Research Article

Gülce Kiren*, Çınar Severcan, Suzan Muratoğlu Severcan and Hatice Paşaoğlu

The effect of curcumin on PI3K/Akt and AMPK pathways in insulin resistance induced by fructose

https://doi.org/10.1515/tjb-2024-0027
Received February 13, 2024; accepted May 23, 2024;
published online June 14, 2024

Abstract

Objectives: Excessive fructose consumption is recognized to elevate insulin resistance in animals and humans. In our study, we aimed to assess the possible consequences of curcumin (curc) treatment applied to rat models of fructose-induced insulin resistance on adenosine monophosphate-activated protein kinase (AMPK) and phosphatidylinositol-3 kinase (PI3K)/protein kinase B (Akt) pathways in skeletal muscle and adipose tissue.

Methods: We established four distinct rat groups: corn oil (negative control group), 20 % fructose (positive control group), 20 % fructose and 100 mg/kg curc (100 mg/kg curc group), and 20 % fructose and 200 mg/kg curc (200 mg/kg curc group). The ELISA method was used to determine serum insulin levels, an auto-analyzer was used to measure serum glucose levels, and homeostatic model assessment of insulin resistance (HOMA-IR) values were calculated. In the rat's skeletal muscle and adipose tissues, the ELISA method was used to determine the following parameters: insulin receptor substrate-1 (IRS-1), phosphorylated insulin receptor substrate-1 (p-IRS-1), PI3K, phosphatidylinositol 3,4,5-trisphosphate (PIP3), phosphoinositide-dependent kinases (PDK-1), phosphorylated Akt (p-Akt), AMPK and glucose transporter type 4 (GLUT4) levels.

Results: The positive control group exhibited a significant increase in serum glucose, insulin, and HOMA-IR levels, confirming the establishment of the insulin resistance model. In the curcumin dose groups, these values significantly decreased. Additionally, compared to the positive control groups, curcumin dose groups demonstrated a significant increase in the parameters of the Akt/PI3K pathway, AMPK activation, and GLUT4 levels in skeletal muscle and adipose tissues.

Conclusions: We observed that curcumin demonstrates potential ameliorative effects on the insulin signaling pathway through PI3K/Akt and AMPK pathways.

Keywords: insulin resistance; curcumin; AMPK; PI3K; p-Akt; GLUT4

***Corresponding author: Gülce Kiren**, MD, Department of Medical Biochemistry, Gazi University Faculty of Medicine, 06560, Ankara, Türkiye, E-mail: glce.koca@hotmail.com. https://orcid.org/0000-0002-2646-1003
Çınar Severcan, Department of Biochemistry, Zonguldak Bulent Ecevit University Faculty of Pharmacy, Zonguldak, Türkiye. https://orcid.org/0000-0002-3806-6406
Suzan Muratoğlu Severcan, Department of Medical Biochemistry, Gazi University Institute of Health Sciences, Ankara, Türkiye. https://orcid.org/0000-0001-5978-8144
Hatice Paşaoğlu, Department of Medical Biochemistry, Gazi University Faculty of Medicine, Ankara, Türkiye. https://orcid.org/0000-0001-8343-7432

Introduction

Reduced sensitivity to circulating insulin levels in insulin-dependent cells, such as skeletal muscle and adipose tissues, is a hallmark of insulin resistance [1, 2].

It has been reported that diets rich in free sugars, particularly those abundant in fructose, lead to increased fat accumulation in skeletal muscles and liver. Consequently, this raises the risk of insulin resistance and type 2 diabetes mellitus [3, 4]. Studies have indicated that an 8-week fructose supplementation has effects on inducing hyperinsulinemia and insulin resistance in rat models [5, 6].

Skeletal muscle, which is vital for controlling glucose metabolism and energy homeostasis, is where insulin-stimulated utilization of glucose occurs mostly [7]. Adipose tissue is a crucial endocrine organ that provides factors regulating systemic insulin sensitivity and energy homeostasis [8]. Through the phosphatidylinositol-3 kinase/protein kinase B (PI3K/Akt) signaling pathway, insulin governs the metabolic processes in skeletal muscle and adipose tissue by enhancing protein synthesis, glucose transport and lipogenesis [9].

