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European Journal of Pharmacology xx (2007) xxx–xxx
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# Direct adipotropic actions of atorvastatin: Differentiation state-dependent induction of apoptosis, modulation of endocrine function, and inhibition of glucose uptake

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Received 9 August 2006; received in revised form 31 January 2007; accepted 1 February 2007

**Abstract**

Statins exert anti-inflammatory, anti-atherogenic actions. The mechanisms responsible for these effects remain only partially elucidated. Diabetes and obesity are characterized by low-grade inflammation. Metabolic and endocrine adipocyte dysfunction is known to play a crucial role in the development of these disorders and the related cardiovascular complications. Thus, direct modulation of adipocyte function may represent a mechanism of pleiotropic statin actions. We investigated effects of atorvastatin on apoptosis, differentiation, endocrine, and metabolic functions in murine white and brown adipocyte lines. Direct exposure of differentiating preadipocytes to atorvastatin strongly reduced lipid accumulation and diminished protein expression of the differentiation marker CCAAT/enhancer binding protein-β (CEBP-β). In fully differentiated adipocytes, however, lipid accumulation remained unchanged after chronic atorvastatin treatment. Furthermore, cell viability was reduced in response to atorvastatin treatment in proliferating and differentiating preadipocytes, but not in differentiated cells. Moreover, atorvastatin induced apoptosis and inhibited protein kinase B (AKT) phosphorylation in proliferating and differentiating preadipocytes, but not in differentiated adipocytes. On the endocrine level, direct atorvastatin treatment of differentiated white adipocytes enhanced expression of the pro-inflammatory adipokine interleukin-6 (IL-6), and downregulated expression of the insulin-mimetic and anti-inflammatory adipokines visfatin and adiponectin. Finally, these direct adipotropic endocrine effects of atorvastatin were paralleled by the acute inhibition of insulin-induced glucose uptake in differentiated white adipocytes, while protein expression of the thermogenic uncoupling protein-1 (UCP-1) in brown adipocytes remained unchanged. Taken together, our data for the first time demonstrate direct differentiation state-dependent effects of atorvastatin including apoptosis, modulation of pro-inflammatory and glucostatic adipokine expression, and insulin resistance in adipose cells. These differential interactions may explain variable clinical observations.


*Keywords:* Adipocyte; Adipokine; Apoptosis; Differentiation; Statin; Insulin resistance

## 1. Introduction

Statins, inhibitors of 3-hydroxy-3-methylglutaryl co-enzyme A reductase, control both hypercholesterolemia and hypertriglyceridemia. In addition, pleiotropic anti-inflammatory, anti-oxidative, and anti-atherogenic actions have been described (Arnaud et al., 2005; Jain and Ridker, 2005). These appear to be integral part of the beneficial activity profile of statins. However, the tissue and cell-specific mechanisms mediating these effects remain only partially elucidated.

Direct statin interactions with a number of cell types including smooth muscle cells (Baetta et al., 1997; Blanco-Colio et al., 2002), cardiac myocytes (Demyanets et al., 2006), and endothelial cells (Muck et al., 2004a) have been reported. Yet, little is known about direct statin interactions with preadipose and adipose cell functions. Several reports have focused on the effects of statins on adipocyte differentiation and lipid metabolism (Li et al., 2003; Nishio et al., 1996; Saiki et al., 2005; Song et al., 2003), but influences on apoptosis, endocrine,

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doi:10.1016/j.ejphar.2007.02.024

and gluco-metabolic functions remain almost unexplored. However, such functional interactions are likely to be of special interest for a number of reasons: 1) Visceral adipose tissue mass is closely associated with cardiovascular morbidity (Dagenais et al., 2005; Rexrode et al., 1998). 2) Adipocyte-secreted factors, 'adipokines', such as adiponectin, interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and visfatin, are emerging as important modulators of energy and glucose homeostasis, as well as inflammatory processes and atherosclerosis (Kershaw and Flier, 2004; Rajala and Scherer, 2003; Sethi and Vidal-Puig, 2005). 3) Adipocyte-specific impairment of glucose metabolism results in systemic alterations of glucose and energy homeostasis (Minokoshi et al., 2003). Therefore, dysregulation of endocrine and metabolic adipose function is considered a crucial component in the pathogenesis of insulin resistance, diabetes, and obesity. Moreover, adipocyte-based therapies may provide new treatment strategies for disorders constituting the metabolic syndrome and its related cardiovascular complications (Klein et al., 2006).

Atorvastatin is considered one of the most potent statins. Its anti-atherogenic actions have been demonstrated in large-scale interventional clinical studies (Colhoun et al., 2004; Sever et al., 2003). In the present study, we investigated direct effects of atorvastatin on cell proliferation, apoptosis, and differentiation, as well as endocrine and metabolic functions of preadipose and adipose cells. Our data suggest differential, multi-level interactions with adipose functions that may contribute to their clinical activity profile.

## 2. Materials and methods

### 2.1. Materials

Antibodies against phospho-Akt (Ser473) and CCAAT/enhancer binding protein-β (C/EBP-β) were from Cell Signaling Technology Inc. (Beverly, MA, USA) and Santa Cruz Biotechnology Inc. (CA, USA), respectively. The uncoupling protein-1 (UCP-1)-specific antibody was purchased from Chemicon International (Temecula, CA, USA). Glucose uptake assays were performed with 2-deoxy-[3H]glucose from NEN Life Technologies (Deirich, Germany). Primers for expression analysis were ordered from Biometra (Göttingen, Germany). All other chemicals were from Sigma-Aldrich Co. (St. Louis, MO, USA), unless stated otherwise. Atorvastatin was kindly provided by Pfizer (New York, NY, USA). A 10 mmol/l stock solution in 100% methanol was freshly prepared for all experiments.

