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LETTERS

Association of C-peptide with novel hormones in children with type 1 diabetes: A rising potentials for more reliable biomarkers

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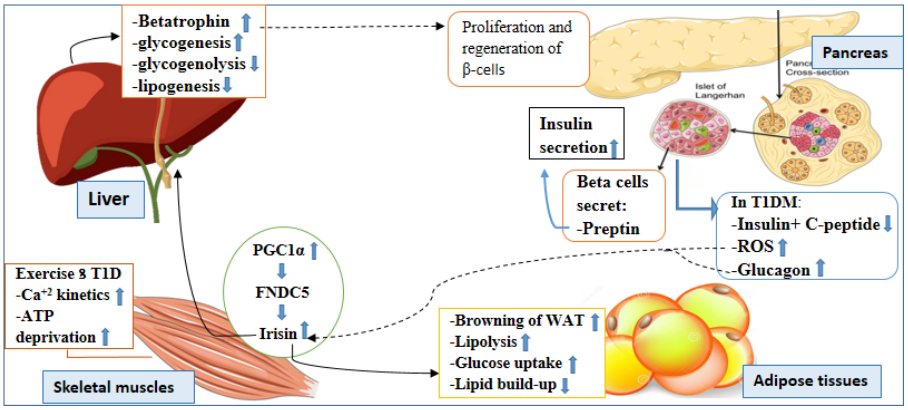
Article



ABSTRACT

Type 1 diabetes is a heterogeneous disorder caused by reduced β-cell mass as a result of T-cell mediated autoimmune destruction. C-peptide is a linker chain cleaved from proinsulin to produce the mature, functional insulin hormone. Irisin is a novel adipo-myokine plays a crucial role in glucose homeostasis regulation. Preptin is a peptide hormone synthesized in β-cells and plays a role in augmenting insulin secretion. The current study aims to investigate preptin and irisin levels in diabetic children and determine their correlation with C-peptide and the development of this disease. This study recruited 90 children, divided into two groups: 45 patients and 45 controls. Commercial ELISA kits were used to measure C-peptide, irisin, and preptin. C-peptide levels were significantly decreased among the patients' group (P<0.05). Preptin and irisin levels were significantly increased in the patients' group (P<0.05). C-peptide was noticeably correlated with preptin, irisin and RBS (P <0.05). Preptin and irisin levels also had a positive correlation with RBS (P<0.05). In regression analysis, irisin had a strong association with C-peptide. In conclusion, irisin was a considerable predictive marker for the residual β-cells through its association with preptin and C-peptide in regression analysis. Preptin might be an indicator of insulin resistance.

Keywords: C-peptide, Preptin, Irisin, Diabetes, Biomarkers

INTRODUCTION

Type 1 diabetes mellitus is a chronic multifactorial metabolic disease characterized by hyperglycaemia and metabolic imbalance, mostly affects children, adolescents, and young adults. The auto-reactive T-lymphocytes are considered a key pathogenic trigger of pancreatic β-cells destruction that subsequently leads to insulin autoimmune deficiency.[1] The reduced β-cell mass and insulin deficiency result in hyperglycemia which eventually results in numerous metabolic derangements after a long period. Persistent hyperglycemia results in the activation of the polyol pathway that, in turn, leads to the formation of reactive oxygen species (ROS), and subsequently osmotic stress, glycation of proteins, and vascular impairment. These pathways lead to the development of micro- and macrovascular complications (i.e. retinopathy, nephropathy, neuropathy, cardiovascular disease, cerebrovascular disease, foot ulcers, and amputations of the lower extremities).[2,3] Different risk factors are implicated in the development of this disease including genetic susceptibility, autoimmunity, and environmental factors.[4] T1D incidence and prevalence have increased recently, with a global incidence rate of 15/100,000 population and a prevalence rate of 9.5/10,000 people.[5] In Iraq, the prevalence of T1DM was found to be 159 per 100,000.[6] Since patients with type 1 diabetes are prone to metabolic disturbances, it is crucial to find a new therapy that aids in glucose homeostasis regulation with insulin, thus reducing the global burden of this disease.

