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Serum leucine as a potential biomarker of insulin resistance in diabetic, prediabetic, and leucine-supplemented individuals

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Abstract

Background and objective: Diabetes Mellitus type 2 (T2DM) represents a serious risk to public health known as hyperglycemia. High levels of serum leucine a branched chain amino acid have been associated with early insulin resistance and metabolic imbalances. This study investigates the relationship between serum leucine levels and insulin resistance, and to evaluate its potential as a biomarker alongside standard blood sugar markers in diabetic, prediabetic, and leucine-supplemented individuals undergoing training.

Methods: A cross-sectional study, conducted at the (Galiawa hospital for diabetes and endocrinology and (AKA Fitness) center)/ Erbil city / Iraq. A total of 155 participants, aged between 30 and 69, were included, composed of 46 with T2DM, 38pre-diabetic patients, 36 non-diabetic and 35 healthy young active training individuals who received leucine supplementation. Data was collected by interview questionnaire about; sociodemographic, life style and results of biochemical tests.

Results: High levels of serum Leucine were significantly increase with age, obesity (OR=2.4, 95% CI. 1.7 - 2.8) and increased risk of high fasting blood sugar (OR=1.14,95% of CI 1.09-1.18), HbA1c (OR=1.24, 95% of CI 1.12-1.37), and insulin resistance (OR= 1.06, 95% of CI 1.02-1.1) in diabetic and prediabetic. Excessive supplementation caused excess serum leucine levels and increase potential insulin resistance (OR=1.3, 95% CI I,07-1.6) with advancing age.

Conclusion: High serum leucine levels can cause insulin resistance in elderly, obese, prediabetic, and diabetic patients, suggesting its potential use as a biomarker for early diagnosis. A low dose of leucine supplementation should be considered in individuals undergoing training.

Keywords: Glycaemic markers, Amino acid, Leucine supplementation, Age, Obesity, Vigorous physical activity.

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Introduction

Diabetes and its precursor, prediabetes, represent significant public health challenges worldwide. Diabetes mellitus (DM) refers to a group of metabolic disorders marked by high blood sugar levels resulting from issues with insulin production or its effectiveness (1). Type 2 diabetes (T2DM) is a chronic condition characterized by insulin resistance and relative insulin deficiency, leading to elevated blood glucose levels. It is commonly associated with obesity, poor diet, physical inactivity, and genetic factors (2). Prediabetes, affecting 352 million globally, increases the risk of type 2 diabetes, necessitating effective interventions to improve glucose metabolism and insulin sensitivity (3).

Prediabetes is linked to obesity, especially abdominal or visceral fat, elevated triglyceride levels and/or low HDL cholesterol, as well as high blood pressure (4).

Leucine is an essential branched-chain amino acid (BCAA) that plays a pivotal role in protein synthesis, metabolic regulation, and insulin signaling. Protein-rich foods and exercise can increase blood leucine levels, which are often elevated in individuals with insulin resistance and type 2 diabetes (5). Inadequate protein intake, chronic illness, liver disease, metabolic disorders, and aging-related muscle mass decline can also decrease leucine levels (6).

There are several hypotheses regarding the role of leucine in the development and progression of diabetes. Some researchers found that leucine's has unique structure effectively stimulates the mammalian target of rapamycin (mTOR) pathway, crucial for protein synthesis and muscle growth (7). Leucine regulates blood sugar levels by enhancing insulin secretion and improving insulin sensitivity, while maintaining energy balance by promoting fatty acid oxidation and reducing fat accumulation (8).

Mechanistically, leucine is known to activate the mTORC1 pathway, which can impair insulin signaling and contribute to β-cell dysfunction. Furthermore, studies have shown that individuals with obesity and insulin resistance often exhibit increased circulating levels of leucine, suggesting a role in the pathogenesis of diabetes (9). Leucine induces insulin resistance through mTORC1 pathway activation, inhibiting insulin signaling, impairing glucose uptake, and promoting lipid accumulation and suppressing autophagy, exacerbating the condition (10). While leucine is not currently used as a standard diagnostic marker, its inclusion in metabolomic profiles may enhance early detection and risk assessment of T2DM, especially when

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used alongside traditional glucose-based tests (11).

