Journal of Functional Foods 60 (2019) 103430

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Curcumin exerts beneficial role on insulin resistance through modulation of SOCS3 and Rac-1 pathways in type 2 diabetic rats

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ARTICLE INFO

Keywords:
Curcumin
SOCS3
IRS1
STAT3
Type 2 diabetes mellitus

ABSTRACT

The present study examined the anti-diabetic effect of curcumin (cur) on insulin resistance (IR) through modulation of SOCS3 and Rac-1 pathways in rats with type 2 diabetes (T2D) induced by high-fat diet (HFD) and streptozotocin (STZ). The expression pattern of proteins and genes associated with insulin signaling pathways was measured by western blot and real-time PCR. Moreover, biomarkers of the oxidative stress in skeletal muscles were measured. Diabetic rats showed increased malondialdehyde (MDA) in skeletal muscles with a concomitant decrease in superoxide dismutase (SOD), and catalase (CAT) activities. Moreover, diabetic rats showed heightened levels of SOCS-3 and STAT-3 expression, as well as decreased IRS-1, Rac1, and Rac-GTP in skeletal muscles. The level of antioxidant enzyme activities reached the baseline after the treatment with cur. The anti-diabetic effect of cur was exerted by a reduction in the expression of STAT-3, SOCS3, as well as the elevation in IRS-1, Rac1, and Rac-GTP.

1. Introduction

Diabetes mellitus is defined as a group of chronic metabolic disorders characterized by the presence of a hyperglycemic state and subsequent complications in many tissues due to dysfunction of insulin-producing pancreatic beta cells and a peripheral IR in skeletal muscle, adipose tissue, and liver (Bouzakri, Koistinen, & Zierath, 2005; Esser, Legrand-Poels, Piette, Scheen, & Paquot, 2014). Environmental factors, including obesity, the consumption of a high caloric diet, physical inactivity, and genetic predisposition are considered potential risk factors for the development of type 2 diabetes mellitus (T2DM) (Liu et al., 2015; Sharma et al., 2011). Skeletal muscle is the principal tissue for the glucose uptake and considered one of the major sites of IR in obesity and T2DM (DeFronzo & Tripathy, 2009; Esser et al., 2014). Indeed, skeletal muscle IR is a primary defect in the pathogenesis of T2DM.

IR, characterized by impaired insulin action in adipose tissue, muscle, and liver, leads to the disturbance in the metabolism of glucose, fat, and protein (Feng, Tang, Leng, & Jiang, 2014). Although the molecular mechanism underlying IR in human is largely unknown, current research indicates an essential role for Insulin Receptor Substrate (IRS) proteins (Lavin, White, & Brazil, 2016). Insulin signaling pathway involves a family of cellular scaffold proteins (IRS proteins), playing a significant regulatory role in insulin signaling which translates the extracellular signals into the intracellular responses, ultimately leading to cellular consequences. IRS proteins include at least four distinct members namely IRS-1-4. Among them, IRS-1 and IRS-2 are broadly expressed in several human tissues (Cruz et al., 2013; Lavin et al., 2016). The phosphorylation of serine residues in IRS proteins incites the up-regulation of various cytokines and metabolites preventing the signal transduction. For example, circulating free fatty acids, diacylglycerol, fatty acyl-CoAs, glucose, or ceramides increase the phosphorylation of serine residues in IRS-1/IRS-2 (Lavin et al., 2016). Since SOCS family members can bind to certain tyrosine-phosphorylated proteins, such as IRS1/2 (Inagaki-Ohara & Yoshimura, 2014), they are capable of inhibiting the insulin signaling pathway by different mechanisms including the inhibition of tyrosine phosphorylation in IRS proteins, induction of the proteasomal degradation of IRS, and the suppression of insulin receptor kinase (Lebrun & Van Obberghen, 2008). An increasing body of evidence suggests that SOCS proteins play a crucial role in the progression of T2D (Feng et al., 2014), and among them, SOCS3 is predominantly involved in the development of obesity and diabetes (Inagaki-Ohara & Yoshimura, 2014). In vitro studies have shown SOCS3 impairs insulin action by targeting IRS1 and IRS2 thereby ubiquitin-mediated degradation or the oppression of the phosphorylation of

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https://doi.org/10.1016/j.jff.2019.103430
Received 3 December 2018; Received in revised form 19 June 2019; Accepted 20 June 2019

tyrosine receptors (Emanuelli et al., 2000; Rottenberg & Carow, 2014; Rui, Yuan, Frantz, Shoelson, & White, 2002). Cytokine-inducible SH2 protein (CIS)/SOCS has been shown to prevent the activation of the JAK-STAT pathway (Inagaki-Ohara & Yoshimura, 2014). Among the negative regulators of the JAK/STAT signaling pathway, SOCS3 protein is a classical negative inhibitor of the corresponding pathway. In particular, SOCS3 is a classic negative feedback inhibitor of the JAK2/STAT3 pathway (Xiang et al., 2013). The predominant function of SOCS3 is the repression of the signaling pathway induced by IL-6 resulting in hindrance of the activation of JAK/STAT3 (Yin, Liu, & Dai, 2015).

