Curcumin ameliorates protein expression changes involved in mitochondrial fatty acids metabolism in heart of mice fed a high-fructose diet

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Research

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Abstract

Background: It has been proposed that curcumin modulates the gene expression of different signaling pathways, improve the fatty acids metabolism and exerts a potential beneficial effect on cardiometabolic disease, but this has not been thoroughly demonstrated. In the present study, we evaluated the effect of curcumin upon the expression of PPARα, CPT1, MCAD, VLCAD and ACAA2 in heart of mice fed a high-fructose diet (HFD).

Methods: Twenty-four mice C57BL/6 were divided into four groups (n=6) and treated for 15 weeks. Control group (C) received standard diet (SD), Curcumin group (C+Cur), Fructose group (F) and Fructose with Curcumin group (F+Cur). The groups were treated with 0.75% w/w curcumin mixed in the SD and 30% w/v fructose in water, respectively. Heart proteins expression were analyzed by Western Blot.

Results: Curcumin supplementation increased PPARα and ACAA2 expression and decreased CPT1 and MCAD expression in heart of mice fed a HFD. However, it did not modify the VLCAD expression.

Conclusions: Curcumin regulated PPARα, CPT1 and MCAD expression and increased ACAA2 expression; suggesting a therapeutic potential in the prevention of alterations in mitochondrial fatty acids metabolism in heart of mice fed a HFD.

Background

Consumption of high-fructose diet (HFD) has increased considerably in recent years, primarily as result of excessive intake of beverages with high-fructose corn syrup. Epidemiological studies have associated high-fructose ingestion with metabolic disorders as hypertension, dyslipidemia, obesity, insulin resistance, fatty liver and cardiovascular diseases [1, 2]. Fructose catabolism and its regulation are quite different from glucose [3]. Fructose is metabolized in liver by fructolysis pathway that targets de novo lipogenesis. Primary metabolites and by-products of fructose metabolism include glucose, free fatty acids (FFA), very low-density lipoprotein (VLDL), uric acid (UA) and methylglyoxal (MG) [4]. These metabolites are considered direct dangerous factors, with the potential to disturb functions of extrahepatic tissues and organs as the heart [5].

The FFA are the main energy source of heart, but an increase in the FFA absorption by the cardiomyocyte has been associated with excessive oxidation and higher production of reactive oxygen species (ROS), leading to cardiometabolic disease [6]. In cardiac tissue, the Peroxisome proliferator-activated receptor (PPAR) regulates genes expression involved in fatty acids metabolism [7]. PPARs are a family of fatty acids-activated transcription factors composed by 3 different isoforms (α, β/δ, and γ) that regulate transcription of a large variety of genes involved in metabolism, inflammation, proliferation and differentiation [8].

PPARα is expressed in tissues with high rates of fatty acid oxidation like the heart. In the cardiomyocyte, PPARα regulates the malonyl-CoA decarboxylase (MCAD) expression, enzyme involved in the conversion

of malonyl-CoA to acetyl-CoA [9, 10]. This transcriptional factor also regulates the expression of the carnitine palmitoyl transferase 1 (CPT1) that transports fatty acids to the mitochondrial matrix [11], and enzymes involved in β-oxidation like the very long chain acyl-CoA dehydrogenase (VLCAD) and the acetyl-CoA acyl transferase 2 (ACAA2) [12, 13]. Previous studies suggest that the pathogenesis of fructose-induced metabolic syndrome is related to a reduced PPARα expression due to alterations in the state of DNA methylation [12, 14].

In the clinical practice are used two principal synthetic agonists for the PPARs; the fibrates that activate PPARα and produce a lipid-lowering effect, and the thiazolidinediones or glitazones that activate PPARγ, which are used clinically in the treatment of type 2 diabetes mellitus (DM2) [15]. However, these drugs have negative side effects such as an increase of food intake and increase adipogenesis in heart and liver. Some plant origin compounds, such as polyphenols, exert an activity similar to PPARs synthetic agonists without side effects, therefore, they are of key interest [16]. Curcumin is a natural polyphenol extracted from the turmeric rhizome (Curcuma longa), which is widely used as a spice in food preparation in Asian countries. In vitro and in vivo studies have reported that curcumin exhibits potent antioxidant, anti-inflammatory and anti-cancer activities [17, 18].

