Calcium and 1,25-Dihydroxyvitamin D_3 Regulation of Adipokine Expression

Xiaocun Sun and Michael B. Zemel

Abstract

SUN, XIAOCUN AND MICHAEL B. ZEMEL. Calcium and 1,25-dihydroxyvitamin D_3 regulation of adipokine expression. *Obesity.* 2007;15:340–348.

Objective: Obesity is associated with elevated oxidative stress and low-grade systemic inflammation. We have demonstrated recently that 1α,25-$(OH)_2$-D_3 promotes reactive oxygen species production in cultured adipocytes, whereas suppression of 1α,25-$(OH)_2$-D_3 by increasing dietary calcium down-regulates diet-induced oxidative stress in aP2-agouti transgenic mice. However, whether the anti-obesity effect of dietary calcium plays a role in regulation of obesity-associated inflammation is not clear.

Research Methods and Procedures: We investigated the role of dietary calcium in the regulation of inflammatory cytokine production in aP2-agouti transgenic mice fed low- and high-calcium obesigenic diets and in the modulation of cytokine production by 1α,25-$(OH)_2$-D_3 in cultured murine and human adipocytes.

Results: The high-calcium diet inhibited the expression of pro-inflammatory factors tumor necrosis factor α and interleukin (IL)-6 by 64% and 51%, respectively ($p < 0.001$), in visceral fat, stimulated the expression of the anti-inflammatory factors IL-15 and adiponectin by 52% ($p = 0.001$) and 54% ($p = 0.025$), respectively, in visceral fat, and induced a 2-fold increase in IL-15 expression in soleus muscle ($p = 0.01$) compared with litter mate controls on a low-calcium diet. 1α,25-$(OH)_2$-D_3 also markedly stimulated the expression of tumor necrosis factor α ($p < 0.001$) and IL-6 ($p = 0.016$) in differentiated 3T3-L1 adipocytes and increased IL-6 ($p = 0.004$) and IL-8 ($p < 0.001$) production in differentiated human adipocytes. These effects were blocked by calcium channel antagonism with nifedipine.

Discussion: These data demonstrate that 1α,25-$(OH)_2$-D_3 favors inflammatory cytokine expression and inhibits anti-inflammatory cytokine expression; accordingly, suppression of 1α,25-$(OH)_2$-D_3 by dietary calcium inhibits adipocyte-derived inflammation associated with obesity.

Key words: 1α,25-$(OH)_2$-D_3, inflammation, calcium, reactive oxygen species, cytokines

Received for review February 13, 2006.
Accepted in final form September 21, 2006.
The costs of publication of this article were defrayed, in part, by the payment of page charges. This article must, therefore, be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Department of Nutrition, University of Tennessee, Knoxville, Tennessee.
Address correspondence to Michael B. Zemel, Department of Nutrition, University of Tennessee, 1215 West Cumberland Avenue, 229, Knoxville, TN 37996-1900.
E-mail: mzemel@utk.edu
Copyright © 2007 NAASO

Introduction

Obesity is a principle causative factor in the development of metabolic disorders such as insulin resistance, hyperglycemia, dyslipidemia, hypertension, and atherosclerosis (1–3). Excess adipose tissue, particularly in the visceral compartment, is associated with systemic oxidative stress in humans and mice (4–6), and increased oxidative stress in accumulated fat appears to be an important contributor to the pathogenesis of obesity-associated metabolic syndrome (6). Visceral adiposity is characterized by low-grade systemic inflammation, and obese subjects exhibit elevated production of inflammatory markers (7–9). Adipocytes produce a variety of biological molecules, including both inflammatory cytokines such as tumor necrosis factor (TNF)[1] α, interleukin (IL)-6, and IL-8 and anti-inflammatory factors such as adiponectin and IL-15 (10–12). Dysregulated production of these adipocytokines contributes to the pathogenesis of obesity-associated metabolic syndrome. Although the mechanism responsible for the development of systemic inflammation is not clear, multiple previous studies have indicated that increased oxidative stress in accumulated fat is an underlying cause of dysregulation of adipocytokines (6,13–15).

[1] Nonstandard abbreviations: TNF, tumor necrosis factor; IL, interleukin; ROS, reactive oxygen species; H_2O_2, hydrogen peroxide; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; PCR, polymerase chain reaction; SE, standard error; NADPH, nicotinamide adenosine dinucleotide phosphate.

Table 1. Body weight and fat pad weight at baseline and three weeks after diet treatment in aP2-agouti transgenic mice fed low- and high-calcium diets

	Baseline		Three weeks after		
	Low calcium diet	High calcium diet	Low calcium diet	High calcium diet	p
Body weight (g)*	25.28 ± 0.39	24.47 ± 0.47	32.96 ± 0.95	28.56 ± 0.57*	0.023
Body fat (g)	N/A	N/A	4.47 ± 0.37	2.44 ± 0.23*	0.007
Subcutaneous fat† (g)	N/A	N/A	1.76 ± 0.17	0.94 ± 0.11*	0.015
Visceral fat‡ (g)	N/A	N/A	2.48 ± 0.19	1.31 ± 0.11*	0.004

* Values are mean ± standard deviation, $n = 10$.
† Subscapular fat pad.
‡ Sum of perirenal and abdominal fat pads.
p indicates significant level between animals on the basal diet and those on the high-Ca diet.