In mammals, the Rho family includes about 20 members into subfamilies. Rac1 is a small Rho family GTPase modulating several cellular processes. Rac1-GTP biologically is an active form of Rac1 and is converted into the inactive conformation following the hydrolysis of GTP to GDP by means of its intrinsic GTPase activity (Chiu, Jensen, Sylow, Richter, & Klip, 2011).

Rac1 is a regulator of insulin-stimulated translocation of GLUT4 to the skeletal muscle and also a new influential factor which is involved in the IR in skeletal muscle. With regard to recent reports, dysregulated Rac1 confers the risk for the progression of insulin resistance (Chiu et al., 2011; Sylow et al., 2013; Sylow et al., 2014).

The combination of high-fat feeding and low-to-moderate doses of STZ to induce mild-to-moderate IR may serve as an alternative way for the production of animal models to mimic the pathology of type 2 diabetes that is also suitable for the analysis and monitoring the anti-diabetic agents for the treatment of type 2 diabetes (Furman, 2015). HFD induces the intracellular accumulation of lipid metabolites in the liver and skeletal muscles giving rise to IR via decreased tyrosine phosphorylation of IRS (Sharma et al., 2011).

Recent *in vivo* studies revealed that cur might have anti-hyperglycemic impacts and improve IR (El-Moselhy, Taye, Sharkawi, El-Sisi, & Ahmed, 2011; Song et al., 2015). In addition, some reports have spotlighted that cur decreased the body weight in obese rats (Yu, Hu, & Yan, 2008). Several lines of evidence suggest that cur has pleiotropic effects on various biological targets. The targets consisted of transcription factors, a transcriptional coactivator, cytokines, enzymes, and growth factors along with their cognate receptors. Cur also hampers the activity of several protein kinases (Soetikno et al., 2012). In these studies, animals can be preferentially selected and recognized as good models to survey general principles, and the results can then be compared with those obtained from humans. Accordingly, in the present study, we hypothesised the efficacy of cur on the amelioration of hyperglycemia and the IR in experimental type 2 diabetic rats induced by a high fat-diet and STZ through the IRS-1/SOCS3/JAK-STAT3 pathway and Rac1 signaling.

2. Materials and methods

2.1. Chemicals and materials

The high-fat diet (HFD) was purchased from Royan Institute (Isfahan, Iran). The curcumin used in animal experiments was purchased from Sigma Chemical Co. (St. Louis, MO, Purity [high-performance liquid chromatography] > 80%, USA). Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). TRIzol reagent was purchased from Invitrogen. (Carlsbad, CA). Rat Leptin ELISA kit was purchased from Zell Bio GmbH Co. (Germany). Rat insulin ELISA kit was purchased from Mercodia Co. (Sweden) Serum lipid profile and serum glucose were analyzed using the commercial assay kits Pars Azmon Diagnostic Co (Iran). The RT-PCR assay kit was purchased from Takara Bio Inc. (Japan).

For immunoblots, polyclonal rabbit anti-SOCS3 primary antibody (ab16030), polyclonal rabbit anti-STAT3 primary antibody (ab68153), polyclonal rabbit anti-IRS-1 primary antibody (ab27712), monoclonal rabbit anti-Rac1 primary antibody (Abcam, Cambridge, UK), monoclonal mouse anti-Rac1-GTP (Newest biosciences) and polyclonal rabbit anti-β-actin (ab8227) were purchased from Abcam. Goat anti-rabbit IgG secondary antibody conjugated to horseradish peroxidase was purchased from Cell Signaling (Munich, Germany). The antibody detection kit (Enhanced Chemiluminescence) was purchased from Amersham (GE Healthcare, Life Sciences, UK).

2.2. Animal care and treatment

A total of 36 male Wistar rats (weighing 180–220 g, 6 weeks old) obtained from the Pasteur Institute (Tehran, Iran) were housed 6 per cage under the conditions of constant temperature 22 ± 2.0 °C, 50 ± 5% humidity, a light/dark cycle of 12 h with free access to food (normal pellet diet) and water. All of the study's protocols followed the current ethical considerations of local ethical committee of animal use. After adaptation for one week, rats were randomly divided into two groups consisting of 12 (group A) and 24 (group B) rats by feeding either a normal pellet diet or HFD (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal), respectively, for 4 weeks. After 4 weeks, the group B fed a high-fat diet, were kept fasting overnight and received a single intraperitoneal injection of streptozotocin at a low dose (35 mg/kg body weight in 0.1 mmol/L cold citrate buffer pH 4.5) while the respective control rats were given only citrate buffer. After 72 h, rats possessed a non-fasting blood glucose level above 300 mg/dl were coined as diabetic and selected for further pharmacological studies (Srinivasan, Viswanad, Asrat, Kaul, & Ramarao, 2005).

Experimental diabetic rats were successfully established and randomly divided into two groups: untreated diabetic (HFD/STZ, n = 12) and cur-treated (100 mg/kg BW/day) diabetic [(HFD/STZ) + cur, n = 12] groups (Soetikno et al., 2012). The cur-treated diabetic group received curcumin in 0.5% carboxymethylcellulose buffer solution through oral gavage. Rats in the control group were given 1% carboxymethylcellulose buffer only. Rats were allowed to continue feeding and treating on their respective diets until the end of the study up to 4 weeks. After 4 weeks of the treatment, animals were sacrificed under diethyl ether anesthesia. The blood sample was collected directly from the heart, and serum was separated and used for biochemical analysis. Skeletal muscles were quickly excised off and rinsed with a cold saline solution, then immediately frozen at −80 °C for until analysis.