Curcumin action mechanisms include modulation of signal transduction cascades and gene expression regulation. However, these mechanisms have not been fully elucidated [19]. Researches performed in diabetic mice have reported that curcumin and its tetrahydrocurcumin metabolite prevent oxidative stress, lipid peroxidation and reducing serum lipids by PPARγ activation in a similar way as others synthetic drugs, but without negative side effects [20, 21]. Although, curcumin hypolipidemic properties have been reported in certain studies, the molecular mechanism that underlies its protective effects on disorders in fructose-induced mitochondrial fatty acids metabolism is still unclear. Therefore, the present study was designed to analyze the effect of curcumin upon the expression of PPARα, CPT1, MCAD, VLCAD and ACAA2 in heart of mice fed a HFD.

Methods

Reagents and Antibodies

Fructose, potassium chloride, sodium chloride, ethylenediamine-tetraacetic acid (EDTA) and phenol were obtained from Sigma Chemical (St. Louis, MO, USA). TRIS reagent, sodium dodecyl sulfate (SDS), tween-20, glycerol, glycine and 2 β-mercaptoethanol were purchased from Bio-Rad (Mexico City, Mexico). Curcumin was obtained from the Botanimex Company (Guanajuato, Mexico). The standard rodent feed LabDiet® was purchased from PMI Nutrition® (St. Louis, MO, USA). The primary rabbit anti-mouse antibodies: anti-PPARα (ab178865), anti-CPT1 (ab234111), anti-MCAD (ab95945), anti-VLCAD (ab155138) and anti-ACAA2 (ab128911) were purchased from Abcam (Cambridge, UK).

Animals and Treatment

Male C57BL/6 mice (6 weeks old) were separately housed in cages and maintained under standard laboratory conditions (temperature 25°C ± 2°C, 12 h dark/light cycle), according to current Mexican legislation (NOM-062-ZOO-1999) and in accordance with the Guide for the care and use of laboratory animals of the National Institutes of Health (Bethesda, MD, USA).

All animals had free access to diet and water. All experimental procedures were approved by the Bioethics Committee of the University of Guanajuato (CIBIUG-P28-2017). Twenty-four mice were assigned to four groups (n=6) and treated for 15 weeks: Control group (C), which received standard diet. Curcumin group (C+Cur), Fructose group (F) and Fructose + Curcumin group (F+Cur). F groups were administered with 30% (w/v) fructose in the water [22] and Cur groups were administered with 0.75% (w/w) curcumin in the diet [20]. At the end of the treatment, the mice fasted for 8 h (after weighing), subsequently were sacrificed by decapitation, then the heart was removed and blood sample was obtained for measure serum glucose and cholesterol concentration, through a commercial kit (Spinreact, SA, Girona, Spain).

Heart proteins extraction

Heart proteins were obtained by homogenization at 4 °C in a potter in the presence of a protease inhibitor (Mini Complete, Roche, Mexico). To further limit proteolysis, protein isolation was performed using phenol extraction [23]. To solubilize and obtain completely denatured and reduced proteins, pellets were dried and resuspended in Laemli buffer (Tris-HCl 0.125 M, SDS 4%, Glycerol 20%, 2β-mercaptoethanol 10%, pH 6.8). To determinate protein concentration, the modified Bradford procedure was used [24]. A pool was made for every 2 random samples, obtaining 3 samples per group, which were used for Western Blot analysis.

Western Blot Analysis

Total protein was separated by 12% SDS-PAGE and transferred to nitrocellulose membrane in a Mini Trans-Blot cell (Bio-Rad, CA, USA) for 2 h at 120 V/h. The nitrocellulose membranes were blocked with TBS-Tween blocking buffer (25 mmol/L Tris, pH 7.6, 154 mmol/L NaCl, 0.1% Tween-20 and 4% milk) for 3 h. Subsequently, the membranes were incubated with the primary antibodies: anti-PPARα (1:12000/4 h), anti-CPT1 (1:1000/1 h), anti-MCAD (1:1000/12 h), anti-VLCAD (1:2000/4 h) and anti-ACAA2 (1:4000/4 h) at 4 °C. After 3 washes with TBS-Tween, the complexes were detected by goat anti-rabbit IgG secondary antibody (1:50000/1 h) at room temperature using an enhanced chemiluminescence protein detection kit (Wester Lightninh™ Plus-ECL, Perkin Elmer, Waltham, MA, USA), and with the use of the XRS+System photodocumenter (Bio Rad, CA, USA). The optical density of the bands was quantified with the Image Lab software (Bio Rad, Mexico). The nitrocellulose membranes were stained with Amido black to quantify the total protein, as a load control. The results were reported as the relation protein of interest/total protein and, each experiment was performed in triplicate.

