abbreviation: *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

[a] *t* value

[b] χ^2 value

Statistical analysis

Continuous variables and categorical variables were presented as the mean ± SD and absolute value with percentage, respectively. The comparisons of continuous or categorical characteristics were examined using the *t*-tests or Chi-square tests.

Conditional logistic regression models were used to calculate odds ratios (*ORs*) and 95% confidence intervals (95% *CIs*) for the relationships of BCAAs and mTOR concentrations with the risk of GDM. The levels of BCAAs and mTOR were analyzed as categorical variables based on the tertile distributions among GDM and controls (the lowest tertile was defined as the referent group). Additionally, we conducted trend tests by using the median value of each tertile of BCAAs and mTOR. Covariates were retained in the models if they altered a > 10% change of regression parameter in the unadjusted model or had been associated with BCAAs or mTOR and GDM in previous studies. Finally, three models were fitted as follows: model 1 was an unadjusted analysis; model 2 was adjusted for age and pre-pregnancy BMI; model 3 was further adjusted for levels of HDL, LDL and TG.

Statistical analyses were performed using SPSS software (IBM SPSS 25.0, SPSS Inc), and two-sided *P*-values < 0.05 were considered statistically significant in all of the tests.

Table 2 The serum index of case and control group in the second trimester of pregnancy

Variables	Case ($n=66$)	Control ($n=66$)	*t*	*P*
FPG (mmol/L)	4.94 ± 0.55	4.60 ± 0.35	4.348	< 0.001
OGTT 1hPG (mmol/L)	9.82 ± 1.95	7.25 ± 1.46	8.181	< 0.001
OGTT 2hPG (mmol/L)	8.56 ± 2.12	6.57 ± 1.39	6.839	< 0.001
$AUC_{Glucose}$[mmol/(L • h)]	16.20 ± 3.82	12.84 ± 1.98	6.426	< 0.001
Fasting insulin (μIU/mL)	13.26 ± 10.72	13.19 ± 8.65	0.039	0.969
HOMA-IR	2.98 ± 2.51	2.41 ± 1.93	1.457	0.150
TC (mmol/L)	5.70 ± 1.04	6.19 ± 1.35	-2.101	0.024
TG (mmol/L)	2.82 ± 1.16	2.46 ± 0.84	1.992	0.051
HDL-C (mmol/L)	1.62 ± 0.33	1.78 ± 0.43	-2.315	0.256
LDL-C (mmol/L)	3.12 ± 0.76	3.30 ± 1.00	-1.147	0.051
Val (μmol/L)	171.18 ± 50.42	154.20 ± 39.40	2.110	0.039
Ile (μmol/L)	96.58 ± 47.70	88.89 ± 42.99	0.979	0.331
Leu (μmol/L)	130.68 ± 42.34	129.02 ± 42.36	0.221	0.826
mTOR (ng/mL)	20.13 ± 6.97	17.84 ± 4.95	2.090	0.041

Abbreviation: *FPG* fasting plasma glucose, *OGTT* oral glucose tolerance test, *1hPG* 1 h plasma glucose, *2hPG* 2 h plasma glucose, *AUC* area under curve, *HOMA-IR* homeostasis model assessment of insulin resistance, *TC* total cholesterol, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *Val* valine, *Ile* isoleuline, *Leu* leucine, *mTOR* mammalian target of rapamycin

Results

Table 1 presented the baseline characteristics of case and control group. The average age and pre-pregnancy BMI of the 132 participants were 30.7 ± 5.9 years and 22.2 ± 3.2 kg/m^2, respectively. Compared with the controls, GDM cases had higher waist-hip ratio (0.88 ± 0.05 vs. 0.85 ± 0.06, $P=0.002$). The proportion of pregnant women from urban in GDM group were higher than that in control group (68.2% vs. 40.9%, $P=0.002$). No significant differences were observed between the cases and controls in age, pre-pregnancy BMI, parity, educational level, pregnancy times, miscarriage times, history of previous miscarriages, systolic blood pressure (SBP) and diastolic blood pressure (DBP) (all $P > 0.05$).

Table 2 showed the biochemical index of case and control group in the second trimester of pregnancy. In the 75 g oral glucose tolerance test, the mean glucose levels of fasting (4.94 ± 0.55 mmol/L vs. 4.60 ± 0.35 mmol/L), 1 h (9.82 ± 1.95 mmol/L vs. 7.25 ± 1.46 mmol/L), and 2 h (8.56 ± 2.12 mmol/L vs. 6.57 ± 1.39 mmol/L) among cases were significantly higher than that among controls, respectively (all $P < 0.001$). The average of $AUC_{Glucose}$ (16.20 ± 3.82 mmol/ (L • h) vs. 12.84 ± 1.98 mmol/ (L • h)), TC (5.70 ± 1.04 mmol/L vs. 6.19 ± 1.35 mmol/L), Val (171.18 ± 50.42 μmol/L vs. 154.20 ± 39.40 μmol/L) and mTOR (20.13 ± 6.97 ng/mL vs. 17.84 ± 4.95 ng/mL) in serum concentrations among cases were significantly higher than that among controls, respectively (all $P < 0.05$). There were no statistically significant differences between two groups on Fasting insulin, HOMA-IR, HDL-C, LDL-C, TG, Ile and Leu (all $P > 0.05$).

