17 Vitamin D Modulation of Adipocyte Function

Michael B. Zemel and Xiaocun Sun

Abstract Calcitriol has recently been demonstrated to play an important role in modulating adipocyte function by regulating adipocyte lipid metabolism and energy homeostasis via both genomic and non-genomic actions. Physiological concentrations of calcitriol dose-dependently inhibit adipocyte apoptosis, although supra-physiological concentrations stimulate adipocyte apoptosis; the former is mediated by inhibition of mitochondrial uncoupling and the latter by mitochondrial calcium overload. Calcitriol also regulates adipose tissue fat depot location and expansion by promoting glucocorticoid production and release. Finally, calcitriol also modulates the cross talk between adipose tissue and both skeletal muscle and macrophages. Calcitriol modulation of adipocyte–macrophage cross talk results in a synergistic increase in expression and release of reactive oxygen species and inflammatory cytokines from both cell types, while calcitriol regulation of adipocyte–skeletal muscle cross talk results in inhibition of skeletal muscle fatty acid oxidation and preferential energy storage in adipocytes. Accordingly, conditions which chronically increase calcitriol levels, such as low-calcium diets, increase obesity risk, decrease metabolic flexibility, and increase oxidative and inflammatory stress.

Key Words: Adipocyte; adipokine; calcitriol; calcium; energy metabolism; obesity

1. INTRODUCTION

Although vitamin D and its metabolites are not generally considered to have a major role in the control of energy metabolism, evidence accumulated over the past 8 years indicates that calcitriol does play a key regulatory role in adipocyte lipid metabolism and thereby modulates energy homeostasis and obesity risk *(1)*. This concept emerged from the search for a mechanism to explain an apparent "anti-obesity" effect of dietary calcium first noted in epidemiological studies and subsequently confirmed in some clinical trials. Data from the 1987–1988 US Department of Agriculture's Nationwide Food Consumption Survey showed an inverse relationship between dietary calcium intake and body weight *(2, 3)*; notably, when stratified by ethnicity, non-Hispanic blacks had the lowest mean daily calcium intake and the highest obesity prevalence. Similarly, in the data from the first National Health and Nutrition Examination Survey (NHANES I) McCarron demonstrated a significant inverse association between calcium intake and

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body weight *(3)*, and data from NHANES III demonstrated a strong inverse association between calcium intake and relative risk of obesity *(4)*. Subsequent support emerged from retrospective and prospective epidemiological and observational studies, secondary analysis of past clinical trials originally conducted with other primary endpoints (e.g., skeletal, cardiovascular), and prospective clinical trials; these effects are supported by a clear mechanistic framework based upon emerging data demonstrating a key role for calcitriol in modulating energy metabolism *(5)*; this role is mediated through both genomic and non-genomic actions of calcitriol, as discussed in the remainder of this chapter.