We have shown previously that dietary calcium attenuates obesity by inhibiting lipogenesis, stimulating lipolysis and thermogenesis, and increasing adipocyte apoptosis (16,17). We have also recently demonstrated that diet-induced obesity per se promotes oxidative stress and inhibition of obesity by increasing dietary calcium suppresses oxidative stress (18). These effects are mediated by suppression of 1α,25-$(OH)_2$-D_3 production, resulting in inhibition of 1α,25-$(OH)_2$-D_3-induced stimulation of Ca^{2+} influx and suppression of metabolic uncoupling status in adipose tissue (16,18). Accordingly, we have now investigated the role of 1α,25-$(OH)_2$-D_3 in regulating adipocytokine expression in murine and human adipocytes and the effects of dietary calcium modulation of 1α,25-$(OH)_2$-D_3 on cytokine expression in an animal model of obesity. We have previously described aP2 agouti transgenic mice as a useful model for diet-induced obesity in a genetically susceptible human population in that they are not obese on a standard AIN-93G diet (19) but develop mild to moderate obesity and hyperglycemia when fed high-sucrose and/or high-fat diets (20). We have also shown that aP2 agouti transgenic mice exhibit significantly greater baseline reactive oxygen species (ROS) production compared with wild-type controls before the feeding period and that the consumption of the obesity-promoting diet significantly increases adipose tissue ROS production (18), indicating that aP2 agouti transgenic mice may be utilized as an animal model to study diet-induced obesity and oxidative stress. We, therefore, utilized aP2 transgenic mice as the animal model to investigate the effect of obesity and diet-induced oxidative stress on adipose tissue inflammatory cytokine production after a 3-week obesity induction period on high-sucrose/high-fat diets with either low-calcium (0.4% from $CaCO_3$) (basal diet) or high-calcium (1.2% from $CaCO_3$) (high-calcium diet) content.

Research Methods and Procedures

Chemicals

1α,25-$(OH)_2$-D_3, $\alpha \pm$ tocopherol, hydrogen peroxide (H_2O_2), and nifedipine were obtained from Sigma (St. Louis, MO).

Animals and Diets

At 6 weeks of age, 20 male aP2-agouti transgenic mice from our colony were randomly divided into two groups (10 mice/group) and fed a modified AIN 93 G diet with sub-optimal calcium (0.4% from calcium carbonate) or high calcium (1.2% from calcium carbonate), respectively. Sucrose was the sole carbohydrate source, providing 64% of energy, and fat was increased to 25% of energy with lard. Mice were studied for 3 weeks, during which food intake and spillage were measured daily and body weight, fasting blood glucose, and food consumption were assessed weekly. At the conclusion of the study, all mice were killed under isoflurane anesthesia, and blood was collected by cardiac puncture; visceral fat pads (perirenal and abdominal), subcutaneous fat pads (subscapular), and soleus muscle were immediately excised, weighed, and used for further study, as described below. This study was approved from an ethical standpoint by the Institutional Care and Use Committee of The University of Tennessee.

Cell Culture

3T3-L1 preadipocytes were incubated at a density of 8000 cells/cm^2 (10 cm^2 dish) and grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and antibiotics (adipocyte medium) at 37 °C in 5% CO_2 in air. Confluent preadipocytes were induced to differentiate with a standard differentiation medium consisting of DMEM-F10 (1:1, v/v) medium supplemented with 1% FBS, 1 μM dexamethasone, 0.5 mM

isobutylmethylxanthine, and antibiotics (1% penicillin-streptomycin). Preadipocytes were maintained in this differentiation medium for 3 days and subsequently cultured in adipocyte medium. Cultures were re-fed every 2 to 3 days to allow 90% cells to reach full differentiation before chemical treatment.

Human preadipocytes used in this study were supplied by Zen-Bio (Research Triangle, NC). Preadipocytes were inoculated in DMEM/Ham's F-10 medium (1:1, v/v) containing 10% FBS, 15 mM N-[2-hydroxyethyl]piperazine-N′-2-ethanesulfonic acid, and antibiotics at a density of 30,000 cells/cm^2. The cells were isolated from the stromal vascular fraction of human subcutaneous adipose tissue and differentiated in vitro as follows. Confluent monolayers of preadipocytes were induced to differentiate with a standard differentiation medium consisting of DMEM-F10 (1:1, v/v) medium supplemented with 15 mM N-[2-hydroxyethyl] piperazine-N′-2-ethanesulfonic acid, 3% FBS, 33 μM biotin, 17 μM pantothenate, 100 nM insulin, 0.25 μM methylisobutylxanthine, 1 μM dexamethasone, 1 μM BRL49653, and antibiotics. Preadipocytes were maintained in this differentiation medium for 3 days and subsequently cultured in adipocyte medium in which BRL49653 and 1-methyl-3-isobutylxanthine were omitted. Cultures were re-fed every 2 to 3 days until fully differentiated.

Cells were incubated in serum-free medium overnight before chemical treatment. Chemicals were freshly diluted in adipocyte medium before treatment. Cells were washed with fresh adipocyte medium, re-fed with medium containing the different treatments [control; 10 nM 1α,25-$(OH)_2$-D_3; 10 μM nifedipine; 10 nM 1α,25-$(OH)_2$-D_3 plus 10 μM nifedipine; 100 nM H_2O_2; 1 μM $\alpha \pm$ tocopherol; or 100 nM H_2O_2 plus 1 μM $\alpha \pm$ tocopherol], and incubated at 37 °C in 5% CO_2 for 48 hours before analysis. Cell viability was measured by trypan blue exclusion.

Total RNA Extraction

A total cellular RNA isolation kit (Ambion, Austin, TX) was used to extract total RNA from cells according to the manufacturer's instructions.

Quantitative Real-Time Polymerase Chain Reaction (PCR)

Adipocyte and muscle 18s, TNFα, IL-6, IL-8, IL-15, and adiponectin were quantitatively measured using a smart cycler real-time PCR system (Cepheid, Sunnyvale, CA) with a TaqMan 1000 Core Reagent Kit (Applied Biosystems, Branchburg, NJ). The primers and probe sets were obtained from Applied Biosystems TaqMan Assays-on-Demand Gene Expression primers and probe set collection and utilized according to the manufacturer's instructions. Pooled adipocyte total RNA was serial-diluted in the range of 1.5625 to 25 ng and used to establish a standard curve, and total RNAs for the unknown samples were also diluted in this range. Reactions of quantitative reverse transcription-PCR for standards and unknown samples were also performed according to the instructions of Smart Cycler System (Cepheid) and TaqMan Real Time PCR Core Kit (Applied Biosystems). The mRNA quantitation for each sample was further normalized using the corresponding 18S quantitation.