Statistical Analysis

All results were presented as ~~the~~ mean ± SD. The differences among the groups were analyzed by one-way ANOVA followed by Tukey's post hoc test considering a value of $p<0.05$ as significant. All statistical analysis was performed in SPSS 23 software (IMB ® SPSS ® Statistics).

Results

Curcumin prevents high-fructose diet-induced body weight gain

It has been reported that high-fructose consumption for a long time leads to metabolic syndrome that includes dyslipidemia, obesity and diabetes and their associated cardiometabolic risks [1, 25]. Here, we used an animal model fed a HFD in order to induce alterations of mitochondrial fatty acids metabolism to explore whether curcumin ameliorate these fructose-induced alterations. The HFD caused higher body weight gain in the F group (11 ± 1.3 g) than the C and C+Cur groups (6.7 ± 0.6 and 8.1 ± 2.2 g, respectively; $p<0.01$). In F+Cur group, the body weight gain was significantly lower (8.1 ± 0.8 g, $p<0.05$) compared to the F group (Fig. 1).

In addition, total cholesterol concentration was higher in the F and F+Cur groups (156.5 ± 10 and 136.6 ± 6.5 mg/dL; $p<0.01$ and $p<0.05$, respectively) compared with the C group (110.3 ± 7.3 mg/dL). Similarly, total cholesterol concentration was higher in the F group compared with the C+Cur group (118.3 ± 14.3 mg/dL, $p<0.01$) (Fig. 2A). Fructose had not any effect on the glucose concentration (Fig. 2B).

Curcumin modulates proteins expression changes induced by high-fructose diet

While the relationship between HFD and heart disease development has been proposal, the underlying mechanisms are not yet completely understood. Therefore, to estimate the effects of curcumin on fructose-induced alterations, the expression of PPARα, CPT1, MCAD, VLCAD and ACAA2 were evaluated. The PPARα expression was lower in the F group compared to the C group ($p<0.05$). Of note, curcumin supplementation in the F+Cur group not only prevented the fructose-induced decrease, curcumin also induced a PPARα overexpression compared to F, C+Cur and C groups ($p<0.01$) (Fig. 3A). As for CPT1, a remarkable difference was found, where curcumin significantly increased CPT1 expression in the C+Cur group ($p<0.01$). Similarly, the CPT1 expression was higher in the F group ($p<0.05$) compared to the C group, while the CPT1 expression was lower in the F+Cur group compared to the C+Cur group ($p<0.05$) (Fig. 3B).

As shown in Figure 4A, MCAD expression increased in the F group compared to C and C+Cur groups ($p<0.01$), while curcumin supplementation prevented this fructose-induced increase in the F+Cur group ($p<0.01$) compared to the F group. With respect to ACAA2, curcumin administration increased its expression in the F+Cur group compared to the F and C+Cur groups ($p<0.01$) (Fig. 4B). With respect to the VLCAD expression, no statistically significant differences were observed between the groups at the end of the treatment.

Discussion

Curcumin has been shown to attenuate several aspects of metabolic syndrome by improving insulin sensitivity, suppressing adipogenesis, reducing high blood pressure, inflammation and oxidative stress [26, 27]. There is evidence that curcuminoids modulate the gene expression and enzymes activity, regulating the lipid homeostasis [28]. Here, the HFD increased total cholesterol concentration. However, although curcumin supplementation reduced its concentration, the difference was not significant. Probably if curcumin treatment is given for longer, a favorable effect would be observed. In addition, a complete lipid profile was not performed, so it is not known which lipoprotein is elevated. Previously it has been reported that curcumin improves abnormal lipid metabolism, reducing the serum TG and LDL concentrations and increasing the HDL concentration in diabetic rats [26].

The HFD promotes de novo lipogenesis that resulting in dyslipidemia [4]. Here, we used an animal model to induce changes in lipid metabolism without inducing diabetes. In the present study, the HFD after 15 weeks, did not induce changes in glucose concentration, so we did not expect an effect of curcumin. The same effect was reported by Yoo et al. [22]; where the administration of 30% fructose for 20 weeks does not induce changes in blood glucose. Another study reported that the HFD induced body weight gain in rats, while curcumin supplementation prevented an increase in fructose-induced body weight [29]. Consistent with our present findings, curcumin supplementation significantly prevented body weight gain in mice fed a HFD. To understand the molecular mechanisms by which curcumin exhibits beneficial effects on the lipid metabolism, we investigated the expression of key proteins involved in the lipid homeostasis.