2. Ca^{2+} SIGNALING

Calcitriol is well recognized to modulate Ca^{2+} signaling in numerous cell types *(6–10)*, including adipocytes, and earlier work on the *agouti* gene demonstrated that Ca^{2+} signaling plays a pivotal role in adipocyte lipid metabolism *(11, 12)*. *Agouti* is normally expressed in mouse melanocytes, where it is involved in modulation of hair pigmentation via melanocortin-1 receptor antagonism *(13)*; normal expression of *agouti* produces the characteristic wild-type pigmentation pattern of mouse hair, with a predominately black hair shaft with a subapical yellow segment. However, dominant mutations in the mouse *agouti* gene confer a pleiotropic syndrome characterized by obesity and insulin resistance, and expressing the wild-type *agouti* cDNA under control of either a ubiquitous promoter or an adipose tissue-specific promoter (aP2) recapitulates this syndrome *(14, 15)*. Notably, the human homolog of this gene is primarily expressed in white adipose tissue. Agouti protein exerts significant paracrine/autocrine effects by targeting ion channels, thereby causing an increase in adipocyte intracellular free calcium ($[Ca^{2+}]_i$) *(16, 17)*. Strong correlation between the degree of agouti expression and both $[Ca^{2+}]_i$ levels and body weight has been demonstrated in mice, indicating that agouti may modulate adiposity via a $[Ca^{2+}]_i$-dependent mechanism *(18)*. Indeed, an agouti/Ca^{2+} response sequence has been mapped to the fatty acid synthase (FAS) promoter region *(19)*, and increasing $[Ca^{2+}]_i$ in adipocytes via either receptor- or voltage-mediated Ca^{2+} channel activation stimulated FAS gene expression and consequently resulted in stimulation of FAS activity *(20)*; notably, there is a significant association between adipose tissue agouti expression and both FAS expression and body mass index in normal humans *(21)*. The role of Ca^{2+} signaling in obesity was confirmed by the observation that calcium channel inhibition resulted in significant decreases in adipose tissue mass and adipocyte lipogenesis in obese *agouti*-transgenic mice *(22)*. Agouti protein also plays a role in regulation of adipocyte lipolysis via a Ca^{2+}-dependent mechanism *(23, 24)*. Recombinant agouti protein inhibits both basal and agonist-stimulated lipolysis in human adipocytes. Increasing Ca^{2+} influx through either voltage- or receptor-operated Ca^{2+} channels also inhibits lipolysis, and this effect is blocked by Ca^{2+} channel antagonists, indicating that this anti-lipolytic effect is mediated by calcium signaling. The mechanism underlying the anti-lipolytic effect of Ca^{2+} has been demonstrated to be mediated by increased activation of phosphodiesterase 3B, resulting in reduced cAMP levels and consequently inhibition of hormone sensitive lipase activity. Although calcitriol and

Ca^{2+} signaling inhibit the early stages of adipogenesis, they serve to accelerate late-stage differentiation and lipid filling of existing adipocytes by stimulating expression of peroxisome proliferator-activated receptor γ (PPAR-γ) and key downstream genes, such as aP2, stearoyl-CoA desaturase (SCD-1), phosphoenolpyruvate carboxykinase (PEPCK), and FAS *(25)*. Thus, increased Ca^{2+} signaling favors a smaller number of hypertrophic adipocytes.

This regulation of lipid metabolism by $[Ca^{2+}]_i$ provides the framework for calcitriol modulation of adiposity and, consequently, the link between dietary calcium and obesity. We found that calcitriol induces rapid Ca^{2+} influx, while a specific membrane vitamin D receptor antagonist (1β,25-dihydroxyvitamin D_3) blocked this effect *(26)*. This indicated a non-genomic action of calcitriol via a putative membrane vitamin receptor, which later was identified as the membrane-associated rapid response to steroid ($1,25(OH)_2$D-MARRS) *(27)*, in modulating $[Ca^{2+}]_i$. Dietary calcium supplementation has been demonstrated to decrease the $[Ca^{2+}]_i$ concentration in various cell types including adipocytes *(4, 28–30)*, and this effect is largely mediated by suppression of calcitriol. We have demonstrated that the increased calcitriol produced in response to low-calcium diets stimulates adipocyte Ca^{2+} influx and, consequently, promotes adiposity by both inhibiting lipolysis and stimulating lipogenesis *(31, 32)*. Accordingly, suppressing calcitriol levels by increasing dietary calcium is an attractive target for obesity interventions. In support of this concept, transgenic mice expressing the agouti gene specifically in adipocytes (a human-like pattern) respond to low-calcium diets with accelerated weight gain and fat accretion, whereas high-calcium diets markedly inhibit lipogenesis, accelerate lipolysis, increase thermogenesis, and suppress fat accretion and weight gain in animals maintained at identical caloric intakes *(31)*. Further, low-calcium diets impede body fat loss whereas high-calcium diets markedly accelerate fat loss in transgenic mice subjected to caloric restriction *(32)*. These concepts are confirmed by both epidemiological and clinical data *(33, 34)*, which demonstrate that increasing dietary calcium results in significant reductions in adipose tissue mass in obese humans in the absence of caloric restriction and markedly accelerates the weight and body fat loss secondary to caloric restriction, whereas dairy products exert significantly greater effects.