Insulin binding to its receptor causes increased receptor tyrosine kinase activity, which in turn causes insulin

Open Access. © 2024 the author(s), published by De Gruyter. This work is licensed under the Creative Commons Attribution 4.0 International License.

receptor substrate-1 (IRS-1) to be tyrosine phosphorylated, initiating the responses of adipose tissue and skeletal muscle to insulin. Subsequently, phosphatidylinositol-3 kinase (PI3K) is activated, and its activation converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3), which leads to activation of phosphoinositide-dependent kinases (PDK1) and Akt. Glucose transporter type 4 (GLUT4) protein, expressed in adipose tissue and skeletal muscle, catalyzes glucose transport across the plasma membrane by a facilitatory diffusion mechanism under the influence of insulin and other stimuli [10, 11]. GLUT4 translocation to the plasma membrane is accelerated following activation of Akt, thereby enhancing glucose transport into adipose tissue and skeletal muscle [12].

Adenosine monophosphate-activated protein kinase (AMPK) is a protein kinase which modulates energy balance [13]. When AMPK is activated, it promotes GLUT4 translocation, enhances autophagy, and inhibits cholesterol and fatty acid synthesis [14].

A plant belonging to the Zingiberaceae (ginger) family is called *Curcuma longa*. Curcumin (curc), the most active ingredient in medicinal remedies, is also present in the extract made from powdered and dried *C. longa* roots. Curc demonstrates anti-diabetic, antioxidant and anti-inflammatory properties by effectively modulating molecular targets associated with the pathogenesis of various diseases pathogenesis, attributed to its polyphenolic structure [15, 16].

The literature remains unclear about the combined effects of curcumin administration to high fructose-fed rat groups on PI3K/Akt signaling pathway proteins, AMPK signaling pathway and GLUT4 levels in skeletal muscle and adipose tissues of rats. In our study, the PI3K/Akt and AMPK signaling pathways, as well as the GLUT4 protein, were evaluated to assess the potential therapeutic benefits of curcumin in insulin resistant rat models.

Materials and methods

Experimental animal models, design and protein quantification

Twenty-four male adult Wistar rats taken from Gazi University Experimental Animal Center were included in the study. Throughout the duration of the experiment, the rats were maintained in an environment with the temperature controlled within the range of 22–24 °C. Standard rodent feed and tap water were given to all groups.

Consistent with our previous studies, we established an insulin-resistant group (positive control group) by administering 20 % D-fructose for 8 weeks. Similarly, to assess the therapeutic effects of curcumin, we chose dosages for the rats that were 100 and 200 mg for each kilogram of body weight [17]. We divided the rats into four groups as follows: a negative control group that received solely corn oil, a positive control group where insulin resistance was induced by administering 20 % fructose and corn oil, a 100 mg/kg curc group that received 20 % fructose and corn oil with dissolved 100 mg/kg curc, and a 200 mg/kg curc group that received 20 % fructose and corn oil with dissolved 200 mg/kg curc. We applied the respective treatments to all groups for 8 weeks.

At the end of these treatments, ketamine–xylazine anesthesia is used to euthanize the rats. The blood samples were collected from the heart to obtain plasma samples. A phosphate-buffered saline (PBS) buffer at a 1:10 ratio (pH=7.4, 150 mg of tissue+1,350 µL of PBS) was used to homogenize adipose tissue and skeletal muscle. The supernatants were obtained after centrifugation of these tissues at 2,500×*g* for 15 min. The plasma samples and the resulting supernatants were stored at −80 °C.

Total protein content in all tissue samples was measured using the BCA protein colorimetric assay kit (Cat. No. E-BC-K318-M, Elabscience), which demonstrated average intra-assay and inter-assay precisions 2.2 and 4.5 % respectively [18].

Measurement of parameters

Serum glucose levels were enzymatically measured at the Central Biochemistry Laboratory of Gazi University Faculty of Medicine Hospital using an autoanalyzer (AU480, Beckman Coulter, Brea, CA, USA). For enzyme-linked immunosorbent assay (ELISA) analysis of serum insulin levels, we used USCN kit (Catalog No: CEA448Ra), which demonstrated an intra-assay precision less than 10 % and an inter-assay precision less than 12 %. The evaluation of insulin resistance was conducted utilizing the “homeostatic model assessment” (HOMA-IR) formula. The formula is as follows: HOMA-IR=Fasting glucose (mg/dL)×Fasting insulin (µIU/mL)/405 [19, 20]. Individuals scoring 3.9 or higher were identified as exhibiting insulin resistance [19].

For the ELISA analysis of PI3K (Catalog no: E0438Ra), PDK-1 (Catalog no: E2888Ra), IRS-1 (Catalog no: E0919Ra), phosphorylated IRS-1 (Catalog no: E2453Ra), phosphorylated Akt (Catalog no: E2452Ra), AMPK (Catalog no: E0436Ra), and GLUT4 (Catalog no: E0499Ra) levels in skeletal muscle and adipose tissue, we used the kits from Bioassay Technology Laboratory. The intra-assay precision for all kits was less than 8 %, and the inter-assay coefficient of variation (CV) was

less than 10 %. For ELISA analysis of PIP3 levels of these tissues, we used USCN kit (Catalog no: CEG856Ge), which demonstrated an intra-assay precision less than 10 % and an inter-assay precision less than 12 %.