Fructose is metabolized by insulin independent pathways, and its excessive intake increases synthesis of pyruvate and acetyl-CoA. The acetyl-CoA is utilized as the major substrate for de novo lipogenesis, inducing the overproduction of FFA [1]. The FFA in circulation are the main energetic substrate of the heart, and the uptake of FFA leads to PPARα activation in the cardiomyocyte. PPARα is a master regulator for fatty acid oxidation in heart. However, it has been reported that the HFD induces epigenetic changes that lead to a decrease in the hepatic PPARα expression, which contributes to metabolic syndrome development [14], including hyperlipidemia. On the contrary, it has been shown that PPARα agonists improve metabolic syndrome in rats [30]. According to our current findings, the HFD decreased the PPARα expression in the heart, while curcumin treatment significantly prevented this decrease, overexpressing PPARα.

The impact that curcumin exerts on PPARs is not fully understood, but it is suggested that exerts a similar effect to other drugs like fibrates that activate PPARα or the thiazolidinediones that increase PPARγ expression [20]. Consequently, the increase of fructose-induced FFA uptake leads to intracellular malonyl-CoA accumulation in heart [18]. The MCAD is an enzyme that degrading malonyl-CoA in acetyl-CoA, evidence suggests that its expression in muscle and liver is increased in conditions with high concentrations of circulating FFAs associated to HFD, high-fat diet (DAG) and obesity, in response to an increase in malonyl-CoA synthesis [31, 32]. Our data showed that the HFD increased MCAD expression, which suggests that the intracellular malonyl-CoA concentration was elevated in the cardiomyocyte.

However, it has been reported that malonyl-CoA is a potent inhibitor of CPT1, an enzyme involved in the transport of long-chain acyl-CoA into the mitochondria for its entry into β-oxidation [9].

Here, we found that the HFD induced CPT1 overexpression. However, there are two structural genes that code for CPT1, the liver enzyme isoform (L-CPT1) and the muscle isoform (M-CPT1), both isoforms are expressed in heart, but the L-CPT1 isoform is least sensitive to malonyl-CoA inhibition with a higher affinity for carnitine [33], and L-CPT1 was the isoform evaluated in this study, whereby its expression was not inhibited. Also, acute L-CPT1 isoform overexpression has been reported in cardiomyocytes that develop pathological hypertrophy [33].

In this study, we did not evaluate cardiac hypertrophy markers, however, it has been reported that feeding with 30% w/v fructose in mice C57BL/6 induces cardiac hypertrophy [34], so it is suggested that CPT1 overexpression could be an adaptive response to a possible HFD-induced hypertrophy. Further, taking into account that CPT1 and MCAD expression are under PPARα regulation, it was expected that reduced expression of fructose-induced PPARα will lead to a reduced expression of CPT1 and MCAD, however, both enzymes were overexpressed, therefor our present findings suggest that there are other mechanisms that regulate the expression or activity of these enzymes.

Curcumin effects on CPT1 expression was reported by Lone et al. [35], in isolated rat adipocytes, in which curcumin increased CPT1 expression in vitro. In contrast to their results, in regards to cardiac tissue, we found that supplementation with 0.75% of curcumin alone in the diet induces CPT1 overexpression, suggesting that the lipid-lowering potential attributed to curcumin is partly due to its effect on the CPT1 enzyme, which would increase the fatty acids transport into the mitochondria, favoring its oxidation and preventing its accumulation in the cardiomyocyte.

Conversely, curcumin supplementation in the F group, had a different effect on the CPT1 and MCAD expression, in which the fructose-induced overexpression of these proteins was prevented. In this case, it is suggested that curcumin may counteract the harmful effects of the HFD, as was reported by Maithilikarpagaselvi et al. [29], where observed a reduction of the fatty acids synthesis and their accumulation in liver. These effects were concomitant with decreased expression of lipogenic transcription factors (LXR-α and SREBP1c) in liver, preventing the body weight gain, as was observed in the present study. In this way, by preventing de novo lipogenesis increase, there is a decrease of serum FFA concentration in circulation and the entry of fatty acids into the heart could be regulated so that the CPT1 and MCAD expression is not altered regarding with the control group.