3. ROLE OF THE NUCLEAR VITAMIN D RECEPTOR

In addition to regulating adipocyte metabolism via $[Ca^{2+}]_i$ through the non-genomic $1,25(OH)_2$D-MARRS, calcitriol also exerts a genomic act via the adipocyte nuclear vitamin D receptor (nVDR) to inhibit the expression of uncoupling protein 2 (UCP2) *(35)*. Polymorphisms in the nVDR are associated with the susceptibility to obesity in humans *(36, 37)*, and several lines of evidence demonstrate alterations in the vitamin D endocrine system in obese humans *(38, 39)*. Interestingly, the nVDR is expressed at very low level in preadipocytes, but is transiently stimulated during adipogenesis and then returns to low levels. However, we have recently shown nVDR expression in mature adipocytes to be subjected to regulation by multiple compounds, including calcitriol, resulting in a positive feedback pathway in response to both agonist (calcitriol) and glucocorticoid *(40)*, as discussed in a subsequent section of this chapter.

Calcitriol acts via the nVDR to inhibit UCP2 expression and to suppress UCP2 responses to both isoproterenol and fatty acids *(35)*. The role of the nVDR in this antagonism is demonstrated by its reversal by antisense oligonucleotide-mediated nVDR knockout in adipocytes and by the failure of $1,25(OH)_2$D-MARRS agonist and antagonist to either mimic or prevent the calcitriol inhibition of UCP2 expression. Uncoupling proteins (UCPs) are mitochondrial transporters present in the inner membrane of mitochondria and have been shown to stimulate mitochondrial proton leak and therefore exhibit a potential role in thermogenesis and energy metabolism *(41)*. Unlike the other isoforms of UCPs, UCP2 is ubiquitously expressed, with the highest level in white adipose tissue while UCP3 is dominantly expressed in skeletal muscles of human and rodents. Suppression of calcitriol by feeding high-calcium diets to energy-restricted mice results in increased adipose tissue UCP2 and skeletal muscle UCP3 expression and attenuates the decline in core temperature which otherwise occurs with energy restriction *(42)*. The high level of homology of UCP2 and UCP3 with UCP1 suggests a possible uncoupling activity which has been shown to stimulate mitochondria proton leak and therefore exhibit a potential role in thermogenesis and energy metabolism *(43–45)*; both are associated with fatty acid transport across the inner mitochondrial membrane and subsequent β-oxidation, and polymorphisms of anonymous markers encompassing the UCP2–UCP3 locus in humans are strongly linked to resting metabolic rate. Accordingly, calcitriol suppression of UCP2 expression may be anticipated to increase energetic efficiency and thereby increase obesity risk.