C-peptide is a connecting chain composed of 31-amino acids, cleaved from proinsulin to produce the functional and mature insulin hormone in pancreatic β-cells. For decades, C-peptide was believed to be an inactive molecule; however, in nineties, it was discovered to have numerous beneficial effects on different

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cell types and tissues. In clinical practice, C-peptide currently mirrors the functionality of beta-cells. Alongside its stability, C-peptide has distinguished features that are represented in its ability to evade first-pass metabolism, having a longer half-life than insulin by 25 minutes, and being secreted in equimolar quantities to insulin, which makes it a reliable biomarker for the vital β-cells function.[7]

Preptin, on the other hand, is a pancreatic hormone synthesized primarily in beta cells and released synchronously with insulin in response to increased blood glucose levels. It can be also secreted from other organs such as the liver, kidney, salivary gland, and mammary tissue.[8] The main metabolic action of preptin is the stimulation of insulin secretion that is not confined in amplifying pathway but also in the triggering pathway through a calcium dependent signaling (figure 1).[9] This hormone is cleaved from proinsulin-like growth factor-II (pro-IGF-II) by proteases at the 21st phenylalanine amino acid fragment, acting on the IGF-II receptor of the β-cells.[10]

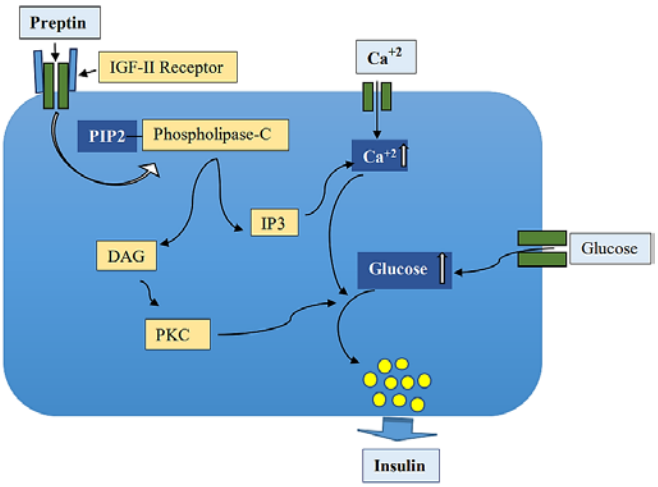

Figure 1. Regulation of insulin secretion by preptin. PIP2-Phosphatidylinositol 4,5-bisphosphate, DAG-diacylglycerol , IP3-inositol trisphosphate, PKC-protein kinase-C.

Irisin is a novel exercise hormone secreted primarily from skeletal muscle in response to physical activity and exercise. It has an important function in the stimulation of muscle growth and regulation of energy expenditure, along with a critical role in glucose homeostasis regulation.[11] The main mechanistic pathways through which irisin elicits its functions are the mitogen-activated protein kinase (MAPK) and ERK1/2 pathways.[12] This polypeptide hormone is produced from the cleavage of fibronectin type III domain-containing protein 5 (FNDC5), which is a membrane-bound protein mainly found in muscles and white adipose tissues. The synthesis of FNDC5 is controlled by a master regulator of metabolic genes, 'peroxisome proliferator activated receptor γ co-activator 1α (PGC1α)', figure (2).[13] As irisin is known to stimulate insulin secretion, β-cell regeneration, β-cell survival, and protection from apoptosis, it is proposed to be used as a therapeutic agent in patients with metabolic diseases such as diabetes.[14]

Regulation of energy expenditure by irisin is represented by its ability to stimulate the browning of white adipose tissues (WAT) through the p38-MAPK-UCP1 pathway. The distinctive features of brown adipose tissues (BAT) are the higher energy expenditure, the presence of higher levels of mitochondria due to increased O_2 consumption, the accumulation of fewer amounts of lipids, and the higher content of GLUT4 than WAT.[12] Therefore, browning of WAT by irisin through the p38-MAPK-UCP1 pathway helps to increase glucose uptake.

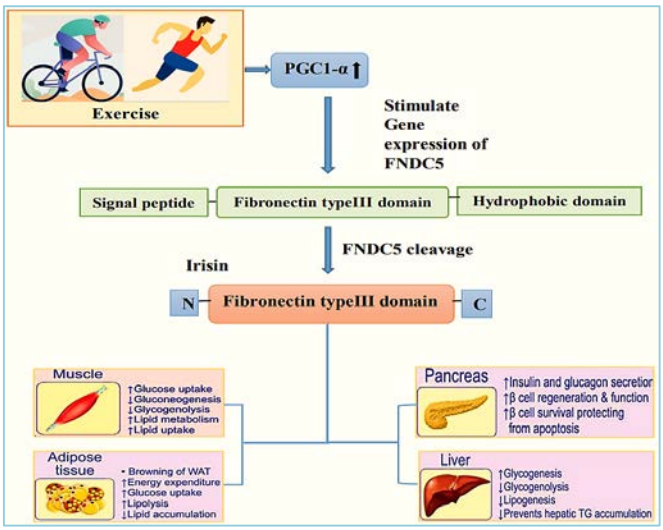

Figure 2. Synthesis of irisin. WAT-white adipose tissue.