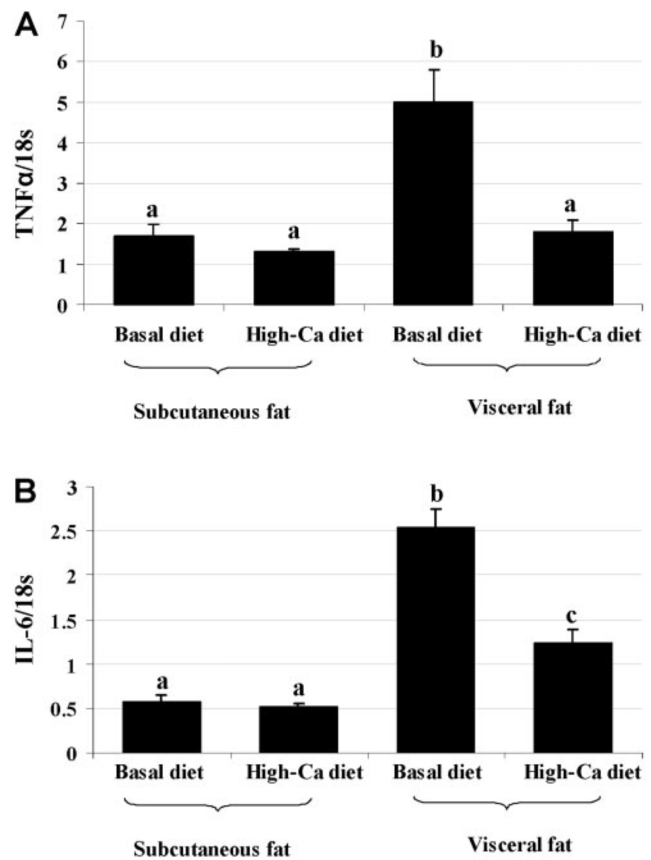

Figure 1: Adipose tissue TNFα expression ratio (A) and IL-6 expression ratio (B) in aP2-agouti transgenic mice fed low-calcium (basal) or high-calcium diets. Data are normalized to 18s expression. Values are presented as mean $\pm$ SE, $n = 6$. Means with different letter differ, $p < 0.001$.

Plasma 1α,25-$(OH)_2$-D_3 Assay

A 1α,25-$(OH)_2$-D_3-vitamin D enzyme-linked immunosorbent assay kit (Alpco Diagnostics, Windham, NH) was used to measure plasma 1α,25-$(OH)_2$-D_3 content according to the manufacturer's instructions.

Statistical Analysis

Data were evaluated for statistical significance by ANOVA or Student's t test, and significantly different group means were then separated by the least significant difference test by using SPSS (SPSS Inc., Chicago, IL). All data presented are expressed as mean $\pm$ standard error (SE).

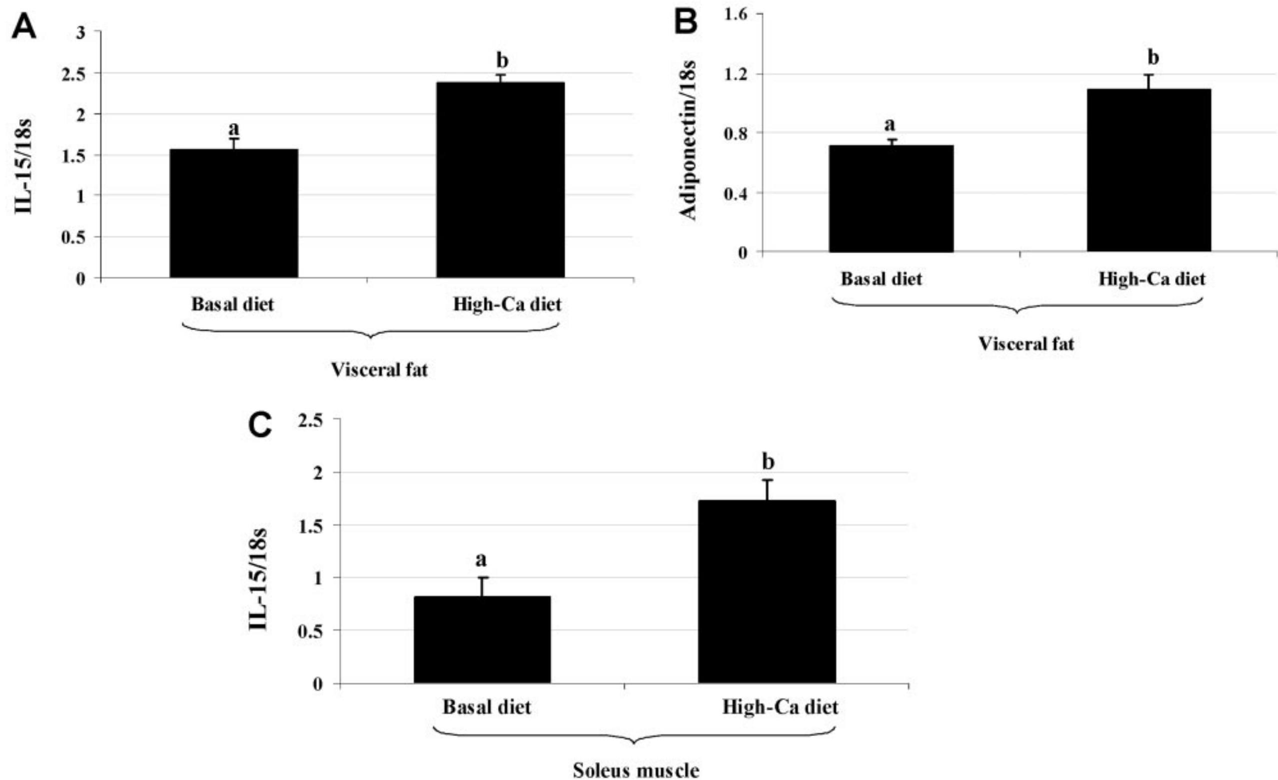

Figure 2: Adipose tissue IL-15 expression (A), adipose adiponectin expression (B), and muscle IL-15 expression (C) in aP2-agouti transgenic mice fed low-calcium (basal) or high-calcium diets. Data are normalized to 18s expression. Values are presented as mean ± SE, $n = 6$. Means with different letter differ, $p < 0.03$.