4. CALCITRIOL REGULATION OF ADIPOCYTE APOPTOSIS

Calcitriol regulation of both UCP2 and $[Ca^{2+}]_i$ appears to exert an additional role in energy metabolism by affecting adipocyte apoptosis *(46)*. Although calcitriol has previously been shown to exert a pro-apoptotic effect in several tissues *(47–49)*, these effects are generally observed with supra-physiological levels of the hormone ($\geq$100 nM), and our data in human adipocytes also demonstrate a pro-apoptotic role of such high concentrations. However, we have also shown that lower doses of calcitriol (0.1–10 nm) dose-dependently inhibit apoptotic gene expression such as caspase-1 and caspase-3 expression but stimulate anti-apoptotic gene expression such as BCL-2 and increase the BCL-2/Bax ratio in wild-type adipocytes. Furthermore, calcitriol dose-dependently induced an increase in mitochondrial potential ($\Delta\psi$) and ATP production, while overexpressing UCP2 in adipocytes exerted the opposite effect, indicating that suppression of UCP2 expression and consequent increases in mitochondrial potential and ATP production may contribute to the anti-apoptotic effect of calcitriol. Notably, high doses of calcitriol also induced a markedly increase in mitochondrial calcium ($[Ca^{2+}]_m$) load while lower, more physiological doses of calcitriol exerted the opposite effect, indicating that the increased $[Ca^{2+}]_m$ is associated with the induction of apoptosis by calcitriol. Mitochondria are often located close to endoplasmic reticulum (ER) and are thereby exposed to the Ca^{2+} released by the inositol-1,4,5-triphosphate receptor (IP3R) and ryanodine receptor (RyR). The high Ca^{2+} levels achieved at these contact sites favor Ca^{2+} uptake into mitochondria. Because of their tight coupling to ER Ca^{2+} stores, mitochondria are highly susceptible to abnormalities in Ca^{2+} signaling *(50)*. Recent evidence

suggests that the amount of Ca^{2+} going through mitochondria is crucial in triggering a Ca^{2+}-dependent apoptotic response, probably by the opening of a sensitized state of permeability transition pore (PTP) *(51)*. Thus, the anti-apoptotic effect of physiological concentrations of calcitriol appears to be mediated primarily by suppression of UCP2, while the pro-apoptotic effects observed with pharmacological concentrations are mediated by mitochondrial Ca^{2+} overload (Fig. 1). The effects of calcitriol on adipocyte apoptosis were further supported by our recent microarray study of human adipocytes *(52)*, as physiological concentrations of calcitriol suppressed the pro-apoptotic gene stanniocalcin 2 (STC2) but stimulated anti-apoptotic gene STC1. Further in vivo data provide additional supporting evidence for a role of calcitriol and of dietary calcium in adipocyte apoptosis *(46)*, with suppression of calcitriol using high-calcium diets resulted in significant, substantial increases in white adipose tissue apoptosis in diet-induced obesity.

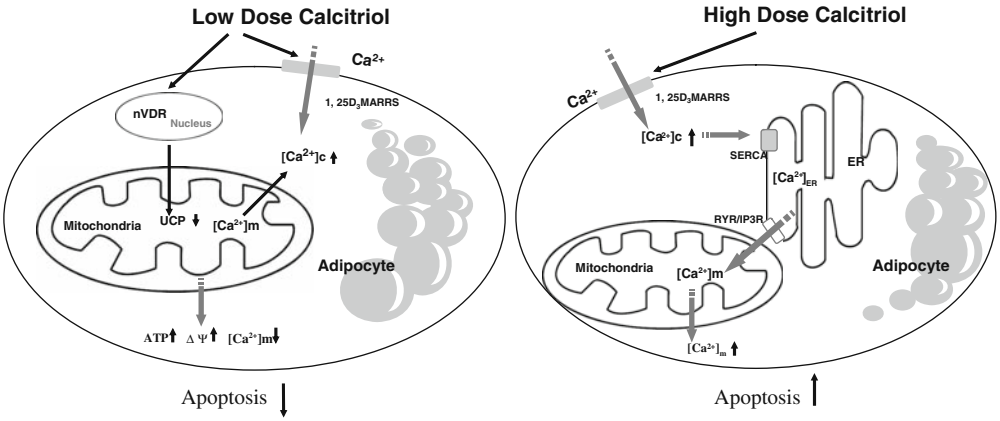

Fig. 1. Proposed mechanism for calcitriol modulation of adipocyte apoptosis.