Leucine supplementation at a dose of 5 grams per day, combined with resistance training, has been shown to improve insulin sensitivity and glucose metabolism in individuals undergoing training. This combination enhances muscle protein synthesis and facilitates better glucose uptake, thereby reducing insulin resistance and supporting overall metabolic health (12).

Leucine supplementation combined with resistance training has been demonstrated to improve insulin sensitivity and glucose metabolism by enhancing muscle protein synthesis and facilitating glucose uptake, thereby reducing insulin resistance and supporting overall metabolic health (13).

In 2007, type 2 diabetes became a major health problem in Iraq, affecting about 2 million people, or 7.43% of the population. Diabetes, can lead to serious health problems (damage to blood vessels) over time. These problems might take 10 to 20 years to show up, but sometimes they happen earlier, even in people who don't know they have diabetes (14) who studied levels of branched chain amino acids among diabetic patients and concluded that, more research is needed to find out if BCAAs can be used to help diagnose, monitor, or track the progress of diabetes and other metabolic diseases, early research in Iraq and the Kurdistan Region remains scarce, with no clinical trials or population-based studies directly addressing its metabolic effects (36). This highlights a significant gap and the need for local investigations to explore leucine's potential role in managing, prediabetic and type 2 diabetes, and also the role in other two groups which are controlled group participants with and without taking supplements. This study conducted to investigate the relationship between serum leucine levels and insulin resistance, and to evaluate its potential as a biomarker alongside standard blood sugar markers in diabetic, prediabetic, and leucine-supplemented individuals undergoing training.

Methods

Across-sectional analytical study. conducted at the (Galiawa technical hospital for diabetes and endocrinology and (AKA Fitness) center), 15 September 2024 to 31 March 2025.

Participant of the study: The study included 155 participants, and consisted of: 46 individuals with Type 2 diabetes (T2DM) including both newly diagnosed and those with an existing diagnosis, 38 individuals classified as prediabetic, 36 individuals free from DM type 2(FT2DM) (only 22% had hypertension) and 35 young healthy (no disease , normal weight were training participants) Which are the main character in our study with leucine supplementation(9-

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22 grams per day before exercise for 2-3 months). They are collected from 15 September 2024 to 15 December 2024. Participants aged between 30 and 69 years of both genders.

Exclusion criteria: Individuals with a history of other endocrine disorders (Thyroid disorder, Parathyroid disorder, Adrenal disorder, Reproductive disorder). Participants with acute illness that could currently undergoing treatment with medications known to influence blood glucose levels, such as corticosteroids or antipsychotics. pregnant or breastfeeding women. also, those who had undergone recent surgery or had significant weight changes in the last three months.

Method and tool of data collection: Data was collected by interview questionnaire constructed by researcher, and composed of the following parts: socio demographic data, medical history, family history. Body Mass Index and physical activity classified according to WHO.

Biochemical Tests: include fasting blood sugar (FBS), Random blood sugar (RBS), Glycated hemoglobin (HbA1c), Serum insulin, Insulin resistance (IR), and serum leucine

Leucin Determination Method: was done using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Double antibody sandwich technique). Normal range of serum leucine of the kit used in analysis ranged between 45 to 150 µmol/L (Mybiosource.com). (plasma) leucine normal range (54.9–205.0 µmol/L) based on age and LabCorp's data 2025.150 µmol/L is well within the normal range for all age groups

Ethical consideration: The permission from the college of Health science/ Hawler Medical University was obtained before data collection. In addition, informed consent obtained from participants (they were free to participate in this study after clarifying and explaining the objective of the study.

Statistical analysis: The Statistical Package for Social Science software (SPSS Version 26) was used for data processing and statistical analysis, include (Frequency & percentage and mean value), Data were tested for normality using the Shapiro–Wilk test. Due to variability between groups (cases and T2DM and supplement group), some data were not normally distributed. Therefore, non-parametric tests (Kruskal–Wallis and Mann–Whitney U) were used for group comparisons, beside ANOVA test for comparison variables with normal distribution. Logistic regression was used for B coefficient and odds ratio determination. P-value of 0.05 and 0.01 are considered significant and high significant respectively.