Figure 1 presented the comparison of BCAAs and mTOR in GDM and healthy pregnant women among different tertiles of oral glucose tolerance. In Fig. 1a, the levels of Val among cases were significantly higher than that among controls in the second tertile of OGTT 1 h PG and third tertile of FPG, OGTT 1hPG and OGTT 2hPG (all $P < 0.05$). In Fig. 1b, the levels of Ile among cases were significantly higher than that among controls in the second tertile of OGTT 1hPG and the first and the third tertile of OGTT 2hPG (all $P < 0.05$). In Fig. 1c, the levels of Leu among cases were significantly higher than that among controls in the first tertile of FPG and the third tertile of OGTT 1hPG and OGTT 2hPG (all $P < 0.05$). In Fig. 1d, the levels of mTOR among cases were significantly higher than that among controls in the first tertile of FPG and OGTT 1hPG, and the second and the third tertile of OGTT 2hPG (all $P < 0.05$).

Multivariate conditional logistic regression analysis *ORs* for GDM across tertiles of BCAAs and mTOR were reported in Table 3. In crude models, compared to the first tertile of mTOR, the crude *ORs* (95% *CIs*) of GDM

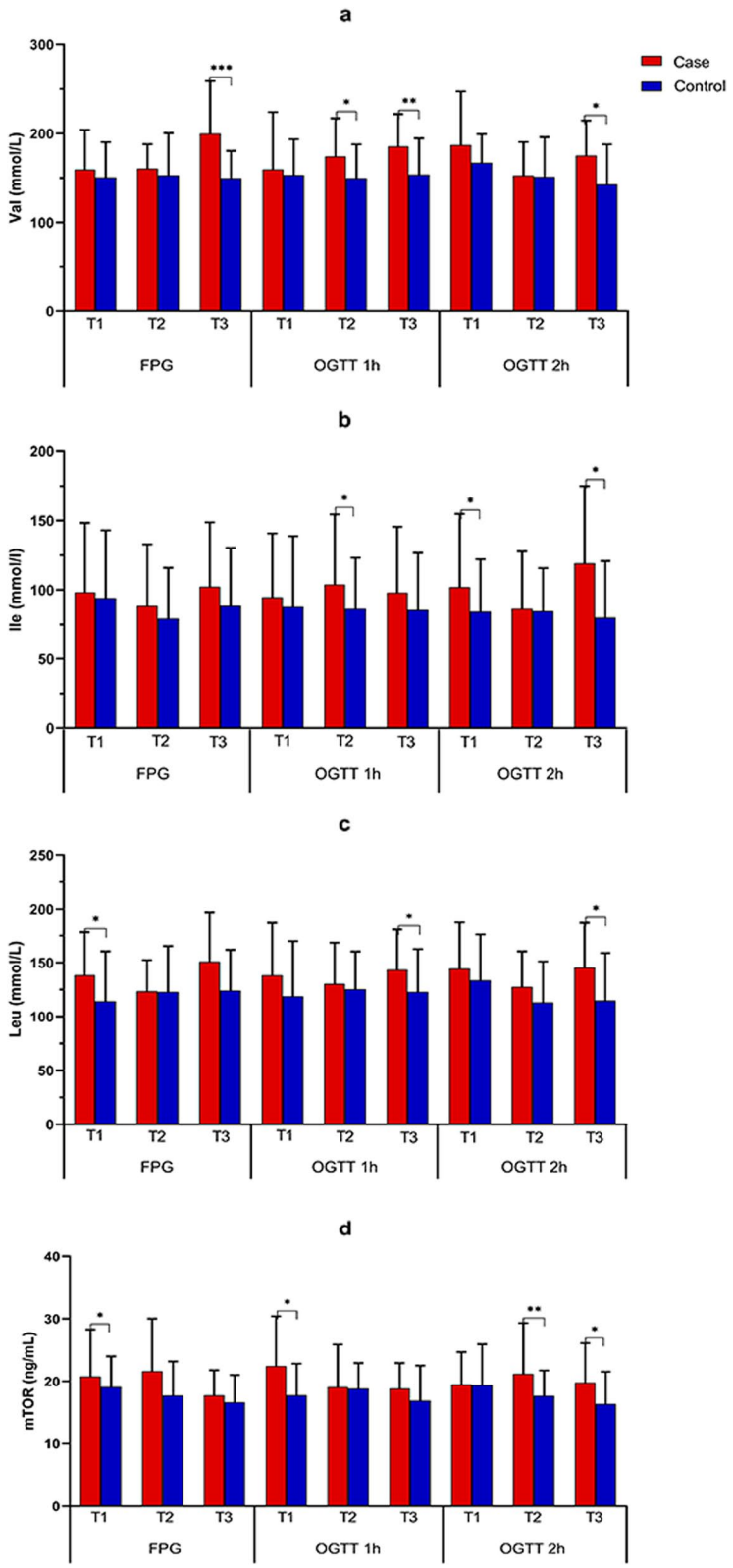

Fig. 1 Comparison of glucose tolerance in GDM and control among different tertiles of BCAAs and mTOR. Abbreviation: GDM, gestational diabetes mellitus; BCAAs, Branched chain amino acids; mTOR, mammalian target of rapamycin; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test, 1hPG, 1 h plasma glucose; 2hPG, 2 h plasma glucose; Val, valine; Ile, isoleuline; Leu, leucine.*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Table 3 Odd ratio for gestational diabetes mellitus in BCAAs and mTOR

		Model 1		Model 2		Model 3	
BCAAs and mTOR levels	**N(case/control)**	***OR* (95% *CI*)**	***P***	***OR* (95% *CI*)**	***P***	***OR* (95% *CI*)**	***P***
Val							
T_1 (< 149.94μmol/L)	21/24	1.00 (Ref)	-	1.00 (Ref)	-	1.00 (Ref)	-
T_2 (149.94–176.37μmol/L)	28/16	0.500 (0.214–1.168)	0.109	0.498 (0.211–1.177)	0.112	0.377 (0.149–0.954)	0.039
T_3 (≥ 176.37μmol/L)	17/26	1.338 (0,574–3.120)	0.500	1.217 (0.533–3.030)	0.589	1.154 (0.473–2.818)	0.753
P for trend			0.073		0.092		0.041
Leu							
T_1 (< 116.69μmol/L)	19/25	1.00 (Ref)	-	1.00 (Ref)	-	1.00 (Ref)	-
T_2 (116.69–144.43μmol/L)	29/15	0.393 (0.166–0.932)	0.034	0.382 (0.160–0.915)	0.031	0.322 (0.129–0.811)	0.016