The results for PI3K, PDK-1, IRS-1, PIP3 and GLUT4 were expressed in ng/mg protein as a ratio to total protein amount. Phospho-Akt and phosphorylated IRS-1 results were expressed in ng/g protein, and AMPK results were expressed in U/g protein.

Statistical analysis

To analyze and identify significant differences among the groups we used IBM SPSS (Statistical Package for Social Sciences) version 25. In the representation of the obtained data, median and quartile range values were used. Under non-parametric test conditions, the significance level of the differences among the groups was determined by the Kruskal–Wallis test. To identify significant differences between the groups we adopted significance level of $p \leq 0.05$. Additionally, to ascertain significant differences between two specific groups, the Mann–Whitney U test was employed. In our study, comparisons were made between the negative control group and the positive control group, the 100 mg/kg curc group and the positive control group, as well as the 200 mg/kg curc group and the positive control group. Since there were a total of three comparisons, the Bonferroni correction suggested to divide the p value ($p \leq 0.05$) by three (0.05/3=0.017). Consequently, p-values that were equivalent or less than 0.017 were regarded statistically significant for the assessment of significance between two groups. Correlation analysis using Spearman's method was conducted among the groups to investigate the correlation between the parameters.

Results

Plasma glucose, insulin, HOMA-IR

The medians and interquartile ranges of serum insulin, glucose, and HOMA-IR parameters for all groups are presented in Table 1.

Significant differences in serum glucose, insulin, and HOMA-IR levels were statistically identified across all groups ($p \leq 0.05$). In our study, the HOMA-IR level of the positive control group was above 3.9, indicating the development of insulin resistance. In contrast, the HOMA-IR values in the 100 mg/kg and 200 mg/kg curcumin groups were below 3.9, indicating a decrease in insulin resistance. The positive control group demonstrated a statistically significant elevation in glucose levels when contrasted with those of the negative control group, with a p-value of ≤0.017. When comparing the curc groups with the positive control group, it was noted that the 100 mg/kg curc group exhibited an increase in glucose levels, whereas the 200 mg/kg curc group displayed a decrease. These changes, however, did not reach statistical significance ($p > 0.017$) (Table 1). The positive control group exhibited a statistically significant increase in both insulin levels and HOMA-IR values when compared to the negative control group. In addition, the insulin levels and HOMA-IR values in both curc groups demonstrated a statistically significant decrease compared to insulin resistance group ($p \leq 0.017$) (Table 1).

Muscle tissue

The medians and interquartile ranges of insulin signaling pathway parameters in skeletal muscle tissue for all groups are presented in Table 2.

The levels of IRS-1, p-IRS-1, PI3K, PIP3, PDK1, p-Akt, GLUT4, and AMPK in the skeletal muscle tissue of the positive control group demonstrated a statistically significant decrease compared to those in the negative control group ($p \leq 0.017$). The levels of IRS-1, p-IRS-1, PIP3, PDK1 and p-Akt in the 100 mg/kg curc group exhibited statistically significant increase compared to the positive control group ($p \leq 0.017$). Nevertheless, although there was a partial increase in PI3K, AMPK and GLUT4 levels, this increase was not statistically significant ($p > 0.017$). In the 200 mg/kg curc group, all parameters, including GLUT4 and AMPK, were observed to significantly increase compared to the positive control group ($p \leq 0.017$) (Table 2).

According to the results of the correlation analysis between skeletal muscle tissue parameters and HOMA-IR, HOMA-IR values showed a very strong negative correlation

Table 1: The medians and interquartile ranges of serum insulin, glucose, and HOMA-IR.

Parameters	Negative control group n=6	Positive control group n=6	100 mg/kg curc group n=6	200 mg/kg curc group n=6
Glucose, mg/dL	142.3 (138.5–148)	212.3 (263.3–183.7)[a]	249.3 (222.7–269.7)	159.3 (141.7–215.2)
Insulin, µU/mL	6.6 (6.02–6.82)	8.13 (7.9–8.94)[a]	5.49 (5.01–5.77)[b]	4.5 (3.37–5.17)[c]
HOMA-IR	2.3 (2.14–2.36)	4.23 (3.82–5.28)[a]	3.31 (3.04–3.54)[b]	1.89 (1.27–2.0)[c]

[a]$p \leq 0.017$ (comparison between the negative control group and the positive control group); [b]$p \leq 0.017$ (comparison between the positive control group and the 100 mg/kg curc group); [c]$p \leq 0.017$ (comparison between the positive control group and the 200 mg/kg curc group). Curc, curcumin; HOMA-IR, homeostatic model assessment of insulin resistance.