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Results

Sociodemographic property of diabetic, prediabetic, FT2DM and supplement group:

Age ranged between 30-69 years with mean ±SD (49.9 ±10. 1). Categorized to four groups. Table 1 showed the diabetic group, 37% were aged 59–69, 52.2% lived in rural areas, 60.9% were female, and 34.8% were illiterate, reflecting older age, rural residence, and lower education levels. The prediabetic group showed a more balanced distribution, with 34.2% aged 50–59, 78.9% urban residents, 52.6% females, 42% employed and 39.5% having secondary education. In the control group, 30.6% were aged 30–39, 66.7% lived in urban areas, 52.8% were female, 50% employed and 41.7% had secondary education, while the leucine-supplemented group was primarily urban (100%), employed (100%), aged 30–39 (88.6%), with 74.3% having university or institute degrees.

Table 1. Sociodemographic properties of the DM, Pre DM, T2DM and supplement group

		Diabetic		Prediabetic		FT2DM		Supplement with Leucine	
		F.	%	F	%	F	%	F.	%
Age Groups	30-39	3	6.5	9	23.7	11	30.6	31	88.6
	40-49	11	23.9	11	28.9	11	30.6	4	11.4
	50-59	15	32.6	13	34.2	10	27.8		
	59-69	17	37.0	5	13.2	4	11.1		
Gender	Male	18	39.1	18	47.4	17	47.2	18	51.4
	Female	28	60.9	20	52.6	19	52.8	17	48.6
Residence	Rural	24	52.2	8	21.1	12	33.3	0	0.0
	Urban	22	47.8	30	78.9	24	66.7	35	100.0
Occupation	Employed	13	28.3	16	42.1	18	50.0	35	100.0
	Non-Employed	25	54.3	15	39.5	14	38.9		
	Retired	8	17.4	7	18.4	4	11.1		
Education	Primary	10	21.7	6	15.8	6	16.7	8	22.9
	Secondary	7	15.2	15	39.5	15	41.7	26	74.3
	University and Institute	13	28.3	13	34.2	13	36.1		
	Illiterate	16	34.8	4	10.5	2	5.6	1	2.9

F= Frequency

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Life style factors

Life style factors of the participants (DM, Pre DM, FT2DM and supplement group)

Table 2 shows that 52.2% of individuals with type 2 diabetes also had hypertension. In contrast, 71.1% of prediabetic individuals and 77.8% of those free from diabetes had no chronic diseases. Regarding medication use, 63% of individuals with T2DM were using metformin, while 73.7% of prediabetics and 83.3% of diabetes-free individuals were not using any medications. In terms of family history, 30.4% of T2DM patients, 26.3% of prediabetics, and 30.6% of diabetes-free participants reported that both parents had diabetes. Smoking patterns indicated that passive smoking was the most common form across all groups, particularly among diabetics (65.2%) and prediabetics (57.9%). Obesity was prevalent in 69.6% of individuals with DM and 63.2% of prediabetics, while 56.2% of the diabetes-free group were overweight. Sedentary lifestyle was common in all groups, affecting 69.6% of DM patients, 71.1% of prediabetics, and 63.9% of those without diabetes The leucine-supplemented group demonstrated the healthiest lifestyle, with 100% having normal BMI and engaging in hard physical activity, and none reporting chronic diseases Although 62.9% passive smokers, 22.9% had no family history of diabetes.

Glycemic markers among all participant:

Normal value of serum fasting blood glucose is ranged between (75-115) mg/ dl according to the kit used in analysis.

Table 3 showed significant differences between groups in mean value of FBS, HbA1c serum and insulin resistance. Diabetic and pre-diabetic groups, had significantly higher FBS levels and FT2DM compare to supplement group.