### 2.2. Cell culture

Immortalized murine epididymal white and brown preadipocytes were cultured as previously described (Klein et al., 1999, 2002). In brief, proliferating preadipocytes were grown to confluence at 5% $CO_2$ in Dulbecco's modified Eagle's medium (Life Technologies, Paisley, Scotland, UK), supplemented with 20% fetal bovine serum, 4.5 g/l glucose, 20 nmol/l insulin, and 1 nM T3 ('differentiation medium'). Adipocyte differentiation was induced by complementing the medium further with 250 μmol/l indomethacine, 500 μmol/l isobutylmethylxanthine and 2 μg/ml dexamethasone ('induction medium') for 24 h when confluence was reached. After this induction period, early differentiating cells were changed back to differentiation medium, and cell differentiation was continued for six more days until cells reached the terminally differentiated state as determined microscopically and by oil red O staining. For mRNA analysis, glucose uptake assays, and immunoblotting experiments, cells were serum-deprived for 24 h prior to carrying out the experiments. For experiments requiring insulin-resistant adipocytes, cells were treated with 100 nm insulin in differentiation medium over the entire differentiation period of six days. Cells were used between passages 10 and 30.

### 2.3. Cell viability assay

Cells were cultured on 6-well plates and treated with atorvastatin as indicated for each experiment. Cell viability was measured using the MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide) colorimetric assay (Ferrari et al., 1990). In brief, MTT, dissolved in phosphate buffered saline (PBS), was given to the medium at a final concentration of 0.5 mg MTT/ml, and cells were incubated for 3 h at 5% $CO_2$ and 37 °C. During that time, formazan crystals were formed by succinatdehydrogenase of viable cells. After 3 h, the medium was removed, and crystals were solved in lysis buffer (10% sodium dodecyl sulfate (SDS), 0.6% acetic acid in dimethyl sulfoxide (DMSO)). Optical density was then determined at the wavelengths of 570 nm and 630 nm. The final results were calculated as the difference between the two extinctions.

### 2.4. Cell proliferation

Preconfluent preadipocytes were cultured on 6-well plates and treated with atorvastatin as indicated. Trypsinized cells were briefly centrifugated and resuspended in 1 ml PBS, and 10 μl of this resuspension was diluted in 90 μl Trypan Blue. Cell number was determined using a Neubauer cell chamber.

### 2.5. Apoptosis

Cells were cultured on 6 well plates and treated with atorvastatin as indicated. Induction of apoptosis was determined based on the immunochemical detection of histone-complexed DNA fragments using the Cell Death Detection ELISA$^{Plus}$ kit according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany).

### 2.6. Immunoblotting

Cells were cultured on 10 cm plates and treated with atorvastatin as indicated. Each experiment was terminated by washing cells with ice-cold PBS. Proteins were isolated using a whole-cell lysis buffer containing 10 μg/ml aprotinin, 10 μg/ml leupeptin, 2 mmol/l vanadate, and 2 mmol/l phenylmethylsulphonyl fluoride. Protein amount was measured by the Bradford method using the dye from Bio-Rad (Hercules, CA, USA).

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Normalized whole cell protein lysates were subjected to 10% SDS polyacrylamid gel electrophoresis and transferred to nitrocellulose membranes. Membranes were blocked overnight with 10 mmol/l Tris, 150 mmol/l NaCl, and 0.05% Tween 100 pH 7.2, and incubated with the respective antibodies for 2 h (C/EBP-β and UCP-1) and overnight (AKT), respectively. Protein bands were visualized using chemiluminescence (Roche Molecular Biochemicals, Mannheim, Germany) and enhanced chemiluminscence film (Amersham Pharmacia Biotech, Freiburg, Germany).

### 2.7. Oil red O staining and microscopy

Preadipocytes and adipocytes were grown on 10 cm plates and treated with atorvastatin as indicated. Cells were washed with PBS and fixed with formalin for at least fifteen minutes. Oil red O staining solution was added to plates for 1 h. Cells were washed several times with distilled water. Photos were taken using a digital camera at 40 fold increase. Stained cells were decolorized with isopropanol, and optical density was measured at a wavelength of 500 nm.

### 2.8. Quantitative Gene Expression Analysis using real-time RT-PCR

Adiponectin, Visfatin, IL-6 and MCP-1 mRNA expression were analyzed by reverse transcription (RT) followed by polymerase chain reaction (PCR). Housekeeping gene control was 36B4. Total RNA isolation was performed using TRIzol reagent (Invitrogen, Karlsruhe, Germany). RNA quality was tested by RNA visualization on an agarose gel and photometric analysis. Up to 4 μg total RNA was reverse-transcribed using Superscript II (Invitrogen, Karlsruhe, Germany) and an oligo p(DT)15 primer (Roche Molecular Biochemicals, Mannheim, Germany). cDNA was diluted 1/10 with RNAse free water. 2 μl of diluted cDNA was amplified in a 25 μl PCR containing 250 nM of each primer, and QuantiTect SYBR Green PCR-mix (Qiagen, Mölden, Germany). PCR was performed on an Abi Prism 7000 detection system (Forster City, CA, USA).

