Biochemical and Biophysical Research Communications 460 (2015) 977–982

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

TNF-α knockdown alleviates palmitate-induced insulin resistance in C2C12 skeletal muscle cells

Karimeh Haghani [a], Somayeh Pashaei [b], Sanaz Vakili [c], Gholamreza Taheripak [c], Salar Bakhtiyari [a, *]

[a] Department of Clinical Biochemistry, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran
[b] Student Research Committee, Ilam University of Medical Sciences, Ilam, Iran
[c] Department of Clinical Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 20 March 2015
Available online 1 April 2015

Keywords:
TNF-α
Short hairpin RNA
Insulin resistance
Knockdown
Palmitate

ABSTRACT

Insulin resistance is a cardinal feature of Type 2 Diabetes (T2D), which accompanied by lipid accumulation and TNF-α overexpression in skeletal muscle. The role of TNF-α in palmitate-induced insulin resistance remained to be elucidated. Here, we assessed effects of TNF-α knockdown on the components of insulin signaling pathway (IRS-1 and Akt) in palmitate-induced insulin resistant C2C12 skeletal muscle cells. To reduce TNF-α expression, C2C12 cells were transduced with TNF-α-shRNA lentiviral particles. Afterwards, the protein expression of TNF-α, IRS-1, and Akt, as well as phosphorylation levels of IRS-1 and Akt were evaluated by western blot. We also measured insulin-stimulated glucose uptake in the presence and absence of palmitate. TNF-α protein expression in C2C12 cells significantly increased by treatment with 0.75 mM palmitate ($P < 0.05$). In TNF-α knockdown cells, the protein expression level of TNF-α was significantly decreased by almost 70% ($P < 0.01$) compared with the control cells. Our results also revealed that, in control cells, palmitate treatment significantly reduced the insulin-induced phosphorylations of IRS-1 (Tyr632) and Akt (Ser473) by 60% and 66% ($P < 0.01$), respectively. Interestingly, these phosphorylations, even in the presence of palmitate, were not significantly reduced in TNF-α knockdown cells with respect to the untreated control cells ($P > 0.05$). Furthermore, palmitate significantly reduced insulin-dependent glucose uptake in control cells, however, it was not able to reduce insulin-stimulated glucose uptake in TNF-α knockdown cells in comparison with the untreated control cells ($P < 0.01$). These findings indicated that TNF-α down-regulation maintains insulin sensitivity, even in the presence of palmitate, therefore, TNF-α inhibition could be a good strategy for the treatment of palmitate-induced insulin resistance.

1. Introduction

Insulin resistance is the key feature of type 2 diabetes, which mainly occurs in skeletal muscle. Although many studies have focused on the underlying mechanisms of Insulin resistance, the exact mechanism is not yet fully understood [1,2]. Skeletal muscle is responsible for almost 80% of whole body insulin-stimulated glucose disposal, so it is important to investigate the possible reasons for insulin resistance in this tissue [3–5]. In the case of insulin resistance, because of reduced insulin sensitivity in the fat cells, the plasma level of free fatty acids (FFAs) increases, which in turn results in accumulation of FFAs and their metabolites in non-adipose tissues. The interference of these metabolites with the insulin signaling pathway leads to the reduced insulin sensitivity and finally development of type 2 diabetes [6–9]. Some evidences suggest that oversupplying FFAs cause intracellular accumulation of FFA-derived metabolites, which ultimately can phosphorylate insulin receptor substrate (IRS) proteins on Ser/Thr residues resulting to the inhibition of insulin receptor (IR)-mediated signaling and subsequent insulin resistance [10–15]. Palmitate is the most abundant FFA in the plasma [3] that has been shown to induce insulin resistance in insulin target tissues [16,17]. High concentrations of palmitate also induce inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) [18,19], which has been shown to be an important contributor to the development of insulin

* Corresponding author. Fax: +98 84 32235745.
E-mail addresses: bakhtiyaribio@gmail.com, bakhtiyari-s@medilam.ac.ir (S. Bakhtiyari).

http://dx.doi.org/10.1016/j.bbrc.2015.03.137
0006-291X/

resistance [20,21]. TNF-α can potently stimulate basal lipolysis in adipocytes which in turn may contribute to hyperlipidemia and peripheral insulin resistance seen in obesity [22–26]. Several studies have been demonstrated that saturated fatty acids, especially palmitate induces TNF-α expression in muscle cell [27–29]. However, the role of TNF-α in palmitate induced insulin resistance and the underlying molecular mechanism by which TNF-α impairs insulin signaling pathway has not been fully understood. Therefore, the aim of the present study was to explore the effect of TNF-α knockdown on the palmitate-induced insulin resistance. To this end, we assessed the beneficial phosphorylations of two key proteins (Tyr632-IRS-1 and Ser473-Akt) in the insulin signaling pathway and glucose uptake in TNF-α knockdown and control C2C12 cells in the presence and absence of palmitate.