Glycated hemoglobin (HbA1c) is a key marker for long-term glycemic control; there was significant difference within groups except FT2DM with the group of supplements. Serum insulin showed no difference among groups. There was significant difference of FT2DM with diabetic group, and prediabetic in insulin resistance and not differ with supplement group.

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Table 2. Life style factors of the study participants

		Diabetic		Prediabetic		Free diabetic individual		Supplement group	
		F.	%	F.	%	F	%	F	%
Med History	No disease	0	0.0	27	71.1	28	77.8	35	100
	Diabetic	22	47.8	0	0.0	0	0.0		
	Hypertension	0	0.0	11	28.9	8	22.2		
	Diabetic + hypertension	24	52.2	0	0.0	0	0.0		
Drug	Insulin	14	30.4	1	2.6	1	2.8	35	100
	Metformin	29	63.0	8	21.1	3	8.3		
	Both	3	6.5	1	2.6	2	5.6		
	No drug	0	0.0	28	73.7	30	83.3		
Family History	Mother	7	15.2	10	26.3	8	22.2	6	17.1
	Father	7	15.2	4	10.5	8	22.2	7	20.0
	Both	14	30.4	10	26.3	11	30.6	7	20.0
	Sister	3	6.5	1	2.6	1	2.8	4	11.4
	Brother	1	2.2	2	5.3	1	2.8	3	8.6
	All	5	10.9	1	2.6	1	2.8	8	22.9
	None	9	19.6	10	26.3	6	16.7		
Smoking	Current Smoker	7	15.2	11	28.9	7	19.4	13	37.1
	Passive Smoker	30	65.2	22	57.9	22	61.1	22	62.9
	Ex-Smoker	9	19.6	5	13.2	7	19.4		
BMI	Under weight	0	0.0	0	0.0	0	0.0		
	Normal	1	2.2	10	26.3	9	25.0	35	100.0
	Over weight	13	28.3	4	10.5	20	55.6		
	Obese	32	69.6	24	63.2	7	19.4		
Physical Activity	Hard work	0	0.0	0	0.0	1	2.8	35	100.0
	Moderate	14	30.4	11	28.9	12	33.3		
	Sedentary	32	69.6	27	71.1	23	63.9		

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Table 3. Levels of Glycemic markers among groups and differences within groups

	F	Mean± SD	Kruskal test	Mean Rank	P-value	Mann-Whitney (U) FT2DM within groups FT2DM	Groups	P-value
Fasting Blood Sugar (75-115) mg/ dl								
Diabetic	46	176.4± 38.9	113.2	130.1	<0.01**	6	Diabetic	<0.01**
Pre-Diabetic	38	121.6± 20.2		83.71		237	Pre-Diabetic	<0.01**
Free T2DM	36	100.34± 1.5		49.93				
Supplement G	35	91.14± 12.4		32.2		71.5	Supplement	<0.01**
Total	155	126.04 ± 2.4						
Serum Insulin 2–10 µIU/mL normal								
Diabetic	46	12.9 ± 8.4	5.84	89.1	0.12	638.5	Diabetic	0.77
Pre-diabetic	38	10.5 ± 4.7		81.1		585.5	Pre-diabetic	0.29
FT2DM	36	9.9 ± 5.4		71.2		587.5	Supplement	0.63
Supplement G	35	9.1 ± 3.9		67.2				
Insulin resistance (IR) 1-2.5 Normal								
Diabetic	46	5.78±3.83	51.9	113.5	<0.01	255	Diabetic	<0.01**
Pre -Diabetic	38	3.28±1.83		81.6		466	Pre-Diabetic	0.018*
FT2DM	36	2.5 ± 1.81		58.4		543.5	Supplement	0.32
Supplement G	35	1.98 ± 0.92		47.6				
<5.7% is normal HbA1c					Post Hoc-LSD test for differences of FT2DM within groups			
	F	Mean ±SD	ANOVA F-test	P-value	FT2DM	Groups		P-value
Diabetic	46	8.73± 1.5	166.7	<0.01		Diabetic		<0.01**
Pre-Diabetic	38	6.14± 0.2				Pre-Diabetic		<0.01**
FT2DM	36	5.22± 0.37				Supplement G		0.436
Supplement G	35	5.06± 36						
Total	155	6.45± 1.75						