5. CALCITRIOL MODULATION OF ADIPOCYTE GLUCOCORTICOID PRODUCTION

Calcitriol may also participate in the energy metabolism by regulating adipose tissue fat depot location and expansion. Excessive central fat deposition in obesity may result from the greater capacity for regeneration of active glucocorticoids in the visceral fat depot. Local adipose tissue glucocorticoid levels and intracellular glucocorticoid availability are controlled by the activity of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD 1) to generate active cortisol from inactive cortisone *(53)*. Overexpression of 11β-HSD 1 specifically in white adipose tissue of transgenic mice recapitulates features of the metabolic syndrome, including central obesity, hypertension, dyslipidemia, and insulin resistance *(54, 55)*, suggesting that tissue-specific dysregulation of glucocorticoid metabolism may promote expansion of adipose tissue stores. Previous studies from this laboratory demonstrate that the anti-obesity effect of dietary calcium is associated with preferential loss of central adipose tissue *(56)*, and we recently demonstrated that calcitriol directly up-regulates adipocyte 11β-HSD 1 expression and

cortisol release and, consequently, correspondingly affects local cortisol levels, indicating a potential role for calcitriol in visceral adiposity *(57, 58)*. This effect is attributable to the rapid non-genomic action of calcitriol mediated through the $1,25(OH)_2D$-MARRS because this response is prevented by $1,25(OH)_2D$, which antagonizes the rapid, membrane-associated signaling events resulting from exposure to calcitriol. These findings are further supported by our recent microarray study *(52)*, as well as by data demonstrating that dietary calcium-induced suppression of calcitriol attenuated adipose tissue 11β-HSD 1 expression in diet-induced obese mice *(59)*.

Recent data from this laboratory also demonstrated an interesting positive feedback regulation of glucocorticoids on calcitriol in adipocytes *(40)*; the cortisol precursor cortisone or the synthetic glucocorticoid dexamethasone each increased nVDR expression, while 11β-HSD 1 knockdown attenuated this effect. We thus propose that the increased cortisol, which results from liganded adipocyte nVDR stimulation of 11β-HSD 1, upregulates nVDR expression, resulting in further potential binding of calcitriol by the nVDR and consequent further stimulation of active glucocorticoid generation (Fig. 2). Notably, calcitriol also exerts additional effect on stimulation of glucocorticoid production by increasing cortisol release. This effect, however, appears to be independent of the nVDR and is instead mediated by intracellular calcium signaling because knockdown 11β-HSD 1 did not affect short-term corticosterone release while modulating calcium influx by KCl, BAYK8644, and $1,25(OH)_2D$-MARRS agonist lumisterol markedly increased corticosterone release.

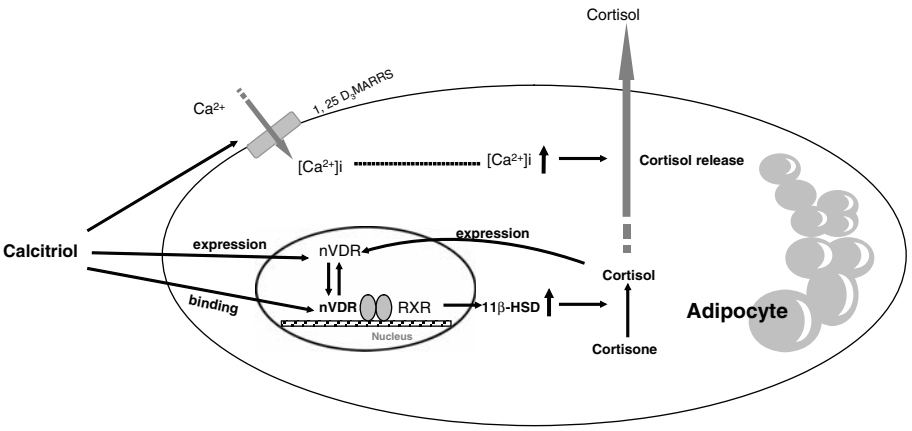

Fig. 2. Calcitriol regulation of adipocyte nVDR expression: interaction with glucocorticoids.