In addition, the HFD caused a lower ACAA2 expression, an enzyme that catalyzes the last reaction of β-oxidation, but the difference was not significant. In contrast to this result, Chan et al. [36]; reported that the administration of a DAG decreased the expression of key enzymes involved in β-oxidation at 10 week of treatment and this could be an adaptive response because there is little energy expenditure. However, Bruce et al. [37] have suggested that excess fatty acid flow into mitochondria is not accompanied by complete β-oxidation due to the inability of the tricarboxylic acid cycle (TCA) to cope to the increased uptake of FFA [37].

Furthermore, in the F group only a percentage of fatty acids could be entering β-oxidation without altering the VLCAD and ACAA2 expression, and probably the rest could be metabolized in fatty acid intermediates, such as diacylglycerol and ceramides, generating mitochondrial stress in the cardiomyocyte, which could contribute to expression changes of the rest of the proteins evaluated in this study [37]. Moreover, curcumin supplemented with the HFD increased ACAA2 expression, which suggests that fatty acids oxidation was not inhibited and this could be a beneficial effect of curcumin given that if the activity of ACAA2 is suppressed, the ability of mitochondria to oxidize fatty acids is limited [38]. In addition, PPARα was overexpressed when the curcumin was administrated together with HFD, this overexpression could upward regulate ACAA2, since its expression is under the transcriptional control of PPARα.

Finally, to our knowledge, the present study is one of the few that has examined the curcumin effects on protein expression that regulate lipid metabolism in heart of mouse fed a HFD. However, the present study did not evaluate the effect of curcumin in cardiac hypertrophy and lipid peroxidation, which would be important to carry out in subsequent studies.

Conclusions

In conclusion, we found that curcumin supplementation prevented PPARα, CPT1 and MCAD expression changes and increased ACAA2 expression in heart of mice fed induced by HFD. Significantly our study suggests that lipid-lowering effect of curcumin is associated with an increase in mitochondrial fatty acid oxidation and plays a key role in the regulation of heart lipid metabolism alterations, which is of great value to find effective therapeutic strategies for the prevention of fructose-induced alterations.

Abbreviations

ACAA2
Acetyl-CoA acyl-transferase 2
C
Control group
C + Cur
Curcumin group
CPT1
Carnitine palmitoyl transferase 1
DAG
High-fat diet
F
Fructose group
F + Cur
Fructose + Curcumin group
FFA
Free fatty acids

HFD
High-fructose diet
MCAD
Malonyl-CoA descarboxylase
MG
Methylglyoxal
PPAR
Peroxisome proliferator-activated receptor
ROS
Reactive oxygen species
SD
Standard diet
T2DM
Type diabetes mellitus
TCA
Tricarboxylic acid cycle
UA
Uric acid
VLCAD
Very long chain acyl-CoA dehydrogenase
VLDL
Very low-density lipoprotein.

Declarations

Ethics approval and consent to participate

The Ethics Committee for Animal Research approved animal care and experimental procedures used in the current study from the University of Guanajuato, Mexico (CIBIUG-P28-2017).

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

All authors participated in the design, interpretation of the studies, analysis of the data, and review of the manuscript. J.R.-E and V.P.-V managed and obtained project financing. V.P-V and J.R.-E participated in the conceptualization of the project. All authors read and approved the final manuscript.

