Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 395–399
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Anti-inflammatory effect of atorvastatin ameliorates insulin resistance in monosodium glutamate–treated obese mice

Daniela T. Furuya[a,*], Ana C. Poletto[a], Rodolfo R. Favaro[b], Joilson O. Martins[c], Telma M.T. Zorn[b], Ubiratan F. Machado[a]

[a]*Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, 05508-900 São Paulo, Brasil*
[b]*Department of Department of Cell and Development Biology, Institute of Biomedical Sciences, University of São Paulo, 05508-900 São Paulo, Brasil*
[c]*Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, 05508-900 São Paulo, Brasil*

Received 14 January 2009; accepted 9 August 2009

Abstract

Considering that inflammation contributes to obesity-induced insulin resistance and that statins have been reported to have other effects beyond cholesterol lowering, the present study aimed to investigate whether atorvastatin treatment has anti-inflammatory action in white adipose tissue of obese mice, consequently improving insulin sensitivity. Insulin sensitivity in vivo (by insulin tolerance test); metabolic-hormonal profile; plasma tumor necrosis factor (TNF)–α, interleukin (IL)-6, and adiponectin; adipose tissue immunohistochemistry; glucose transporter (GLUT) 4; adiponectin; TNF-α; IL-1β; and IL-6 gene expression; and IκB kinase (IKK)–α/β activity were assessed in 23-week-old monosodium glutamate–induced obese mice untreated or treated with atorvastatin for 4 weeks. Insulin-resistant obese mice had increased plasma triglyceride, insulin, TNF-α, and IL-6 plasma levels. Adipose tissue of obese animals showed increased macrophage infiltration, IKK-α (42%, $P < .05$) and IKK-β (73%, $P < .05$) phosphorylation, and TNF-α and IL-6 messenger RNA (mRNA) ($\sim$15%, $P < .05$) levels, and decreased GLUT4 mRNA and protein (30%, $P < .05$) levels. Atorvastatin treatment lowered cholesterol, triglyceride, insulin, TNF-α, and IL-6 plasma levels, and restored whole-body insulin sensitivity. In adipose tissue, atorvastatin decreased macrophage infiltration and normalized IKK-α/β phosphorylation; TNF-α, IL-6, and GLUT4 mRNA; and GLUT4 protein to control levels. The present findings demonstrate that atorvastatin has anti-inflammatory effects on adipose tissue of obese mice, which may be important to its local and whole-body insulin-sensitization effects.

1. Introduction

Obesity is strongly associated with insulin resistance and reduced glucose transporter (GLUT) 4 expression in insulin-sensitive tissues [1]. Furthermore, in obesity, white adipose tissue (WAT) overexpresses several proinflammatory cytokines, such as tumor necrosis factor (TNF)–α and interleukin (IL)-6 [2], which are reported to induce insulin resistance [3,4]. In this regard, the IκB kinase (IKK)–dependent nuclear factor–κB (NF-κB) activation, a major component of the inflammatory pathway, has already been suggested as a repressor of the GLUT4 gene expression [3,5], pointing out a mechanism by which inflammation links obesity and insulin resistance.

Several beneficial effects of the 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors (statins) beyond cholesterol lowering have been identified, including anti-inflammatory and insulin-sensitization effects [6-8]. However, the impact on glucose metabolism of statins is controversial [6,9]; and molecular mechanisms are poorly understood.

In the current study, monosodium glutamate–treated mice, as a model of obesity, were investigated to verify whether atorvastatin (1) ameliorates insulin resistance, (2)

Conflict of interest statement: I, Daniela Tomie Furuya, declare that I have no proprietary, financial, professional, or other personal interest in any product and/or company that could be construed as influencing the position presented in this article.

* Corresponding author. Tel.: +55 11 3091 7494; fax: +55 11 3091 7285.
E-mail address: danifuruya@yahoo.com (D.T. Furuya).