2.3. OGTT

The day before rats were sacrificed, to assess the oral glucose tolerance, HFD–STZ–diabetic rats were kept fasting overnight, and then, their serum glucose response to the oral administration of a solution of 20% glucose (2 g/kg of body weight) dissolved in water via gavage was determined. Blood samples were collected from the tail vein before (time 0) and 30, 60, and 120 min after the administration of glucose, and glucose concentrations were determined by the glucose oxidase method. Animals were not anesthetized for this procedure.

2.4. Determination of serum leptin, insulin, glucose levels, and HOMA-IR

The level of serum leptin was measured using Rat Leptin ELISA kit. Serum glucose concentrations were determined by the glucose oxidase method, using a glucose assay kit. Fasting serum insulin was assayed by an ELISA kit following the manufacturer's protocol. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated to measure the insulin sensitivity of rats fed with HFD using the following formula: [fasting plasma insulin (mU/L) × fasting blood glucose (mmol/L)]/22.5.

2.5. Serum lipid profile

Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and Low-density lipoprotein cholesterol (LDL-C)

Table 1
Primer sequences used for real-time PCR.

Gene	Forward Primer (5′–3′)	Reverse Primer (5′–3′)	Product size (bp)
IRS1	TGGATGCAAGTGGATGACTC	CGGAGGATTGTTGAGATGGT	170
SOCS3	GGGACCAAGAACCTACGC	GCTGCTCCTGAACCTCAAA	186
STAT3	GACGGAGAAGCAGCAGATG	ACGATCCTCTCCTCCAGC	518

were analyzed using the commercial assay kits according to the manufacturer's protocols and recorded by a Roche BT 3000 Auto Analyzer.

2.6. Preparation of homogenates

Skeletal muscle homogenates were prepared using lysis buffer containing 0.1 M phosphate buffer, pH 7.4,1% Triton X-100, 2 mM PMSF, 0.1% SDS. The samples were centrifuged at 1500 rpm for 30 min at 4 °C (Ghoreshi et al., 2017). The supernatants were then collected, and the protein concentration was measured with the Bradford protein assay using Bovine serum albumin (BSA) as a standard.

2.7. Determination of MDA as a proxy for lipid peroxidation in skeletal muscle tissues

MDA level was calculated by the thiobarbituric acid (TBA) method. For the evaluation of MDA in tissue homogenates, 100 µL of homogenized skeletal muscle tissue was incubated with TBA and 8.1% SDS in a boiling water bath for 1 h. After cooling, the samples were centrifuged at 3000 rpm for 10 min, and the absorbance of the supernatant was measured at 532 nm. MDA equivalents were expressed as nmol/mg protein of the tissue.

2.8. Determination of catalase activity in skeletal muscle tissues

Catalase activity was assessed in tissue homogenates by the method of Aebi, in which the disappearance of peroxide is spectrophotometrically recorded at 240 nm. The reaction mixture containing potassium phosphate buffer (50 mM, pH 7.0) and 100 µL sample was incubated at room temperature for 10 min. The reaction was initiated by the addition of H_2O_2 (30 mM prepared in potassium phosphate buffer 50 mM, pH 7.0), and the decrease in the absorbance was measured for 3 min at 240 nm (Kabirifar et al., 2017). The value of 0.0394 $cm^{-1}{\cdot}mol^{-1}$ proposed by Nelson and Kiesow was introduced as the extinction coefficient of H_2O_2, and the results were expressed in IU/mg protein.

2.9. Determination of SOD activity in skeletal muscle tissues

The activity of SOD was measured using the method described by (Winterbourn, Hawkins, Brian, & Carrell, 1975) based on the inhibition of nitro blue tetrazolium (NBT) reduction by superoxide. The reaction mixture contained 0.067 M phosphate buffer (pH 7.8) that was added to 0.1 M EDTA containing 0.3 mM sodium cyanide, 1.5 mM NBT, and 0.1 ml of homogenate. Then, 0.12 mM riboflavin was added to each sample to initiate the reaction and was incubated for 12 min. The absorbance was recorded at 560 nm for 5 min the value of 0.00436 $cm^{-1}{\cdot}mol^{-1}$ was applied as the extinction coefficient. The amount of enzyme required to produce 50% inhibition was taken as 1U and results were expressed as U/mg protein.