Results

Dietary Calcium Regulates Inflammatory Cytokine Production in Adipose Tissue and Skeletal Muscle

Feeding the high-calcium ad libitum for 3 weeks significantly decreased weight and fat gain (Table 1) and suppressed *TNF*α gene expression by 64% in visceral, but not subcutaneous, fat compared with mice on the low-calcium basal diet (Figure 1A) ($p < 0.001$). Similarly, IL-6 expression was decreased by 51% in visceral fat of mice on the high-calcium diet vs. mice on the low-calcium basal diet (Figure 1B) ($p < 0.001$), but this effect was absent in subcutaneous fat. In contrast, dietary calcium up-regulated IL-15 expression in visceral fat, with 52% increases in mice on the high-calcium diet compared with animals on the low-calcium diet (Figure 2A) ($p = 0.001$). Adiponectin expression was similarly elevated in visceral fat of mice on the high-calcium diet vs. mice on the low-calcium diet (Figure 2B) ($p = 0.025$). The high-calcium diet also induced a 2-fold increase in IL-15 expression in soleus muscle compared with mice on the low-calcium diet (Figure 2C) ($p = 0.01$).

Intracellular Calcium and 1α,25-$(OH)_2$-D_3 Regulates Cytokine Production in Cultured Murine and Human Adipocytes

The high-calcium diet suppressed plasma 1α,25-$(OH)_2$-D_3 (Figure 3); consequently, we investigated the role of 1α,25-$(OH)_2$-D_3 and calcium in regulation of adipokine production in vitro. Figure 4A shows that 1α,25-$(OH)_2$-D_3 stimulated TNFα expression by 135% in 3T3-L1 adipocytes, and addition of the calcium channel antagonist nifedipine completely blocked this effect ($p < 0.001$), whereas nifedipine alone exerted no effect. Similarly, 1α,25-$(OH)_2$-D_3 markedly increased IL-6 expression in 3T3-L1 adipocytes, and this effect was reversed by addition of nifedipine ($p = 0.016$) (Figure 4B).

Similar results were observed in differentiated human adipocytes. 1α,25-$(OH)_2$-D_3 stimulated IL-6 and IL-8 expression by 53% and 49%, respectively (Figure 5, A, $p = 0.004$; B, $p < 0.001$), and the addition of nifedipine blocked this effect. We found no effect of 1α,25-$(OH)_2$-D_3 or nifedipine on IL-15 (Figure 5C, $p = 0.473$) or adiponectin expression (Figure 5D, $p = 0.377$) in human adipocytes.

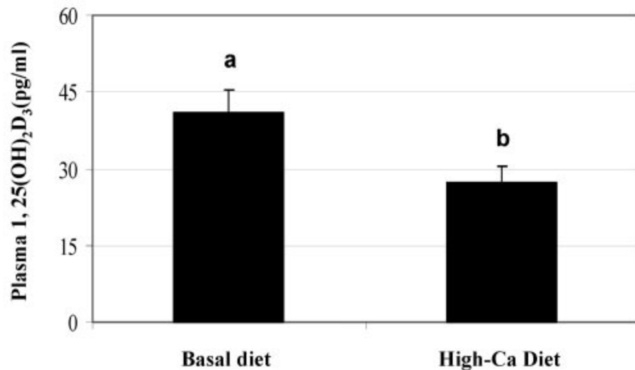

Figure 3: Plasma 1α,25-$(OH)_2$-D_3 in aP2-agouti transgenic mice fed low-calcium (basal) or high-calcium diets. Values are presented as mean ± SE, $n = 10$. Means with different letter differ, $p = 0.005$.

ROS Exerted Direct Impact on Cytokine Production in Cultured Adipocytes

The direct role of ROS in regulation of adipose cytokine production was investigated in differentiated 3T3-L1 adipocytes. Figure 6A shows that H_2O_2 increased IL-6 expression by 167% ($p < 0.001$) and that this effect was attenuated by the addition of antioxidant α ± tocopherol ($p = 0.016$), indicating that ROS exerted a direct role in stimulation of inflammatory cytokine production. α ± Tocopherol also increased adiponectin production ($p = 0.002$), although ROS (H_2O_2) was without significant effect ($p = 0.06$) (Figure 6B). Similarly, there was no direct effect of ROS on IL-15 expression; however, addition of α ± tocopherol markedly increased IL-15 by 2.2-fold ($p = 0.043$) (Figure 6C) compared with H_2O_2-treated cells, providing further evidence that oxidative stress is involved in adipocyte cytokine production.

Discussion

Previous data from our laboratory demonstrated that dietary calcium exerts an anti-obesity effect and suppresses obesity-associated oxidative stress by a 1α,25-$(OH)_2$-D_3-mediated mechanism (16,17). We have demonstrated that 1α,25-$(OH)_2$-D_3 plays a direct role in the modulation of adipocyte Ca^{2+} signaling, resulting in increased lipogenesis and decreased lipolysis (21). In addition, 1α,25-$(OH)_2$-D_3 is also involved in regulation of metabolic efficiency by modulating adipocyte uncoupling protein 2 expression (22). Accordingly, the suppression of 1α,25-$(OH)_2$-D_3 by increasing dietary calcium attenuates adipocyte triglyceride accumulation and causes a net reduction in fat mass in both mice and humans in the absence of caloric restriction (23,24), a marked augmentation of body weight and fat loss during energy restriction in both mice and humans (23,25–27), and a reduction in the rate of weight and fat regain after energy restriction in mice (20). Given that obesity and related disorders are associated with low-grade systemic inflammation (28), it is possible that dietary calcium may also play a role in modulating adipose tissue cytokine production. Data from the present study demonstrate that dietary calcium decreased production of pro-inflammatory factors such as TNFα and IL-6 and increased anti-inflammatory molecules such as IL-15 and adiponectin in visceral fat. We also found that 1α,25-$(OH)_2$-D_3 stimulated TNFα, IL-6, and IL-8 production in cultured human and murine adipocytes and that this effect was completely blocked by a calcium channel antagonist, suggesting that dietary calcium suppresses inflammation factor production in adipocyte and that 1α,25-$(OH)_2$-D_3-induced Ca^{2+} influx may be a key mediator of this effect.