0026-0495/$ – see front matter © 2010 Published by Elsevier Inc.
doi:10.1016/j.metabol.2009.08.011

Table 1
Profile of control mice and obese mice untreated or treated with atorvastatin for 4 weeks

	C	O	OA
Body weight (g)	37.9 ± 1.14	37.9 ± 1.1	38.2 ± 1.3
Lee obesity index (×100)	32.64 ± 0.34	35.98 ± 0.28‡	35.78 ± 0.32‡
Absolute WAT weight (g)	0.47 ± 0.06	1.62 ± 0.08‡	1.36 ± 0.07‡,§
Relative WAT weight (×100) (g)	1.26 ± 0.21	4.21 ± 0.14‡	3.54 ± 0.27‡,§
Food ingestion (g)	6.68 ± 0.31	4.87 ± 0.15‡	5.39 ± 0.09‡
Plasma cholesterol (mmol/L)	1.93 ± 0.09	2.0 ± 0.15	1.31 ± 0.07*,‖
Plasma triglyceride (mmol/L)	1.37 ± 0.17	1.77 ± 0.12*	1.08 ± 0.11‖
Plasma glucose (mmol/L)	8.72 ± 0.47	8.06 ± 0.48	8.03 ± 0.24
Plasma insulin (pmol/L)	659.4 ± 67.7	848.4 ± 7.9*	817.6 ± 27.3*
Plasma adiponectin (μg/mL)	10.8 ± 1.2	9.8 ± 1.7	10.1 ± 0.9
Plasma TNF-α (pg/mL)	112.4 ± 9.4	220.7 ± 21.3†	63.6 ± 36.9‖
Plasma IL-6 (pg/mL)	185.9 ± 10.8	288.3 ± 22.5†	217.8 ± 20.3§
kITT (%/min)	4.37 ± 0.07	3.08 ± 0.23*	5.11 ± 0.57§

Daily food, liquid, and calorie intake data were measured during the 4-week atorvastatin treatment period. Data from plasma cholesterol, triglyceride, glucose and insulin concentration, and kITT were obtained from mice subjected to 4 hours of food deprivation. Data are means ± SE of 8 to 10 (morphologic parameters) and 3 to 6 (metabolic-hormonal parameters) animals. C indicates control; O, obese untreated; OA, obese treated with atorvastatin.
*$P < .05$, †$P < .01$, and ‡$P < .001$ vs C; §$P < .05$ and ‖$P < .01$ vs O; 1-way ANOVA and Student-Newman-Keuls post hoc test.

modulates GLUT4 gene and protein expression in WAT, and (3) has anti-inflammatory effect in WAT.

2. Materials and methods

2.1. Animals

Obesity was induced in male offspring mice (CD1) by subcutaneous injections of monosodium glutamate (2 mg/g body weight) from first to fifth day after birth [1]. Control mice were injected with 0.9% NaCl. Animals were weaned and allowed access to standard rodent chow and water ad libitum. Atorvastatin (Pfizer, Guarulhos, SP, Brazil) was given in chow (0.1% wt/wt) to 19-week-old mice for 4 weeks. Lee obesity index (body weight [in grams]/nasoanal length [in centimeters]) and relative weight of epididymal (tissue weight [in grams]/body weight [in grams]) WAT were used to estimate the obesity degree. Blood and WAT were sampled from 23-week-old mice. All procedures were performed in anesthetized animals (50 mg/kg body weight sodium pentobarbital intraperitoneally) and were approved by the Ethical Committee for Animal Research of the Institute of Biomedical Sciences, University of São Paulo (123/2005).

2.2. Metabolic and hormonal analysis

Plasma glucose, insulin, cholesterol, and triglyceride, as well as the glucose disappearance constant during the insulin tolerance test (kITT), were measured as previously described [10].

2.3. Enzyme-linked immunosorbent assays

Tumor necrosis factor–α, IL-6, and adiponectin concentrations in the plasma samples were determined by enzyme-linked immunosorbent assay using commercially available kits according to the manufacturer's instructions (R & D Systems, Minneapolis, MN).

2.4. GLUT4 messenger RNA and protein

Glucose transporter 4 messenger RNA (mRNA) and protein were analyzed by Northern and Western blotting as previously described [11].