We applied Kruskal Wallis test for all groups and A Mann–Whitney test U value for FT2DM within groups, because FBS, Insulin and Insulin resistance have abnormal statistical distribution. F = Frequency and G = Group, * = P-value ≤ 0.05 is significant, **= P value ≤ 0.01 is highly significant

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Amino acid Leucine

Mean value of leucine equal to 85.67 ±34.35 µmol/L. Statistical analysis showed high significant differences between groups. Free T2DM group has low level of leucine and differ high significantly from other groups, (Table 4).

Association of leucine with sociodemographic and life style of DM, Pre DM, and FT2DM.

There was a significant association between leucine levels, age, and employment. Low leucine levels (<54.9 µmol/L) were more common in the 30–49-year age group compared to old age groups, also increased among employed individuals (FT2DM). Additionally, the low levels (<54.9 µmol/L) of leucine increased significantly with normal and overweight body mass index (BMI) which involve mostly group FT2DM, (Table 5).

Table 4. Levels of amino acid leucine among groups and their differences

Groups	F	Mean±SD µmol/L	Mean Rank	Kruskal-Wallis	P-value	Mann-Whitney U value within groups FT2DM	Groups	P-value
Diabetic	46	87.1 ± 4.7	97.50	142.55	<0.001**	0.0	Diabetic	<0.001**
Pre-Diabetic	38	69.81± 11.4	54.42			41	Pre-Diabetic	<0.001**
Free T2DM	36	47.6 ± 10.4	19.64					
Supplement G	35	140.2± 13.6.				0.0	Supplement G	<0.001**
All groups	155	85.67 ± 34.35						

F= frequency, **= P value ≤ 0.01 is highly significant., G= Group

Table 5. Association of leucine with sociodemographic and life style properties of (DN, pre-DM, and FT2DM)

Leucine µmol/L	Properties				Spearman Correlation	P-Value	Logistic regression OR	95% CI	P-value of regression
	Age groups								
	30-39	40-49	50-59	>59					
< 54.9	9	12	7	2	0.28	<0.001***	7.7	1.46- 40.9	0.016*
54.9-150	14	21	31	24			6.9	1.4- 34.2	0.019*
	Occupation								
	Employed	No	Retired		0.199	0.037*			
< 54.9	17	10	3						
54.9-150	30	44	16						
	Normal	Overweight	Obese						
< 54.9	9	15	6		15.5	<0.001***	6.3	1.8- 21.8	<0.01**
							6.5	2.2- 18.8	<0.01**
54.9-150	11	22	57						

* is significant and P-value ≤0.05, ***= is very highly significant and P value ≤ 0.001

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Association of Leucine with glycemic markers among diabetic, prediabetic and Free T2DM group

Table 6 shows a statistically significant association between fasting blood sugar (FBS), Elevated plasma leucine levels were significantly linked to an increased risk of abnormal glycemic status. Random blood sugar (RBS), and HbA1c with the amino acid leucine. Specifically, high FBS and RBS levels are significantly associated with high leucine levels. Similarly, abnormal HbA1c levels increase with increase leucine concentrations. Additionally, optimal insulin sensitivity decreases as leucine levels increase and there is increase of insulin resistance compare to optimal sensitivity by increase in leucine levels.

Table 6. Association of Leucine with glycemic markers diabetic, prediabetic and free T2DM

Amino acid	Biochemical parameter	Spearman correlation	Sig	OR	95% CI	P-value of regression
Leucine	FBSG	0.59	<0.001**	1.14	1.09-1.18	<0.001***
	RBSG	0.54	<0.001**	1.12	1.08- 1.17	<0.001***
	HbA1c	0.73	<0.001**	1.24	1.12- 1.37	<0.001***
	IR	0.33	<0.001**	1.06	1.02- 1.1	0.006**

*** Very highly significant, P value ≤0.001

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Association of Leucine with sociodemographic and glycaemic markers among the group with supplementation

Mean value was 140.2± 13.6. and has normal distribution statistically. The analysis showed significant association of amino acid leucine with ages and education status. High levels of leucine >150 µmol/Lincrease with increasing age and levels of education Table 7.