The following primers were used: 36B4 (acc. no. NM_007475) AAG CGC GTC CTG GA TTG TCT (sense) and CCG CAG GGG CAG CAG TGG T (antisense); adiponectin (acc. no. NM_009605) CTT AAT CCT GCC CAG TCA TGC (sense) and CCT TCC AAC CTG CAC AAG TTC (antisense); visfatin (acc. no. NM_021524) TCG GTT CTG GTG GCG CTT TGC TAC (sense) and AAG TTC CCC GCT GGT GTC CTA TGT (antisense); interleukin-6 (acc. no. NM_031168) AGC CAG AGT CCT TCA GA (sense) and GGT CCT TAG CCA CTC CT (antisense); MCP-1 (acc. no. NM_011333) GCC CCA CTC ACC TGC TGC TAC T (sense) and CCT GCT GCT GGT GAT CCT CTT GT (antisense). PCR for adiponectin and visfatin was performed as follows: initial denaturation at 95 °C for 600 s, 40 cycles with 95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s. Temperature profile for interleukin-6 and MCP-1 was: initial denaturation at 95 °C for 600 s, 40 cycles with 95 °C for 30 s, 55 °C for 45 s, 72 °C for 60 s. Specific amplification was confirmed by producing melting curve profiles (cooling the samples to 65 °C for 10 s and heating to 95 °C in steps of 0.2 °C/s with continuous measurement of fluorescence). Optimized relative quantification was performed based on the second derivative maximum method normalized to 36B4 as housekeeping gene.

### 2.9. Glucose uptake assay

Glucose uptake assays were essentially carried out as described elsewhere (Klein et al., 1999). In brief, fully differentiated cells were starved overnight in serum-free medium and then washed twice in Krebs-Ringer-HEPES. Cells were then stimulated with or without insulin (100 nM) for 30 min. At the end of the stimulation period, the cells were incubated with 2-deoxy-[3H]glucose for 4 min, washed in phosphated-buffered saline, and lysed with 0.1% sodium dodecyl sulfate (SDS). The incorporated radioactivity was determined by liquid scintillation counting.

### 2.10. Statistical analysis

Statistical analyses were performed with Sigma Plot (SPSS Science, Chicago, IL, USA). Data are presented as means ± S.E.M. Statistical significance was determined using the unpaired Student $t$ test. $P$ values $< 0.05$ were considered significant, those $< 0.01$ highly significant.

## 3. Results

### 3.1. Atorvastatin differentially inhibits lipid accumulation in differentiating preadipocytes, but not in terminally differentiated adipocytes

Early differentiating white preadipocytes and terminally differentiated adipocytes were treated chronically with 1 and 10 μM atorvastatin for 72 h from day 1–3 and from day 4–6 after the induction of differentiation, respectively. In differentiating preadipocytes, lipid accumulation was unaltered at 1 μM atorvastatin, but diminished at 10 μM, as compared to control cells (Fig. 1A, top). By contrast, neither 1 μM nor 10 μM atorvastatin affected lipid accumulation and cell morphology in differentiated adipocytes (Fig. 1A, bottom).

### 3.2. Atorvastatin diminishes CEBP-β protein expression in differentiating adipocytes

To test for underlying differences in the expression of differentiation markers, white preadipocytes were treated for 72 h with atorvastatin (1 and 10 μM) from day 1–3 after induction. Immunoblotting for the intermediate differentiation marker C/EBP-β demonstrated a highly significant decrease in protein expression by 60% after treatment with 10 μM atorvastatin. Treatment with 1 μM resulted in a non-significant 25% reduction of C/EBP-β protein expression (Fig. 1B). Furthermore, insulin-induced lipogenesis in differentiating adipocytes was reduced by 50% in response to 24 h atorvastatin

Please cite this article as: Mäuser, W., et al., Direct adipotropic actions of atorvastatin: Differentiation state-dependent induction of apoptosis, modulation of endocrine function, and inhibition of glucose uptake, Eur. J. Pharmacol. (2007), doi:10.1016/j.ejphar.2007.02.024