6. CALCITRIOL REGULATION OF CROSS TALK BETWEEN ADIPOCYTE AND SKELETAL MUSCLE IN ENERGY METABOLISM

Calcitriol may also modulate energy metabolism via regulation of the expression and production of multiple adipokines, as discussed later in this chapter. In addition to its role as a fuel reservoir, adipose tissue serves as an active endocrine organ which synthesizes and secretes a variety of biological molecules *(60–63)*. The more recent recognition that skeletal muscle may also assume a similar role in response to various

metabolic stimuli suggests a potential interaction between skeletal muscle and adipose tissue *(64, 65)*. Energy partitioning between adipose tissue and skeletal muscle has been previously demonstrated. Animals lacking myostatin exhibit markedly increased skeletal muscle mass *(66, 67)* and reduced body fat accumulation, and stimulation of beta-adrenergic receptor produces a dramatic increase in skeletal muscle mass and a corresponding reduction body fat content *(68, 69)*. Consistent with this concept, data from our laboratory demonstrated an independent role of leucine and calcium antagonism in regulating energy partitioning between adipocytes and muscle cells *(70)*, with both favoring fatty acid oxidation and UCP3 expression in C2C12 muscle cells while calcitriol suppressed fatty acid oxidation and attenuated the effects of leucine and nifedipine, indicating that this increase in muscle fat oxidation is coupled with decreased fat storage and increased fat catabolism in adipocytes. Indeed, we found that leucine inhibited FAS and PPAR gamma expression in differentiated 3T3-L1 adipocytes while calcitriol stimulated the expression of both genes and attenuated the effect of leucine on FAS expression. In addition, we have demonstrated that calcitriol decreases mitochondrial biogenesis and associated regulatory gene expression in both myocytes and adipocytes while leucine exerts the opposite effect *(71)*, indicating that calcitriol modulates both adipocyte and muscle metabolism by antagonizing mitochondrial biogenesis. Moreover, muscle cells treated with conditioned medium derived from adipocytes or co-cultured with adipocytes exhibited suppressed fatty acid oxidation *(70)* and decreased mitochondria biogenesis *(71)*, indicating that one or more factors derived from adipocytes regulate skeletal muscle energy metabolism. Indeed, leucine, nifedipine, and calcitriol also modulate adiponectin production, with leucine and nifedipine increasing adiponectin production while calcitriol exerts the opposite effect *(70)*. Consequently, we further evaluated the role of adiponectin in mediating the response to leucine, nifedipine, and calcitriol *(70)*. Our data to date suggest calcitriol modulation of adipocyte–skeletal muscle cross talk is mediated, in part, by alterations in adipocyte adiponectin and skeletal muscle interleukin-15 (IL-15) and IL-6 *(70)*; however, additional studies are required to clarify the role of specific adipokines in mediating this cross talk between adipose tissue and skeletal muscle (Fig. 3).

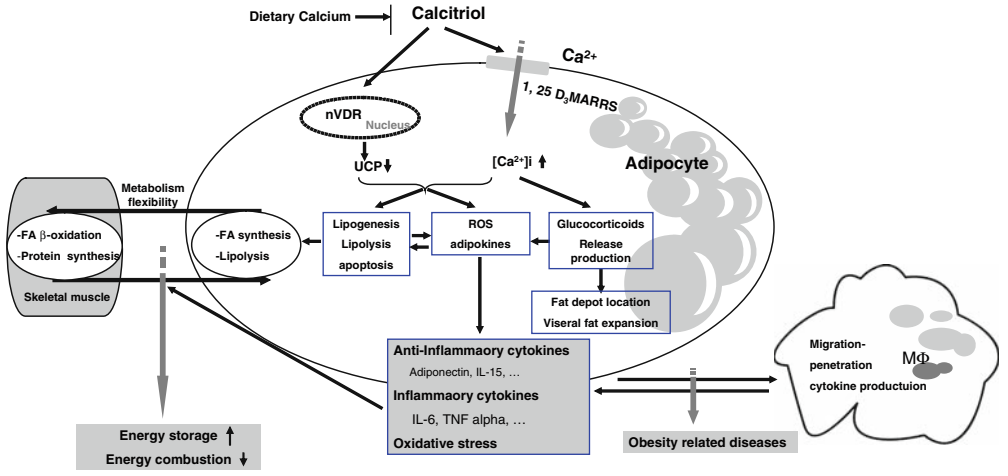

Fig. 3. Calcitriol regulation of adipocyte function and cross talk with bother organs.