T_3 (≥ 144.43μmol/L)	18/26	1.098 (0.471–2.580)	0.829	1.016 (0.426–2.424)	0.972	0.760 (0.302–1.909)	0.559
P for trend			0.038		0.046		0.043
Ile							
T_1 (< 66.03μmol/L)	23/21	1.00 (Ref)	-	1.00 (Ref)	-	1.00 (Ref)	-
T_2 (66.03–95.41μmol/L)	23/23	1.000 (0.433–2.308)	1.000	1.026 (0.440–2.390)	0.953	0.997 (0.409–2.431)	0.995
T_3 (≥ 95.41μmol/L)	20/24	1.314 (0.569–3.038)	0.523	1.286 (0.551–3.000)	0.561	1.058 (0.433–2.582)	0.902
P for trend			0.762		0.815		0.989
mTOR							
T_1 (< 17.84ng/ml)	47/20	1.00 (Ref)	-	1.00 (Ref)	-	1.00 (Ref)	-
T_2 (17.84–20.13ng/ml)	6/29	11.358 (4.084–31.593)	< 0.001	12.344 (4.290–35.515)	< 0.001	11.771 (3.949–35.083)	< 0.001
T_3 (≥ 23.13ng/ml)	13/17	3.073 (1.260–7.497)	0.014	4.200 (1.595–11.061)	0.004	4.869 (1.742–13.611)	0.003
P for trend			< 0.001		< 0.001		< 0.001

Model 1: unadjusted confounders, Model 2: adjusted for age, pre-pregnancy BMI. Model 3: further adjusted for HDL-C, LDL-C and TG, based on Model 2. *P* for trend was calculated by treating tertiles as ordinal predictors in multivariate conditional logistic regression

Abbreviation: *BCAAs* Branched chain amino acids, *mTOR* mammalian target of rapamycin, *Val* valine, *Ile* isoleuline, *Leu* leucine, *T1* the first tertile, *T2* the second tertile, *T3* the third tertile, *OR* odds ratio, *CI* confidence interval, *Ref* reference

risk were 11.358 (4.084–31.593) for the second tertile and 3.073 (1.260–7.497) for the third tertile. Compared to the first tertile of Leu, the crude *OR* (95% *CI*) of GDM risk were 0.393 (0.166–0.932) for the second tertile. In model 3 adjusted for confounding factors, serum mTOR level was still associated with a higher risk of GDM, and the adjusted *OR*s and 95% *CI*s of GDM for the subjects in T2 and T3 compared to the subjects in T1 of serum mTOR level were 11.771 (3.949–35.083) and 4.869 (1.742–13.611), respectively (both $P<0.05$). Serum Val and Leu levels were associated with a lower risk of GDM, and the *OR*s and 95% *CI*s of GDM for the subjects in T2 compared to the subjects in T1 of serum Val and Leu level were 0.377 (0.149–0.954) and 0.322 (0.129–0.811), respectively (both $P<0.05$). However, there was no significant association between the risk of GDM and serum levels of Ile ($P=0.989$).