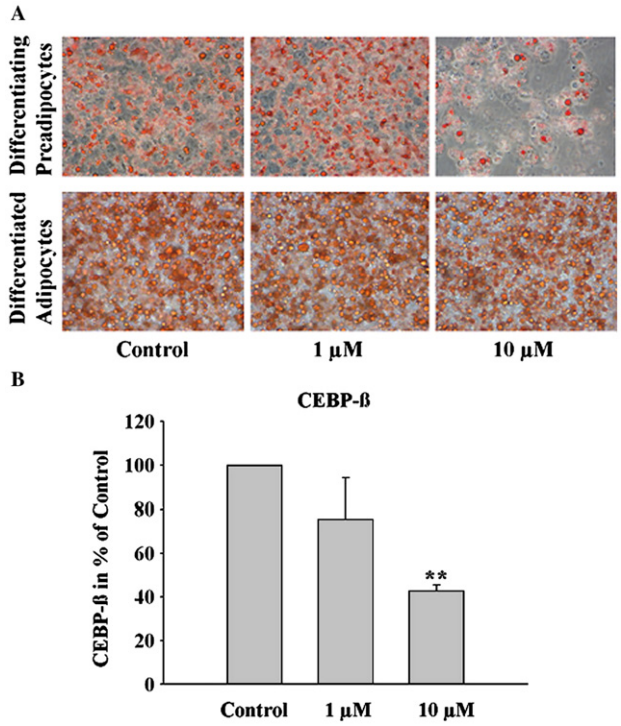


Fig. 1. Atorvastatin differentially affects adipose lipid accumulation and differentiation. Differentiating preadipocytes (top row, day 1–3 after induction) and terminally differentiated white adipose cells (bottom row, day 4–6 after induction) were treated for 72 h with atorvastatin (1 and 10 µM). Lipid accumulation was visualized by Oil Red O staining and microscopy (A). Protein expression of C/EBP-β in differentiating preadipocytes was determined by immunoblotting as described in Materials and Methods. A bar graph analysis of 3 independent experiments, including the SEM, is shown. ** $P<0.01$ comparing non treated (control) to treated cells (B).

treatment at both 1 µM and 10 µM concentrations (data not shown).

### 3.3. Atorvastatin reduces cell viability in proliferating and differentiating preadipocytes, but not in terminally differentiated adipocytes

To explore the differential effects on lipid accumulation and cell differentiation, we assessed atorvastatin's effects on cell viability in pre-confluent, proliferating and in differentiating preadipocytes (day 1–3 after induction), as well as in terminally differentiated adipocytes (day 5 after induction). Exposure of proliferating white preadipocytes to 10 µM atorvastatin for 48 h reduced cell viability by 30% (Fig. 2A). Similarly, atorvastatin treatment (10 µM) of differentiating preadipocytes reduced cell viability to the same extent (Fig. 2B). However, in terminally differentiated cells, atorvastatin treatment (1 and 10 µM) did not influence cell viability (Fig. 2C). Moreover, lactate dehydrogenase (LDH) release and protein expression of the late differentiation marker aP2 was unaltered in terminally differentiated adipocytes in response to atorvastatin treatment for 1 h or 24 h (data not shown). Interestingly, atorvastatin stimulated AMPK phosphorylation in adipocytes in a time-dependent manner after 1 h and 24 h by up to 240% (data not shown).

### 3.4. Atorvastatin inhibits preadipocyte proliferation

Further, we investigated cell proliferation in pre-confluent, proliferating white preadipocytes. As compared to non-treated control cells, 24 h and 48 h of atorvastatin treatment (1 and 10 µM) resulted in a relative reduction in preadipocyte numbers by maximally 35% and 45%, respectively (Fig 3A and B).

### 3.5. Atorvastatin induces apoptosis in proliferating and early differentiating, but not in terminally differentiated adipocytes

Given the inhibition of cell proliferation and the differential reduction in cell viability, we next examined apoptotic cell death in proliferating and early differentiating preadipocytes, as well as in terminally differentiated white adipocytes. Treatment of proliferating and differentiating preadipocytes with 1 and 10 µM atorvastatin for 24 h dose-dependently induced apoptosis by maximally 180% and 70%, respectively, as compared to non-

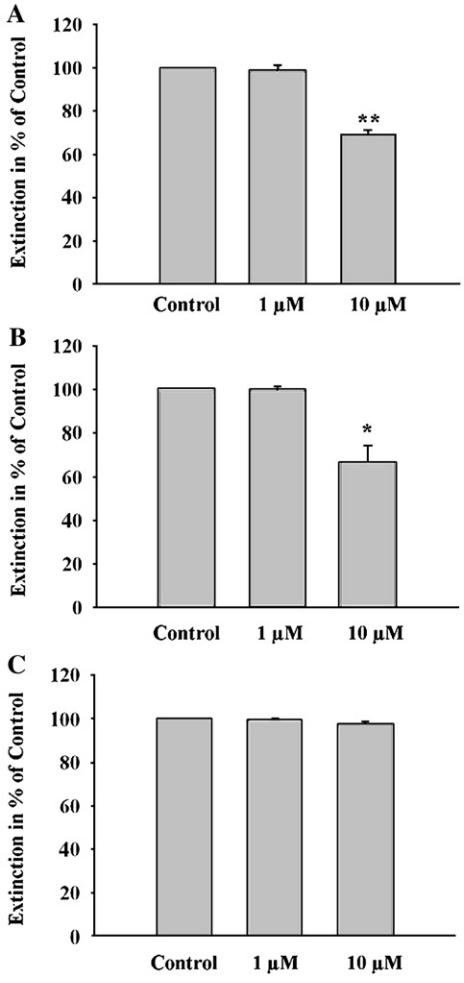


Fig. 2. Atorvastatin reduces cell viability in proliferating and differentiating, but not in terminally differentiated adipocytes. Proliferating (A), early differentiating (day 1 after induction, B) preadipocytes, and terminally differentiated (day 5 after induction, C) white adipocytes were treated with 1 and 10 µM atorvastatin. Cell viability was measured using the MTT assay as described in Materials and Methods. A bar graph analysis of at least 3–4 independent experiments, including the SEM, is shown. * $P\leq 0.05$ and ** $P<0.01$ comparing non treated (control) to treated cells.

