Calcitriol and energy metabolism

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Calcitriol, a calcitrophic hormone that can be suppressed by high dietary calcium, favors fatty acid synthesis and inhibits lipolysis via non-genomic modulation of Ca\(^{2+}\) influx. Calcitriol also suppresses UCP2 expression via the nVDR and thereby increases energy efficiency. Calcitriol 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 the modulation of cytokines, with potential roles in energy metabolism in adipocytes, macrophages, and skeletal muscle.

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INTRODUCTION

Although the multifunctional vitamin D hormone is not commonly thought to have a major effect in energy metabolism, emerging evidence indicates that calcitriol plays a central role in adipocyte lipid metabolism and thereby modulates energy homeostasis and obesity risk.\(^1\) Obesity results from a chronic imbalance between energy intake and expenditure, and risk may be modulated through changes in energy intake, efficiency of energy utilization, or changes in any of the components of energy expenditure (resting metabolic rate, diet-induced thermogenesis, or exercise-induced thermogenesis).\(^2,3\) A large number of food components have been proposed to exert potential effects on energy metabolism and thereby modify obesity risk; these include conjugated linoleic acid, medium chain triglycerides, diacylglycerols, green tea extract, caffeine, and capsaicin.\(^4\) However, the reported effect for each of these is modest and inconsistent.\(^4\) In contrast, a substantial body of data now indicates that dietary calcium modulates adipocyte metabolism, energy efficiency, and energy partitioning between adipose tissue and skeletal muscle, resulting in an anti-obesity effect.\(^5-10\) In addition to 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 reported effects are supported by a clear mechanistic framework based upon emerging data demonstrating a key role for calcitriol [1, 25(OH)\(_2\)D\(_3\)] in modulating energy metabolism.\(^11\) This role is mediated through both genomic and non-genomic actions of calcitriol, as discussed below.

MECHANISMS

Ca\(^{2+}\) Signaling

Calcitriol is well recognized to modulate Ca\(^{2+}\) signaling in numerous cell types,\(^12-16\) including adipocytes,\(^17\) and our earlier work demonstrates that Ca\(^{2+}\) signaling plays a pivotal role in adipocyte lipid metabolism.\(^17,18\) We originally identified this role in our studies of the murine agouti protein.\(^19\) Agouti is normally involved in modulation of hair pigmentation via melanocortin-1 receptor antagonism;\(^19\) 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 the control of a ubiquitous promoter recapitulates this syndrome.\(^19,20\) The human homolog of the agouti gene is 85% identical to the mouse gene and primarily expressed in white adipose tissue.
Recombinant agouti protein stimulates a rapid increase in intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) influx in adipocytes and promotes lipogenesis by increasing fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD) expression and inhibits lipolysis in both murine and human adipocytes.\(^{21-25}\) Moreover, these effects are replicated by direct stimulation of Ca\(^{2+}\) influx and inhibited by Ca\(^{2+}\) entry blockers.\(^{25}\) In addition, the strong correlation between the degree of agouti expression and both [Ca\(^{2+}\)]\(_i\) levels and body fat in mice suggested that agouti may modulate adiposity via a [Ca\(^{2+}\)]\(_i\)-mediated mechanism.

This regulation of lipid metabolism by [Ca\(^{2+}\)]\(_i\) via calcitriol initially provided the framework for dietary calcium modulation of adiposity and energy metabolism. An agouti/Ca\(^{2+}\) response sequence has been mapped to the FAS promoter region,\(^{26}\) 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.\(^{15,21}\) In addition, elevated [Ca\(^{2+}\)]\(_i\) also inhibits lipolysis, leading to an expansion of adipocyte triglyceride storage.\(^{18,27}\) Dietary calcium supplementation has been demonstrated to decrease the [Ca\(^{2+}\)]\(_i\) concentration in various cell types including adipocytes.\(^{28-30}\) This effect is largely mediated by reducing calcitriol. We have found calcitriol to stimulate rapid increases in human adipocyte [Ca\(^{2+}\)]\(_i\). Accordingly, the decreases in [Ca\(^{2+}\)]\(_i\) observed with increasing calcium intake appear attributable to a reduced [Ca\(^{2+}\)]\(_i\) influx resulting from the suppression of circulating calcitriol.

Data from our laboratory have demonstrated that calcitriol induces rapid [Ca\(^{2+}\)]\(_i\) influx, while a specific membrane vitamin D receptor antagonist (1\(\beta\), 25-dihydroxyvitamin D\(_3\)) blocked this effect.\(^{15}\) 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 (1,25D\(_3\)-MARRS),\(^{31}\) in modulating [Ca\(^{2+}\)]\(_i\).