The restricted cubic spline indicated that after adjusted age, pre-pregnancy BMI, LDL-C, HDL-C, TC and TG, there was a significant nonlinear association between the serum levels of mTOR and the risk of GDM ($P_{\text{overall}}=0.001$, $P_{\text{non-linearity}}=0.005$). However, no statistically significant non-linear association was found between the serum levels of Val, Ile, Leu and the risk of GDM (all $P>0.05$. Figure 2).

The correlation heat map showed that there was significantly positive correlation between the serum levels of Val and FPG ($r=0.293$, $P=0.001$), OGTT 1hPG ($r=0.218$, $P=0.212$). However, no statistically significant association was found between the serum levels of Ile, Leu, mTOR and the oral glucose tolerance (all $P>0.05$. Figure 3 and Supplementary Table 1).

Discussion

In this study, we conducted an exploratory analysis on 132 pregnant women including 66 GDM cases and 66 normal controls. The present study found that the serum levels of val and mTOR were higher in GDM women than that in the control group. And the higher concentrations of serum BCAAs and mTOR levels were association with the higher glucose according to the OGTT results. The conditional logistic regression models showed that the higher serum level of mTOR in pregnant women was

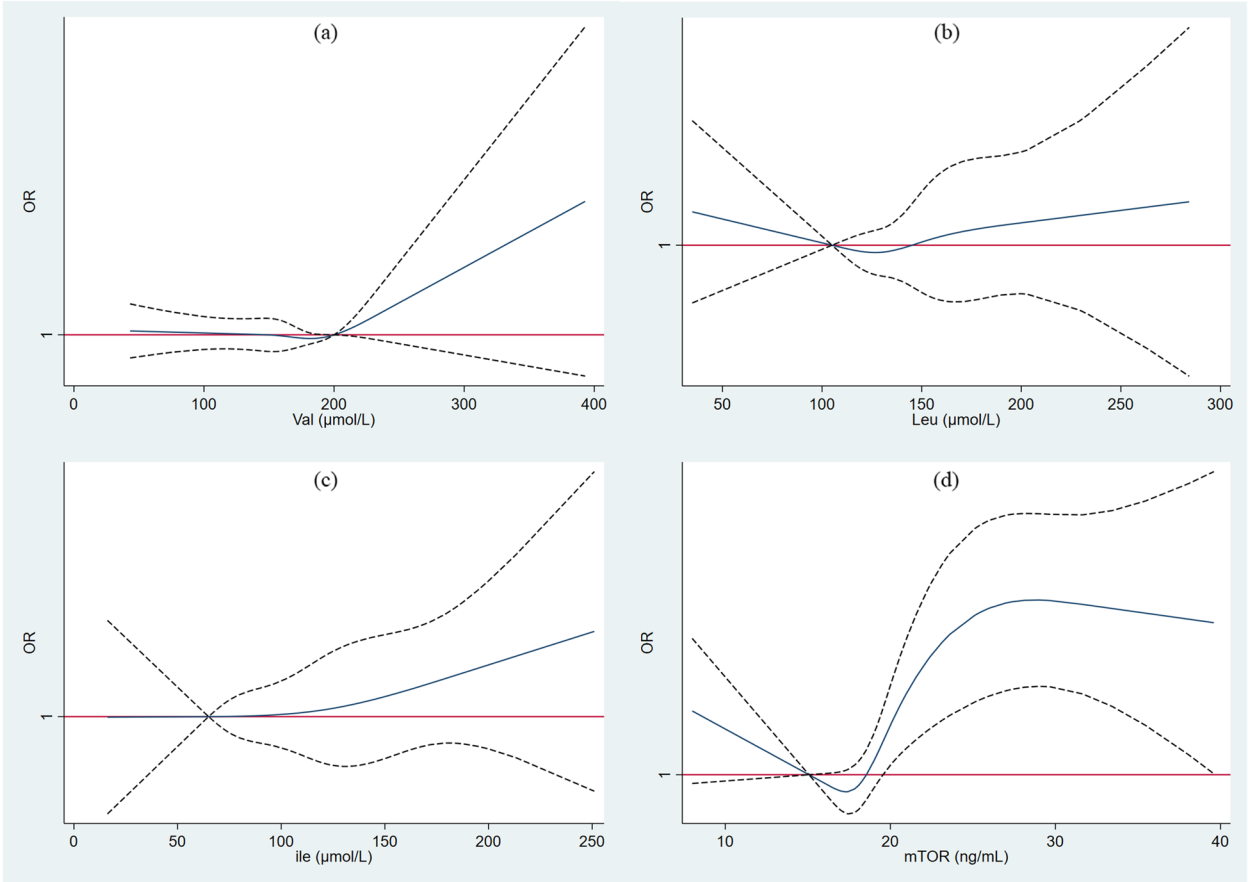

Fig. 2 The restricted cubic spline for the association between Val, Ile, Leu, mTOR concentration and risk of GDM. Abbreviation: GDM, gestational diabetes mellitus; Val, valine; Ile, isoleucine; Leu, leucine; mTOR, mammalian target of rapamycin; *OR* were displayed as a blue solid line, and the upper and lower limit of 95% confidence intervals were displayed as a dotted line. Adjustment factors were age, pre-pregnancy BMI, HDL-C, LDL-C, TC, TG

significantly correlated with increased risk of GDM. However, serum levels of Val and Leu were negatively associated with risk of GDM within a certain range.