Table 2: The medians and interquartile range values of insulin signaling pathway parameters in muscle tissue.

Parameters	Negative control group n=6	Positive control group n=6	100 mg/kg curc group n=6	200 mg/kg curc group n=6
p-IRS-1 (ng/g protein)	630.3 (540.2–1,057.8)	355.5 (246.7–430.3)[a]	609.8 (457.3–807.7)[b]	785.4 (727.3–943.4)[c]
IRS-1 (ng/mg protein)	4.39 (4.05–6.19)	2.73 (2.31–3.44)[a]	4.18 (3.99–4.45)[b]	5.54 (5.14–8.36)[c]
PI3K (ng/mg protein)	4.12 (2.91–6.95)	1.22 (0.74–2.29)[a]	2.99 (1.36–4.04)	4.42 (3.93–6.83)[c]
PIP3 (ng/mg protein)	1.61 (1.22–4.64)	0.7 (0.56–0.87)[a]	1.65 (1.62–1.85)[b]	2.53 (1.5–3.37)[c]
PDK1 (ng/mg protein)	4 (3.54–8.98)	1.91 (0.86–3.08)[a]	4.33 (3.12–5.8)[b]	7.05 (6.35–10.7)[c]
p-Akt (ng/g protein)	24.2 (49–70.4)	7.4 (3.1–14)[a]	31.3 (23.7–50)[b]	49.1 (29.8–86.1)[c]
GLUT4 (ng/mg protein)	4.36 (3.72–8.35)	2.31 (1.6–2.77)[a]	2.59 (2.32–3.43)	4.61 (3.46–6.89)[c]
AMPK (U/g protein)	119.1 (97.2–163.2)	73.9 (60.1–86.9)[a]	95.9 (80.7–131.5)	136.1 (98.4–184.6)[c]

[a]$p \leq 0.017$ (comparison between the negative control group and the positive control group); [b]$p \leq 0.017$ (comparison between the positive control group and the 100 mg/kg curc group); [c]$p \leq 0.017$ (comparison between the positive control group and the 200 mg/kg curc group). Curc, curcumin; p-IRS-1, phosphorylated insulin receptor substrate-1; IRS-1, insulin receptor substrate-1; PI3K, phosphatidylinositol-3, kinase; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PDK1, phosphoinositide-dependent kinases; p-Akt, phosphorylated Protein kinase B; GLUT4, glucose transporter type 4; AMPK, adenosine monophosphate-activated protein kinase.

with IRS-1, PDK1, and GLUT4 (r=−0.838, −0.780, −0.787; respectively). HOMA-IR values also showed a strong negative correlation with p-IRS-1, PI3K, PIP3, p-Akt, and AMPK (r=−0.633, −0.652, −0.670, −0.657, −0.676; respectively).

Adipose tissue

The medians and interquartile ranges of insulin signaling pathway parameters in adipose tissue for all groups are presented in Table 3.

The levels of p-IRS-1, IRS-1, PI3K, PIP3, PDK1, p-Akt, GLUT4, and AMPK in the adipose tissue of the positive control group demonstrated a statistically significant decrease compared to those in the negative control group ($p \leq 0.017$). However, in contrast, the levels of these parameters in the positive control group significantly increased in both curc groups ($p \leq 0.017$).

According to the results of the correlation analysis between adipose tissue parameters and HOMA-IR, HOMA-IR values showed a strong negative correlation with p-IRS-1, IRS-1, PI3K, PIP3, PDK1, p-Akt, GLUT4, and AMPK (r=−0.730, −0.697, −0.666, −0.737, −0.717, −0.559, −0.636, −0.647; respectively).

Discussion

In our study, we demonstrated that 100 mg/kg and 200 mg/kg curcumin treatment at both doses had potential beneficial effects on the reduction of insulin resistance in rat groups in which insulin resistance was induced by 20 % fructose administration (Table 1). According to our findings, these effects of curcumin were mediated by increased PI3K/Akt signaling pathway proteins and AMPK activation in skeletal muscle and adipose tissue (Tables 2 and 3).

Table 3: The medians and interquartile range values of insulin signaling pathway parameters in adipose tissue.