2.10. Gene expression analysis by quantitative real-time PCR (qPCR)

Total RNA was purified from freshly isolated skeletal muscle tissue using 1 ml of the TRIzol reagent according to the manufacturer's protocol. The RNA purity and concentration were determined by a Nanodrop spectrophotometer (Epoch BioTek, USA) at A260/A280 nm absorbance ratio. Briefly, one microgram of total RNA was reverse-transcribed to cDNA using RT-PCR kit according to the manufacturer's instructions, and then qPCR was performed triplicate using SYBR-green (Takara, Japan) in the Rotor-Gene system (Corbett Research 2004, Australia). The program was set to run for one cycle at 95 °C for 10 min, followed by 40 cycles at 95 °C for 30 s, at 60 °C for 30 s, and at 72 °C for 30 s. For the PCR analysis, normalization was achieved against β-actin, and relative quantity of gene expression was calculated based on the $2^{-\Delta\Delta CT}$ method. Oligonucleotide primer sequences used for real-time PCR are shown in Table 1.

2.11. Western blot analysis

The protein concentration of skeletal muscle tissue homogenates was determined using the Bradford method. Equal amounts of protein samples (50 µg) were separated by SDS polyacrylamide gel electrophoresis and transferred onto a nitrocellulose (NC) filter membranes. The membranes were blocked with 5% bovine serum albumin (BSA) in TBST for 1 h at room temperature, then probed with polyclonal rabbit anti-SOCS3 primary antibody, polyclonal rabbit anti-STAT3 primary antibody, polyclonal rabbit anti-IRS-1 primary antibody, monoclonal rabbit anti-Rac1 primary antibody, monoclonal mouse anti-Rac1-GTP, and polyclonal rabbit anti-β-actin as a reference overnight at 4 °C. The membranes were washed and incubated with goat anti-rabbit IgG secondary antibody conjugated to horseradish peroxidase (1:4000) for 2 h at room temperature. Specific bands for antibodies were visualized by enhanced chemiluminescence on a ChemiDoc system (Syngene GBOX, 680X). The blot images were scanned and the intensity of the bands was densitometrically quantified by means of the ImageJ software (National Institutes of Health, Bethesda, MD, USA) (Kabirifar et al., 2018).

2.12. Statistical analysis

Data obtained from three groups (n = 10) were represented as the mean ± standard deviation (SD). Normality was tested by Shapiro-Wilk test. Difference between groups (mean ± SD) was calculated by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons using GraphPad Prism software version 6.01. The level of significance was set at $p < 0.05$.

3. Results

3.1. OGTT

OGTT acts as a sensitive marker to measure the changes in glucose tolerance in diabetic rats after curcumin treatment. The results of glucose changes are shown in Table 2. Glucose tolerance was impaired in HFD/STZ rats when compared with the control group ($P < 0.05$). As shown in Table 2, curcumin treatment showed a significant ($P < 0.05$) reduction in levels of blood glucose levels at all four time-points during OGTT.

3.2. Serum glucose, serum insulin, HOMA-IR, and serum leptin

The concentration of serum glucose, serum insulin, HOMA-IR, and serum leptin of the control, diabetic, and curcumin-treated diabetic groups are depicted in Table 3. Serum glucose, HOMA-IR, and serum leptin were significantly elevated in the diabetic rats as compared with

Table 2
OGTT results.

Time (min)	Control	HFD/STZ	(HFD/STZ) + Cur
0	76.57 ± 16.39	112.42 ± 53.23[a]	125.12 ± 53.97[b]
30	102.42 ± 8.94	298.21 ± 137.81[a]	140.74 ± 9.91[b]
60	129.71 ± 13.22	311.64 ± 179.70[a]	170.16 ± 69.01[b]
120	108.85 ± 6.96	266.81 ± 184.12[a]	115.98 ± 19.95[b]

Data are expressed as mean ± SD. [a] $P < 0.05$, compared with the control group; [b] $P < 0.05$, compared with the HFD/STZ group. OGTT: oral glucose tolerance test; HFD: High fat diet; STZ: Streptozotocine; Cur: Curcumin.

Table 3
The effects of curcumin on fasting blood glucose, insulin, HOMA-IR, and leptin in the serum of three groups.

Parameters	Control	HFD/STZ	(HF(HFD/STZ) + Cur
FBG (mmol/L)	3.82 ± 0.22	13.61 ± 0.47[a]	8.13 ± 0.47[b]
FBI (mU/L)	54.53 ± 0.51	27.08 ± 0.61[a]	38.49 ± 0.94[b]
HOMA-IR	9.26 ± 0.53	16.36 ± 0.44[a]	13.87 ± 0.86[b]
Leptin (ng/ml)	1.64 ± 0.36	2.99 ± 0.15[a]	1.42 ± 0.29[b]

Data are expressed as mean ± SD. [a]Represents $P < 0.0001$, compared with the control group; [b]Represents $P < 0.0001$, compared with the HFD/STZ group. FBG; fasting blood glucose, FBI; fasting blood insulin, HOMA-IR; homeostatic model assessment of insulin resistance, Cur; curcumin.

the control rats ($P < 0.0001$). Treatment with curcumin for 4 weeks resulted in a significant reduction in serum glucose and HOMA-IR as compared with the diabetic rats. Diabetic rats showed a significant reduction in serum insulin in comparison with the control rats. The results showed that the treatment with curcumin caused a significant elevation in serum insulin ($P < 0.0001$).