The association between circulating BCAAs and the risk of type 2 diabetes (T2DM) was described, previously. A nested case–control study in the Framingham Offspring Study including 189 cases and 189 controls reported that for the branched-chain and aromatic amino acids, each s.d. increment in log marker was associated with a 57–102% increased odds of future diabetes [11]. Another two studies including a nested case–control study and a cohort study found that BCAAs were potential markers to be evaluated as predictors of T2DM after pregnancy complicated by GDM [22, 23]. A cohort study conducted in African-American women showed that amino acids and their derivatives were increased significantly in T2DM subjects [10]. What's more, an animal experimentation showed that adding BCAAs to a high-fat diet contributed to development of obesity-associated insulin resistance in the context of a dietary pattern that includes high fat consumption in rodents [9].

Several previous studies investigated the association between levels of BCAAs and GDM in pregnant women. A case–control study found the association between plasma amino acids levels and higher risk of GDM in Iran [24]. A nest case–control study ($n=486$) found that serum Val, Leu, Ile and total BCAAs in early pregnancy were positively associated with the risk of GDM in Chinese pregnant women [25]. A meta-analysis including eight studies with 432 subjects showed that the increase of individual plasma BCAAs concentration, as potential biomarkers, might be related to the increased risk of GDM [26]. In our present study, univariate analysis showed that BCAAs were the risk factors of GDM. However, the result of conditional logistic regression analysis showed that levels of Val and Leu were negatively associated with risk of GDM in the range of 149.94–176.37 μmol/L and 116.69–144.43 μmol/L,

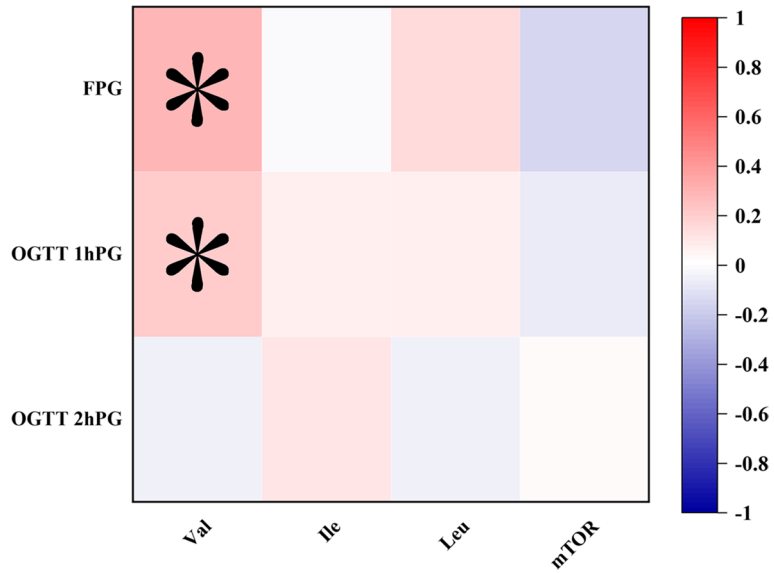

Fig. 3 The correlation heat map between BCAAs, mTOR and oral glucose tolerance. Abbreviation: BCAAs, Branched chain amino acids; mTOR, mammalian target of rapamycin; Val, valine; Ile, isoleucine; Leu, leucine; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test, 1hPG, 1 h plasma glucose; 2hPG, 2 h plasma glucose.*: $P < 0.05$