2. Materials and methods

2.1. Cell culture

C2C12 myoblasts (Pasteur Institute, Iran) were cultured in Dulbecco's modified Eagle's Medium (DMEM; Gibco, Berlin, Germany) containing 10% fetal bovine serum (FBS), 2 mM glutamine, 100 unit/ml penicillin, and 100 μg/ml streptomycin. The cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 pressure. When the cells had achieved 70–80% confluence, differentiation of myoblasts into myotubes was induced by replacing the media with DMEM containing 2% horse serum, 2 mM glutamine, 100 unit/ml penicillin, and 100 μg/ml streptomycin. Four days after fusion, the differentiated myotubes were used for the experiments.

2.2. Lentivirus-mediated shRNA targeting mouse TNF-α

The shRNA-mediated knockdown of TNF-α was performed using shRNA lentiviral particles (Santa Cruz Biotechnology, Inc., Heidelberg, Germany), which were designed to suppress the production of TNF-α in mouse cells. C2C12 cells that were transduced with shRNA lentiviral particles with noneffective scrambled shRNA sequences were used as the control (scrambled control). The cells at a density of 3×10^5 cells per dish were seeded onto 35 mm dishes. After 1 day of seeding, 200 μl of lentiviral particles in 2 ml DMEM medium containing 10% FBS were added to the cultures, which were then incubated for 24 h in 5% CO_2 at 37 °C. The cells that were successfully infected by lentiviral particles were selected using 3 μg/ml puromycin in the presence of 10% FBS for 48 h. The cells were then harvested and subjected to western blot analysis to determine the efficiency of the used lentiviral particles for suppressing TNF-α gene expression.

2.3. Palmitate treatment

Palmitate was conjugated with the fatty acids-free BSA. Briefly, different concentrations of sodium palmitate were solved in 50% ethanol and added to DMEM containing 1% BSA. Then, this medium, incubated at 37 °C for 2 h on a shaker. After 2 h, the prepared medium was filtered and used for the treatment of C2C12 myotubes.

2.4. Western blot analysis

C2C12 cell lysate was prepared by homogenization in modified RIPA buffer (50 mM Tris–HCl pH 7.4, 1% Triton X-100, 0.2% sodium deoxycholate, 0.2% SDS, 1 mM Na-EDTA, 1 mM PMSF) supplemented with protease inhibitor cocktail (Roche, Mannheim, Germany). For detection of phospho protein, a buffer consisting of 50 mM HEPES pH 7.5, 150 mM NaCl, 100 mM NaF, 10 mM EDTA, 10 mM Na4P2O7, 2 mM NaVO4, and protease inhibitor cocktail was used. Protein concentration was determined using the Bradford's method [30]. 20–30 μg of total protein was fractionated by SDS-PAGE. The gel was then transferred onto a PVDF membrane (Millipore, Schwalbach, Germany), blocked in blocking buffer overnight (5% skimmed milk in TBST buffer), and incubated for 1 h with primary antibodies diluted in TBST containing 1% BSA. Primary antibodies used were as follows: TNF-α, p-IRS-1 (Tyr632), and IRS-1 (Santa Cruz Biotechnology, Santa Cruz, USA), Akt, and phospho-Akt (Ser473) (Cell Signaling Technology, Beverly, MA, USA), β-actin (Abcam, Cambridge, MA, USA). The membrane was then incubated with secondary antibody conjugated to HRP (Santa Cruz Biotechnology, Santa Cruz, USA) for 1 h and detection was performed using ECL reagents (Amersham Pharmacia Corp, Piscataway, NJ, USA). Films were scanned and protein bands quantified using Scion Image software. Each experiment was performed at least three times.

2.5. Glucose uptake assay

Glucose uptake was assayed by measuring the uptake of [3H]2-DOG ([^{3}H]2-deoxyglucose) (Amersham Pharmacia Corp, Piscataway, NJ, USA) in C2C12 cells. Glucose uptake measurements were performed in three independent experiments. Briefly, after 4 days of differentiation, myotubes were treated with 0.75 mM palmitate for 16 h followed by a serum starvation for 2–3 h in DMEM plus 0.1% BSA. Myotubes were then treated with or without 100 nM insulin for 30 min and washed two times with wash buffer [20 mM HEPES (pH 7.4), 140 mM NaCl, 5 mM KCl, 2.5 mM MgSO4, and 1 mM CaCl2]. These cells incubated in the transport buffer (wash buffer containing 0.5 mCi [^{3}H]2-DOG/ml and 10 μM 2-DOG) for 10 min. Afterwards, cells were lysed in 0.05 M NaOH and [^{3}H]2-DOG levels were counted in the cell lysate using a scintillation counter. Nonspecific uptake was determined by incubating the cells in the presence or absence of 10 μM cytochalasin B.

2.6. Statistical analysis

All data were expressed as the means ± standard deviation (SD) of at least three independent experiments. Analysis of data was performed using SPSS ver. 18.0. Comparisons among groups were performed with the one-way analysis of variance (ANOVA) and Student's t-tests, where appropriate. If statistical significance was found, the Tukey post hoc test was performed. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Palmitate induces TNF-α expression in C2C12 skeletal muscle cells

As shown in Fig. 1(A), treatment by 0.75 mM palmitate at all times markedly increased the protein expression levels of TNF-α in C2C12 cells ($P < 0.05$). These increments in TNF-α protein expression at times 8 h, 16 h, and 24 h were approximately 1.5-, 2.56-, and 2.48-fold, respectively, compared with the control cells. According to our statistical analysis, significant differences in TNF-α protein levels between treatment time 8 h and other times were found; however, there was no significant difference between the times 16 h and 24 h. Hence, we selected time 16 h for all following palmitate treatments.