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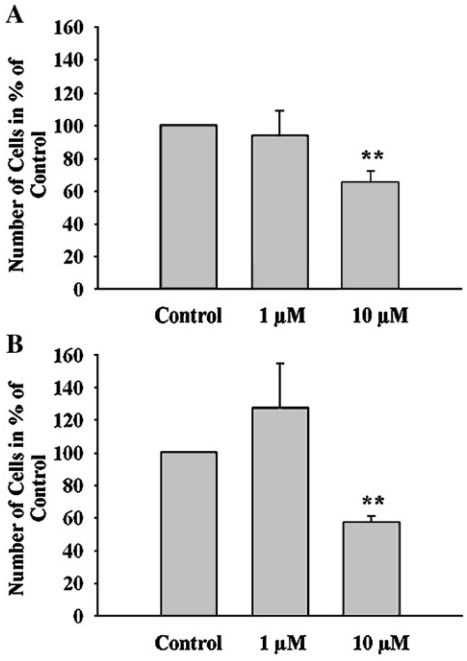


Fig. 3. Atorvastatin inhibits cell proliferation. Pre-confluent, proliferating white preadipocytes were treated with atorvastatin (1 and 10 µM) for 24 h (A) and 48 h (B). Total number of cells was determined as described in Materials and Methods. A bar graph analysis of 4 independent experiments, including the SEM, is shown. $*P \leq 0.05$ and $**P < 0.01$ comparing non treated (control) to treated cells.

treated control cells (Fig. 4A and B). However, in accord with the previous findings of undisturbed lipid accumulation and cell viability, atorvastatin treatment failed to induce apoptosis in terminally differentiated adipocytes (Fig. 4C).

### *3.6. Atorvasatin inhibits AKT activation in early differentiating adipocytes*

AKT acts as an anti-apoptotic serine/threonine kinase. Consistent with its differential apoptosis-inducing effects, acute treatment for 5 min with 10 µM atorvastatin inhibited AKT phosphorylation by 40% in early differentiating pre-adipocytes as compared to non-treated control cells, but this treatment did not alter basal AKT phosphorylation in terminally differentiated adipocytes (Fig. 5A and B).

### *3.7. Atovastatin directly influences the pro-inflammatory, glucostatic adipokine gene expression pattern in terminally differentiated white adipocytes*

Differentiated adipocytes contribute to systemic inflammatory processes and glucose homeostasis by virtue of secretion of adipocyte-derived factors, so-called adipokines. We, therefore, investigated next direct atorvastatin-induced alterations in the gene expression of classic and novel glucostatic, as well as pro- and anti-inflammatory adipokines.

IL-6 mRNA expression was enhanced by 230% after atorvastatin treatment with 10 µM for 24 h in terminally differentiated white adipocytes as compared to non-treated control cells (Fig. 6A). Treatment with 1 µM did not show a significant effect. MCP-1 mRNA expression, however, remained unaltered after atorvastatin treatment for the same time (1 µM and 10 µM) (Fig. 6B). By contrast, atorvastatin at 1 µM and 10 µM suppressed gene expression of the anti-inflammatory, insulin-sensitizing adiponectin by 60% and 50%, respectively (Fig. 6C). Furthermore, gene expression of the insulin-mimetic visfatin was reduced by 50% and 30% after 1 and 10 µM of atorvastatin treatment, respectively, as compared to control cells (Fig. 6D).

### *3.8. Differential metabolic effects of atorvastatin in white and brown adipocytes: Inhibition of glucose uptake without alteration of thermogenic UCP-1 expression*

Adipose endocrine function is closely intertwined with metabolic adipose functions. We assessed insulin-induced

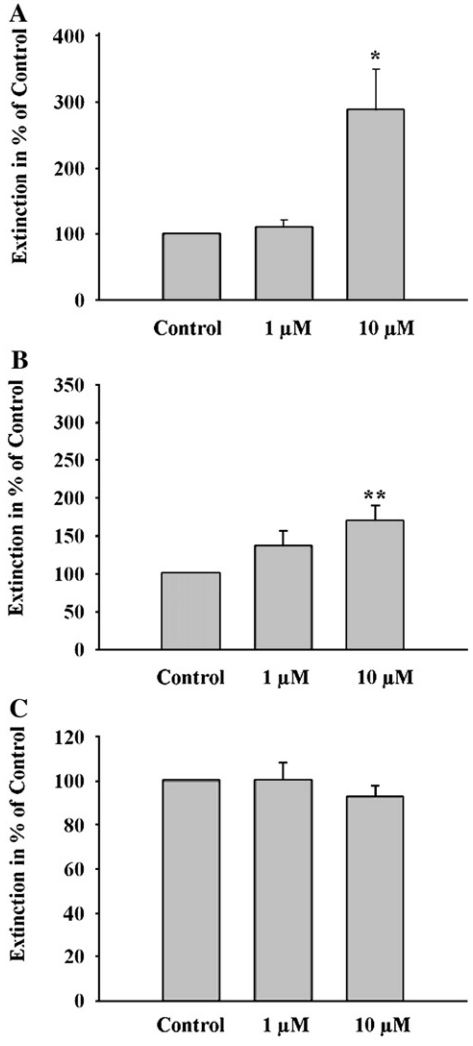


Fig. 4. Atorvastatin induces apoptosis in proliferating and differentiating, but not in terminally differentiated adipocytes. Proliferating (A) and differentiating (B) preadipocytes, as well as terminally differentiated adipocytes (C) were treated with atorvastatin for 24 h at the concentrations indicated. Induction of apoptosis was measured by immunochemical determination of histone-complexed DNA fragments as described in Materials and Methods. A bar graph analysis of 6 (A), 10 (B), and 4 (C) independent experiments, including the SEM, is shown. $*P \leq 0.05$ and $**P < 0.01$ comparing non-treated (control) to treated cells.