7. CALCITRIOL REGULATION OF ADIPOCYTE OXIDATIVE STRESS

Adipose tissue excess is an important pathogenic mechanism underlying the obesity-related disorders, and this effect is attributable, in part, to the increased production of reactive oxygen species (ROS) and inflammatory cytokines by adipose tissue *(72–76)*. It has been postulated that hyperglycemia and hyperlipidemia, key clinical manifestation of obesity and diabetes, may promote ROS production through several pathways *(77)*. Indeed, the generation of ROS as byproducts of the mitochondrial electron transport chain has long been attributed to the high rates of glucose and lipid metabolism. Additional mechanism may be operated to produce ROS under high-glucose and high-lipid conditions, including the formation of advanced glycation end products *(78)*, altered polyol pathway activity *(79)*, activation of oxidases *(80, 81)*, and/or reduction of antioxidant enzymes *(82, 83)*. The interaction between ROS and calcium/uncoupling has been intensively investigated. There is a bidirectional interaction wherein ROS regulates cellular calcium signaling while manipulation of calcium signaling may also regulate cellular ROS production. Calcium signaling is essential for production of ROS *(84)*, and elevated $[Ca^{2+}]_i$ activates ROS-generating enzymes, such as NADPH oxidase and myeloperoxidase, as well as the formation of free radicals by the mitochondrial respiratory chain. Increased ROS production also stimulates $[Ca^{2+}]_i$ by activating calcium channels on both the plasma membrane and the endoplasmic reticulum *(85)*. Mitochondrial respiration is associated with production of ROS, and mitochondria produce a large fraction of the total ROS made in cells *(86)*. Mild uncoupling of respiration thus diminishes mitochondrial ROS formation by dissipating mitochondrial proton gradient and potential *(87)*.

We have recently shown that ROS production is modulated by mitochondrial uncoupling status and cytosolic calcium signaling *(88)* and that calcitriol regulates ROS production in cultured murine and human adipocytes via its modulation of both Ca^{2+} signaling and mitochondrial uncoupling. Consistent with this, our recent in vivo data showed that dietary calcium-induced suppression of calcitriol reduced adipose tissue ROS production *(89)*. Interestingly, animals on a low-calcium diet showed markedly higher visceral fat gain than subcutaneous fat versus mice on a high-calcium diet and exhibited strikingly enhanced ROS production and NADPH oxidase expression in visceral fat versus subcutaneous fat, indicating that higher visceral fat predisposes to enhanced ROS production. We also demonstrated that 11β-HSD 1 expression in visceral fat was markedly higher than subcutaneous fat in mice on basal low-calcium group whereas no difference was observed between the fat depots in mice on the high-calcium diet. We also found the high-calcium diet suppressed 11β-HSD 1 expression in visceral adipose tissue compared to mice on the low-calcium diet. These findings demonstrated that suppression of ROS production by dietary calcium may be mediated, at least in part, by the regulation of glucocorticoid-associated fat distribution.