3.3. Serum lipid profile

As shown in Table 4, a significant alteration in the lipid profile of HFD-STZ induced diabetic rats was observed. The diabetic rats statistically had higher levels of TG, TC, and LDL-c and lower levels of HDL-c levels ($P < 0.0001$) than the control group. Curcumin treatment significantly diminished the concentrations of TG, TC, and LDL-c and increased the levels of HDL-c as compared with the diabetic group receiving saline ($P < 0.05$). All of these factors approximately reached to the normal levels.

3.4. Curcumin attenuated HFD/STZ-induced oxidative stress

Fig. 1 illustrate the alteration of antioxidant parameters in skeletal muscle tissue. HFD/STZ-induced diabetic rats exhibited significant reductions in activity of SOD (Fig. 1A) and CAT (Fig. 1B), as well as an increase in MDA level ($P < 0.05$) (Fig. 1C). Curcumin-treated rats indicate a significant increase in activity of both SOD and CAT and a decrease in the concentration of MDA when compared with the diabetic rats ($P < 0.05$).

Table 4
The effects of curcumin on serum lipid profile.

Parameters	Control	HFD/STZ	(HFD/STZ) + Cur
Total cholesterol (mg/dl)	67.75 ± 6.39	129.02 ± 9.05[a]	82.33 ± 6.77[b]
TG (mg/dl)	56.54 ± 5.68	162.52 ± 8.06[a]	120.34 ± 12.56[b]
LDL (mg/dl)	85.45 ± 8.01	129.74 ± 7.27[a]	101.52 ± 8.04[b]
HDL (mg/dl)	40.85 ± 2.84	29.18 ± 2.33[a]	36.45 ± 2.89[b]

Data are expressed as mean ± SD. [a]Represents $P < 0.0001$, compared with the HFD/STZ group. [b]Represents $P < 0.0001$, compared with the HFD/STZ group. TC: total cholesterol, TG: total glycerol, LDL: low-density lipoprotein; HDL: high-density lipoprotein.

3.5. mRNA expression of IRS-1, SOCS3, and STAT3 in skeletal muscle

In the present study, we measured the mRNA expression levels of IRS-1, SOCS3, and STAT3 in skeletal muscle tissue by means of the qPCR method. The level of IRS-1 expression was significantly lower in the diabetic rats as compared with the control group ($P < 0.05$) (Fig. 2A). In contrast, the expression of SOCS3 and STAT3 was significantly increased in the skeletal muscle of the diabetic rats ($P < 0.05$) (Fig. 2B and C). However, curcumin treatment increased IRS-1 expression and decreased the gene expression of SOCS3 and STAT3 in skeletal muscle.

3.6. Protein expression of IRS-1, SOCS3, STAT3, Rac1, and Rac1-GTP in skeletal muscle

We measured the level of protein expression of IRS-1, SOCS3, STAT3, Rac1, and Rac1-GTP in skeletal muscle by western blot analysis to confirm the mRNA expression profile of the genes mentioned above and the results were normalized to β-actin expression. Our results demonstrated that there were significant decreases in protein expressions of IRS-1, Rac1, and Rac1-GTP. Contrarily, the expression of SOCS3 and STAT3 was heightened in the diabetic rats as compared with the control rats ($P < 0.05$). On the other hand, the expression levels of IRS-1, Rac1, and Rac1-GTP in the diabetic rats were increased, and the levels of SOCS3 and STAT3 were decreased in the curcumin-treated diabetic group as compared with the diabetic group ($P < 0.05$). The results are shown in (Fig. 3A–C).

4. Discussion

Nowadays, there is a global concern regarding the increased incidence of diabetes just like overweight and obesity, affecting millions of people and impose an enormous economic burden. T2DM accounts for more than 95% of diabetes (Antony, Sivasankaran, Ignacimuthu, & Al-Dhabi, 2017). Skeletal muscle is the most key tissue involved in maintaining glucose homeostasis (Bouzakri et al., 2005). The IR in skeletal muscle is an important hallmark for the pathogenesis of T2DM. High-fat diets induce IR in rodents and also promote obesity which leads to impaired glucose tolerance and an increased risk of T2DM (Antony et al., 2017). Obesity is associated with the resistance of insulin, dyslipidemia, hyperglycemia, and hypertension (Esser et al., 2014). Reed et al. reported that a high-fat diet combined with a relatively moderate amount of STZ in rats provide an alternative experimental animal model to mimic the pathophysiology of T2D (Reed et al., 2000). In the present study, the induction of diabetes was performed according to the modified method of Srinivasan, et al., by which rats are fed with HFD (58% of calories as fat) and administrated a low dose of STZ (35 mg/kg), resulted in a significant increase in serum glucose, HOMA-IR, and dyslipidemia, but a decrease in serum insulin. Although in our study, HFD/STZ-treated rats with a dose of 35 mg/kg STZ exhibited hyperglycemia, our results were in contrast to the findings of a study carried out by Zhang et al. They reported that STZ (35 mg/kg, i.p, once injection) failed to generate a significant hyperglycemia in HFD-fed rats and demonstrated that a combination of HFD and multiple low doses of STZ injection could induce a rat model that mimics the characteristics of T2D in humans (Zhang, Lv, Li, Xu, & Chen, 2009). On the other hand, Srinivasan et al. have reported that STZ (35 mg/kg, once injected) could be used as the optimum dose for the development of the diabetic model (Srinivasan et al., 2005), which was reflected in our study.