respectively. But the association of Val and Leu with GDM would disappear if the value of Val and Leu was out of the range, which might indicate that the range of 149.94–176.37 μmol/L for Val and 116.69–144.43 μmol/L for Leu was suitable for the pregnant women. And other studies also reported the normal range of Val and Leu, which supported our findings. For example, Li et al. [25] reported that the normal range of Val and Leu is 155.3–200.8 μmol/L and 122.0–155.3 μmol/L in pregnant women, respectively. And Kalliopi et al. [27] reported that the normal range of Val and Leu is 146.65–244.43 μmol/L and 81.44–142.08 μmol/L, respectively. Moreover, null results were also observed in two case–control studies involving the European population and American white person [12, 14]. The null results of the study in European may be due to small sample size ($n = 56$), especially the size of the GDM group ($n = 11$), and the sample source from two different countries in European [12]. Furthermore, the study results conducted in American white person was not suitable for Chinese region because of the variation of genetics and ethnic [14].

In the current study, we provided evidence that the level of serum mTOR is closely associated with the risk of GDM. And several published studies might corroborate our findings. Min Shang et al. found macrosomia born from women with GDM were associated with increased placental mTOR activity in Chinese pregnant women [28]. Kary Tsai et al. concluded that regulation of the mTOR pathway was uniquely involved in the development of some obstetric complications including GDM [29]. What's more, Ke Xu et al. found that MiR-503 regulated functions of pancreatic β-cells by targeting the mTOR pathway, suggesting that targeting miR-503/mTOR axis could serve as a novel therapeutic target for GDM [30]. At the same time, Jie Wen and Xiaoxia Bai confirmed that the expression of mTOR in the GDM cell model was correlated with cell viability and cell apoptosis, including pancreatic islet β cells [31].

Pregnancy entails an increased demand for energy, including amino acids, to enable the fetus and placenta to grow. Thus, normal pregnancy might reduce the BCAAs levels in the circulation, potentially increase protein synthesis aimed at conservation and accretion of nitrogen by the woman and the fetus [32]. Increased levels of BCAAs, or other mitotoxic/lipotoxic metabolites from these amino acids, might increase risk for GDM. Two possible mechanisms might explain the relationship of BCAAs and GDM. Firstly, the higher levels of BCAAs might activate mammalian target of rapamycin complex 1 (mTORC1) through the phosphorylation of insulin receptor substrate 1 (IRS-1), leading to insulin resistance, and then increasing risk for GDM [33]. An experimental study found that BCAAs deprivation for 7 days could improve insulin sensitivity in wild type and insulin-resistant mice models, which is possibly mediated by decreasing mTOR pathway and increasing AMP-activated protein kinase (AMPK) signaling pathways [34]. Another possible mechanism is that the accumulation of precursor amino acids leucine, isoleucine and valine and their metabolites might result

in induction of oxidative stress, mitochondrial dysfunction, impaired insulin action, and ultimately to perturbation of glucose homeostasis [35, 36].

The present study had several strengths. Firstly, it was the first study to investigate the association between serum mTOR level and glucose metabolism. What's more, the cases and controls were matched by pregnant women's age in order to reduce the potential effects of individual's susceptibility and confounder. In addition, we not only collected detailed information of all participants on demographic characteristics, lifestyle factors and medical records, but also used the conditional logistic regression models to adjust for potential confounders and verify the stability of results. Several limitations should also be noted. Firstly, the possible causal relationship could not be established owing to the observational design. Moreover, the BCAA levels were influenced by dietary intake, but the information on dietary habits were not collected. Additionally, the small size of the study was another limitation. Lastly, since the participants in our study were enrolled from in Henan province of China, extrapolated results might not be an accurate description of the wider Chinese population.

Conclusions

In summary, our investigation confirmed the positive association between the serum level of mTOR and risk of GDM. And a certain range serum Val and Leu in second trimester of pregnancy were protective for the risk of GDM. These findings provided epidemiological evidence for prevention and control of GDM. However, future prospective researches on the correlation of circulation BCAAs and mTOR in GDM population with larger sample size and longer follow-up are still expected.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12884-024-06815-2.

Supplementary Material 1.

Acknowledgements

The authors thank nurses and participants for their contributions to the study. The authors acknowledge the financial support received from the Scientific and Technological projects in Henan province (Grant No. SBGJ202103090), the Key Research Programs for Institutions of Higher Education in Henan Province (Grant No.22A320058) and the Key Research and Development Project of Henan Province in 2022 (Grant No.221111310700).

Authors' contributions

Lingling Cui and Zhiqian Li carried out the analysis of data and interpretation of data and drafted the manuscript. Zhonglei Li and Xiaoli Yang participated in the study design, data collection, analysis of data and preparation of the manuscript. Jiaxin Li and Yingying Guo revised critically for the manuscript. Zhengya Zhang, Yuting Gao, Lina Ren and Huijun Zhou participated in the design and coordination of the research, and acquisition of data. Linlin Hua and Xinxin Liu carried out the study design, the analysis and interpretation of data and reviewed the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Scientific and Technological projects in Henan province (Grant No. SBGJ202103090), the Key Research Programs for Institutions of Higher Education in Henan Province (Grant No.22A320058) and the Key Research and Development Project of Henan Province in 2022 (Grant No.221111310700).