Parameters	Negative control group n=6	Positive control group n=6	100 mg/kg curc group n=6	200 mg/kg curc group n=6
p-IRS-1 (ng/g protein)	2,254 (1871–2,468)	1,377 (1,323–1,530)[a]	2,473 (2,185–2,910)[b]	4,945 (2,967–7,501)[c]
IRS-1 (ng/mg protein)	13.1 (11.98–15.47)	10.27 (8.78–10.9)[a]	14.81 (12.64–18.51)[b]	24.4 (15.96–35.33)[c]
PI3K (ng/mg protein)	17.67 (13.58–20.77)	11.78 (10.52–12.76)[a]	19.98 (17.15–22.44)[b]	33.02 (24.27–64.8)[c]
PIP3 (ng/mg protein)	4.53 (3.8–5.33)	2.35 (1.75–2.56)[a]	4 (3.67–4.66)[b]	5.08 (4.28–7.4)[c]
PDK1 (ng/mg protein)	16.57 (12.56–19.41)	9.8 (2.34–11.55)[a]	19.39 (16.11–33.62)[b]	50.68 (26.76–79.73)[c]
p-Akt (ng/g protein)	134.9 (118.3–189.2)	101.6 (88.4–106.7)[a]	233.3 (143–363)[b]	219.2 (136.1–493.1)[c]
GLUT4 (ng/mg protein)	14.1 (12.4–16.8)	11.3 (9.8–12.03)[a]	18.7 (18.6–23.3)[b]	36.8 (21.7–50.7)[c]
AMPK (U/g protein)	266 (230–392)	181 (144–200)[a]	407 (362–463)[b]	617 (336–1,184)[c]

[a]$p \leq 0.017$ (comparison between the negative control group and the positive control group); [b]$p \leq 0.017$ (comparison between the positive control group and the 100 mg/kg curc group); [c]$p \leq 0.017$ (comparison between the positive control group and the 200 mg/kg curc group). Curc, curcumin; p-IRS-1, phosphorylated insulin receptor substrate-1; IRS-1, insulin receptor substrate-1; PI3K, phosphatidylinositol-3, kinase; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PDK1, phosphoinositide-dependent kinases; p-Akt, phosphorylated Protein kinase B; GLUT4, glucose transporter type 4; AMPK, adenosine monophosphate-activated protein kinase.

In order to determine insulin resistance in rat groups, the HOMA-IR cut-off value was estimated as 3.9. A cut-off value of 3.9 was previously mentioned in another study [19]. Our study aligns with the design of Antunes et al. [19]. Both our study and the study by Antunes et al. [19] involved Wistar rats, a common model for such experiments. In both cases, rats were maintained in a controlled environment with a consistent light/dark cycle and temperature range. The HOMA-IR score was calculated using a similar formula in both studies. Considering these similarities, we used a cut-off point of 3.9 for the HOMA-IR value when determining whether the rats in our study were insulin resistant [19]. Different cut-off values have been reported in other studies [20–23]. In numerous studies, it has been reported that 100 mg/kg and 200 mg/kg curcumin doses in experimental animal models has shown advantageous effects on carbohydrate and lipid metabolism by contributing to an anti-inflammatory and antioxidant response [24, 25]. Kelany et al. [26] assessed insulin, glucose, and HOMA-IR levels in rat models subjected to a high fructose diet and administered 200 mg/kg of curc for a duration of 8 weeks. Their results demonstrated that the injection of curc decreased blood glucose levels and enhanced insulin signaling.

In our study, blood glucose, and insulin levels were measured high in the positive control group fed with 20 % fructose, which resulted in an increase in the HOMA-IR value [4.23 (3.82–5.28)], and insulin resistance was successfully detected (Table 1). In our investigation, in addition to the study of Kelany et al. [26], it was shown that plasma insulin level decreased in the 100 mg/kg curc group and blood glucose did not decrease (Table 1). However, it was observed that insulin resistance was significantly reduced in the 100 mg/kg curc group compared to the positive control group that develop insulin resistance ($p \leq 0.017$). Similar to the findings in the literature, blood glucose levels, insulin levels, and insulin resistance decreased in the 200 mg/kg curcumin group (Table 1) [26].

The PI3K/Akt signaling pathway mediates the effects of insulin in skeletal muscle and adipose tissue, which contributes to regulate glucose metabolism [27]. In this signaling pathway, GLUT4 translocation increases as a result of phosphating Akt. Increased GLUT4 translocation leads to the integration of GLUT4 proteins into the plasma membrane, thus, glucose influx into skeletal muscle or adipocytes occurs [28]. Chauhan et al. [29] observed that administering curcumin and curcumin-loaded chitosan nanoparticles to insulin-resistant L6myc myotubes, led to enhanced translocation of GLUT4 to the plasma membrane and increased p-Akt protein levels.