The result showed a significant association of leucine with FBS, RBS, and HbA1c, and their levels increased significantly by increasing leucine level. Optimal insulin levels (<5–10 mIU/L) decreased with increasing leucine. Also, insulin resistance increased with increasing leucine level.

Table 7. Association of Leucine with sociodemographic and glycaemic markers in supplementation group

	Properties	**Pearson s R**	**P-value**	**Multinomial regression**		**P-value of regression**
				OR	**95% CI**	
Leucine	Age	0.36	0.036*	1.24	1-1.6	0.049*
	Education	0.39	0.020*	11.3	1.9-20.6	0.020*
	GI		**P-value**	**β-coefficients**	**95% CI**	
Leucine	FBSG	0.54	0.001**	0.49	0.22-0.76	0.001**
	RBSG	0.42	0.013*	0.423	0.095- 0.75	0.013*
	HbA1c	0.92	<0.001**	0.24**	0.21-0.28	<0.001**
	Insulin	0.49	0.003**	0.14**	0.051- 0.29	0.003**
	Insulin resistance	0.52	0.001***	OR	95% CI	
				1.3	1.07-0.1.6	0.01**

* P ≤0.05 is significant, ** ≤0.01is highly significant,<0.001*** very high significant

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Discussion

The diabetic group was mainly composed of older adults, rural residents, and individuals with lower education levels, reflecting a higher vulnerability linked to limited access to health resources. The prediabetic group showed a more even distribution in age and gender, with most participants living in urban areas and having moderate educational backgrounds as shown in table 1 (15), who also observed a balanced distribution in age and gender among prediabetic individuals, particularly in urban settings with moderate educational levels. The group free of DM2 shared similar urban and educational characteristics but included more young adults, indicating a potentially lower risk profile. The leucine-supplemented group had the most favourable sociodemographic traits, being young, urban, employed, and highly educated, which may enhance the effectiveness of preventive interventions (16).

The treatment of type 2 diabetes mellitus (T2DM) as shown in table 2 with metformin, suggesting that the patient likely has early to moderate stage T2DM, managed without insulin, often in combination with dietary and lifestyle interventions (17). Also showed that obesity, and sedentary life style are risk factor for diabetes and prediabetes. This result conforms (15) who concluded that participants in Erbil city were physically inactive and, high BMI and high body fat are risk factors of metabolic syndrome which is a known precursor to both prediabetes and type 2 diabetes in Erbil city. The significant elevation in FBS among diabetic and pre-diabetic groups and elevated HbA1c levels confirm impaired glycaemic control as shown in table 3. This study aligns with another research in Kurdistan Region showed significant fluctuations in FBS among diabetic patients, with marked differences between fasting and post-meal values (19) and also aligns with a study conducted in Kurdistan region, which reported a significant elevation in fasting blood glucose levels among pre-diabetic individuals, reinforcing the presence of early glycaemic dysregulation within this population (20).

Table 3 also reveals difference insulin resistance between the FT2DM group and the diabetic group. These findings align with the study conducted by (Rashad B, et al) (21) who reported that elevated insulin levels, increased insulin resistance (via HOMA IR), higher glucose and HbA1c values, and greater BMI among males with complications from uncontrolled type 2 diabetes. These results are attributed to older age and increased abdominal obesity—both of which tend to rise with age in diabetic patients. Furthermore, it is consistent with the study conducted by (Abbas F, et al) (22) who found that diabetes and

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impaired fasting glucose were significantly associated with higher age, BMI, and waist circumference as risk factors.