The identification of irisin receptor is still a challenge; however, recent studies suggest that the αV family of integrin receptors are likely to be the irisin receptors in thermogenic fats and osteocytes.[15]

MATERIALS AND METHODS

In this case-control study, 90 children were divided into two groups: 45 patients and 45 apparently healthy volunteers. The diabetic patients were taken from the diabetes center of Marjan City Hospital and Babylon Teaching Hospital for Children and Maternity in Babylon Province. Venous blood samples were collected in gel tubes and centrifuged at 3000 xg for 20 minutes, then stored in the deep freezer of the central blood bank at -60 C°. The measurement of random blood sugar was performed manually by a calorimetric method using a spectrophotometer (glucose kit, Taytec, Canada). Hemoglobin A1C was measured by an automated analyzer (HbA1C Turbidimetric, Linear, Cromatest, Barcelona, Spain).

The investigation of C-peptide, preptin, and irisin was performed using an ELISA technique (Bioassay Technology Laboratory, China). This type of enzyme-linked immunosorbent assay is a sandwich technique, where the plate is pre-coated with a specified antibody for each protein, instead of marker. The principle of this technique is based on an antigen-antibody reaction, in which the indicated protein in the serum reacts with the specified antibody coated on the plate. Subsequently, a biotinylated human antibody is added to bind the human protein, followed by the addition of streptavidin-HRP and then incubated

for 60 min at 37 C^{O}. After the incubation, any unattached antibodies were removed by washing the plate five times with a wash buffer in an automated washer. Then, a substrate solution was added to each well, which was accompanied by a colour development that was proportional to the protein amount in the sample. The plate was incubated for 10 minutes at 37 ℃in the dark. After incubation, an acidic stop solution was added to terminate the reaction, and the optical density was measured at 450 nm.

Inclusion and exclusion criteria

The patient group in the current study included T1DM patients (already diagnosed by paediatric diabetologist) under age 17 without diabetes complications. Patients who developed complications, with thyroid dysfunction, liver disease, growth hormone deficiency, Cushing syndrome, T2DM, and nephrotic syndrome were excluded.

The apparently healthy volunteers were taken without apparent disorders in the liver, skin, kidneys, or having coeliac disease.

Ethical approval: Based on the Declaration of Helsinki's ethical principles, this study was agreed upon it. Verbal agreement was obtained from the parents of all participants before sample collection. The protocol of the study, the information about the subjects, and the consent form were reviewed and approved by a local ethics committee according to document number 14 on July 6, 2022 to get this approval.

Statistical analysis

The statistical tests were performed using SPSS v. 28 (IBM, version 28.0). The data were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The comparison between groups is expressed as mean ± standard deviation for normally distributed data and as median (minimum-maximum) for non-normally distributed data using the Student's t-test and Mann-Whitney U test, respectively. Results with extreme values were excluded. Levels of preptin were transformed by inverse distribution to obtain normally distributed data for comparison. Spearman correlation was used to find the association between variables, because the data are mostly non-normally distributed. A linear regression analysis was performed to find the strong associations and predictions between variables. P-value of ≤ 0.05 was considered statistically significant.

RESULTS

Demographical characteristics and anthropometric measurements of the studied groups

The anthropometric measurements and demographic characteristics are summarized in table-1. There was no difference in age between the studied groups (P>0.05), with an average age ranging between 2 and 17 years. There were no differences in the median BMI percentile (%), BMI (kg/m^2), and BMI Z-score between the studied groups. Among the patients' group, there were 52.38% boys and 47.62% girls, whereas in the control group, there were 40.48% girls and 59.52% boys. Regarding residence, most of the patients came from rural areas 61.90%, while only 38.10% came from urban areas, and this distribution may be related to the higher exposure to risk factors that trigger the development of the disease, such as environmental factors (i.e. chemicals, viruses, etc.) [16]. There were 61.90% of patients with a family history of T2DM; 14.29% with a family history of both T1D and T2DM; 19.1% with no family history; and only 4.76% with a family history of T1DM.