3.2. TNF-α knockdown in C2C12 cells

Western blot analysis was carried out to determine the protein expression level of TNF-α in C2C12 cells. As demonstrated in

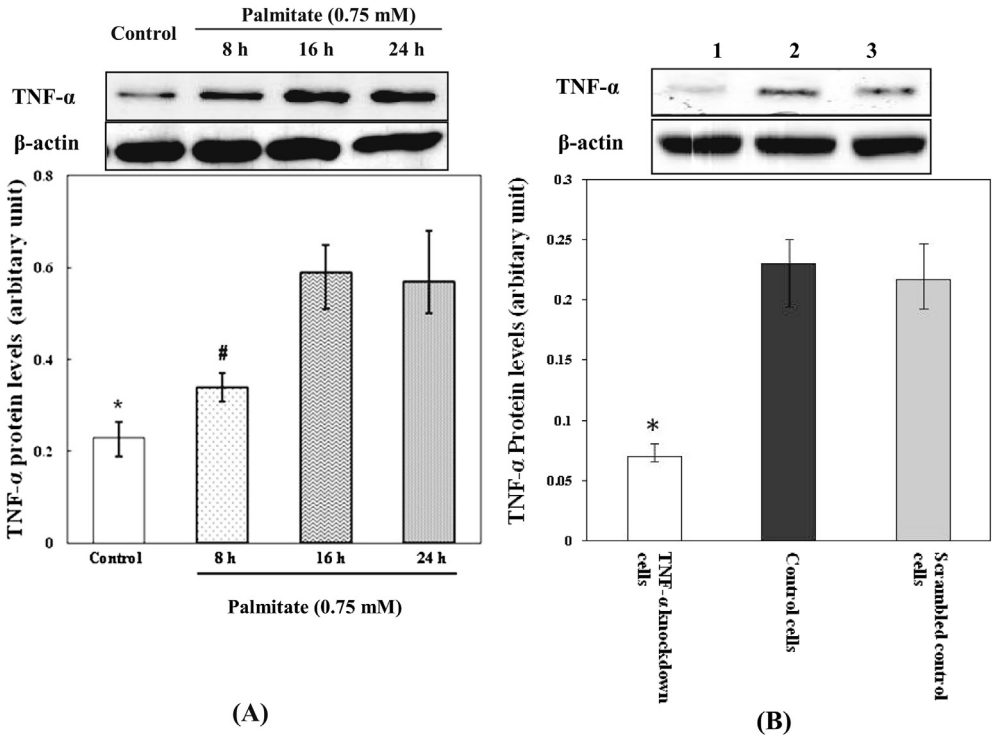

Fig. 1. (A). Effect of palmitate on TNF-α protein levels. Time course of TNF-α protein expression in the presence of 0.75 mM palmitate. **(B)** Levels of TNF-α protein expression in C2C12 myotubes after transduction with lentiviral particles. (1) Myotubes were transduced with TNF-α-shRNA lentiviral particles. (2): Untransduced control myotubes. (3) Myotubes were transduced with lentiviral particles carrying noneffective scrambled shRNA (scrambled control cells). Western blot analysis was performed using antibodies against TNF-α and β-actin (internal control). Levels of TNF-α protein were normalized by β-actin protein. *, $P < 0.01$ vs. all other groups. #, $P < 0.05$ vs. 16 h and 24 h.

Fig. 1(B), the protein expression level of TNF-α was significantly decreased by nearly 70% ($P < 0.01$) in C2C12 cells that transduced with a specific TNF-α-shRNA lentiviral particles in comparison with that of the control and scrambled control cells. No significant difference was detected in TNF-α protein level between control and scrambled control cells ($P > 0.05$).

3.3. Effects of palmitate treatment and TNF-α knockdown on insulin signaling

The results showed that TNF-α knockdown cells had no significant difference in IRS-1 (Tyr632) phosphorylation with respect to the control, in the presence and absence of insulin ($P > 0.05$) (Fig. 2). Furthermore, compared with the untreated control cells, treatment with 0.05 mM palmitate significantly ($P < 0.01$) reduced the insulin-induced phosphorylation of IRS-1 (Tyr632) in C2C12 cells. Interestingly, even in the presence of palmitate, this phosphorylation did not significantly reduce in TNF-α knockdown, as compared with the untreated control cells ($P > 0.05$). We also evaluated the effects of palmitate on Akt phosphorylation in both TNF-α knockdown and control cells (Fig. 3). Our finding also indicated that TNF-α knockdown cells had no significant difference in Akt (Ser473) phosphorylation with respect to the control, in the presence and absence of insulin ($P > 0.05$). However, compared with untreated control cells, palmitate treatment resulted in a marked reduction (66%) in insulin-induced Akt phosphorylation (Ser473) in C2C12 cells ($P < 0.01$), while there was no significant decrease in the level of Akt phosphorylation in TNF-α knockdown cells under this condition ($P < 0.05$). Taken together, these data showed that TNF-α down-regulation maintains the activity of key elements of the insulin signaling pathway even in the presence of palmitate.