2.5. Phosphorylated IKK-α/β

Phosphorylated IKK-α (Ser180) and β (Ser181) were assayed by Western blot [10] using anti-phospho-IKK-α/β antibody (1:1000; Cell Signaling, Beverly, MA) followed by standard chemiluminescence detection.

2.6. Semiquantitative reverse transcriptase polymerase chain reaction

Total RNA (1 μg) was previously treated by RNase-free DNase and then reverse transcribed following manufacturer's instructions (Improm; Promega, Madison, WI). Complementary DNA was amplified by polymerase chain reaction (PCR) in triplicate using GoTaq DNA Polymerase (Promega). Reverse transcriptase PCR primers, designed to amplify bases 187 to 387 of mouse TNF-α (National Center for Biotechnology Information accession no. NM_013693), 599 to 803 of mouse IL-1β (NM_008361), 121 to 340 of mouse IL-6 (NM_031168), 86 to 515 of mouse adiponectin (NM_009605), and 56 to 349 of mouse glyceraldehyde-3-

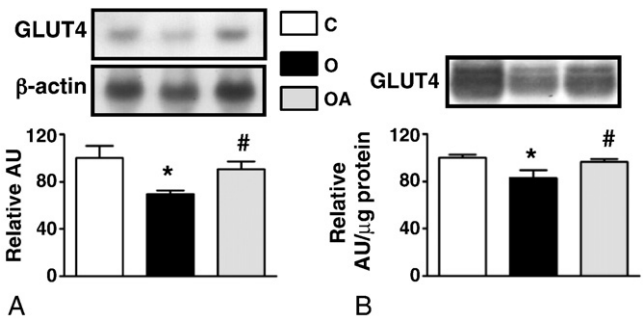

Fig. 1. Glucose transporter 4 mRNA (A) and GLUT4 protein (B) expression of epididymal WAT from control (white bars) and obese untreated (black bars) or atorvastatin-treated (gray bars) mice. A and B, Images of one typical experiment and relative values of mRNA or protein content are shown. The GLUT4 mRNA expression was analyzed by Northern blotting (A). Forty micrograms of protein was immunoblotted for GLUT4 protein (B). The values are means ± SE of 5 to 6 (GLUT4 mRNA) and 6 (GLUT4 protein) animals per group. *$P < .05$ vs control; #$P < .05$ vs obese untreated; 1-way ANOVA and Student-Newman-Keuls post hoc test. C indicates control; O, obese untreated; OA, obese treated with atorvastatin.

phosphate dehydrogenase (GAPDH) (NM_008084), comprised the following: TNF-α forward (FW) (5′-GAACTGGCAGAAGAGGCACT-3′), TNF-α reverse (RV) (5′-GGTCTGGGCCATAGAACTGA-3′), IL-1β FW (5′-GGGCCTCAAAGGAAAGAATC-3′), IL-1β RV (5′-CTCTGCTTGTGAGGTGCTGA-3′), IL-6 FW (5′-CCGGAGAGGAGACTTCACAG-3′), IL-6 RV (5′-TCCAGTTTGGTAGCATCCATC-3′), adiponectin FW (5′-TGGATCTGACGACACCAAAA-3′), adiponectin RV (5′-CGAATGGGTACATTGGGAAC-3′), GAPDH FW (5′-GAAGGTCGGTGTGAACGGATT), and GAPDH RV (5′-AAGACACCAGTAGACTCCACGA-3′). Annealing temperature was 58°C with 34 cycles for adiponectin and GAPDH and 40 cycles for the other genes.

2.7. Morphologic and immunohistochemical analysis

White adipose tissue was fixed in methacarn (60% methanol, 30% chloroform, and 10% acetic acid). Five-micrometer Paraplast-embedded sections (Merck, São Paolo, Brazil) were submitted to hematoxylin-eosin or immunoperoxidase staining. Sections were treated with H_2O_2 to block endogenous peroxidase and incubated with normal donkey serum to reduce nonspecific staining. Sections were incubated with rat antibody raised against the mouse F4/80 macrophage antigen (1:500; Serotec, Oxford, United Kingdom), followed by biotin-conjugated donkey anti-rat immunoglobulin G (1:2000; Rockland, Gilberstsville, PA), and finally streptavidin/peroxidase complex. Peroxidase was visualized using 3,3′-diaminobenzidine (Roche, Indianapolis, IN) and counterstained with hematoxylin.