Table.1. Demographic characteristics and anthropometric measurements of the studied groups**.**

	T1DM group	Control group	P-value
Gender (male/female) (%)	52.38% / 47.62%	59.52%/ 40.48%	N.S
Residence (Rural, Urban)	61.90%, 38.1%	12%, 88%	--
Family history (T1D,T2D,T1,2D,No)	4.8%, 61.9%, 14.3%, 19.1%	T2D (64%), No (36%)	--
Age (years)	10.1 ± 3.6	10.1 ± 3.9	N.S
BMI percentile (%)	51.89 (4.57-94.73)	69.51 (5-97.7)	0.133
BMI (kg/m^2)	17.1 (13.9-25)	18.6 (13.8-27)	N.S
BMI Z score	0.045 (-1.69-1.62)	0.51 (-4.39-1.99)	0.133

*Normally distributed data are expressed as mean± SD while non-normally distributed data expressed as median (Min-Max), using Student's t-test and Mann-Whitney U test, respectively. N.S: non-significant.

Clinical characteristics of the studied groups

Serum levels of C-peptide were significantly decreased in children with T1D when compared with healthy children (P<0.05). In terms of disease duration, C-peptide levels were slightly lower in patients with less than five years of disease compared to patients with more than five years (P > 0.05). As well, the current study showed a significant increase in irisin and preptin levels in the patient group with a p-value of (P=0.03) and (P=0.04) respectively, as shown in Table-2. The levels of hemoglobin A1C were (10.1 ± 3) in the patient group. Diabetic children had higher levels of random blood sugar than the control group (P<0.001). The median duration of the disease was 2 years (Table 2).

In addition, there were non-significant differences in serum levels of C-peptide, irisin, and preptin between boys and girls in the patients' group (P>0.05), as revealed in Table 3.

Table.2. Clinical characteristics of the studied groups

Variables	T1D group	Control group	P-value
C-peptide (ng/ml)	2.0 ± 0.5	3.1 ± 1.5	**0.049**
Preptin (ng/ml)	308.9 (117.1-1331.5)	301.4 (119.1-1320.4)	**0.04**
Irisin (ng/ml)	9.6 ± 4.5	8.1 ± 2.4	**0.03**
RBS (mmol/dl)	13.2 (4.4-36.9)	4.2 (2.7-7.5)	**<0.001**
HbA1C (%)	9.95 ± 2.6	--	--
Duration of Disease (years)	2 (0.02-14)	--	--

*Results are expressed as mean± SD for normally distributed data and median (Min-Max) for non-normally distributed data. Student's t-test and Mann-Witney U test were used for comparison, respectively.

Table 3. Comparison of C-peptide, preptin and irisin levels in the patient group according to gender**.**

Variables	Gender		P-value
	Girls	Boys	
C-peptide (ng/ml)	2.1 ± 0.5	1.9 ± 0.4	0.2
Preptin (ng/ml)	311.1 (139.2-1331.5)	296.8 (117.1-1274.1)	0.6
Irisin (ng/ml)	8.3 ± 3.4	8.5 ± 4.6	0.9

*Mean ± SD and median (Min-Max) were used to describe normally and non- normally distributed data, respectively.

Spearman correlation between C-peptide, preptin and irisin with study variables

In the present study, serum C-peptide levels were positively correlated with random blood sugar (P=0.007, r=0.41), irisin (P<0.001, r=0.69), and preptin (P<0.001, r=0.66), as shown in Table-4. While C-peptide correlated negatively with body mass index and duration of the disease (P>0.05, Table 4). Serum irisin levels showed a positive correlation with preptin (P<0.001, r=0.81) and RBS (P=0.02, r=0.41) while negative correlation with body mass index (P>0.05). Serum preptin levels showed a significant positive correlation with random blood sugar (P=0.03, r=0.35) and a negative correlation with body mass index and duration of the disease, as revealed in Table 4.

Table 4. Spearman's correlations between C-peptide, irisin, and preptin with study variables.