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Clinical Trial Ethics Committee of the Third Affiliated Hospital of Zhengzhou University, and the study had been registered with on the Chinese Clinical Trial Registry (ChiCTR2000028811). Informed consent was provided by all participants before they were recruited the study. All methods were carried out in accordance with relevant guidelines and regulations and data were analyzed anonymously.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Nutrition and Food Hygiene, College of Public Health, Zhengzhou University, Zhengzhou, Henan 450001, China. [2]Department of Nutrition, Huaihe Hospital of Henan University, Kaifeng, Henan 475000, China. [3]Department of Advanced Medical Research, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450001, China.

Received: 17 April 2023 Accepted: 10 September 2024
Published online: 02 October 2024

References

1. Kim C. Gestational diabetes: risks, management, and treatment options. Int J Womens Health. 2010;2:339–51.
2. Nezami N, Safa J, Eftekhar-Sadat AT, Salari B, Ghorashi S, Sakhaee K, et al. Lovastatin raises serum osteoprotegerin level in people with type 2 diabetic nephropathy. Clin Biochem. 2010;43:1294–9.
3. Wang H, Li N, Chivese T, Werfalli M, Sun H, Yuen L, et al. IDF diabetes atlas: estimation of global and regional gestational diabetes mellitus prevalence for 2021 by international association of diabetes in pregnancy study group's criteria. Diabetes Res Clin Pract. 2022;183: 109050.
4. Zhang Q, Cheng Y, He M, Li T, Ma Z, Cheng H. Effect of various doses of vitamin D supplementation on pregnant women with gestational diabetes mellitus: A randomized controlled trial. Exp Ther Med. 2016;12:1889–95.
5. Aittasalo M, Raitanen J, Kinnunen TI, Ojala K, Kolu P, Luoto R. Is intensive counseling in maternity care feasible and effective in promoting physical activity among women at risk for gestational diabetes? Secondary analysis of a cluster randomized NELLI study in Finland. Int J Behav Nutr Phys Act. 2012;9: 104.
6. Xin G, Du J, Wang YT, Liang TT. Effect of oxidative stress on heme oxygenase-1 expression in patients with gestational diabetes mellitus. Exp Ther Med. 2014;7:478–82.
7. Noctor E, Crowe C, Carmody LA, Kirwan B, O'Dea A, Glynn LG, et al. ATLANTIC-DIP: prevalence of metabolic syndrome and insulin resistance in women with previous gestational diabetes mellitus by International Association of Diabetes in Pregnancy Study Groups criteria. Acta Diabetol. 2015;52:153–60.