2.8. Statistical analysis

All data are reported as means ± SEM. Statistical analysis was 1-way analysis of variance (ANOVA) (Student-Newman-Keuls as a post hoc test).

3. Results

Although obese animals consumed less chow, they became obese when compared with controls (C), as evident from higher Lee obesity index and absolute and relative weights of epididymal (Table 1) and retroperitoneal (data not shown) WATs. Obese animals had higher plasma triglyceride, insulin, TNF-α, and IL-6 levels, as well as lower kITT, revealing the in vivo insulin-resistant and inflammatory state (Table 1). In contrast, atorvastatin treatment completely reversed these parameters (Table 1) and impacted glucose metabolism by increasing insulin sensitivity (66% vs obese untreated mice, $P < .05$). No significant difference among all groups was found in plasma adiponectin (Table 1). White adipose tissue contribution to whole-body insulin sensitivity was assessed by GLUT4 gene and protein analysis. The GLUT4 mRNA (30%, $P < .05$ vs C, Fig. 1A) and protein (27%, $P < .05$ vs C, Fig. 1B) expression decreased in obese animals.

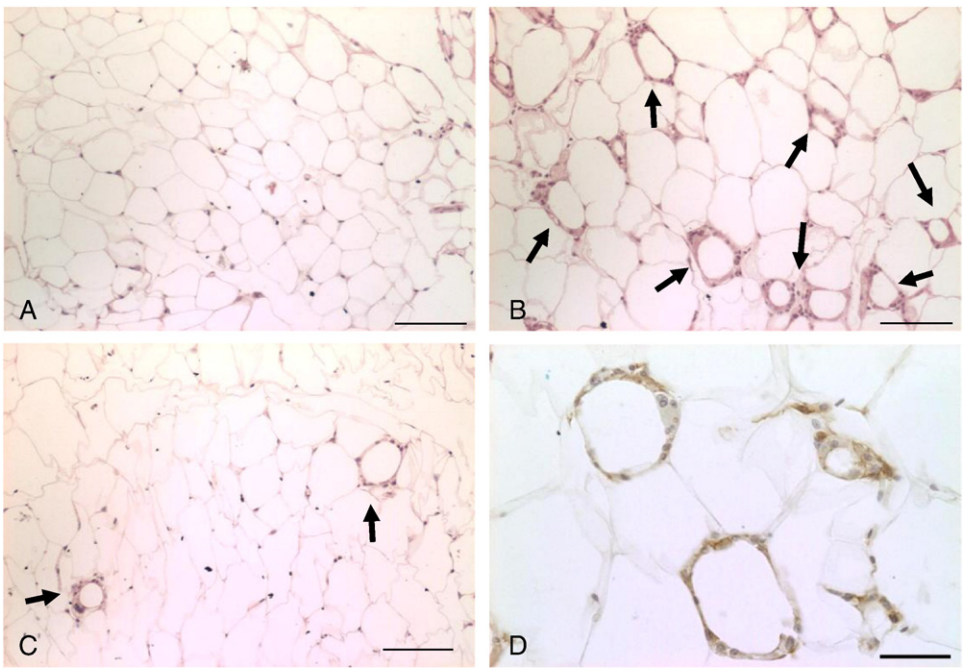

Fig. 2. Morphologic and immunohistochemical analyses of epididymal WAT. Hematoxylin and eosin staining of epididymal WAT from control (A), obese untreated (B), or atorvastatin-treated mice (C). Frequency of crown-like structures around adipocytes, indicated by arrows, was reduced after atorvastatin treatment. Immunohistochemistry analysis for the macrophage-specific antigen F4/80 (D) confirmed the nature of crown-like structures. Calibration mark = 100 μm for A, B, and C and 50 μm for D. AU indicates arbitrary unit.

Interestingly, atorvastatin treatment restored GLUT4 expression to control levels.