Study Variables	C-peptide		Irisin		preptin	
	r	P-value	r	P-value	r	P-value
Age (years)	0.01	0.97	0.09	0.59	0.05	0.73
BMI (kg/m^2)	-0.15	0.78	0.06	0.73	-0.16	0.33
Duration	-0.08	0.61	-0.06	0.71	-0.03	0.86
RBS	0.41	**0.007**	**0.41**	**0.02**	**0.35**	**0.03**
Irisin	0.69	**<0.001**	--	--	**0.81**	**<0.001**
C-peptide	--	--	**0.69**	**<0.001**	**0.66**	**<0.001**
Preptin	0.66	**<0.001**	**0.81**	**<0.001**	--	--
HbA1c	0.06	0.71	0.22	0.17	0.14	0.38

Linear regression analysis

Irisin was an independent positive predictor for C-peptide and preptin with a P-value <0.001 in linear regression analysis, as shown in Table 5.

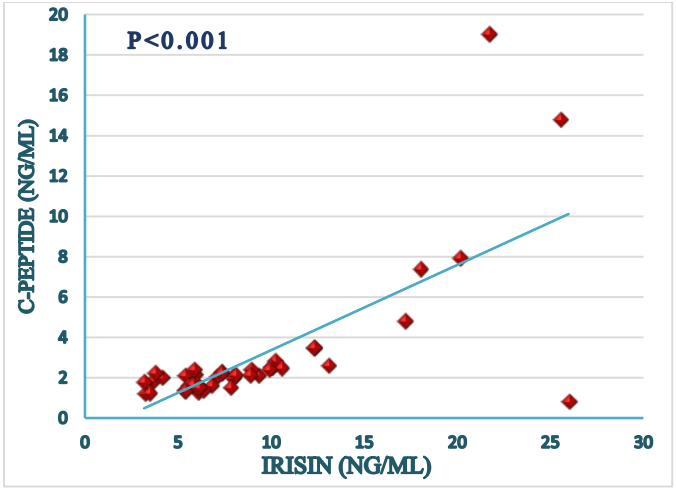

Figure 3. Correlation between irisin and C-peptide.

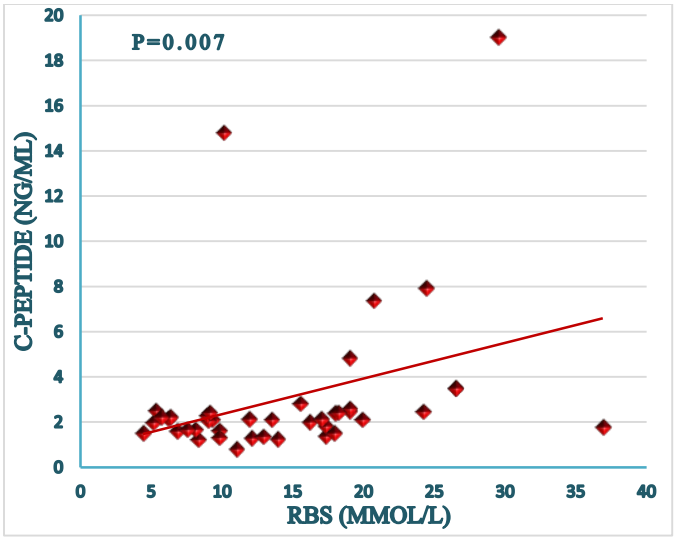

Figure 4. Correlation between C-peptide and RBS.