Obesity is associated with increased expression of inflammatory markers (29), whereas weight loss results in decreased expression and secretion of pro-inflammatory components in obese individuals (30). Accordingly, modulation of the adipose tissue mass appears to result in corresponding modulation of cytokine production. TNFα and IL-6 are two intensively studied cytokines in obesity and have been consistently found to be increased in the white

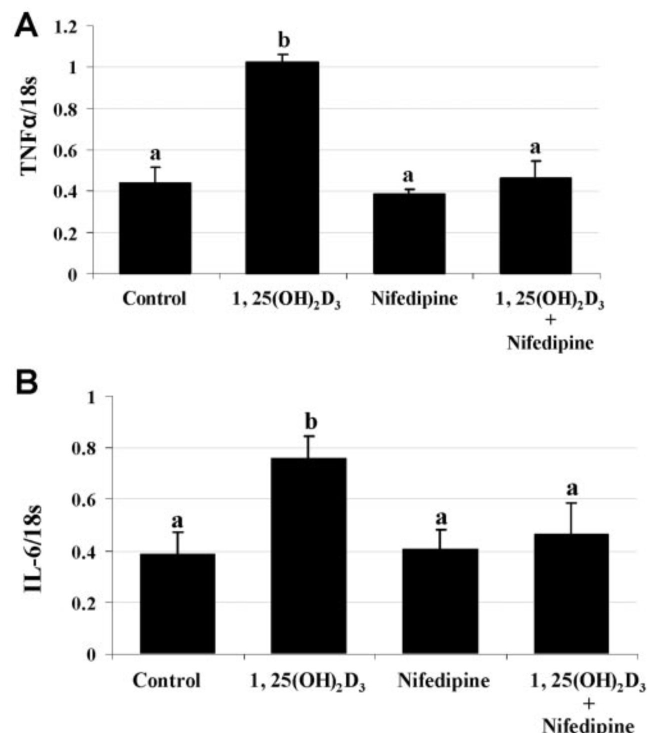

Figure 4: TNFα expression (A) and IL-6 expression (B) in differentiated 3T3-L1 adipocytes. Adipocytes were treated with 10 nM 1α,25-$(OH)_2$-D_3, 10 μM nifedipine, and 10 nM 1α,25-$(OH)_2$-D_3 plus 10 μM nifedipine, respectively, for 48 hours. Data are normalized to 18s expression. Values are presented as mean ± SE, $n = 6$. Means with different letter differ, $p < 0.02$.

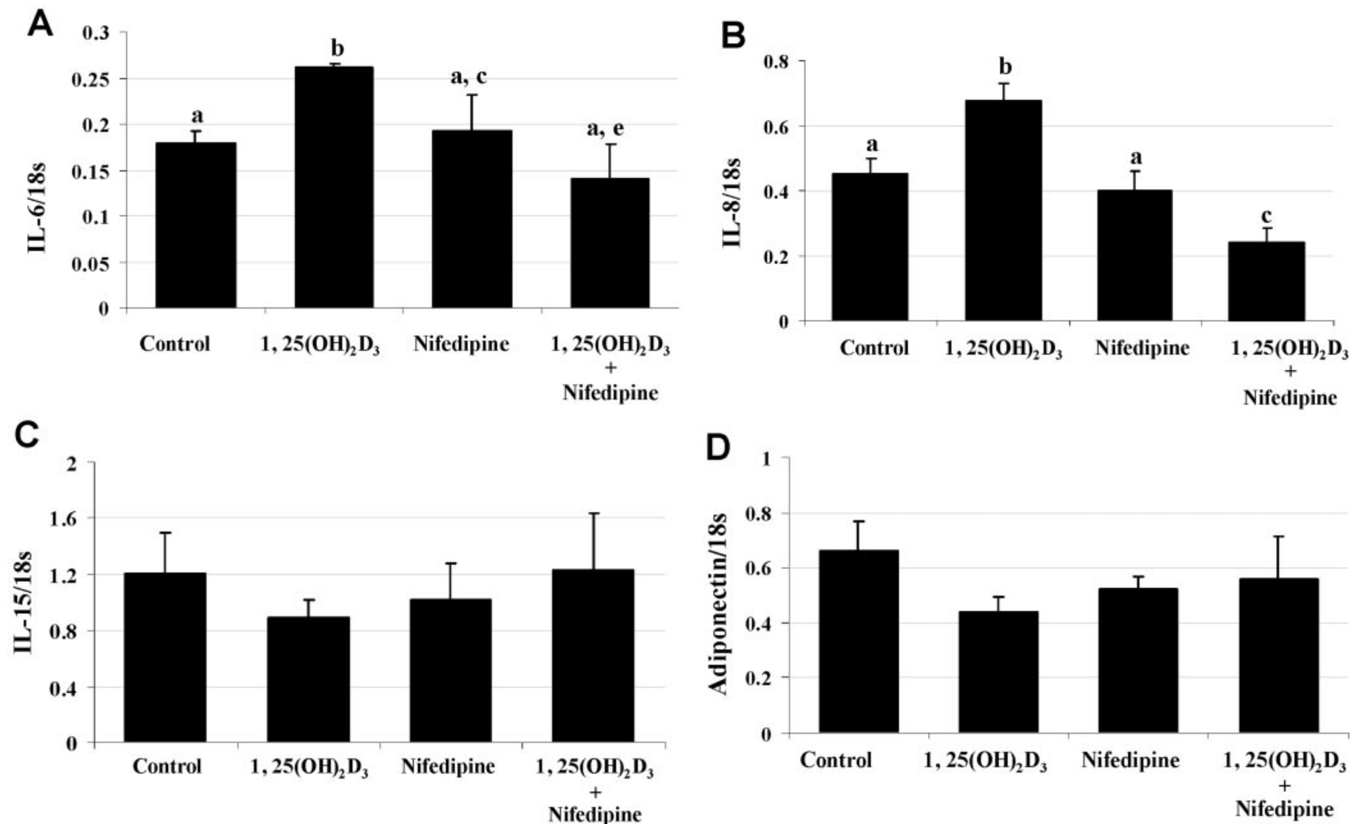

Figure 5: IL-6 expression (A), IL-8 expression (B), IL-15 expression (C), and adiponectin expression (D) in differentiated Zen-Bio human adipocytes. Adipocytes were treated with 10 nM 1α,25-$(OH)_2$-D_3, 10 μM nifedipine, and 10 nM 1α,25-$(OH)_2$-D_3 plus 10 μM nifedipine, respectively, for 48 hours. Data are normalized to 18s expression. Values are presented as mean ± SE, $n = 6$. Means with different letter differ, $p < 0.005$.

adipose tissue of obese subjects (31). Previous studies suggest that white adipose tissue contributes a considerable portion of circulating IL-6, with visceral fat contributing markedly more IL-6 than subcutaneous fat (32,33). Expression of TNFα is increased in inflammatory conditions such as obesity and cachexia and is considered a likely mediator of insulin resistance associated with visceral adiposity (34,35). Consistent with this, diet-induced obesity in the present study resulted in increased expression of TNFα and IL-6 in visceral fat, and dietary calcium attenuated these effects.