To assess whether atorvastatin has any effect in WAT inflammatory state, a characteristic feature of obesity, histologic analysis was performed. The presence of crown-like structures was markedly found around obese adipocytes (Fig. 2B compared with A), and atorvastatin greatly reduced this infiltration (Fig. 2C). Immunohistochemical analysis for F4/80 antigen (Fig. 2D) confirmed that the crown-like structures were composed of macrophages.

Afterward, to address the molecular mechanisms of inflammatory pathway, NF-κB signaling and proinflammatory cytokines were investigated. Fig. 3A clearly shows that obese animals had an increase of IKK-α (42% vs C, $P < .05$) and IKK-β (73% vs C, $P < .05$) phosphorylation, and atorvastatin completely reversed these parameters to control levels.

Fig. 3B shows that WAT of obese animals was unaltered in adiponectin and IL-1β mRNA expression. In contrast, TNF-α and IL-6 mRNA significantly increased in obese mice (~15% vs C, $P < .05$) and decreased to control levels after atorvastatin treatment (Fig. 3C).

Atorvastatin treatment did not alter AST and ALT plasma levels, suggesting that the drug did not cause functional liver injury.

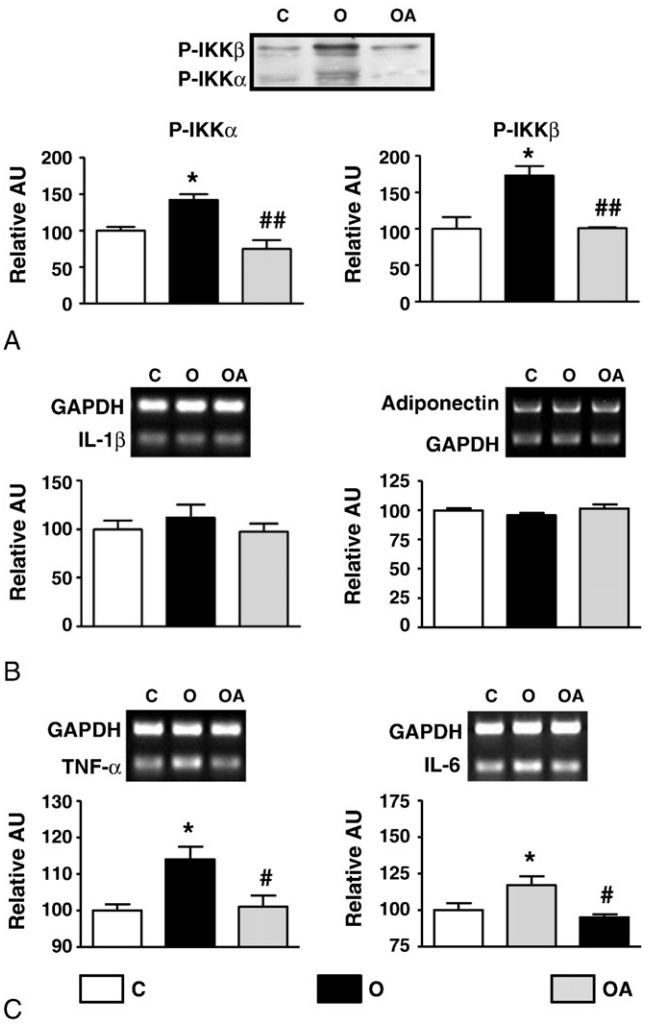

Fig. 3. Phospho-IKK-α/β (A), IL-1β and adiponectin (B), and TNF-α and IL-6 mRNA (C) content of epididymal WAT from control (white bars) and obese untreated (black bars) or atorvastatin-treated (gray bars) mice. A and B, Images of one typical experiment and relative values of protein or mRNA content are shown. Fifty micrograms of protein was immunoblotted for phospho-IKK-α/β (A) analysis. The mRNA expressions of IL-1β, adiponectin, TNF-α, and IL-6 mRNA (B and C) were analyzed by semiquantitative reverse transcriptase PCR. The values are means ± SE of 3 (P-IKK-α/β) and 5 to 7 (TNF-α, IL-1β, and IL-6) animals per group. $^{*}P < .05$ vs control; $^{\#}P < .05$ and $^{\#\#}P < .01$ vs obese untreated; 1-way ANOVA and Student-Newman-Keuls post hoc test. C indicates control; O, obese untreated; OA, obese treated with atorvastatin.