3.4. Effects of palmitate treatment and TNF-α knockdown on glucose uptake

To determine the possible inhibitory effect of TNF-α on glucose uptake in C2C12 cells, [3H]2-DOG uptake was measured. In the absence of palmitate, insulin-stimulated glucose uptake was not significantly different between TNF-α knockdown and control cells, and insulin stimulation led to significant increases in glucose uptake in both groups (Fig. 4). Palmitate treatment reduced Insulin-stimulated glucose uptake in C2C12 cells by nearly 38% ($P < 0.01$) with respect to the untreated control cells. However, surprisingly, palmitate was not able to reduce insulin-stimulated glucose uptake in TNF-α knockdown cells in comparison with the untreated control cells ($P < 0.01$). These results indicate that knockdown of TNF-α maintains insulin-stimulated glucose uptake in C2C12 cells even in the presence of palmitate.

4. Discussion

Insulin resistance is a metabolic disorder in which target cells fail to respond to normal levels of circulating insulin. Many studies have been carried out to determine the genetic and environmental factors involved in insulin resistance. In this connection, several genes involved in insulin resistance have been investigated, of which TNF-α has attracted much attention in recent years. It has been reported that insulin resistance is associated with elevated plasma levels of TNF-α [31], and its expression is increased in adipose tissue [25–27,32–34] and skeletal muscle of insulin-resistant cases [22–24,29,35,36]. Several studies have shown that increasing the concentration of the fatty acid palmitate, the most abundant saturated fatty acids in plasma, leads to insulin resistance in insulin

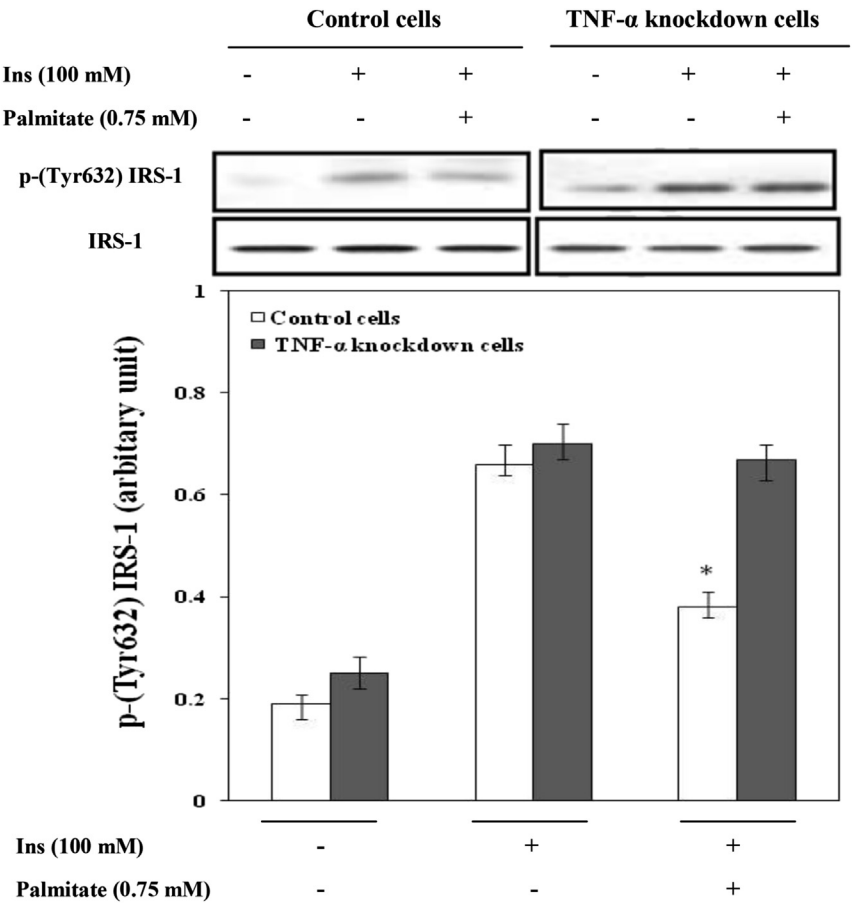

Fig. 2. The effect of palmitate on Tyr632 phosphorylation of IRS-1 in TNF-α knockdown C2C12 and control cells. Western blot analysis was done against IRS-1 (internal control) and phospho-Tyr632 of IRS-1 molecules. Phospho-Tyr632 level was normalized using its corresponding IRS-1 protein level. Ins: Insulin. *, $P < 0.01$.

target tissues [10,16,17,35,37,38]. To determine, the possible role of TNF-α as a mediator for the inhibitory effects of FFAs on insulin signaling, we studied the time course of TNF-α protein levels in C2C12 myotubes under treatment with palmitate. Consistent with previous studies reporting elevated expression of TNF-α in skeletal muscle in palmitate-induced insulin resistance states [29,34,39–41], our results demonstrated that the high concentration of palmitate (0.75 mM) for 16 h induced TNF-α protein levels. This implies that the pathological concentration of palmitate may partly be responsible for TNF-α overexpression observed in insulin resistance muscle.