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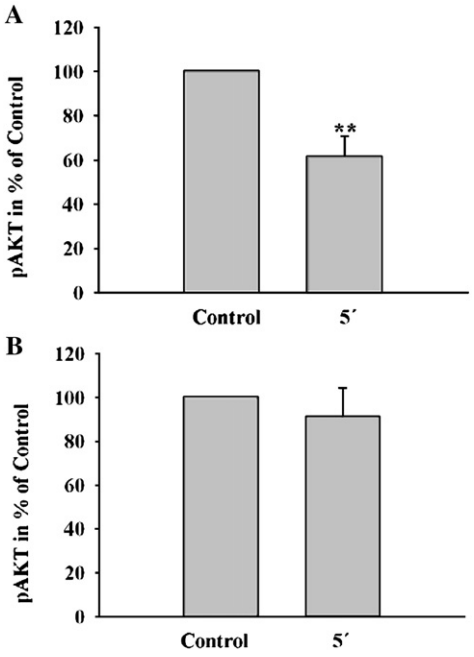


Fig. 5. Atorvastatin decreases AKT phosphorylation in early differentiating, but not in terminally differentiated adipocytes. Differentiating (day 1 after induction) (A) and terminally differentiated adipocytes (day 7 after induction) (B) were treated with 10 μM atorvastatin as indicated. Immunoblot analyses using a phospho-specific AKT antibody were performed as described in Materials and Methods. A bar graph analysis of 4–5 independent experiments, including the SEM, is shown. $^{**}P<0.01$ comparing non-treated (control) to treated cells.

glucose uptake in terminally differentiated white adipocytes, as well as protein expression of the thermogenic UCP-1 in brown adipocytes. Treatment of white adipocytes with 1 and 10 μM atorvastatin for 1 h resulted in a highly significant, dose-dependent reduction in insulin-induced glucose uptake by maximally 20% (Fig. 7A). This negative effect of atorvastatin was more pronounced at higher insulin concentrations suggesting an insulin signaling element-mediated mechanism of insulin resistance induction (data not shown). Conversely, atorvastatin-induced insulin resistance was only seen in insulin-sensitive, terminally differentiated adipocytes, but was abolished in a model of insulin-resistant cells, which had been chronically pretreated with high insulin concentrations (Fig. 7B). Basal glucose uptake did not change significantly in response to atorvastatin treatment. Furthermore, there was no significant difference in the basal glucose uptake comparing insulin-sensitive and insulin-resistant states of the cells. In brown adipocytes, exposure to atorvastatin (1 μM and 10 μM) for 24 h did not alter protein expression of UCP-1 (Fig. 7C).

## 4. Discussion

In this study, we demonstrate direct, multi-level interactions of atorvastatin with adipose cell functions. These effects include the differentiation state-dependent induction of apoptosis, modulation of pro-inflammatory and glucostatic adipokine gene expression, and acute inhibition of insulin-induced glucose uptake.

To our knowledge, this study is the first to describe a differential induction of apoptosis in adipose cells by atorvastatin. We found a strong induction of apoptosis in proliferating and early differentiating preadipocytes, but not in terminally differentiated adipocytes. In accord with these findings, cell proliferation was reduced in pre-confluent cells, and cell viability was decreased in preadipose, but not in

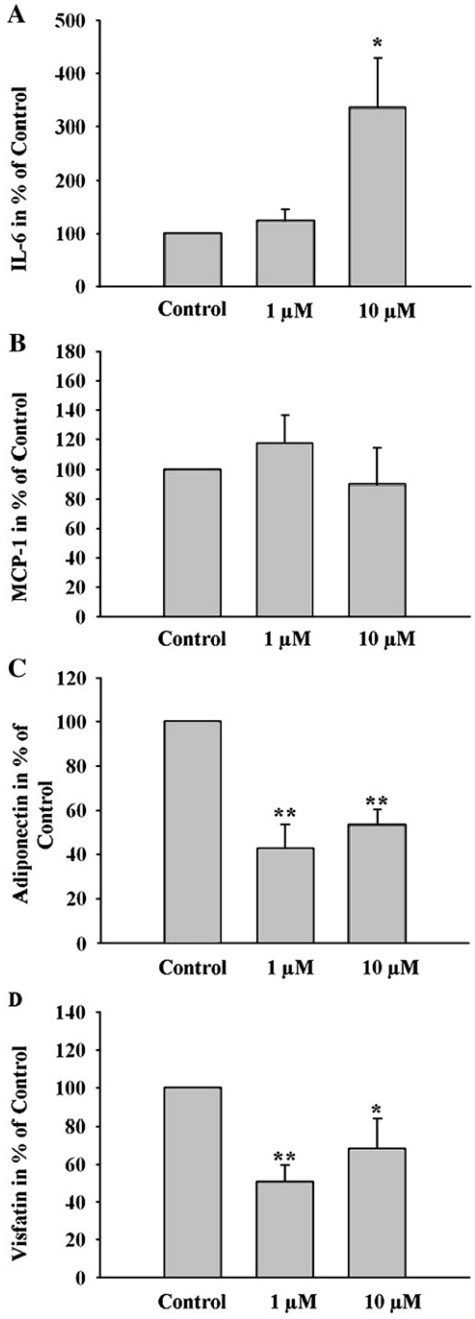


Fig. 6. Atorvastatin directly influences the pro-inflammatory, glucostatic adipokine gene expression pattern. Terminally differentiated white adipocytes were treated with atorvastatin (1 and 10 μM) for 24 h. Quantitative mRNA gene expression analysis of IL-6 (A), MCP-1 (B), adiponectin (C), and visfatin (D) was determined using quantitative real-time RT-PCR as described in Materials and Methods. A bar graph analysis of at least 4–6 independent experiments, including the SEM, is shown. $^{*}P\leq 0.05$ and $^{**}P<0.01$ comparing non-treated (control) to treated cells.