AMPK is a regulator of glucose and fatty acid catabolism. In skeletal muscle, AMPK activation increases GLUT4 translocation independently of insulin, leading to an increase in glucose uptake [30]. Additionally, a recent study proposes that AMPK plays a role in regulating glucose uptake stimulated by insulin [31]. Studies have shown that high-dose fructose consumption attenuates the AMPK signaling pathway, causing insulin resistance in skeletal muscle and adipose tissue [32, 33].

While examining skeletal muscle tissue parameters, in the positive control group where insulin resistance was induced, the parameters in the IRS-1/PI3K/Akt insulin signaling pathway (p-IRS-1, IRS-1, PI3K, PIP3, PDK1, p-Akt, GLUT4) and AMPK activation in skeletal muscle tissue significantly decreased, resulting in a decrease in GLUT4 levels (Table 2). The decreased muscle tissue AMPK and GLUT four levels in the positive control group are consistent with findings documented in the literature [32, 33]. In addition to demonstrating that curcumin increases GLUT4 and p-Akt protein levels in muscle tissue similar to the study by Chauhan et al. [29], our study also conducted a detailed examination of the PI3K/Akt signaling pathway proteins. Considering IRS-1/PI3K/Akt insulin signaling pathway parameters in skeletal muscle tissue, significant increases were observed in the groups administered 100 and 200 mg/kg curcumin (Table 2). In the group treated with 200 mg/kg curcumin, there were significant increases in AMPK activation and GLUT4 levels in skeletal muscle tissue. However, only partial increases in AMPK activation and GLUT4 levels were observed in skeletal muscle tissue in the group treated with 100 mg/kg curcumin (Table 2). Based on the results of our study, in rat groups fed with a high fructose diet, skeletal muscle insulin signaling pathway parameters and membrane GLUT4 protein levels decreased, contributing to insulin resistance. The non-significant increase in GLUT4 protein in the group administered 100 mg/kg curc may be explained by the fact that it was not effective in lowering blood glucose (Table 1). In contrast, our study demonstrated that the administration of curcumin at doses of 100 mg/kg and 200 mg/kg improved skeletal muscle insulin signaling. Furthermore, the 200 mg/kg dose group significantly increased membrane GLUT4 protein levels ($p \leq 0.017$) (Table 2). This suggests that curcumin contributed to the reduction of insulin resistance, possibly by enhancing the IRS-1/PI3K/Akt pathway and, in the case of the 200 mg/kg dose, by significantly increasing membrane GLUT4 protein levels, thus facilitating glucose uptake into skeletal muscle cells, and improving insulin sensitivity.

Ding et al. [34] administered 40 mg/kg and 80 mg/kg of curc treatment for 12 days to obese rats induced by a high-fructose diet. The researchers illustrated that curcumin reduced lipid accumulation in mouse liver and

adipose tissues while enhancing insulin sensitivity. Wang et al. [35] demonstrated that AMPK activation increased with curc treatment in mice in which insulin resistance was induced with a high-fat diet for 10 days. Consequently, the protein kinase C (PKC)/Akt pathway prevented the release of free fatty acids from adipose tissue, which in turn reduced hepatic lipid absorption and prevented the development of hepatic insulin resistance.

In our study, p-IRS-1, IRS-1, PI3K, PIP3, PDK1, p-AKT, GLUT4 and AMPK levels in adipose tissue were significantly decreased in the positive control group in which insulin resistance was induced (Table 3). In both 100 mg/kg and 200 mg/kg curc groups, the parameters in the IRS-1/PI3K/Akt insulin signaling pathway and GLUT4 levels were increased in adipose tissue (Table 3). In addition, AMPK activation was significantly increased in both curcumin dose groups, similar to the study of Wang et al. [35]. These changes are aligned with observations made in previous studies regarding the beneficial effects of curcumin on insulin sensitivity via adipose tissue effects [34, 35]. Based on the results of our study, in rat groups fed with a high-fructose diet, there was a decrease observed in adipose tissue insulin signaling pathway parameters and membrane GLUT4 protein levels, thereby contributing to insulin resistance (Table 3). However, our study also demonstrated that the administration of curc at doses of 100 mg/kg and 200 mg/kg improved adipose tissue insulin signaling, AMPK activation and significantly increased membrane GLUT4 protein levels, thus contributing to the reduction of insulin resistance (Table 3).

One of the strengths of our study is that the effect of curcumin on insulin resistance was examined in both muscle and adipose tissue. Furthermore, the detailed presentation of the results of both PI3K/Akt signaling pathway proteins and AMPK activation as well as the decrease in insulin resistance under the effect of curcumin highlights the importance of our study. However, the small sample size in our study constitutes a limitation in terms of generalizing our findings.