TNF-α-induced insulin resistance appears to involve down-regulation of several known proteins required for insulin signaling. A major negative regulatory role to insulin action is attributed to agents that enhance Ser/Thr phosphorylation of either the receptor itself or of its downstream effectors such as Akt [36,42–44]. TNF-α induces Ser/Thr phosphorylation of IRS-1 while it diminishes insulin-induced Tyr phosphorylation of IRS-1 [20,44]. In agreement with these reports, we also showed that 0.75 mM palmitate can significantly reduce insulin-stimulated Tyr632 IRS-1 and Ser473 Akt phosphorylations in C2C12 skeletal muscle cell. Consequently, we measured the glucose uptake in these cells to determine whether these reduced phosphorylations can cause the inhibition of insulin stimulated glucose uptake. Our finding showed that palmitate treatment reduced Insulin-stimulated glucose uptake in C2C12 cells by nearly 38%. Here, we demonstrated insulin resistance-inducing effect of palmitate is mediated, at least partly, via increasing TNF-α protein expression, which can reduce insulin signaling and finally insulin stimulated glucose uptake in skeletal muscle. Furthermore, TNF-α has been proposed to stimulate the production of diacylglycerol (DAG) and ceramide, which have been implicated in the pathogenesis of skeletal muscle insulin resistance [14,45,46].

To rule out the simple coincidence between increased TNF-α protein levels and inhibition of insulin signaling and glucose uptake, we specifically decreased TNF-α protein expression in myotubes using TNF-α-shRNA lentiviral particles and then treated them with palmitate. Transduction of myotubes with this shRNA resulted in nearly 70% reduction in TNF-α protein levels in comparison with that of the control cells. Our results showed that reduction of TNF-α protein expression in C2C12 myotubes improves insulin-stimulated Tyr632 IRS-1 and Ser473 Akt phosphorylations even in the presence of palmitate, while treatment with palmitate resulted in about 60% reduction of insulin-induced IRS-1 and Akt phosphorylation in control cells. In addition, surprisingly, palmitate was not able to reduce insulin-stimulated glucose uptake in TNF-α knockdown cells and these myotubes remained sensitive to insulin even in the presence of pathologic dose of palmitate. Here, we showed that TNF-α inhibition attenuates insulin resistance induced by palmitate, suggesting the role for PTP1B as a mediator in palmitate induced insulin resistance. These results are in line with the other studies showing that the reduction of TNF-α activity through different approaches, including disruption of TNF-α gene and neutralizing antibodies resulted in improved insulin sensitivity in animal models [47–49]. However, the direct effect of TNF-α knockdown, specifically in skeletal muscle cells, on the palmitate-