A potential role of calcitriol in regulating energy metabolism and contributing to obesity risk is also suggested by other data. Polymorphisms in the nuclear vitamin D receptor (nVDR) are associated with susceptibility to obesity in humans,\(^{32,33}\) and several lines of evidence demonstrate alterations in the vitamin D–endocrine system in obese humans, with an increase in the circulating calcitriol level.\(^{34,35}\) These observations, coupled with the direct effects of calcitriol on adipocyte metabolism, strongly indicated an increase in calcitriol found on low-calcium diets as a contributory factor to excess adiposity.

**Role of the nVDR**

In addition to regulating adipocyte metabolism via [Ca\(^{2+}\)]\(_i\) through the non-genomic 1,25D\(_3\)-MARRS, calcitriol also acts via the adipocyte nVDR to inhibit the expression of uncoupling protein2 (UCP2).\(^{36}\) Uncoupling proteins (UCPs) are mitochondrial transporters present in the inner membrane of mitochondria; they have been shown to stimulate mitochondrial proton leak and therefore exhibit a potential role in thermogenesis and energy metabolism.\(^{37}\) UCP2 is expressed ubiquitously, with the highest level in white adipose tissue, while UCP3 is mainly expressed in skeletal muscle.\(^{38}\) Although the role of UCP2 in thermogenesis is unclear, the possibility that UCPs participate in basal thermogenesis is supported by the demonstration that polymorphism of anonymous markers encompassing the UCP2-UCP3 locus in humans is strongly genetically linked to the resting metabolic rate. Furthermore, UCP2 and UCP3 are associated with fatty acid transport across mitochondrial inner membrane and \(\beta\)-oxidation, indicating an additional potential role in energy metabolism. Calcitriol suppression of UCP2 expression was demonstrated by our early work in human adipocytes.\(^{36}\) This effect appears to be mediated by the adipocyte nVDR, as 1\(\beta\), 25-dihydroxyvitamin D\(_3\) was unable to block this effect, while nVDR knockout successfully inhibited it. Suppression of calcitriol by feeding high-calcium diets to mice results in increased adipose tissue UCP2 and skeletal muscle UCP3 expression,\(^{8}\) and attenuates the decline in core temperature that otherwise occurs with energy restriction.\(^{39}\) Although the observed thermogenic effect induced by a high-calcium diet may be mediated by other unidentified mechanisms, the direct modulation of UCP2 by calcitriol may, nonetheless, contribute to fat oxidation and lipid storage.

**Adipocyte apoptosis**

Calcitriol regulation of both UCP2 and [Ca\(^{2+}\)]\(_i\) appears to exert an additional role in energy metabolism by affecting adipocyte apoptosis.\(^{39}\) Calcitriol is generally thought to exert a pro-apoptotic effect in several tissues.\(^{40-42}\) However, these effects are observed with supra-physiological levels of the hormone (\(\geq 100\) nM),\(^{40-42}\) and our data in human adipocytes also demonstrate a pro-apoptotic role of such high concentrations.\(^{39}\) However, we have also shown that lower doses of calcitriol (0.1 nm–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. In contrast, overexpressing UCP2 in adipocyte significantly attenuated this 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. Indeed, our data demonstrate a calcitriol dose-dependent increase in mitochondrial potential (\(\Delta\psi\)) and ATP production, while...
overexpressing UCP2 in adipocytes exerted the opposite effect. In vivo data provide further supporting evidence for a role of calcitriol and of dietary calcium in adipocyte apoptosis, as suppression of calcitriol with high-calcium diets resulted in significant, substantial increases in white adipose tissue apoptosis in mice with diet-induced obesity. As noted above, very high doses of calcitriol (≥100 nM) exert the opposite effect. We found 100 nM calcitriol to stimulate caspase-1 and caspase-3 expression and inhibit Bcl-2/Bax ratio, a complete reversal of the effect of lower doses of calcitriol on apoptosis. Notably, high-dose calcitriol also induced a marked 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 the ryanodine receptor (RyR). The high Ca\(^{2+}\) levels achieved at these contact sites favors 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. Recent evidence suggests that the amount of Ca\(^{2+}\) going through mitochondria is crucial in triggering a Ca\(^{2+}\)-dependent apoptosis response, probably by the opening of a sensitized state of permeability transition pore (PTP). Accordingly, 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 (Figure 1). The effects of calcitriol on adipocyte apoptosis were further supported by our recent microarray study of human adipocytes, as physiological concentrations of calcitriol suppressed the pro-apoptotic gene stanniocalcin 2 (STC2) and stimulated anti-apoptotic gene STC1.