8. CALCITRIOL REGULATION OF ADIPOCYTE INFLAMMATORY CYTOKINE PRODUCTION

Adipocytes produce a variety of biological molecules, including both inflammatory cytokines such as tumor necrosis factor alpha (TNFα), IL-6, and IL-8 and

anti-inflammatory factors such as adiponectin and IL-15 *(60–63)*. Dysregulated production of these adipocytokines contributes to the pathogenesis of obesity-associated metabolic syndrome. Given that obesity and related disorders are associated with low-grade systemic inflammation and oxidative stress, and that both Ca^{2+} signaling and ROS production modulate cytokine expression and release, we considered the possibility that calcitriol, and by extension dietary calcium, may also play a role in modulating adipose tissue cytokine production and systemic inflammatory stress. We found that calcitriol 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 *(90)*, suggesting that dietary calcium suppresses inflammatory factor production in adipocyte and that calcitriol-induced Ca^{2+} influx may be a key mediator of this effect. Moreover, 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, as well as systemic biomarkers of both oxidative and inflammatory stress *(90, 91)*. Our recent clinical evidence support these observations *(91)*, as high-calcium diets suppressed circulating C-reactive protein and increased adiponectin levels during both weight loss and weight maintenance in obese subjects. In a recent follow-up randomized crossover study in overweight and obese subjects, we found that suppressing calcitriol levels with high- versus low-calcium diets suppressed both oxidative and inflammatory stress within 7 days of initiation of supplementation and that these effects increased in magnitude with increased duration of supplementation *(92)*. Collectively, these findings indicate an important role of both calcitriol in inducing oxidative and inflammatory stress and, consequently, of dietary calcium in attenuating these risk factors.

9. CALCITRIOL REGULATION OF ADIPOCYTE–MACROPHAGE CROSS TALK IN INFLAMMATION

Adipose tissue also includes a stromal vascular fraction that contains blood cells, endothelial cells, and macrophages *(93–95)*. Although adipocytes directly generate inflammatory mediators, adipose tissue-derived cytokines also originate substantially from these non-fat cells *(96)*, among which the infiltrated macrophages play a prominent role. Infiltration and differentiation of adipose tissue-resident macrophages are under the local control of chemokines, many of which are produced by adipocytes. Accordingly, cross talk between adipocytes and macrophages may be a key factor in mediating inflammatory and oxidative changes in obesity. Calcitriol stimulates production of adipokines associated with macrophage function and increases inflammatory cytokine expression in both macrophages and adipocytes *(97)*; these include CD14, migration inhibitory factor (MIF), macrophage colony-stimulating factor (M-CSF), macrophage inflammatory protein (MIP), TNFα, IL-6, and monocyte chemotactic protein-1 (MCP-1) in adipocytes, and TNFα and IL-6 in macrophages. Moreover, a cytokine protein array identified multiple additional inflammatory cytokines that were up-regulated by calcitriol in both macrophages and adipocytes *(97)*. Further, calcitriol also regulated cross talk between macrophages and adipocytes, as shown by augmentation of expression and production of inflammatory cytokines from adipocytes and macrophages in co-culture versus individual culture. These effects were attenuated by either calcium channel antagonism

or increasing mitochondrial uncoupling, indicating that the pro-inflammatory effect of calcitriol is mediated by calcitriol-induced stimulation of Ca^{2+} signaling and attenuation of mitochondrial uncoupling.

10. CONCLUSION

Accumulating evidence suggests a key role for calcitriol in regulating adipocyte function. Calcitriol favors lipogenesis and inhibits lipolysis via non-genomic modulation of Ca^{2+} influx. In addition, calcitriol suppresses UCP2 expression via the nVDR and thereby increases energy efficiency and directly stimulates nVDR expression in mature adipocyte to induce a positive feedback loop between calcitriol and its receptor. Calcitriol also exerts a dose-dependent impact on adipocyte apoptosis and regulates adipose tissue fat depot location and expansion by promoting glucocorticoid production and release. Recent data also demonstrate a pivotal role of calcitriol in modulation of adipokine production resulting in significant roles in cross talk between adipocytes and macrophages in oxidative and inflammatory stress and between adipocytes and skeletal muscle in metabolic flexibility (Fig. 3).

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