In the conclusion of our study, we identified that the administration of curcumin exhibited ameliorative effects on insulin signaling and AMPK activation in rats where insulin resistance was induced through a 20 % fructose administration. In our study, it was demonstrated that both 100 mg/kg and 200 mg/kg curcumin administrations were effective in eliminating insulin resistance. These results contribute significantly to both the existing literature and provide novel insights into potential measures for addressing insulin resistance within the community.

Acknowledgments: We would like to thank all the staff of Gazi University Experimental Research Unit (GUDAM) and Gazi University Scientific Research Projects Coordination Unit (project code TTU-2021-7153).

Research ethics: Research ethics was obtained from the Animal Experiments Local Ethics Committee, Gazi University (G.Ü.ET-21.012).

Informed consent: Not applicable.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Research funding: None declared.

Data availability: The raw data can be obtained on request from the corresponding author.

References

1. Yaribeygi H, Farrokhi FR, Butler AE, Sahebkar A. Insulin resistance: review of the underlying molecular mechanisms. J Cell Physiol 2019; 234:8152–61.
2. McCracken E, Monaghan M, Sreenivasan S. Pathophysiology of the metabolic syndrome. Clin Dermatol 2018;36:14–20.
3. Mazzoli A, Porzio AD, Gatto C, Crescenzo R, Nazzaro M, Spagnuolo MS, et al. Skeletal muscle insulin resistance and adipose tissue hypertrophy persist beyond the reshaping of gut microbiota in young rats fed a fructose-rich diet. J Nutr Biochem 2023;113:109247.
4. Said MA, Nafeh NY, Abdallah HA. Spexin alleviates hypertension, hyperuricaemia, dyslipidemia and insulin resistance in high fructose diet induced metabolic syndrome in rats via enhancing PPAR-ɣ and AMPK and inhibiting IL-6 and TNF-α. Arch Physiol Biochem 2023;129: 1111–6.
5. Khanal P, Patil BM. Reversal of insulin resistance by Ficus benghalensis bark in fructose-induced insulin-resistant rats. J Ethnopharmacol 2022; 284:114761.
6. Ramos VW, Batista LO, Albuquerque KT. Effects of fructose consumption on food intake and biochemical and body parameters in Wistar rats. Rev Port Cardiol 2017;36:937–41.
7. Vlavcheski F, Den Hartogh DJ, Giacca A, Tsiani E. Amelioration of high-insulin-induced skeletal muscle cell insulin resistance by resveratrol is linked to activation of AMPK and restoration of GLUT4 translocation. Nutrients 2020;12:914.
8. Luo L, Liu M. Adipose tissue in control of metabolism. J Endocrinol 2016; 231:R77–99.
9. Huang X, Liu G, Guo J, Su Z. The PI3K/AKT pathway in obesity and type 2 diabetes. Int J Biol Sci 2018;14:1483–96.
10. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. Physiol Rev 2018;98:2133–223.
11. Savova MS, Mihaylova LV, Tews D, Wabitsch M, Georgiev MI. Targeting PI3K/AKT signaling pathway in obesity. Biomed Pharmacother 2023; 159:114244.
12. Satoh T. Molecular mechanisms for the regulation of insulin-stimulated glucose uptake by small guanosine triphosphatases in skeletal muscle and adipocytes. Int J Mol Sci 2014;15:18677–92.