4. Discussion

The present study shows that obese mice develop whole-body insulin resistance, which is associated with reduced GLUT4 expression in adipose tissue, similar to that described in obese humans [12]. Moreover, adipose tissue of obese animals had increased macrophage infiltration and local TNF-α and IL-6 expression, also similar to that described in obese subjects [13-15], highlighting the inflammatory condition. Atorvastatin treatment has been shown to be able to reverse all these findings, providing evidences that, acting as an anti-inflammatory drug, this statin can ameliorate obesity-induced insulin resistance.

Nuclear factor–κB nuclear translocation (activation), a major component of the inflammatory pathway, involves phosphorylation of the inhibitor of NF-κB (IκB) by IκB kinase complex (IKK[1]-α, IKK[2]-β, and IKK[3]-γ). The present study demonstrated that activation of both IKK-β and IKK-α was increased in adipose tissue of obese mice and that atorvastatin treatment repressed phosphorylation of these factors. Moreover, increased expression of NF-κB target genes, such as TNF-α and IL-6, was observed in obese animals; and that decreased in response to atorvastatin, in a parallel modulation of IKK activity. Statins have already been described as able to both prevent NF-κB activation in several cell types [16-19] and repress inducers of NF-κB activation, such as TNF-α and IL-6 [16,20].

Improvement of insulin sensitivity in obese mice has already been shown to involve increased GLUT4 expression in adipose tissue [1], similar to that observed in the present study in response to atorvastatin treatment. Opposite effect of statins upon GLUT4 expression was described in 3T3-L1 adipocytes, in which decreased cell maturation was accompanied by decreased GLUT4 expression [9]. However, it is important to note that there is no inflammatory state in this in vitro model, differently from our in vivo study. As suggested by few studies, inflammatory signals such as increased TNF-α expression and NF-κB activity [3,5] can repress GLUT4 gene expression. Thus, atorvastatin treatment, by reducing inflammatory activity in adipose tissue, was able to enhance GLUT4 gene expression, contributing to improve whole-

body insulin sensitivity. Furthermore, considering that IKK-β can induce serine phosphorylation of insulin receptor substrate–1[21], resulting in insulin signaling inhibition, it is plausible to suppose that atorvastatin-induced reduction of IKK-β phosphorylation would result in increased insulin signaling and GLUT4 translocation to the plasma membrane, additionally contributing to amelioration of insulin resistance. In addition, the amelioration of insulin resistance and inflammatory state could also be due to reduced WAT mass observed in statin-treated animals. For this, other studies should be addressed to figure out if statin plays a role in lipogenesis- or lipolysis-related proteins such as the cell death–inducing DNA fragmentation factor–α–like effector A [22]. In summary, the results show that adipose tissue of insulin-resistant obese mice decreased the GLUT4 gene expression, which was accompanied by inflammatory signals such as increased local macrophage infiltration, TNF-α and IL-6 expression, and IKK phosphorylation, as well as increased plasma levels of TNF-α and IL-6, pointing out the association among obesity, inflammation, and insulin resistance. In addition, atorvastatin treatment was shown to be able to reverse all these findings, providing support that it has anti-inflammatory effects that can ameliorate insulin resistance in obesity.

Acknowledgment

This research was supported by grant from FAPESP (São Paulo State Foundation for Research) 07/050554-1. The authors thank Dr Adauri Brezolin for careful English revision of the manuscript.

References

[1] Papa PC, Seraphim PM, Machado UF. Loss of weight restores GLUT 4 content in insulin-sensitive tissues of monosodium glutamate–treated obese mice. Int J Obes Relat Metab Disord 1997;21:1065-70.

[2] Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest 2005;115:1111-9.

[3] Ruan H, Hacohen N, Golub TR, Van Parijs L, Lodish HF. Tumor necrosis factor–alpha suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes: nuclear factor–kappaB activation by TNF-alpha is obligatory. Diabetes 2002;51:1319-36.