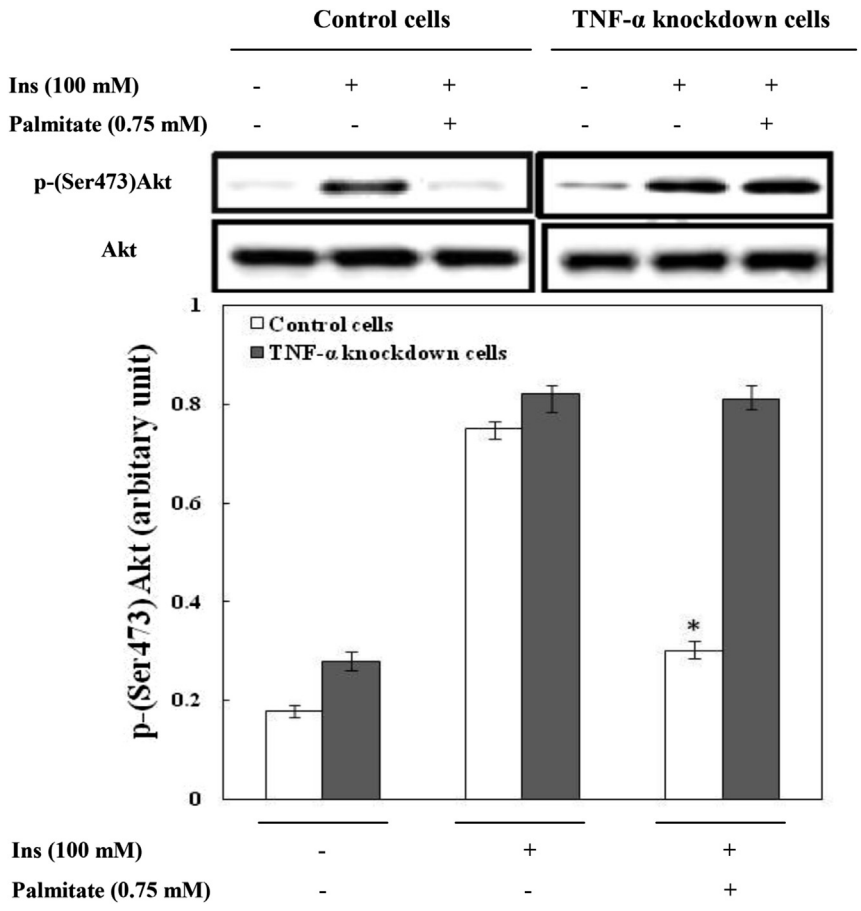

Fig. 3. The effect of palmitate on Ser473 phosphorylation of Akt in TNF-α knockdown C2C12 and control cells. Western blot analysis was done against Akt (internal control) and phospho-Ser473 of Akt molecules. Phospho-Ser473 level was normalized using its corresponding Akt protein level. Ins: Insulin. *, $P < 0.01$.

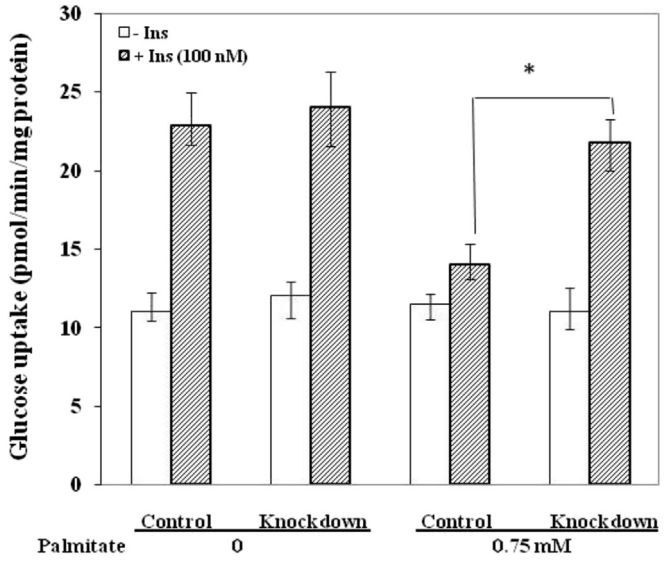

Fig. 4. The effect of palmitate on glucose uptake in TNF-α knockdown and control cells. Myotubes were grown in the absence and presence of 0.75 mM palmitate for 16 h. Glucose uptake was then measured in the presence or absence of insulin (Ins). The figure shows representative data gained from mean ± SD of three independent experiments. *, $P < 0.01$.

induced insulin resistance has not been previously reported and the present study is the first report in this regard.

In summary, the data presented here indicate that palmitate can dysregulate insulin signaling, at least partly, by increased expression of TNF-α in skeletal muscle cells. In addition, TNF-α down-regulation maintains insulin sensitivity in these cells, even in the presence of palmitate. Taking these findings together, TNF-α inhibition could be a good strategy for the treatment of lipid-induced insulin resistance and type 2 diabetes.

Conflict of interest

All authors declared to have no conflicts of interest.

Acknowledgments

This project was supported financially by deputy for research and technology of Ilam University of Medical Sciences, Iran (Grant No. 90101162).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.03.137.

References

[1] G. Boden, X. Chen, J. Ruiz, J.V. White, L. Rossetti, Mechanisms of fatty acid-induced inhibition of glucose uptake, J. Clin. Invest 93 (1994) 2438–2446.