IL-15 is highly expressed in skeletal muscle, where it exerts anabolic effects (36). IL-15 administration reduces muscle protein degradation and inhibits skeletal muscle wasting in degenerative conditions such as cachexia (37). Interestingly, IL-15 exerts the opposite effect in adipose tissue; administration of IL-15 reduced fat deposition without altering food intake and suppressed fat gain in growing rats (38,39). IL-15 also stimulates adiponectin secretion in cultured 3T3-L1 adipocytes (12), indicating a role for IL-15 in regulating adipocyte metabolism. These observations suggest that IL-15 might be involved in a muscle-fat endocrine axis and may regulate energy use between the two tissues (40). We previously found calcium-rich diets to suppress fat gain and accelerate fat loss while protecting muscle mass in diet-induced obesity and during energy restriction, indicating that dietary calcium may similarly regulate energy partitioning in a tissue-selective manner. In the present study, we provide, to our knowledge, the first in vivo evidence that dietary calcium up-regulates IL-15 expression in visceral adipose tissue and skeletal muscle and stimulates adiponectin expression in visceral adipose tissue in aP2 agouti transgenic mice. This suggests that dietary calcium may also regulate energy metabolism, in part, by modulating these cytokines in both adipose tissue and skeletal muscle, thereby favoring elevated energy expenditure in adipose tissue and preserving energy storage in skeletal muscle. However, we found no effect of 1α,25-$(OH)_2$-D_3 on IL-15 expression in human adipocytes. Because these human adipocytes were originally developed from subcutaneous fat, these results further support our in vivo observations of dietary calcium regulation of adipocyte cytokine production in a depot-specific manner, although we do not have data from human visceral adipocytes for comparison.

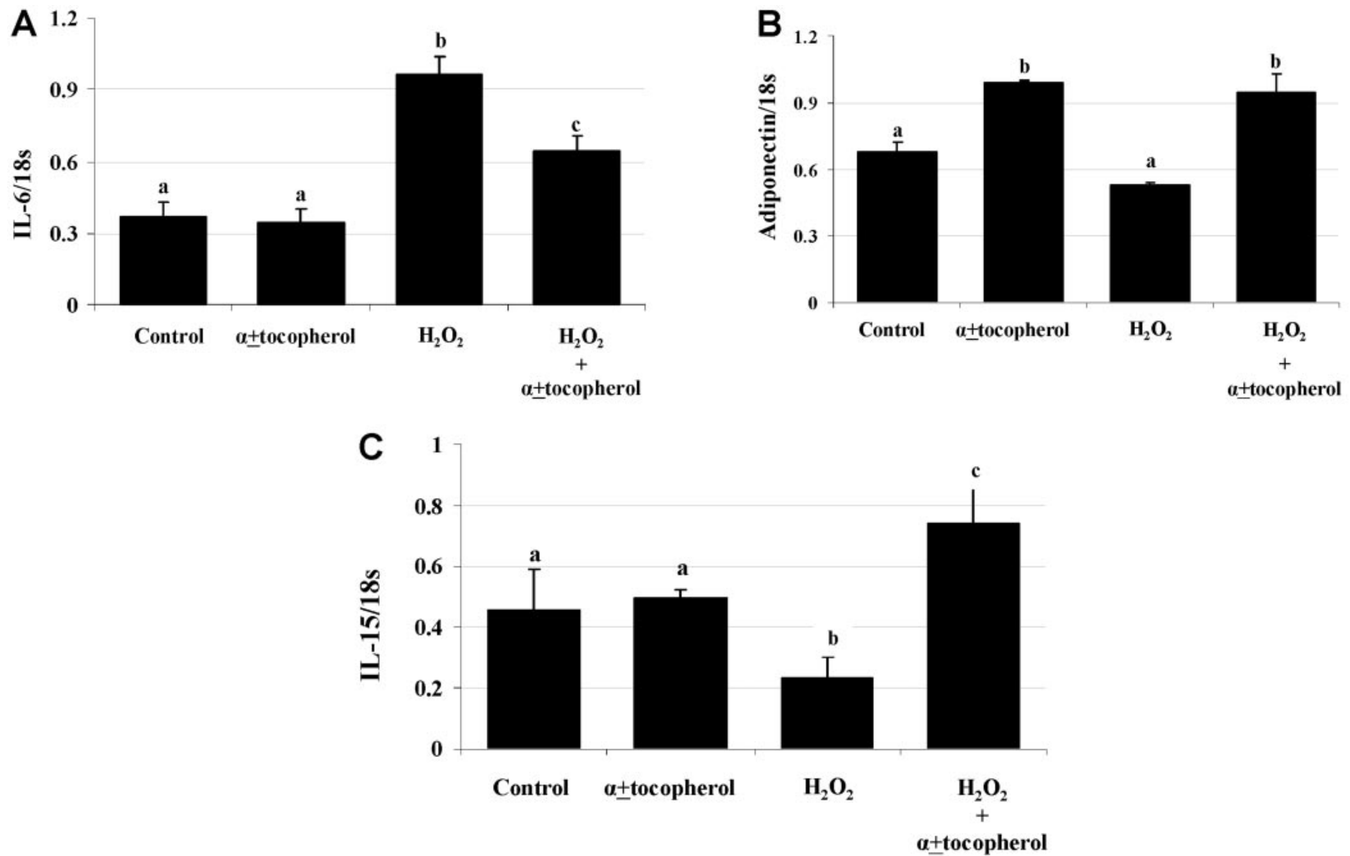

Figure 6: IL-6 expression (A), adiponectin expression (B), and IL-15 expression (C) in differentiated 3T3-L1 adipocytes. Adipocytes were treated with 100 nM H_2O_2, 1 μM $\alpha \pm$ tocopherol, and 100 nM H_2O_2, 1 μM $\alpha \pm$ tocopherol, respectively, for 48 hours. Data are normalized to 18s expression. Values are presented as mean $\pm$ SE, $n = 6$. Means with different letter differ, $p < 0.05$.