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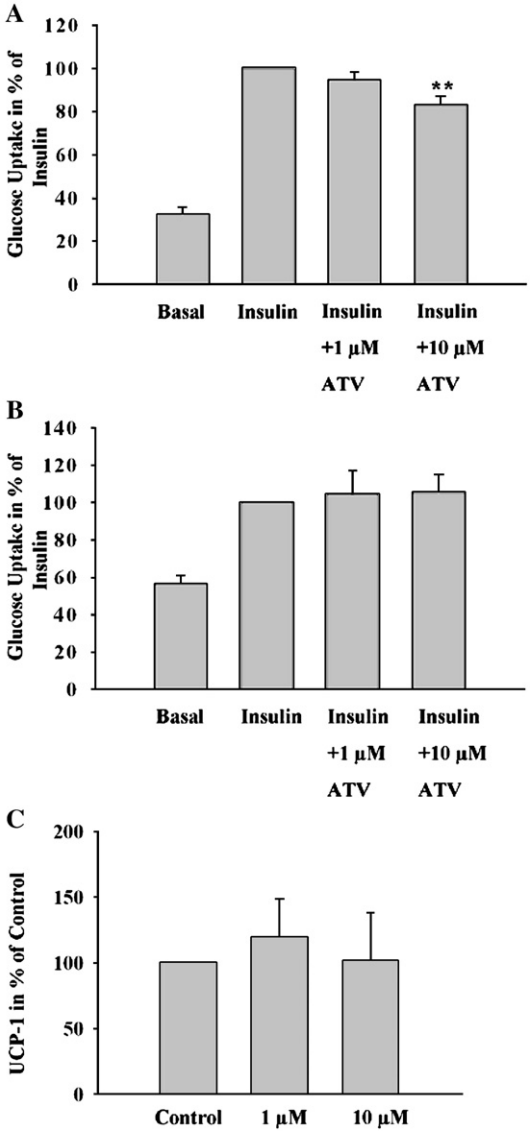


Fig. 7. Atorvastatin and metabolic adipose functions. Terminally differentiated white insulin-sensitive (A), insulin-resistant (B), and brown (C) adipocytes were treated with atorvastatin for 1 h (A, B) and 24 h (C). Glucose uptake assays and UCP-1 immunoblotting were performed as described in Materials and Methods. A bar graph analysis of 7 (A), 3 (B) and 5 (C) independent experiments, including the SEM, is shown. $*P \leq 0.05$ and $**P < 0.01$ comparing insulin-treated cells with and without atorvastatin pretreatment.

differentiated adipose cells. Finally, acute activation of the anti-apoptotic AKT was only inhibited in differentiating preadipocytes, but not in fully differentiated adipocytes. In general, atorvastatin concentrations used here are equal to those commonly reported in similar *in-vitro* studies (Bey et al., 2002; Ichida et al., 2006; Li et al., 2006; Zhao et al., 2005, 2006; Zhao and Zhang, 2003b, 2004). However, our findings have to be interpreted with caution regarding the *in-vivo* situation where concentrations of this lipophilic compound in adipose tissue, although certainly higher than measured plasma concentrations, are most likely below the micromolar range. Atorvastatin-induced apoptosis has been reported in a number of cell systems including smooth muscle cells (Baetta et al., 1997; Blanco-Colio et al., 2002), endothelial cells (Muck et al., 2004a), cardiac myocytes (Demyanets et al., 2006), fibroblasts (Connor et al., 2006), lymphoblasts (Yasuda et al., 2005), hepatocytes (Kubota et al., 2004), and breast cancer cells (Muck et al., 2004b). Given atorvastatin's anti-atherosclerotic *in-vivo* effects, an interaction with adipose cell proliferation and viability may be of particular importance, since a differential induction of apoptosis will change adipose tissue composition and shift the balance between preadipose and adipose cells. This, in turn, may alter metabolic and endocrine functions and, thereby, modify inflammatory processes including atherogenesis.

We are not aware of clinical studies which have addressed such statin-induced modulations of this 'adiposopathy' (Bays and Dujovne, 2006; Klein et al., 2006). In general, both hypertrophy and – possibly beyond a critical level of overweight – hyperplasia are believed to be in effect in the development of obesity (van Harmelen et al., 2003b). Serving as a proof of principle for differential adipotropic effects and an altered preadipose–adipose balance, a differential induction of apoptosis has been described to be induced in human adipose cells after treatment with the HIV protease inhibitor ritonavir (Grigem et al., 2005). Furthermore, changes in adipose tissue composition are well known in response to treatment with the insulin-sensitizing thiazolidinediones and are believed to contribute to their mechanism of action (Mori et al., 1999; Okuno et al., 1998).