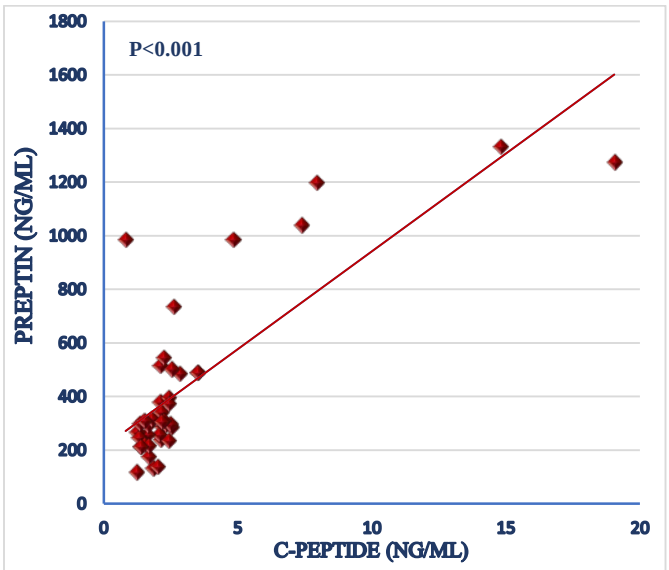

Figure 5. Correlation between prpetin and C-peptide

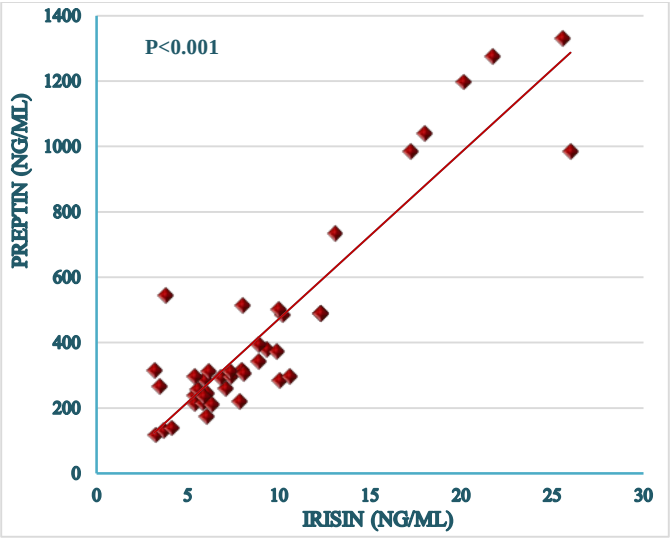

Figure 6. Correlation between prpetin and irisin

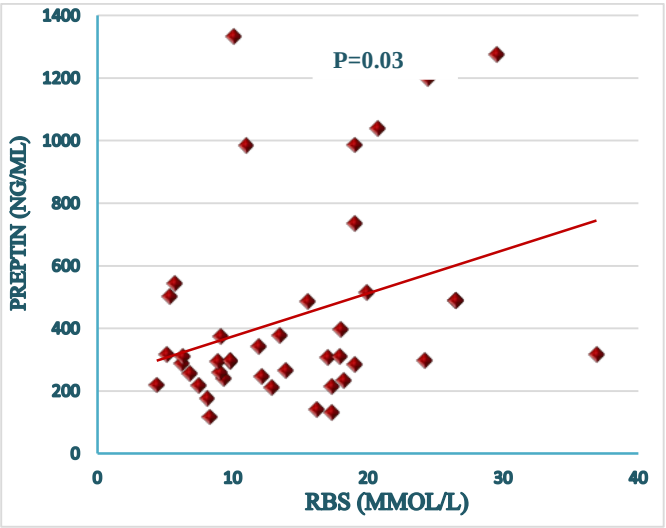

Figure 7. Correlation between preptin and RBS.

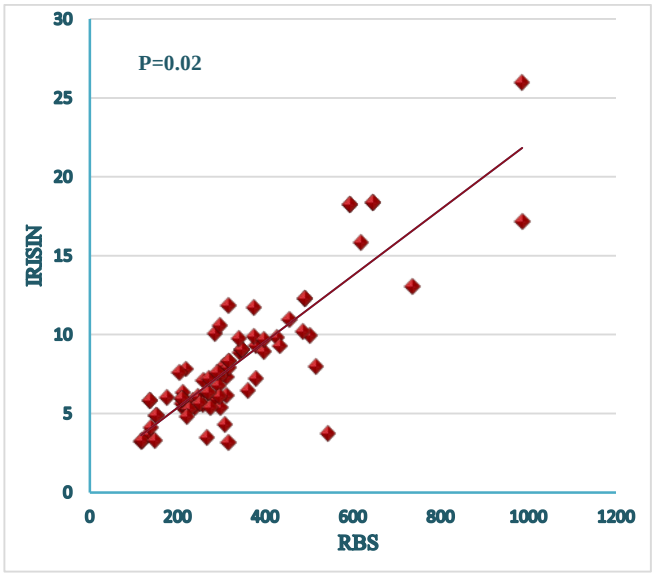

Figure 8. Correlation between irisin and RBS.