We have shown recently that 1α,25-$(OH)_2$-D_3 stimulates ROS production in cultured adipocytes and that suppression of 1α,25-$(OH)_2$-D_3 by dietary calcium also attenuates adipose oxidative stress (18), suggesting a potential connection between oxidative stress and production of inflammatory factors. The present data demonstrate that H_2O_2 stimulates adipocyte IL-6 expression, and $\alpha \pm$ tocopherol inhibits this effect. Although H_2O_2 showed no direct effect on the expression of anti-inflammatory factors adiponectin and IL-15, addition of $\alpha \pm$ tocopherol markedly elevated the expression of both, suggesting a direct role of oxidative stress in regulating inflammation. Indeed, previous studies have demonstrated that oxidative stress is augmented in adiposity, with ROS elevated in blood and tissue in various animal model of obesity (6,41), whereas markers of systemic oxidative stress are inversely related to plasma adiponectin in human subjects (6,11). Moreover, addition of oxidants has been shown to suppress expression of adiponectin and increase expression of IL-6, monocyte chemoattractant protein-1, and plasminogen activator inhibitor-1 (11). These results indicate that a local increase in oxidative stress in accumulated fat causes dysregulated production of adipocytokines. The role of adiposity in upregulation of oxidative stress and inflammation has been investigated intensively. Fat accumulation stimulates nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase expression in white adipose tissue (42,43). Furthermore, NADPH oxidase 4, an isoform of NADPH oxidase, is expressed in adipocytes, but not in macrophage (44,45). Xu et al. (46) and Weisberg et al. (47) also reported that ROS stimulated macrophage infiltration of obese adipose tissue by ROS-induced monocyte chemoattractant protein-1 production and stimulated local NADPH oxidase expression and ROS production, indicating that both adipocytes and macrophages contribute to elevated oxidative stress in obesity.

Notably, the anti-inflammatory effect of dietary calcium is greater in visceral than in subcutaneous fat. We have observed previously a similar pattern in adipocyte ROS production (18) in that ROS production and NADPH oxidase expression were markedly higher in visceral fat than in subcutaneous fat, suggesting that there may be an association between oxidative stress and inflammation in diet-induced obesity. Indeed, it was postulated that because

visceral fat is more sensitive to lipolytic stimuli than adipose tissue stored at other sites, turnover of triacylglycerols and release of fatty acids into the portal circulation are increased (48). Free fatty acids can also stimulate ROS production by stimulating NADPH oxidase expression and activation (11). Accordingly, obesity associated with oxidative stress and inflammation may occur in a depot-specific manner in adipose tissue, with significantly higher ROS and inflammatory cytokines produced in visceral fat than in subcutaneous fat (49). In summary, the present study demonstrates that dietary calcium suppresses obesity-associated inflammatory status by modulating pro-inflammatory and anti-inflammatory factor expression, providing evidence for the first time that increasing dietary calcium may contribute to suppression of obesity-associated inflammation.

Acknowledgments

This research was supported by unrestricted research grants from The Bell Institute for Human Nutrition and The National Dairy Council.

References

1. **Sharma AM, Chetty VT.** Obesity, hypertension and insulin resistance. *Acta Diabetol*. 2005;42(Suppl 1):S3–8.
2. **Abate N.** Obesity and cardiovascular disease: pathogenetic role of the metabolic syndrome and therapeutic implications. *J Diabetes Complications*. 2000;14:154–74.
3. **Lebovitz HE.** The relationship of obesity to the metabolic syndrome. *Int J Clin Pract Suppl*. 2003;134:18–27.
4. **Urakawa H, Katsuki A, Sumida Y, et al.** Oxidative stress is associated with adiposity and insulin resistance in men. *J Clin Endocrinol Metab*. 2003;88:4673–6.
5. **Couillard C, Ruel G, Archer WR, et al.** Circulating levels of oxidative stress markers and endothelial adhesion molecules in men with abdominal obesity. *J Clin Endocrinol Metab*. 2005;90:6454–9.
6. **Furukawa S, Fujita T, Shimabukuro M, et al.** Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest*. 2004;114:1752–61.
7. **Mertens I, Van Gaal LF.** Visceral fat as a determinant of fibrinolysis and hemostasis. *Semin Vasc Med*. 2005;5:48–55.
8. **Park KG, Park KS, Kim MJ, et al.** Relationship between serum adiponectin and leptin concentrations and body fat distribution. *Diabetes Res Clin Pract*. 2004;63:135–42.
9. **Piche ME, Lemieux S, Weisnagel SJ, Corneau L, Nadeau A, Bergeron J.** Relation of high-sensitivity C-reactive protein, interleukin-6, tumor necrosis factor-alpha, and fibrinogen to abdominal adipose tissue, blood pressure, and cholesterol and triglyceride levels in healthy postmenopausal women. *Am J Cardiol*. 2005;96:92–7.
10. **Aldhahi W, Hamdy O.** Adipokines, inflammation, and the endothelium in diabetes. *Curr Diab Rep*. 2003;3:293–8.
11. **Soares AF, Guichardant M, Cozzone D, et al.** Effects of oxidative stress on adiponectin secretion and lactate production in 3T3–L1 adipocytes. *Free Radic Biol Med*. 2005;38:882–9.
12. **Quinn LS, Strait-Bodey L, Anderson BG, Argiles JM, Havel PJ.** Interleukin-15 stimulates adiponectin secretion by 3T3–L1 adipocytes: evidence for a skeletal muscle-to-fat signaling pathway. *Cell Biol Int*. 2005;29:449–57.
13. **Lin Y, Berg AH, Iyengar P, et al.** The hyperglycemia-induced inflammatory response in adipocytes: the role of reactive oxygen species. *J Biol Chem*. 2005;280:4617–26.
14. **Ozcan U, Cao Q, Yilmaz E, et al.** Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science*. 2004;306:457–61.
15. **Kobayashi K.** Adipokines: therapeutic targets for metabolic syndrome. *Curr Drug Targets*. 2005;6:525–9.
16. **Zemel MB.** The role of dairy foods in weight management. *J Am Coll Nutr*. 2005;24(Suppl 6):537–46S.
17. **Zemel MB.** Role of calcium and dairy products in energy partitioning and weight management. *Am J Clin Nutr*. 2004;79:907–12S.
18. **Sun X, Zemel MB.** Dietary calcium regulates ROS production in aP2-agouti transgenic mice on high-fat/high-sucrose diets. *Int J Obesity (Lond)*. 2006;30:1341–6.
19. **Reeves PG.** Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr*. 1997;127:838–41S.
20. **Sun X, Zemel MB.** Calcium and dairy products inhibit weight and fat regain during ad libitum consumption following energy restriction in Ap2-agouti transgenic mice. *J Nutr*. 2004;134:3054–60.
21. **Shi H, Norman AW, Okamura WH, Sen A, Zemel MB.** 1alpha,25-Dihydroxyvitamin D3 modulates human adipocyte metabolism via nongenomic action. *FASEB J*. 2001;15:2751–53.
22. **Shi H, Norman AW, Okamura WH, Sen A, Zemel MB.** 1alpha,25-dihydroxyvitamin D3 inhibits uncoupling protein 2 expression in human adipocytes. *FASEB J*. 2002;16:1808–10.
23. **Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC.** Regulation of adiposity by dietary calcium. *FASEB J*. 2000;14:1132–38.
24. **Zemel MB, Richards J, Milstead A, Campbell P.** Effects of calcium and dairy on body composition and weight loss in African-American adults. *Obes Res*. 2005;13:1218–25.
25. **Thompson WG, Rostad Holdman N, Janzow DJ, Slezak JM, Morris KL, Zemel MB.** Effect of energy-reduced diets high in dairy products and fiber on weight loss in obese adults. *Obes Res*. 2005;13:1344–53.
26. **Zemel MB, Thompson W, Milstead A, Morris K, Campbell P.** Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. *Obes Res*. 2004;12:582–90.
27. **Zemel MB, Richards J, Mathis S, Milstead A, Gebhardt L, Silva E.** Dairy augmentation of total and central fat loss in obese subjects. *Int J Obes (Lond)*. 2005;29:391–7.
28. **Lee YH, Pratley RE.** The evolving role of inflammation in obesity and the metabolic syndrome. *Curr Diab Rep*. 2005;5:70–5.