The group receiving leucine supplementation also showed normal glucose status and lower insulin resistance compared to both the diabetic and pre-diabetic groups. This result may be attributed to the younger age (88.6% were in age group 30-40 year) of this group, which is consistent with the findings of (Croall ID, et al) (23) who hypothesized that age is an important risk factor for Type 2 diabetes mellitus (T2DM). Additionally, this group maintained a normal weight and engaged in vigorous physical activity, aligning with the findings of (Fazeli PK, et al) (24) who demonstrated that sedentary behaviour is associated with an increased risk of T2DM and impaired glycaemic homeostasis, potentially mediated by obesity, lipid metabolism disorders, and lower educational attainment.Additionally, the effect of supplementation on serum leucine levels depends on both the dose and duration of supplementation, as well as its influence on glucose metabolism. Although glucose levels may initially remain within the normal range, as seen in FT2DM), elevated leucine levels may still indicate early signs of metabolic imbalance or a predisposition to impaired glucose regulation. Importantly, serum leucine levels tend to increase gradually with age and over time, which may contribute to a more noticeable impact on metabolic health. This trend is consistent with findings from the study of (Jacob KJ, et al) (25), suggesting that higher doses and longer durations of exposure may be necessary to produce measurable effects on glucose homeostasis and insulin resistance—possibly through mechanisms such as mTOR/S6K1 pathway activation.

Statistical analysis table 4 revealed a significant difference among groups in mean value of leucine. Free T2DM group showing the lowest mean leucine level compared to all others. Notably, the leucine-supplemented group exhibited the highest levels, (140.2± 13.6) confirming the effectiveness of supplementation as a direct consequence of exogenous leucine intake, this result conforms the study of (Jia H, et al) (26) who found, higher dietary BCAAs were significantly associated with higher plasma levels of these amino acids and (Mirmiran P, et al) (27) who concluded that higher leucine intakes were associated with increased plasma leucine, The free T2DM group had the lowest serum leucine levels likely because they did not receive supplementation and had no underlying metabolic stress that might elevate amino acid levels. Unlike diabetic and pre-diabetic individuals, who may experience altered protein metabolism or increased amino acid circulation due to insulin resistance,

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healthy controls maintain more balanced amino acid regulation. This study aligns with the study conducted by (Chapman-Lopez TJ, et al) (11) which reported elevated branched-chain amino acids (BCAAs), including leucine, in individuals with type 2 diabetes due to impaired metabolism and insulin resistance. This supports the idea that diabetic and pre-diabetic individuals have altered protein and amino acid metabolism, contributing to higher leucine levels.

A significant association between serum leucine levels with age and employment status and lifestyle factors (BMI and medical history) among diabetic, pre-diabetic, and free T2DM groups as shown in table 5. Low plasma leucine levels (<54.9 µmol/L) were more frequently observed among individuals aged 30–49 years in the non-diabetic group who did not receive leucine supplementation. Most of these individuals were employed suggesting that lower leucine levels in mid-life associated to efficient metabolism and high insulin sensitivity, which promotes rapid uptake of leucine into muscle for protein synthesis, resulting in lower circulating levels (Crozier SJ, et al) (28) who reported BCAAs serve anabolic roles, being rapidly taken up for protein synthesis via mTOR activation, thereby not accumulating in the blood.

Higher BMI, especially in obese individuals, was significantly associated with normal or elevated leucine levels as well as with medical history reflecting altered amino acid metabolism in those with excess weight and disease of diabetes, and potentially due to changes in insulin sensitivity and protein metabolism. This was in line with the study conducted by (Yoshihara T, et al) (29) who concluded that increased BCAA plasma level is associated with a high risk of metabolic disorder and future insulin resistance, or type 2 diabetes mellitus (T2DM). The activation of mammalian target of rapamycin complex 1 (mTORC1) by BCAAs has been suggested to cause insulin resistance. In addition, defective BCAA oxidative metabolism might occur in obesity, leading to a further accumulation of BCAAs and toxic intermediates. Significant association between serum leucine levels and glycaemic indicators as shown in table 6, Low leucine levels were significantly linked to normal FBS, RBS, and HbA1c, suggesting better glycaemic control in individuals with reduced leucine concentrations. The study conducted by (Xu B, et al) (14) and (Yoon MS) (30) found that BCAA were linked with higher levels of HbA1c, insulin, C-peptide, and insulin resistance. This may help explain how BCAAs are connected to insulin resistance in people with type 2 diabetes. High leucine levels association with increased insulin resistance may due to deficiency of enzymes involved in metabolism of leucine and consistence with the study conducted