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Figures

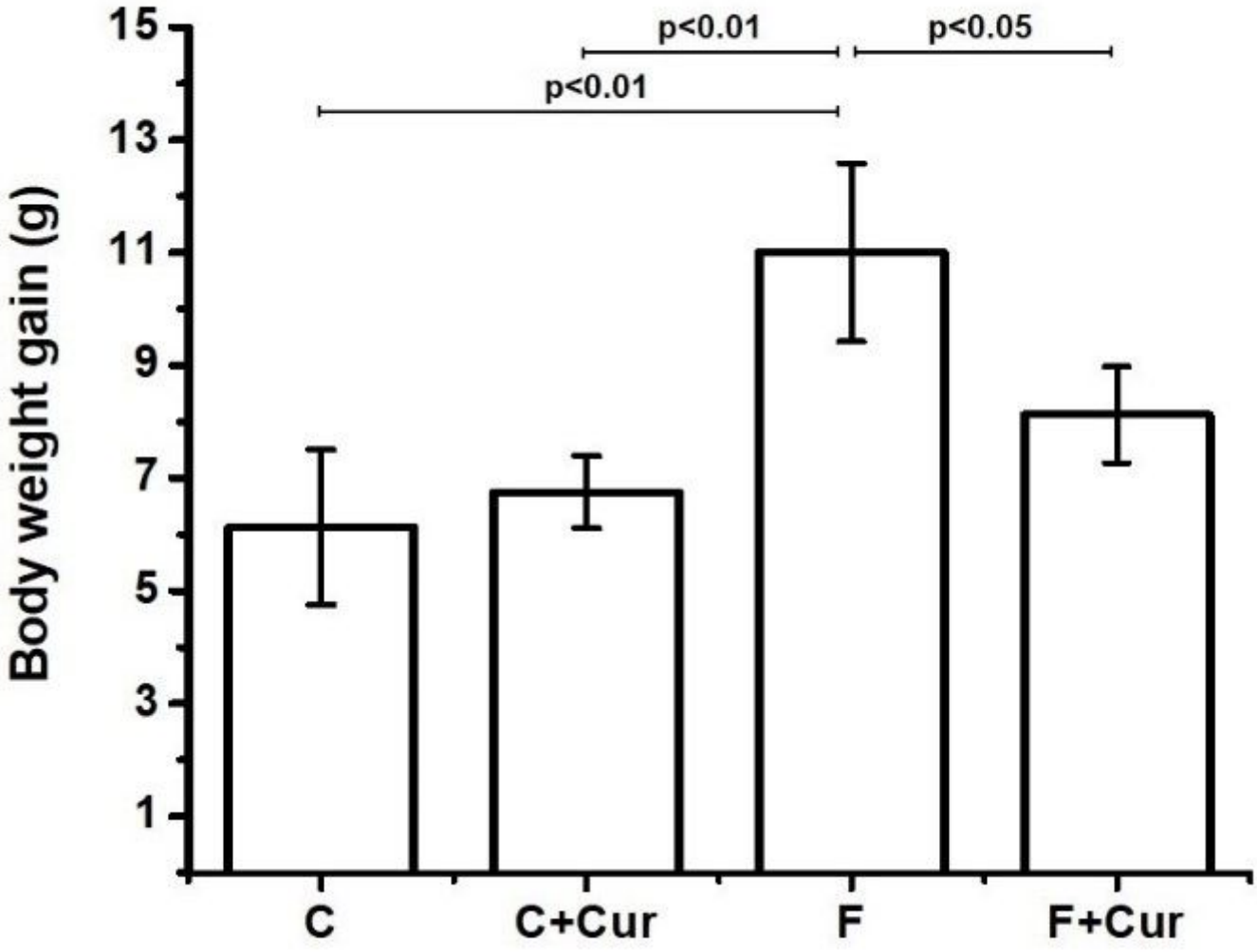

Figure 1

Effect of curcumin on body weight gain in high-fructose-fed mice. Results represent the mean ± SD (n=6). C: Control group, C+Cur: Curcumin group, F: Fructose group, F+Cur: Fructose + Curcumin group.