Moreover, in this study, the fasting serum insulin level was decreased in the diabetic group. The result was consistent with some studies performed by Feng, Tang, Jiang and Zhao (2017), but opposed with the results obtained in a study conducted by Zhang, Xu, Guo, Meng, and Li (2008). This contradiction might be due to the time of sampling in which the level of insulin is increased (hyperinsulinemia) at

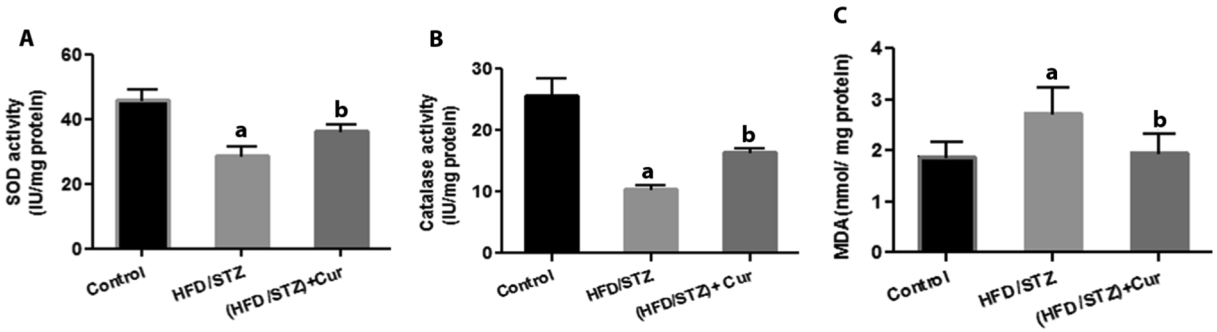

Fig. 1. The effects of curcumin on the oxidative stress parameters in skeletal muscle extracts. SOD activity (A), CAT activity (B), MDA level (C). [a] P value < 0.05 compared with the control group. [b] P value < 0.05 compared with the HFD/STZ group. HFD, High fat diet. STZ, Streptozotocine. Cur, Curcumin.

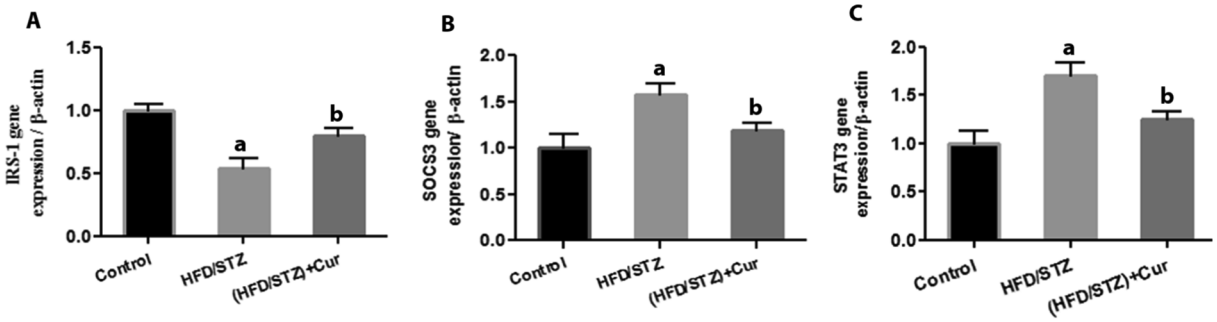

Fig. 2. The gene expression of IRS1 (A), SOCS3 (B), and STAT3 (C) in three studied groups (Control, HFD/STZ, (HFD/STZ) + Cur). [a] P value < 0.05 compared with the control group. [b] P value < 0.05 compared with the HFD/STZ group. HFD, High fat diet. STZ, Streptozotocine. Cur, Curcumin.

the early stage of T2DM, playing a compensatory role in response to IR. However, the level of insulin is gradually decreased in parallel with the progression of diabetes since massive destruction occurs in pancreatic β cells.

Accumulating evidence suggests that curcumin influences the glucose and lipid metabolism in muscle (Seo et al., 2008) and has a potential anti-hyperglycemic effect on experimental diabetic animal models, resulting in improved fasting plasma glucose, insulin levels, insulin sensitivity, and glucose disposal (El-Moselhy et al., 2011). In this study, curcumin treatment not only significantly reduced fasting serum glucose in HFD/STZ-induced diabetic rats, but also remarkably decreased HOMA-IR. Curcumin treatment also reduced fasting blood insulin to some extent, indicating the amelioration of IR in consideration of decreased fasting serum glucose and HOMA-IR by curcumin. In addition to hyperglycemia in HFD/STZ-induced diabetic rats, dyslipidemia was also observed. Dyslipidemia is characterized by an increase in triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and a decrease in high-density lipoprotein cholesterol (HDL-c) levels, and it was shown to be associated with T2DM (Gong et al., 2009). In the present study, a significant alteration in lipid profile in HFD/STZ-induced diabetic rats was found, in which an increase in TG, TC, and LDL-c and a decrease in HDL-c levels were observed. The results denote the onset of dyslipidemia, and the obtained data are in line with the previous studies performed in this area (Irudayaraj et al., 2016; Khan, Vinayagam, Moorthy, Palanivelu, & Panchanatham, 2013; Pushparaj, Low, Manikandan, Tan, & Tan, 2007; Sharma et al., 2011). Upon the treatment with curcumin, the concentrations of TG, TC, and LDL-c were strikingly reduced, and the levels of HDL-c was increased. Interestingly, the above-mentioned factors, which were dysregulated in response to diabetes induction reached to the baseline levels.