8. Yong HY, Mohd Shariff Z, Mohd Yusof BN, Rejali Z, Appannah G, Bindels J, et al. The association between dietary patterns before and in early pregnancy and the risk of gestational diabetes mellitus (GDM): Data from the Malaysian SECOST cohort. PLoS One. 2020;15: e0227246.
9. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab. 2009;9:311–26.
10. Fiehn O, Garvey WT, Newman JW, Lok KH, Hoppel CL, Adams SH. Plasma metabolomic profiles reflective of glucose homeostasis in non-diabetic and type 2 diabetic obese African-American women. PLoS One. 2010;5: e15234.
11. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, et al. Metabolite profiles and the risk of developing diabetes. Nat Med. 2011;17:448–53.
12. Chorell E, Hall UA, Gustavsson C, Berntorp K, Puhkala J, Luoto R, et al. Pregnancy to postpartum transition of serum metabolites in women with gestational diabetes. Metabolism. 2017;72:27–36.
13. Park S, Park JY, Lee JH, Kim SH. Plasma levels of lysine, tyrosine, and valine during pregnancy are independent risk factors of insulin resistance and gestational diabetes. Metab Syndr Relat Disord. 2015;13:64–70.
14. Bentley-Lewis R, Huynh J, Xiong G, Lee H, Wenger J, Clish C, et al. Metabolomic profiling in the prediction of gestational diabetes mellitus. Diabetologia. 2015;58:1329–32.
15. Shimobayashi M, Hall MN. Making new contacts: the mTOR network in metabolism and signalling crosstalk. Nat Rev Mol Cell Biol. 2014;15:155–62.
16. Dimasuay KG, Boeuf P, Powell TL, Jansson T. Placental responses to changes in the maternal environment determine fetal growth. Front Physiol. 2016;7:12.
17. Muralimanoharan S, Maloyan A, Myatt L. Mitochondrial function and glucose metabolism in the placenta with gestational diabetes mellitus: role of miR-143. Clin Sci (Lond). 2016;130:931–41.
18. Hung TH, Wu CP, Chen SF. Differential changes in Akt and AMPK phosphorylation regulating mTOR activity in the placentas of pregnancies complicated by fetal growth restriction and gestational diabetes mellitus with large-for-gestational age infants. Front Med (Lausanne). 2021;8: 788969.
19. Zhang Y, Liang Y, Liu H, Huang Y, Li H, Chen B. Paeoniflorin attenuates gestational diabetes via Akt/mTOR pathway in a rat model. Food Nutr Res. 2020;64:4362.
20. Capobianco E, Gomez Ribot D, Fornes D, Powell TL, Levieux C, Jansson T, et al. Diet Enriched with olive oil attenuates placental dysfunction in rats with gestational diabetes induced by intrauterine programming. Mol Nutr Food Res. 2018;62:e1800263.
21. Li G, Lin L, Wang YL, Yang H. 1,25(OH)2D3 protects trophoblasts against insulin resistance and inflammation via suppressing mTOR signaling. Reprod Sci. 2019;26:223–32.
22. Andersson-Hall U, Gustavsson C, Pedersen A, Malmodin D, Joelsson L, Holmäng A. Higher concentrations of BCAAs and 3-HIB are associated with insulin resistance in the transition from gestational diabetes to type 2 diabetes. J Diabetes Res. 2018;2018:4207067.
23. Allalou A, Nalla A, Prentice KJ, Liu Y, Zhang M, Dai FF, et al. A predictive metabolic signature for the transition from gestational diabetes mellitus to type 2 diabetes. Diabetes. 2016;65:2529–39.
24. Rahimi N, Razi F, Nasli-Esfahani E, Qorbani M, Shirzad N, Larijani B. Amino acid profiling in the gestational diabetes mellitus. J Diabetes Metab Disord. 2017;16:13.
25. Li N, Li J, Wang H, Liu J, Li W, Yang K, et al. Branched-chain amino acids and their interactions with lipid metabolites for increased risk of gestational diabetes. J Clin Endocrinol Metab. 2022;107:e3058–65.
26. Zhao L, Wang M, Li J, Bi Y, Li M, Yang J. Association of circulating branched-chain amino acids with gestational diabetes mellitus: a meta-analysis. Int J Endocrinol Metab. 2019;17: e85413.
27. Pappa KI, Vlachos G, Theodora M, Roubelaki M, Angelidou K, Antsaklis A. Intermediate metabolism in association with the amino acid profile during the third trimester of normal pregnancy and diet-controlled gestational diabetes. Am J Obstet Gynecol. 2007;196(65):e61-65.
28. Shang M, Wen Z. Increased placental IGF-1/mTOR activity in macrosomia born to women with gestational diabetes. Diabetes Res Clin Pract. 2018;146:211–9.
29. Tsai K, Tullis B, Jensen T, Graff T, Reynolds P, Arroyo J. Differential expression of mTOR related molecules in the placenta from gestational diabetes mellitus (GDM), intrauterine growth restriction (IUGR) and preeclampsia patients. Reprod Biol. 2021;21: 100503.
30. Xu K, Bian D, Hao L, Huang F, Xu M, Qin J, et al. microRNA-503 contribute to pancreatic beta cell dysfunction by targeting the mTOR pathway in gestational diabetes mellitus. Excli J. 2017;16:1177–87.
31. Wen J, Bai X. miR-520h Inhibits cell survival by targeting mTOR in gestational diabetes mellitus. Acta Biochim Pol. 2021;68:65–70.
32. Kalhan SC. Protein metabolism in pregnancy. Am J Clin Nutr. 2000;71:1249s–55s.
33. Wolfson RL, Chantranupong L, Saxton RA, Shen K, Scaria SM, Cantor JR, et al. Sestrin2 is a leucine sensor for the mTORC1 pathway. Science. 2016;351:43–8.
34. Xiao F, Yu J, Guo Y, Deng J, Li K, Du Y, et al. Effects of individual branched-chain amino acids deprivation on insulin sensitivity and glucose metabolism in mice. Metabolism. 2014;63:841–50.
35. Bridi R, Braun CA, Zorzi GK, Wannmacher CM, Wajner M, Lissi EG, et al. alpha-keto acids accumulating in maple syrup urine disease stimulate lipid peroxidation and reduce antioxidant defences in cerebral cortex from young rats. Metab Brain Dis. 2005;20:155–67.
36. Funchal C, Latini A, Jacques-Silva MC, Dos Santos AQ, Buzin L, Gottfried C, et al. Morphological alterations and induction of oxidative stress in glial cells caused by the branched-chain alpha-keto acids accumulating in maple syrup urine disease. Neurochem Int. 2006;49:640–50.

Publisher's Note