[4] Lagathu C, Bastard JP, Auclair M, Maachi M, Capeau J, Caron M. Chronic interleukin-6 (IL-6) treatment increased IL-6 secretion and induced insulin resistance in adipocyte: prevention by rosiglitazone. Biochem Biophys Res Commun 2003;311:372-9.

[5] Silva JL, Giannocco G, Furuya DT, et al. NF-kappaB, MEF2A, MEF2D and HIF1-a involvement on insulin- and contraction-induced regulation of GLUT4 gene expression in soleus muscle. Mol Cell Endocrinol 2005;240:82-93.

[6] Wong V, Stavar L, Szeto L, et al. Atorvastatin induces insulin sensitization in Zucker lean and fatty rats. Atherosclerosis 2006;184: 348-55.

[7] Lalli CA, Pauli JR, Prada PO, et al. Statin modulates insulin signaling and insulin resistance in liver and muscle of rats fed a high-fat diet. Metabolism 2008;57:57-65.

[8] Kleemann R, Verschuren L, De Rooij BJ, et al. Evidence for anti-inflammatory activity of statins and PPARalpha activators in human C-reactive protein transgenic mice in vivo and in cultured human hepatocytes in vitro. Blood 2004;103:4188-94.

[9] Nakata M, Nagasaka S, Kusaka I, Matsuoka H, Ishibashi S, Yada T. Effects of statins on the adipocyte maturation and expression of glucose transporter 4 (SLC2A4): implications in glycaemic control. Diabetologia 2006;49:1881-92.

[10] Tomie Furuya D, Binsack R, Onishi ME, Monteiro Seraphim P, Fabres Machado U. Low ethanol consumption induces enhancement of insulin sensitivity in liver of normal rats. Life Sci 2005;77:1813-24.

[11] Zanquetta MM, Nascimento MEC, Mori RCT, D'Agord Schaan B, Young M, Machado UF. Participation of beta-adrenergic modulation of GLUT4 expression during fasting and refeeding in rats. Metabolism 2006;55:1538-45.

[12] Björnholm M, Al-Khalili L, Dicker A, et al. Insulin signal transduction and glucose transport in human adipocytes: effects of obesity and low calorie diet. Diabetologia 2002;45:1128-35.

[13] Cancello R, Henegar C, Viguerie N, et al. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. Diabetes 2005;54:2277-86.

[14] Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor–alpha in human obesity and insulin resistance. J Clin Invest 1995;95: 2409-15.

[15] Bastard JP, Maachi M, Van Nhieu JT, et al. Adipose tissue IL-6 content correlates with resistance to insulin activation of glucose uptake both in vivo and in vitro. J Clin Endocrinol Metab 2002;87:2084-9.

[16] van Harmelen V, Skurk T, Röhrig K, Hauner H. HMG-CoA reductase inhibitor cerivastatin inhibits interleukin-6 expression and secretion in human adipocytes. Horm Metab Res 2003;35:466-70.

[17] Planavila A, Laguna JC, Vázquez-Carrera M. Atorvastatin improves peroxisome proliferator–activated receptor signaling in cardiac hypertrophy by preventing nuclear factor-kappa B activation. Biochim Biophys Acta 2005;1687:76-83.

[18] Hilgendorff A, Muth H, Parviz B, et al. Statins differ in their ability to block NF-kappaB activation in human blood monocytes. Int J Clin Pharmacol Ther 2003;41:397-401.

[19] Dichtl W, Dulak J, Frick M, et al. HMG-CoA reductase inhibitors regulate inflammatory transcription factors in human endothelial and vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2003;23:58-63.

[20] Youssef S, Stüve O, Patarroyo JC. The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. Nature 2002;420:78-84.

[21] Gao Z, Hwang D, Bataille F, et al. Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. J Biol Chem 2002;277:48115-21.

[22] Nordström EA, Rydén M, Backlund EC, et al. A human-specific role of cell death–inducing DFFA (DNA fragmentation factor–alpha)–like effector A (CIDEA) in adipocyte lipolysis and obesity. Diabetes 2005;54:1726-34.