29. **Valle M, Martos R, Gascon F, Canete R, Zafra MA, Morales R.** Low-grade systemic inflammation, hypoadiponectinemia and a high concentration of leptin are present in very young obese children, and correlate with metabolic syndrome. *Diabetes Metab*. 2005;31:55–62.
30. **Clement K, Viguerie N, Poitou C, et al.** Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. *FASEB J*. 2004;18:1657–69.
31. **Cottam DR, Mattar SG, Barinas-Mitchell E, et al.** The chronic inflammatory hypothesis for the morbidity associated with morbid obesity: implications and effects of weight loss. *Obes Surg*. 2004;14:589–600.
32. **Fried SK, Bunkin DA, Greenberg AS.** Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab*. 1998;83:847–50.
33. **Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW.** Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans, *Endocrinology*. 2004;145:2273–82.
34. **Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM.** Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proc Natl Acad Sci U S A*. 1994;91:4854–8.
35. **Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor R.** Effects of an engineered human anti-TNF-alpha antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes*. 1996;45:881–5.
36. **Busquets S, Figueras MT, Meijsing S, et al.** Interleukin-15 decreases proteolysis in skeletal muscle: a direct effect. *Int J Mol Med*. 2005;16:471–6.
37. **Carbo N, Lopez-Soriano J, Costelli P, et al.** Interleukin-15 antagonizes muscle protein waste in tumour-bearing rats. *Br J Cancer*. 2000;83:526–31.
38. **Alvarez B, Carbo N, Lopez-Soriano J, et al.** Effects of interleukin-15 (IL-15) on adipose tissue mass in rodent obesity models: evidence for direct IL-15 action on adipose tissue. *Biochim Biophys Acta*. 2002;1570:33–7.
39. **Carbó N, Lopez-Soriano J, Costelli P, et al.** Interleukin-15 mediates reciprocal regulation of adipose tissue and muscle mass: a potential role in body weight control. *Biochim Biophys Acta*. 2001;1526:17–24.
40. **Argiles JM, Lopez-Soriano J, Almendro V, Busquets S, Lopez-Soriano FJ.** Cross-talk between skeletal muscle and adipose tissue: a link with obesity. *Med Res Rev*. 2005;25:49–65.
41. **Suzuki K, Ito Y, Ochiai J, et al.** Relationship between obesity and serum markers of oxidative stress and inflammation in Japanese. *Asian Pac J Cancer Prev*. 2003;4:259–66.
42. **Sun XC, Zemel MB.** Reactive oxygen species stimulate cell proliferation and down-regulate UCP2 expression in 3T3–L1 adipocytes. *Obes Res*. 2004;11:A37 (abstr).
43. **Inoguchi T, Li P, Umeda F, et al.** High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes*. 2000;49:1939–45.
44. **Mahadev K, Motoshima H, Wu X, et al.** The NAD(P)H oxidase homolog Nox4 modulates insulin-stimulated generation of H2O2 and plays an integral role in insulin signal transduction. *Mol Cell Biol*. 2004;24:1844–54.
45. **Sorescu D, Weiss D, Lassegue B, et al.** Superoxide production and expression of nox family proteins in human atherosclerosis. *Circulation*. 2002;105:1429–35.
46. **Xu H, Barnes GT, Yang Q, et al.** Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest*. 2003;112:1821–30.
47. **Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr.** Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003;112:1796–808.
48. **Wajchenberg BL.** Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev*. 2000; 21:697–738.
49. **Li Y, Bujo H, Takahashi K, et al.** Visceral fat: higher responsiveness of fat mass and gene expression to calorie restriction than subcutaneous fat. *Exp Biol Med (Maywood)*. 2003;228:1118–23.