The data suggest that curcumin enhance dyslipidemia and anti-

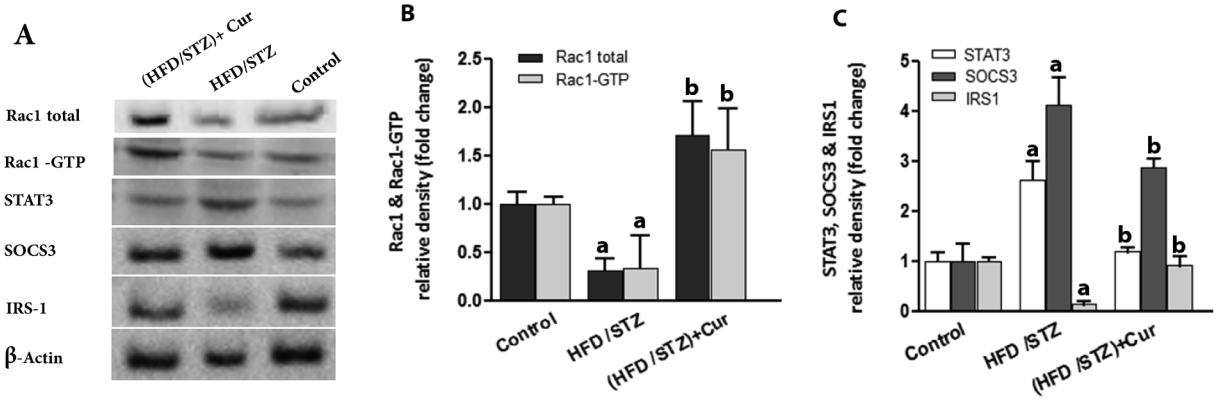

Fig. 3. Western blotting pattern of Rac1, Rac1-GTP, STAT3, SOCS3 and IRS1 proteins expression (A); the relative density of protein expression levels of Rac1, Rac1-GTP which involve in ROS generation and insulin-stimulated Glucose Uptake (B) and STAT3, SOCS3, IRS1 which contribute to insulin resistance (C) in three studied groups (Control, HFD/STZ, (HFD/STZ) + Cur. Each bar represents the mean value of six rats ± S.D. [a] P value < 0.05 compared with the control group. [b] P value < 0.05 compared with the HFD group. HFD, High fat diet. STZ, Streptozotocine. Cur, Curcumin.

insulin resistance effect in rats with T2DM. In addition to dyslipidemia and hyperglycemia, the oxidative stress is also considered a significant risk factor for the onset and progression of T2DM (Rains & Jain, 2011). On the other hand, the elevations in glucose and possibly FFA levels, which are phenomena occurred in IR, induce the oxidative stress through the increased generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and/or by the alteration of the redox balance which can activate stress-sensitive signaling pathways (Evans, Goldfine, Maddux, & Grodsky, 2003; Rains & Jain, 2011). SOD and CAT are the major antioxidant enzymes involved in the direct scavenging oxygen free radicals (Matés, Pérez-Gómez, & De Castro, 1999). According to the previous reports, T2DM is allegedly associated with a decrease in the activity of key antioxidant enzymes and an increase in lipid peroxidation (Zhou et al., 2009). MDA is a bona fide biomarker of ROS-induced lipid peroxidation. In accordance with our previous research (Ren et al., 2015), we observed a significant reduction in activity of SOD, CAT, and an increase in the levels of MDA in skeletal muscle in HFD/STZ-induced diabetic rats. As expected, treatment with curcumin maintained SOD and CAT activity, and also reverted the increased MDA levels to near normal levels in diabetic rats, thus indicating a decreased rate of lipid peroxidation. There is a great deal of evidence indicating that lipid accumulation within skeletal muscle interferes with insulin signaling and contribute to IR in obesity and T2DM (Goodpaster, He, Watkins, & Kelley, 2001; Kelley & Mandarino, 2000; Pan et al., 1997; Stein, Dobbins, Szczepaniak, Malloy, & McGarry, 1997). Recent studies have indicated a direct relationship between the protein expression of SOCS and IR due to the downregulation of the insulin transduction pathway (Lebrun & Van Obberghen, 2008). A number of studies revealed that SOCS-3 is increased (at the gene and protein expression) in patients with T2DM and animal models of insulin resistant (Feng et al., 2014). Ueki et al. reported that in skeletal muscle of obese insulin-resistant db/db mice, SOCS-3 mRNA was markedly increased. Furthermore, in skeletal muscle of LPS-induced insulin resistant mice, SOCS3 mRNA expression was up-regulated (Mooney et al., 2001). Specifically, deletion of SOCS-3 in skeletal muscle inhibits IR in mice, and because of increasing skeletal muscle glucose uptake, the lack of SOCS3 improves systemic insulin sensitivity (Jorgensen et al., 2013). The above evidence suggests a close correlation among SOCS-3, T2D, and IR (Feng et al., 2014). Moreover, SOCS family members were shown to have a strong regulatory role in insulin signaling by various mechanisms (Suchy, Łabuzek, Machnik, Kozłowski, & Okopień, 2013). SOCS-1 and SOCS-3 can impede the insulin signaling pathway thereby controlling the level of IRS-1 and IRS-2 proteins (Lebrun & Van Obberghen, 2008). Indeed, SOCS-1 and SOCS-3 target IRS-1 and IRS-2 for ubiquitination, thus promoting the degradation of both IRS-1/2 (Feng et al., 2014). SOCS3 also modulates the cytokine receptor signaling by several mechanisms. The major role of SOCS3 is to prevent the commencement of signaling pathways which are induced by IL-6 via the inhibition of JAK/STAT signaling (Xiang et al., 2013). Moreover, SOCS3 acts as a linker between the JAK/STAT pathway and insulin signaling pathway (Rieusset et al., 2004). Previous studies have demonstrated that STAT3 is capable of regulating IR and involved in the pathogenesis of skeletal muscle IR in obesity and T2DM. It has also been implicated that STAT3 is activated by the increased circulating levels of adipocytokines, such as leptin resulted from heightened levels of nutrients and inflammation (Du & Wei, 2014; Wunderlich, Hövelmeyer, & Wunderlich, 2013). As earlier mentioned, mice lacking *socs3* gene in their skeletal muscle are resistant to develop hyperinsulinemia and IR. The lack of socs3 gene increases the phosphorylation of IRS-1 and Akt in skeletal muscle, subsequently improving the glucose uptake by the muscles (Jorgensen et al., 2013). Furthermore, Yang et al. demonstrated that the overexpression of SOCS-3 in mice skeletal muscle leads to the exacerbation of both skeletal muscle-specific and systemic IR by the suppression of IRS-1 phosphorylation (Yang et al., 2012).