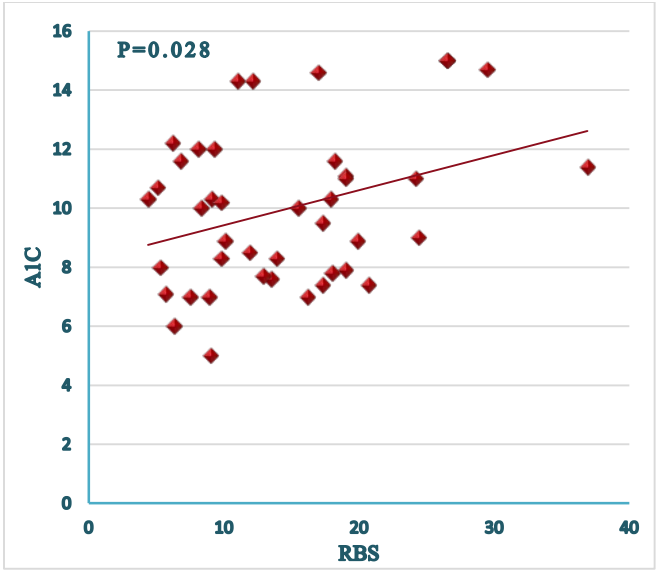

Figure 9. Correlation between HbA1c and RBS

Table 5. Linear regression analysis with C-peptide and preptin as a dependent variable

Study parameters	C-peptide		Preptin	
	β-coefficient	P-value	β-coefficient	P-value
Irisin	0.812	<0.001	0.919	<0.001
RBS	0.233	0.077	0.297	0.072
HbA1c	-0.162	0.349	0.077	0.633

DISCUSSION

The present study observed a significant difference in the levels of C-peptide between the studied groups (P<0.05). The diabetic children had decreased levels of C-peptide in comparison with the healthy children (2.0 ± 0.5 versus 3.1 ± 1.5, P=0.049). This result is related to the fact that type 1 diabetes mellitus is caused by an autoimmune damage to the pancreatic beta cells. Therefore, as C-peptide is synthesized in beta cells and cleaved from insulin prohormone, a reduced beta cell mass results in reduced insulin levels and thus C-peptide levels.[17] Regarding gender, there was a non-significant difference in C-peptide levels between girls and boys in the patient group (P>0.05), as shown in Table-3.

On the other hand, preptin was significantly increased in the patients' group (P<0.05) compared with the control group, and this result is in agreement with a study done by Abd El Dayem et. al. 2015 [18] that showed a P-value of 0.0001. Preptin was found to be high in patients with type 2 diabetes[10] and polycystic ovary syndrome[19,20], whom are at high risk of insulin resistance and yet showed a significant positive correlation with HOMA-IR[10]. Even though it is common to have insulin resistance in type 2 diabetes, it was found that this state as well prominent in patients with type 1 diabetes, which is induced by glucose toxicity, lipotoxicity, and defective mitochondrial function[21,22]. From the shared aspect between T1D and these disorders, the increased susceptibility of

having glucose toxicity and defective mitochondrial function raise the probability of higher preptin levels. A study done by Elsaeed W. et. al. 2021[23] disagrees with the result of this conducted study, where preptin was significantly reduced in type 1 diabetic patients compared to healthy individuals, yet gender implication had no effect on preptin levels in all of the studies (Table-3).

In addition, irisin levels were significantly higher in the diabetic patient in comparison to the control (9.6 ± 4.5 versus 8.1 ± 2.4, P=0.03) that is in accordance with the results of studies done by **Faienza** et. al. 2018 and **Ates** et. al. 2017 [24,25], where T1D patients were recruited. The result of this study contradicts **Tentolouris** et. al. study [26] where the patients showed a reduced level of irisin compared to the control group, yet; recruiting adult diabetic patients with a longer duration of disease could possibly affect their results. There is still no obvious reason related to the higher levels of irisin in patients with T1D; however, different factors might be implicated. Noteworthy, one of these factors is glucagon, which stimulates PGC1α expression through glucagon-Ca^{+2}-CREB, PKA pathways.[27,28] As a result, this pathway leads to increased irisin expression, synthesis, and secretion through PGC1α-FNDC5-irisin pathway. Moreover, another stimulus for irisin syntheses and secretion is ATP deprivation in skeletal muscles that is known to be increased in type 1 diabetic patients. Hyperactive Ca^{+2} kinetics were found to increase Ca^{+2} exposure exist[29,30] in type 1 diabetes, so irisin synthesis might be elevated in the skeletal muscles through Ca^{+2}-AMPK-PGC1α or Ca^{+2}-calcineurin/ CaMKs-CREB, NFAT, MEF2C, MEF2D pathways. Regarding gender, there was no difference between boys and girls in irisin levels in the patient's group (P>0.05), and this is in agreement with another study done by Faienza et al. 2018 [24], as shown in table-3.