13. Entezari M, Hashemi D, Taheriazam A, Zabolian A, Mohammadi S, Fakhri F, et al. AMPK signaling in diabetes mellitus, insulin resistance and diabetic complications: a pre-clinical and clinical investigation. Biomed Pharmacother 2022;146:112563.
14. Lin W, Jin Y, Hu X, Huang E, Zhu Q. AMPK/PGC-1α/GLUT4-mediated effect of icariin on hyperlipidemia-induced non-alcoholic fatty liver disease and lipid metabolism disorder in mice. Biochemistry 2021;86:1407–17.
15. Li R, Yao Y, Gao P, Bu S. The therapeutic efficacy of curcumin vs. metformin in modulating the gut microbiota in NAFLD rats: a comparative study. Front Microbiol 2021;11:555293.
16. Zou T, Li S, Wang B, Wang Z, Liu Y, You J. Curcumin improves insulin sensitivity and increases energy expenditure in high-fat-diet–induced obese mice associated with activation of FNDC5/irisin. Nutrition 2021; 90:111263.
17. Severcan SM, Koca G, Severcan Ç, Yilmaz C, Paşaoğlu ÖT, Paşaoğlu H. Effect of curcumin on plasma TBARS and IL-6, adipose tissue AFABP-4 and liver FGF-21 in fructose-induced metabolic syndrome rats. Indian J Exp Biol 2023;61:640–5.
18. Brown RE, Jarvis KL, Hyland KJ. Protein measurement using bicinchoninic acid: elimination of interfering substances. Anal Biochem 1989;180:136–9.
19. Antunes LC, Elkfury JL, Jornada MN, Foletto KC, Bertoluci MC. Validation of HOMA-IR in a model of insulin-resistance induced by a high-fat diet in Wistar rats. Arch Endocrinol Metab 2016;60:138–42.
20. Orhan C, Kucuk O, Tuzcu M, Sahin N, Komorowski JR, Sahin K. Effect of supplementing chromium histidinate and picolinate complexes along with biotin on insulin sensitivity and related metabolic indices in rats fed a high-fat diet. Food Sci Nutr 2018;7:183–94.
21. Sahin K, Orhan C, Kucuk O, Tuzcu M, Sahin N, Ozercan IH, et al. Effects of magnesium picolinate, zinc picolinate, and selenomethionine co-supplementation on reproductive hormones, and glucose and lipid metabolism-related protein expressions in male rats fed a high-fat diet. Food Chem 2022;4:100081.
22. Van Dijk TH, Laskewitz AJ, Grefhorst A, Boer TS, Bloks VW, Kuipers F, et al. A novel approach to monitor glucose metabolism using stable isotopically labelled glucose in longitudinal studies in mice. Lab Anim 2013;47:79–88.
23. Mi Q, Li Y, Wang M, Yang G, Zhao X, Liu H, et al. Circulating C1q/TNF-related protein isoform 15 is a marker for the presence of metabolic syndrome. Diabetes Metab Res Rev 2019;35:e3085.
24. Shamsi-Goushki A, Mortazavi Z, Mirshekar MA, Mohammadi M, Moradi-Kor N, Jafari-Maskouni S, et al. Comparative effects of curcumin versus nano-curcumin on insulin resistance, serum levels of apelin and lipid profile in type 2 diabetic rats. Diabetes Metab Syndr Obes 2020;13: 2337–46.
25. Duan J, Yang M, Liu Y, Xiao S, Zhang X. Curcumin protects islet beta cells from streptozotocin-induced type 2 diabetes mellitus injury via its antioxidative effects. Endokrynol Pol 2022;73:942–6.
26. Kelany ME, Hakami TM, Omar AH. Curcumin improves the metabolic syndrome in high-fructose-diet-fed rats: role of TNF-α, NF-κB, and oxidative stress. Can J Physiol Pharmacol 2017;95:140–50.
27. Zhang Z, Liu H, Liu J. Akt activation: a potential strategy to ameliorate insulin resistance. Diabetes Res Clin Pract 2019;156:107092.
28. Norton L, Shannon C, Gastaldelli A, DeFronzo RA. Insulin: the master regulator of glucose metabolism. Metabolism 2022;129:155142.
29. Chauhan P, Tamrakar AK, Mahajan S, Prasad G. Chitosan encapsulated nanocurcumin induces GLUT-4 translocation and exhibits enhanced anti-hyperglycemic function. Life Sci 2018;213:226–35.
30. Prabha B, Sini S, Sherin DR, Neethu S, Rameshkumar KB, Manojkumar TK, et al. Promalabaricone B from Myristica fatua Houtt. seeds demonstrate antidiabetic potential by modulating glucose uptake via the upregulation of AMPK in L6 myotubes. Nat Prod Res 2019;35:1–6.
31. Jaiswal N, Gavin MG, Quinn WJ, Luongo TS, Gelfer RG, Baur JA, et al. The role of skeletal muscle Akt in the regulation of muscle mass and glucose homeostasis. Mol Metabol 2019;28:1–13.
32. Tan L, Song A, Ren L, Wang C, Song G. Effect of pioglitazone on skeletal muscle lipid deposition in the insulin resistance rat model induced by high fructose diet under AMPK signaling pathway. Saudi J Biol Sci 2020; 27:1317–23.
33. Li Y, Wang B, Shen J, Bai M, Xu E. Berberine attenuates fructose-induced insulin resistance by stimulating the hepatic LKB1/AMPK/PGC1α pathway in mice. Pharm Biol 2020;58:385–92.
34. Ding L, Li J, Song B, Xiao X, Zhang B, Qi M, et al. Curcumin rescues high fat diet-induced obesity and insulin sensitivity in mice through regulating SREBP pathway. Toxicol Appl Pharmacol 2016;304: 99–109.
35. Wang L, Zhang B, Huang F, Liu B, Xie Y. Curcumin inhibits lipolysis via suppression of ER stress in adipose tissue and prevents hepatic insulin resistance. J Lipid Res 2016;57:1243–55. TnQ