[2] P.J. Randle, E.A. Newsholme, P.B. Garland, Regulation of glucose uptake by muscle. 8. Effects of fatty acids, ketone bodies and pyruvate, and of alloxan-diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscles, Biochem. J. 93 (1964) 652–665.
[3] M.P. Corcoran, S. Lamon-Fava, R.A. Fielding, Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise, Am. J. Clin. Nutr. 85 (2007) 662–677.
[4] R.A. DeFronzo, E. Jacot, E. Jequier, E. Maeder, J. Wahren, J.P. Felber, The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization, Diabetes 30 (1981) 1000–1007.
[5] G.I. Shulman, D.L. Rothman, T. Jue, P. Stein, R.A. DeFronzo, R.G. Shulman, Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy, N. Engl. J. Med. 322 (1990) 223–228.
[6] Y.D. Chen, A. Golay, A.L. Swislocki, G.M. Reaven, Resistance to insulin suppression of plasma free fatty acid concentrations and insulin stimulation of glucose uptake in noninsulin-dependent diabetes mellitus, J. Clin. Endocrinol. Metab. 64 (1987) 17–21.
[7] G. Paolisso, P.A. Tataranni, J.E. Foley, C. Bogardus, B.V. Howard, E. Ravussin, A high concentration of fasting plasma non-esterified fatty acids is a risk factor for the development of NIDDM, Diabetologia 38 (1995) 1213–1217.
[8] G.M. Reaven, C. Hollenbeck, C.Y. Jeng, M.S. Wu, Y.D. Chen, Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with NIDDM, Diabetes 37 (1988) 1020–1024.
[9] R.H. Unger, Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications, Diabetes 44 (1995) 863–870.
[10] J.A. Chavez, T.A. Knotts, L.P. Wang, G. Li, R.T. Dobrowsky, G.L. Florant, S.A. Summers, A role for ceramide, but not diacylglycerol, in the antagonism of insulin signal transduction by saturated fatty acids, J. Biol. Chem. 278 (2003) 10297–10303.
[11] Y.B. Kim, G.I. Shulman, B.B. Kahn, Fatty acid infusion selectively impairs insulin action on Akt1 and protein kinase C lambda/zeta but not on glycogen synthase kinase-3, J. Biol. Chem. 277 (2002) 32915–32922.
[12] K.F. Petersen, S. Dufour, D. Befroy, R. Garcia, G.I. Shulman, Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes, N. Engl. J. Med. 350 (2004) 664–671.
[13] F. Tremblay, C. Lavigne, H. Jacques, A. Marette, Defective insulin-induced GLUT4 translocation in skeletal muscle of high fat-fed rats is associated with alterations in both Akt/protein kinase B and atypical protein kinase C (zeta/lambda) activities, Diabetes 50 (2001) 1901–1910.
[14] C. Yu, Y. Chen, G.W. Cline, D. Zhang, H. Zong, Y. Wang, R. Bergeron, J.K. Kim, S.W. Cushman, G.J. Cooney, B. Atcheson, M.F. White, E.W. Kraegen, G.I. Shulman, Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle, J. Biol. Chem. 277 (2002) 50230–50236.
[15] Y. Zick, Uncoupling insulin signalling by serine/threonine phosphorylation: a molecular basis for insulin resistance, Biochem. Soc. Trans. 32 (2004) 812–816.
[16] J.A. Chavez, S.A. Summers, Characterizing the effects of saturated fatty acids on insulin signaling and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes, Arch. Biochem. Biophys. 419 (2003) 101–109.
[17] C. Schmitz-Peiffer, D.L. Craig, T.J. Biden, Ceramide generation is sufficient to account for the inhibition of the insulin-stimulated PKB pathway in C2C12 skeletal muscle cells pretreated with palmitate, J. Biol. Chem. 274 (1999) 24202–24210.
[18] C. Keller, P. Keller, M. Giralt, J. Hidalgo, B.K. Pedersen, Exercise normalises overexpression of TNF-alpha in knockout mice, Biochem. Biophys. Res. Commun. 321 (2004) 179–182.
[19] C. Weigert, K. Brodbeck, H. Staiger, C. Kausch, F. Machicao, H.U. Haring, E.D. Schleicher, Palmitate, but not unsaturated fatty acids, induces the expression of interleukin-6 in human myotubes through proteasome-dependent activation of nuclear factor-kappaB, J. Biol. Chem. 279 (2004) 23942–23952.
[20] G.S. Hotamisligil, P. Peraldi, A. Budavari, R. Ellis, M.F. White, B.M. Spiegelman, IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance, Science 271 (1996) 665–668.
[21] H. Inadera, The usefulness of circulating adipokine levels for the assessment of obesity-related health problems, Int. J. Med. Sci. 5 (2008) 248–262.
[22] A.T. Cheung, D. Ree, J.K. Kolls, J. Fuselier, D.H. Coy, M. Bryer-Ash, An in vivo model for elucidation of the mechanism of tumor necrosis factor-alpha (TNF-alpha)-induced insulin resistance: evidence for differential regulation of insulin signaling by TNF-alpha, Endocrinology 139 (1998) 4928–4935.
[23] G.S. Hotamisligil, N.S. Shargill, B.M. Spiegelman, Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance, Science 259 (1993) 87–91.
[24] K. Morino, K.F. Petersen, S. Dufour, D. Befroy, J. Frattini, N. Shatzkes, S. Neschen, M.F. White, S. Bilz, S. Sono, M. Pypaert, G.I. Shulman, Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents, J. Clin. Invest 115 (2005) 3587–3593.
[25] M. Ryden, A. Dicker, V. van Harmelen, H. Hauner, M. Brunnberg, L. Perbeck, F. Lonnqvist, P. Arner, Mapping of early signaling events in tumor necrosis factor-alpha -mediated lipolysis in human fat cells, J. Biol. Chem. 277 (2002) 1085–1091.