The main outcomes of this study is the significant positive correlation between C-peptide and irisin (P<0.001, r=0.686, figure 3). This result advocates the studies that found that irisin stimulates the expression and synthesis of betatrophin, which in turn enhances the proliferation and regeneration of pancreatic beta cells through the p38-PGC1α pathway [13]. In consequence, irisin promotes C-peptide secretion indirectly from pancreatic β-cells (muscle-liver-pancreas). The present study also revealed a positive correlation between C-peptide and random blood sugar (r=0.407, P=0.007, figure-4), while no correlation observed with age, BMI, duration of disease and HbA1c. This result coincide with a study done by Crisman et al. 2017.[31] C-peptide is released concomitantly with insulin in response to elevated glucose levels, thus it is correlated positively with random blood sugar.

Furthermore, C-peptide correlated positively with preptin levels (r=0.656, P<0.001, figure-5) and this result may be related to that both preptin and C-peptide are secreted from pancreatic β-cells in response to hyperglycaemia. Therefore, as C-peptide released with insulin in response to increased glucose levels, preptin released at the same time to augment insulin secretion. And this result is supported with the positive association between preptin and RBS that we have conducted in the present study (r=0.35, P=0.03, figure-7), and this is in agreement with Kalayci et al. study.[10]

In the present study, there was also a positive association between irisin and preptin (r=0.681, P<0.001, figure-6). This is the first study that determines a correlation between irisin and preptin in children with type 1 diabetes. The result of this association might be related to the ability of irisin to promote betatrophin synthesis and secretion, a recently discovered protein mainly expressed in adipose tissues and liver[32], which in turn induce beta cell regeneration and proliferation[33] through the p38-PGC-1α pathway [34]. So, irisin indirectly promotes preptin secretion through the stimulation of pancreatic beta cell regeneration.

Moreover, irisin had a positive correlation with RBS (r=0.352, P=0.022, figure 8), and this finding is in agreement with the study of De Meneck et. al.[35], while conflicting with the study of Çatlı et. al.[36] that was conducted on obese children. Irisin has the ability to regulate glucose homeostasis and promote glucose uptake by the skeletal muscles and fatty tissues, so its positive association with RBS might represent a state of a compensatory mechanism [37] in response to hyperglycemia. In regression analysis, irisin showed a significant association with preptin and C-peptide (P<0.001), which may indicate a strong prediction for residual pancreatic beta cells.

This conducted study also observed a significant positive correlation between HbA1c and RBS (P=0.028, r=0.339, figure-9), and this result is in agreement with Beck et. al. 2019 [38] study. Haemoglobin A1c is one of the glycated proteins composed of haemoglobin and glucose, and it indicates how much glucose exists in the blood over the 120 days of the red blood cells' life cycle.

CONCLUSION

This current study has demonstrated a considerable positive correlation between C-peptide, irisin, and preptin in children with type 1 diabetes mellitus. According to the findings of this study, irisin was an independent positive predictor for persistent C-peptide in addition to preptin levels and thus functional pancreatic beta cells. Irisin and preptin were significantly associated with blood glucose levels and, thus, with hyperglycemia. Preptin might be an indicator of insulin resistance. In terms of sex comparison, irisin and preptin levels were not different. We recommend further studies with a larger sample size to find out how much irisin and preptin levels are affected. In addition, we recommend studying irisin and preptin in patients with diabetes complications to explore the influence of diabetes complications on both hormones. As well as, due to the conflicting results about irisin in different studies, it is noteworthy to make a future study to rule out the effect of ethnicity on the results.

Limitations of this study: This present study has several limitations, including the difficulties in sample collection in the fasted state because most diabetic children are prone to hypoglycaemia and have to take their daily morning insulin dose, which requires taking a meal after that. Some of the healthy children appeared to have diabetes after the estimation of random blood sugar without obvious symptoms. Also, it was difficult to

find patients who met the criteria of the study (exclusion and inclusion criteria).

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Author Contributions

Conceived and designed the experiments: the study designed by S. Mohammed and R. Alabedi. Z. Ajam and S. Mohammed performed the experiments. Z. Ajam and R. Alabedi recruited the participants for sample collection. Z. Ajam and S. Mohammed performed the data analysis. Z. Ajam and S. Mohammed wrote the manuscript.

Conflict of Interest: The authors declare that this work has no conflicts of interest with any commercial or financial affiliations.

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