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by (Li N, et al) (31) who attributed high insulin levels can due to reduction in the activity of two key enzymes: branched-chain aminotransferase (BCAT) and branched-chain keto acid dehydrogenase (BCKDH), which are responsible for breaking down branched-chain amino acids (BCAAs). People with type 2 diabetes mellitus (T2DM) often have lower levels of these enzymes. As a result, BCAAs can build up in the body, possibly worsening insulin resistance. Another explanation is that leucine can activate the mammalian target of rapamycin complex 1 (mTORC1). This complex is sensitive to the cell's energy levels. When activated, mTORC1 may block insulin signalling by affecting a protein called insulin receptor substrate-1 (IRS-1), leading to increased insulin resistance (7).

The group receiving leucine supplementation as shown in table 7, serum leucine levels were significantly associated with age and education level. Higher leucine concentrations were observed among older individuals, indicating a potential age-related change in leucine metabolism or retention (As people age, muscle mass often declines (sarcopenia), and metabolic efficiency can decrease, which may slow down the breakdown or use of amino acids like leucine, leading to higher circulating levels, especially when supplemented. Additionally, hormonal and enzymatic changes with age may alter the pathways that regulate amino acid transport, oxidation, or incorporation into proteins (32).

Additionally, individuals with higher levels of education had significantly increased leucine levels, which may reflect differences in health behaviours, awareness, or adherence to supplementation protocols. These findings suggest that both age and educational status can influence the response to leucine supplementation (33).

In the leucine-supplemented group, levels of glucose status were normal not differ with FT2DM. A significant association of leucine levels with glycaemic markers FBS, RBS, HbA1c, and there is increase in normal glucose levels by increasing serum leucine. While57% had border line and high insulin resistance and there is risk of increase insulin resistance by increase leucine level and aging. This result may due to prolonged and excessive intake along with aging which can impair insulin signalling even in healthy individuals and consistence (34) who concluded despite normal fasting and random glucose levels, physically active individuals or athletes with elevated serum leucine may exhibit early signs of insulin resistance. This condition, known as normoglycemic insulin resistance, can occur due to impaired BCAA oxidation, chronic activation of mTOR

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signalling, and mitochondrial stress. This finding suggests that chronic leucine oversupply, especially through supplementation, may disrupt metabolic flexibility in athletes—potentially leading to insulin resistance despite preserved glucose homeostasis. Therefore excessive supplementation must be considered because suppress serum leucine intake by muscle due to accumulation its catabolism products which affect insulin signaling. This result also in line with (35) who concluded that leucine is primarily metabolized in the mitochondria of skeletal muscle, especially during periods of physical activity. However, chronic and excessive leucine supplementation may surpass the body's capacity for oxidation and utilization. This overload can result in the accumulation of metabolic intermediates such as 3-hydroxyisobutyrate (3-HIB) and branched-chain keto acids (BCKAs). these intermediates may promote lipotoxicity, oxidative stress, and mitochondrial dysfunction—key mechanisms underlying the development of insulin resistance (37).

Conclusion

High serum leucine, as a consequence of metabolic stress, obesity, and old age, inhibits the insulin signalling pathway and caused insulin resistance in the study samples of prediabetic and diabetic individuals. Leucine can be used as potential biomarker for diagnosis as it is significantly associated with abnormal levels of glycaemic biomarkers (FBS, RBS, HbA1c), insulin resistance. Controlled, low doses of leucine can stimulate protein synthesis and support muscle growth, particularly when combined with resistance training.

Recommendation

More research is required to generalize the results. Leucine may serve as an early biomarker of metabolic imbalance associated with diabetes and prediabetic individuals, even in the absence of a formal diagnosis. It appears to have biphasic effects—beneficial at moderate levels but potentially harmful when elevated. Monitoring leucine levels and using dose-controlled supplementation is recommended to avoid metabolic disturbances**.**

Competing interest

The authors declare that they have no competing interests.

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