[26] X. Yang, X. Zhang, B.L. Heckmann, X. Lu, J. Liu, Relative contribution of adipose triglyceride lipase and hormone-sensitive lipase to tumor necrosis factor-alpha (TNF-alpha)-induced lipolysis in adipocytes, J. Biol. Chem. 286 (2011) 40477–40485.
[27] K.M. Ajuwon, M.E. Spurlock, Palmitate activates the NF-kappaB transcription factor and induces IL-6 and TNFalpha expression in 3T3-L1 adipocytes, J. Nutr. 135 (2005) 1841–1846.
[28] L. Haversen, K.N. Danielsson, L. Fogelstrand, O. Wiklund, Induction of proinflammatory cytokines by long-chain saturated fatty acids in human macrophages, Atherosclerosis 202 (2009) 382–393.
[29] M. Jove, A. Planavila, R.M. Sanchez, M. Merlos, J.C. Laguna, M. Vazquez-Carrera, Palmitate induces tumor necrosis factor-alpha expression in C2C12 skeletal muscle cells by a mechanism involving protein kinase C and nuclear factor-kappaB activation, Endocrinology 147 (2006) 552–561.
[30] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[31] B. Zinman, A.J. Hanley, S.B. Harris, J. Kwan, I.G. Fantus, Circulating tumor necrosis factor-alpha concentrations in a native Canadian population with high rates of type 2 diabetes mellitus, J. Clin. Endocrinol. Metab. 84 (1999) 272–278.
[32] G.S. Hotamisligil, P. Arner, J.F. Caro, R.L. Atkinson, B.M. Spiegelman, Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance, J. Clin. Invest 95 (1995) 2409–2415.
[33] G.S. Hotamisligil, D.L. Murray, L.N. Choy, B.M. Spiegelman, Tumor necrosis factor alpha inhibits signaling from the insulin receptor, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 4854–4858.
[34] J.M. Stephens, J. Lee, P.F. Pilch, Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction, J. Biol. Chem. 272 (1997) 971–976.
[35] M. Saghizadeh, J.M. Ong, W.T. Garvey, R.R. Henry, P.A. Kern, The expression of TNF alpha by human muscle. Relationship to insulin resistance, J. Clin. Invest 97 (1996) 1111–1116.
[36] P. Storz, H. Doppler, A. Wernig, K. Pfizenmaier, G. Muller, Cross-talk mechanisms in the development of insulin resistance of skeletal muscle cells palmitate rather than tumour necrosis factor inhibits insulin-dependent protein kinase B (PKB)/Akt stimulation and glucose uptake, Eur. J. Biochem. 266 (1999) 17–25.
[37] S. Bakhtiyari, R. Meshkani, M. Taghikhani, B. Larijani, K. Adeli, Protein tyrosine phosphatase-1B (PTP-1B) knockdown improves palmitate-induced insulin resistance in C2C12 skeletal muscle cells, Lipids 45 (2010) 237–244.
[38] G. Taheripak, S. Bakhtiyari, M. Rajabibazl, P. Pasalar, R. Meshkani, Protein tyrosine phosphatase 1B inhibition ameliorates palmitate-induced mitochondrial dysfunction and apoptosis in skeletal muscle cells, Free Radic. Biol. Med. 65 (2013) 1435–1446.
[39] M. Lorenzo, S. Fernandez-Veledo, R. Vila-Bedmar, L. Garcia-Guerra, C. De Alvaro, I. Nieto-Vazquez, Insulin resistance induced by tumor necrosis factor-alpha in myocytes and brown adipocytes, J. Anim. Sci. 86 (2008) E94–E104.
[40] C. Sun, F. Zhang, X. Ge, T. Yan, X. Chen, X. Shi, Q. Zhai, SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B, Cell. Metab. 6 (2007) 307–319.
[41] L. Parvaneh, R. Meshkani, S. Bakhtiyari, N. Mohammadtaghvaie, S. Gorganifiruzjaee, G. Taheripak, A. Golestani, M. Foruzandeh, B. Larijani, M. Taghikhani, Palmitate and inflammatory state additively induce the expression of PTP1B in muscle cells, Biochem. Biophys. Res. Commun. 396 (2010) 467–471.
[42] R.A. Roth, F. Liu, J.E. Chin, Biochemical mechanisms of insulin resistance, Horm. Res. 41 (Suppl. 2) (1994) 51–55.
[43] Y. Zick, The insulin receptor: structure and function, Crit. Rev. Biochem. Mol. Biol. 24 (1989) 217–269.
[44] H. Kanety, R. Feinstein, M.Z. Papa, R. Hemi, A. Karasik, Tumor necrosis factor alpha-induced phosphorylation of insulin receptor substrate-1 (IRS-1). Possible mechanism for suppression of insulin-stimulated tyrosine phosphorylation of IRS-1, J. Biol. Chem. 270 (1995) 23780–23784.
[45] S. Schutze, D. Berkovic, O. Tomsing, C. Unger, M. Kronke, Tumor necrosis factor induces rapid production of 1'2'diacylglycerol by a phosphatidylcholine-specific phospholipase C, J. Exp. Med. 174 (1991) 975–988.
[46] J.M. Adams 2nd, T. Pratipanawatr, R. Berria, E. Wang, R.A. DeFronzo, M.C. Sullards, L.J. Mandarino, Ceramide content is increased in skeletal muscle from obese insulin-resistant humans, Diabetes 53 (2004) 25–31.
[47] G.S. Hotamisligil, A. Budavari, D. Murray, B.M. Spiegelman, Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha, J. Clin. Invest 94 (1994) 1543–1549.
[48] E. Nisoli, L. Briscini, A. Giordano, C. Tonello, S.M. Wiesbrock, K.T. Uysal, S. Cinti, M.O. Carruba, G.S. Hotamisligil, Tumor necrosis factor alpha mediates apoptosis of brown adipocytes and defective brown adipocyte function in obesity, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 8033–8038.
[49] K.T. Uysal, S.M. Wiesbrock, M.W. Marino, G.S. Hotamisligil, Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function, Nature 389 (1997) 610–614.