It has been indicated that the increased basal circulating leptin in HFD-induced obesity can elevate the activity of LepRb-induced STAT3 signaling. The LepRb-induced STAT3 activation sequentially increases the expression of SOCS3, which leads to impaired leptin action and resistance (Münzberg, 2010). Alternatively, an increased level of SOCS-3 can hinder the activation of leptin signaling and result in leptin resistance in peripheral tissues. In fact, SOCS-3 binds to JAK2 and attenuates leptin receptor signaling due to inhibiting leptin-induced tyrosine phosphorylation of JAK2 (Bjørbæk, El-Haschimi, Frantz, & Flier, 1999). These findings reveal that the chronic activation of the JAK-STAT3-SOCS3 pathway in skeletal muscle impairs insulin action and systemic insulin sensitivity.

In the present study, SOCS-3 and STAT3 were significantly increased at the levels of gene and protein expression, and the expression of IRS-1 was decreased in skeletal muscles of rats with type 2 diabetes. Our results displayed that curcumin treatment markedly reduced the expression of SOCS-3 and STAT3, and also increased the levels of IRS-1 in skeletal muscles.

According to the recent reports, insulin activates Rac1 in muscle. In skeletal muscle, Rac1 is required for insulin-stimulated GLUT4 translocation, and dysregulation of Rac1 signaling could result in insulin resistance in muscles of mice and humans. In this context, Sylow et al. reported that the inhibition or knockout of Rac1 protein abolished insulin-stimulated glucose uptake and muscle insulin sensitivity (Sylow et al., 2013).

In other words, the Rac1 expression is decreased in muscles of mice with T2DM and in those fed with HFD. JeBailey et al. showed that Rac1 signaling is less active in L6 myoblasts of insulin-resistant rats (JeBailey et al., 2007). In this regard, Raun et al. recently reported that the lack of Rac1 exacerbates the harmful impact of a high-fat diet in skeletal muscle, which is thought to be caused by a decrease in insulin-stimulated glucose uptake (Raun et al., 2018).

In agreement with a reduction in Rac1 protein expression, in the present study, Rac1 and Rac1-GTP protein levels were significantly decreased in skeletal muscles of HFD/STZ-induced diabetic rats. Curcumin treatment markedly increased the expression of Rac-1 and Rac1-GTP in skeletal muscles. Concerning the dysregulation of Rac1 occurs in response to chronic exposure to insulin resistance condition, it would be expected that in T2DM the expression of Rac1 and Rac1-GTP would be reduced due to the existence of IR.

5. Conclusion

However, the present results show that curcumin improves the detrimental effect of HFD via attenuating SOCS3 expression which, in turn, decreases inflammatory responses. Beside, curcumin increases Rac1 expression in insulin-dependent GluT4 translocation and maintains the normal glucose tolerance and insulin sensitivity in skeletal muscle tissues of rats.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

The results described in this paper were extracted from a Master's thesis and the authors wish to thank Miss Hafizi and Mr. Movahedi for research support. All authors contributed to the literature search, analysis of the data published, manuscript writing and revisions of the article. This article was supported by Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

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