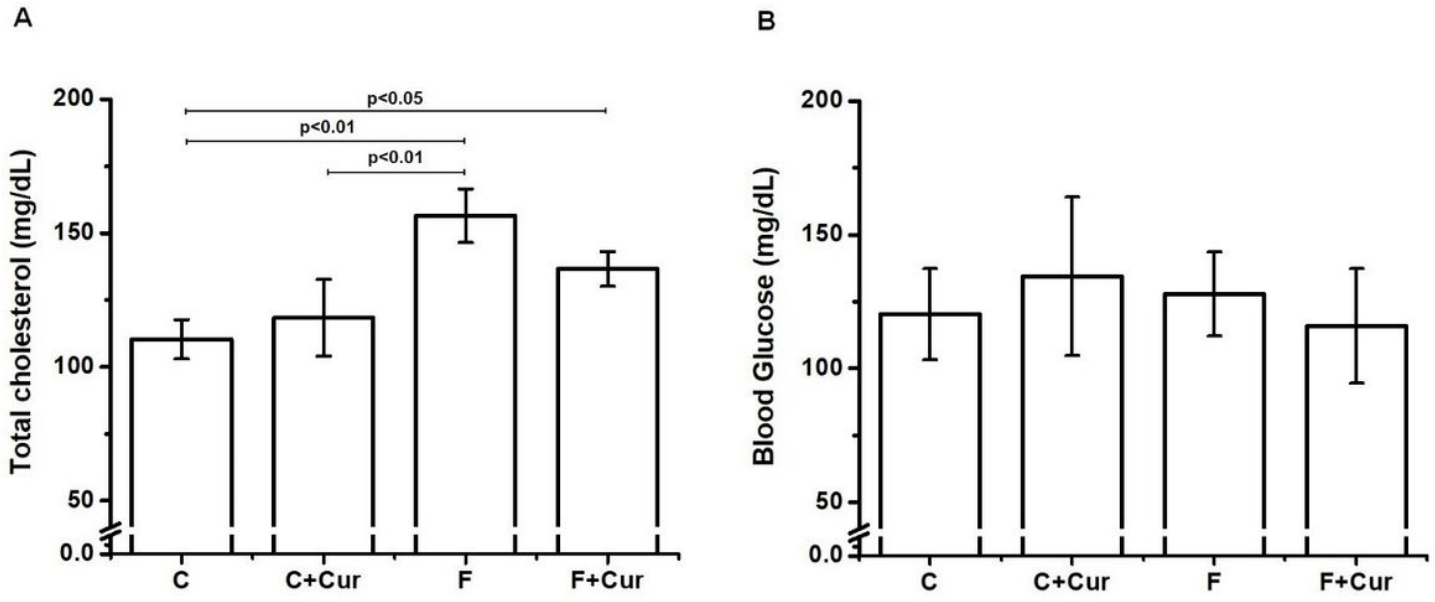

Figure 2

Effect of curcumin on total cholesterol (A) and glucose concentrations (B) in high-fructose-fed mice. Data are presented as the mean ± SD (n=6). C: Control group, C+Cur: Curcumin group, F: Fructose group, F+Cur: Fructose + Curcumin group.