The present study, in concert with others (Li et al., 2003; Nishio et al., 1996; Song et al., 2003), suggests an inhibitory effect of statins on adipocyte differentiation. Although gross effects on adipose tissue mass and distribution appear unlikely, it is conceivable that subtle changes in apoptosis and differentiation, induced by atorvastatin and other members of this drug class, contribute to their beneficial activity profile *in vivo*. Indeed, in an experimental study aiming at defining toxicity, atorvastatin decreased maternal body weight gain and reduced fetal body weight in rats and rabbits (Dostal et al., 1994). In recent clinical studies, either including or not including a concurrent diet, body weight was reported to be non-significantly reduced or constant under atorvastatin therapy (Gomez-Dominguez et al., 2006; Nakata et al., 2006). Of note in this context, our data do not suggest an effect of atorvastatin on energy expenditure, judging from an unaltered expression of the thermogenic UCP-1 in brown adipocytes.

Statin-induced changes in visceral adipose mass have not been formally investigated to our knowledge. However, such alterations may be of special interest, since endocrine highly active, intra-abdominal adipocytes importantly contribute to cardiovascular morbidity and mortality (Dagenais et al., 2005). We, therefore, used differentiated epididymal adipocytes (Ott et al., 2004) to examine direct atorvastatin-induced changes in gene expression of important adipokines that are implicated in the pathogenesis of atherosclerosis (Kershaw and Flier, 2004; Rajala and Scherer, 2003). Surprisingly, we found the induction of a pro-inflammatory and glucostatic adipokine expression profile with an increase in IL-6, but not MCP-1, and a reduction of adiponectin and visfatin. This is the first study describing a direct atorvastatin-induced regulation of adiponectin, visfatin,

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and MCP-1. *In-vitro* and *in-vivo* findings on statin-induced changes in IL-6 levels are conflicting. While a number of clinical studies demonstrate non-significant increases (Huptas et al., 2006) or no changes (Kinlay et al., 2003; van de Ree et al., 2003; Wiklund et al., 2002), others, including experimental *in-vitro* and animal studies, find a decrease in IL-6 concentrations by atorvastatin (Chan et al., 2002; Zhao and Zhang, 2003a) and cerivastatin (van Harmelen et al., 2003a). The role of IL-6 in the regulation of glucose and energy homeostasis is complex. Potentially opposing tissue-specific, paracrine, and endocrine effects may account for apparent discrepancies. Thus, IL-6-knock-out mice develop glucose intolerance and maturity-onset obesity which can be ameliorated only by intracerebroventricular, but not peripheral administration of this pro-inflammatory cytokine (Wallenius et al., 2002). Yet, IL-6 directly induces insulin resistance in adipocytes (Fasshauer et al., 2004; Rotter et al., 2003), which is in contrast to skeletal muscle, where a direct enhancement of insulin sensitivity has been described (Weigert et al., 2005, 2006). Therefore, the overall physiological significance of potential statin-induced IL-6 changes, currently, remains obscure.

Our findings of suppressed adiponectin and visfatin levels lend support to the notion of impaired insulin sensitivity in adipose tissue. In fact, this study demonstrates a direct, atorvastatin-induced inhibition of insulin-mediated glucose uptake in differentiated white adipocytes. Our findings suggest a specific, potentially saturable insulin signaling element-mediated mechanism employed by atorvastatin to inhibit glucose uptake in terminally differentiated cells. *In-vivo* reports vary in their findings on statin-mediated effects on glucose homeostasis. Large-scale interventional trials showed conflicting results on the incidence and control of type 2 diabetes with statin therapy (2001; Colhoun et al., 2004; Collins et al., 2003; Freeman et al., 2001; Sever et al., 2003). Furthermore, a number of smaller studies, both experimental and clinical, were ambiguous with respect to changes in parameters of glucose metabolism (Guclu et al., 2004; Huptas et al., 2006; Ishikawa et al., 2006; Nakata et al., 2006; Satoh et al., 2005; Wong et al., 2006). Our findings of differential atorvastatin effects could provide an intriguing explanation for these inconsistencies. Thus, the balance between differentiating preadipose cells – which are less susceptible to insulin resistance-inducing effects of atorvastatin – and differentiated adipocytes – which may be more 'vulnerable' in this respect – may critically determine the outcome of statin actions on glucose metabolism. In support of this notion, two groups recently reported different outcomes of atorvastatin treatment on glucose metabolism in different patient subgroups depending on the level of obesity (Nakata et al., 2006) or metabolic syndrome components (Huptas et al., 2006). These findings suggest that atorvastatin treatment is associated with less insulin resistance or even enhanced insulin sensitivity in those individuals with a higher degree of obesity (i.e. a higher number of immature preadipose cells).

Taken together, this study demonstrates direct, differentiation state-dependent interactions of atorvastatin on the level of proliferative, endocrine, and metabolic adipose cell responses. These differential effects may contribute to pleiotropic actions of atorvastatin and provide an explanation for variable clinical observations of statin actions on energy and glucose homeostasis.

## Acknowledgments

J.K. is a Feodor-Lynen Fellow of the Humboldt Foundation. This study was supported by a translational research grant from Pfizer and by a grant from the Deutsche Forschungsgemeinschaft to J.K. (Kl 1131/2-5).

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