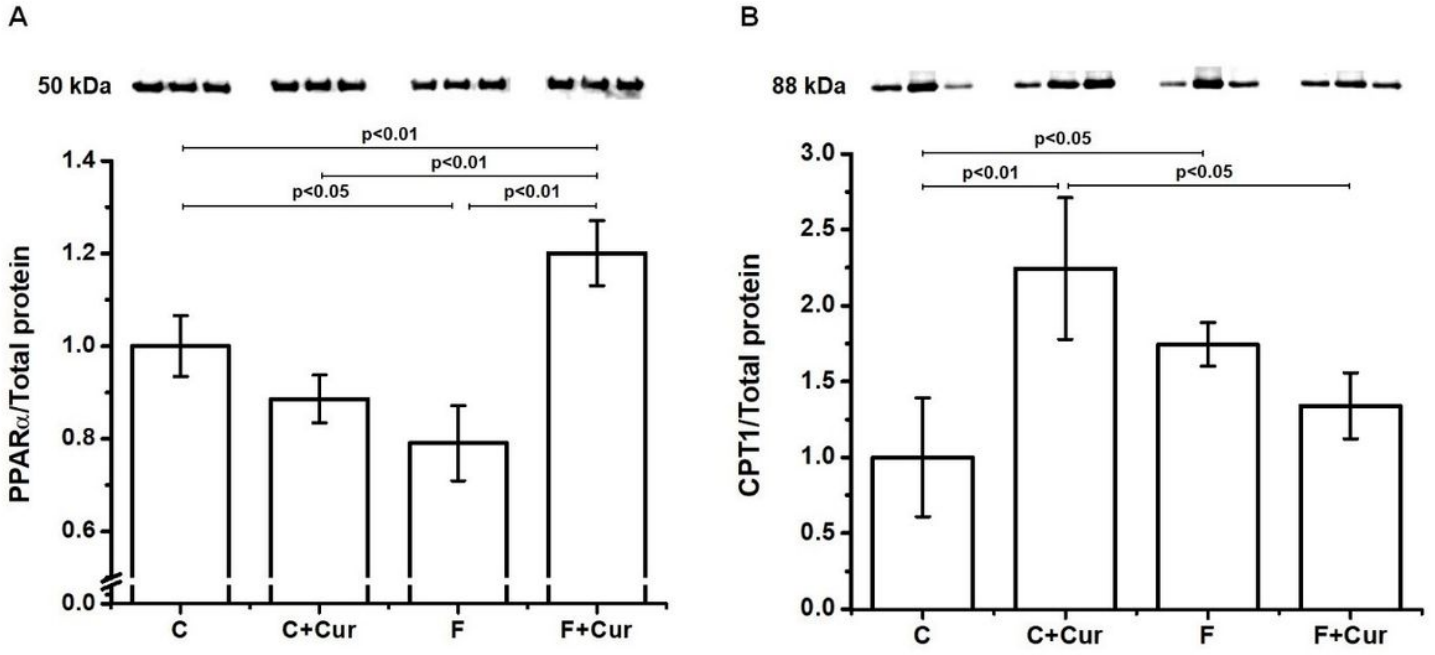

Figure 3

Effect of curcumin on the PPARα and CPT1 expression in heart of mice fed a high-fructose diet. Representative western blot and densitometric analysis of (A) PPARα/Total protein and (B) CPT1/Total protein ratios. Results represent the mean ± SD (n=3). C: Control group, C+Cur: Curcumin group, F: Fructose group, F+Cur: Fructose + Curcumin group.

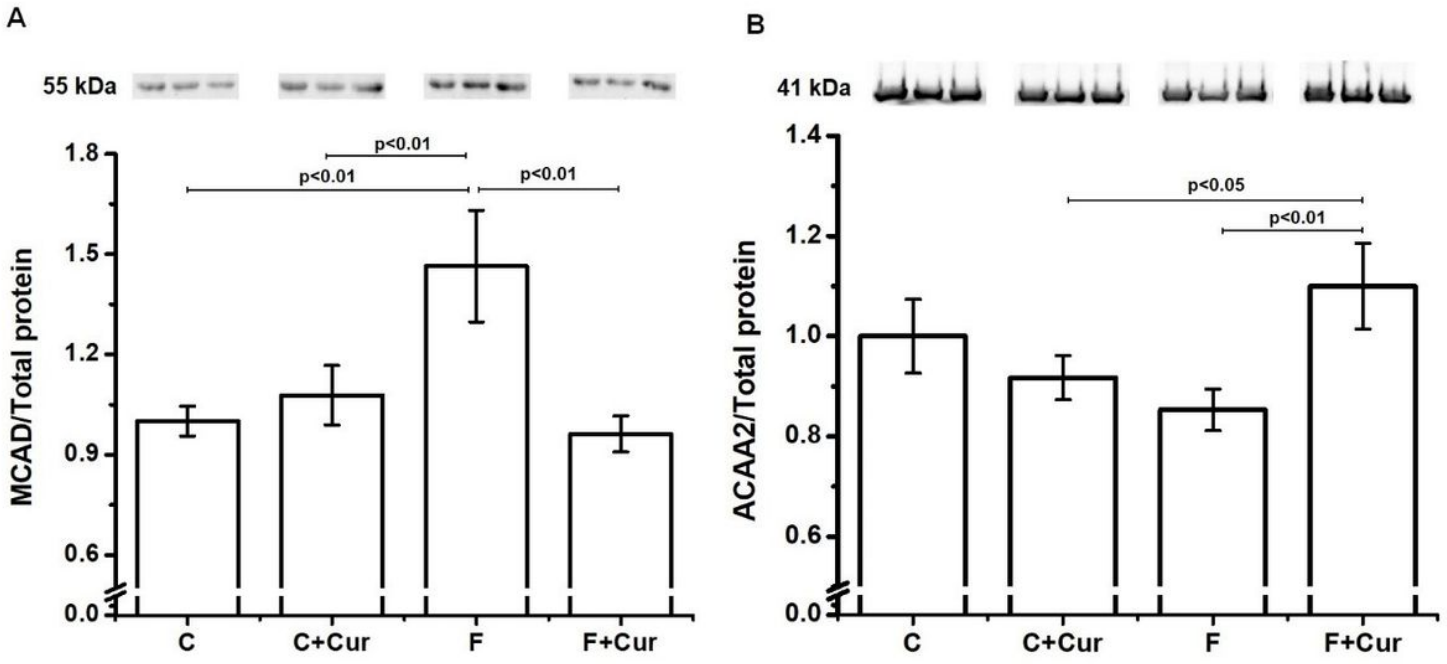

Figure 4

Effect of curcumin on the MCAD and ACAA2 expression in heart of mice fed a high-fructose diet. Representative western blot and densitometric analysis of (A) MCAD/Total protein and (B) ACAA2/Total protein ratios. Results represent the mean ± SD (n=3). C: Control group, C+Cur: Curcumin group, F: Fructose group, F+Cur: Fructose + Curcumin group.