**Modulation of glucocorticoid**

Calcitriol may also participate in energy metabolism by regulating adipose tissue fat depot location and expansion. Previous studies from this laboratory demonstrate that the anti-obesity effect of dietary calcium is associated with preferential loss of central adipose tissue. 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. We recently demonstrated that calcitriol also directly upregulates adipocyte 11β-HSD 1 expression and cortisol release and, consequently, correspondingly affects local cortisol levels, indicating a potential role for calcitriol in visceral adiposity. This effect is attributable to the rapid non-genomic action of calcitriol mediated through the 1,25D3-MARRS because this response is prevented by 1ß-25(OH)\(_2\)-D, which antagonizes the rapid, membrane-associated, signaling events resulting from exposure to calcitriol. These findings are further supported by our recent microarray study, as well as by data demonstrating that dietary calcium-induced suppression of calcitriol resulted in suppression of adipose tissue 11ß-HSD 1 expression in diet-induced obese mice.

Figure 1 Schematic illustration of effects and mechanisms of calcitriol on adipocyte apoptosis. Physiological doses of calcitriol increase mitochondrial potential and ATP production by suppressing UCP2, thereby inhibiting apoptosis. In contrast, pharmacological doses of calcitriol induce mitochondrial calcium overload and stimulate apoptosis.
Calcitriol regulation of adipocyte reactive oxygen species and cytokines: role in energy metabolism

Calcitriol may also modulate energy metabolism via regulation of the expression and production of multiple cytokines. Reactive oxygen species (ROS) production is increased in obesity and associated disorders such as hyperglycemia and hyperlipidemia. We have recently shown that ROS production is modulated by mitochondrial uncoupling status and cytosolic calcium signaling, and that calcitriol regulates ROS production in cultured murine and human adipocytes. Consistent with this in vitro data, we have demonstrated that suppression of calcitriol with dietary calcium attenuated obesity-induced oxidative stress in vivo, with greater effects found in visceral versus subcutaneous adipose tissue. In addition, animals on the basal low-calcium diet showed markedly higher visceral fat gain than subcutaneous fat versus mice on the high-calcium diet and exhibited enhanced ROS production and NADPH oxidase expression in visceral fat versus subcutaneous fat. Conversely, high dietary calcium attenuated visceral fat gain, and mice on the high-calcium diet exhibited similar ROS production in visceral and subcutaneous fat. These results indicate that higher visceral fat predisposes to enhanced ROS production and suggest reciprocal regulation of two pathways by calcitriol, as follows. First, calcitriol favors central adiposity development by modulating glucocorticoid production, as described above, and visceral fat is well known to contribute to elevated oxidative stress in obesity and related disorders. Second, calcitriol stimulates adipocyte ROS production via both genomic and non-genomic actions. Consequently, the greater density of the components of these systems in the visceral depot promote oxidative stress by stimulating factors biased toward adipose tissue deposition while increasing the capacity for generating ROS.

Although ROS may adversely affect cell survival due to membrane damage and irreversible DNA modification, ROS also plays a key signaling role in cell proliferation and growth. Early experiments demonstrated low concentration of superoxide or hydrogen peroxide to be effective in stimulating the in vitro proliferation and growth response in a variety of cultured mammalian cell types, and we demonstrated that a low concentration of H$_2$O$_2$ stimulates cell proliferation in cultured adipocytes. This effect can be augmented by a mitochondrial uncoupling inhibitor and suppressed by a calcium channel antagonist, which are key factors in modulating ROS production. Consistent with this, calcitriol regulation of Ca$^{2+}$ signaling and UCP2 stimulated ROS production and cell proliferation in adipocytes. This concept is supported by evidence that both H$_2$O$_2$ and superoxide anion induce mitogenesis and cell proliferation in several mammalian cell types. Furthermore, reduction of oxidants via supplementation with antioxidants inhibits cell proliferation in vitro. Although the mechanisms for the involvement of oxidative stress in the induction of cell proliferation are not known, it has been demonstrated that ROS and other free radicals influence the expression of a number of genes and transduction pathways involved in cell growth and proliferation. Consistent with this, our recent microarray study of human adipocytes provides further supporting evidence for a potential role of cal-

![Figure 2](image_url)  
**Figure 2** Schematic illustration of the proposed mechanism of the role of calcitriol in coordinately regulating energy metabolism in adipose tissue and skeletal muscle.
Calcitriol in adipogenesis, as calcitriol stimulated gene expression associated with cell proliferation, angiogenesis, cell cycle, neurogenesis, DNA replication, and cell differentiation such as vascular endothelial growth factor (VEGF), insulin-like growth factor 1 receptor, insulin-like growth factor 1, and c-fos-induced growth factor. Although cell proliferation is an energetically costly process, it may also signal the need for expanding energy storage in adipose tissue in response to the positive net energy balance. Calcitriol is also involved in the modulation of cytokine production in multiple cell types, including adipocytes, macrophages, and myocytes. We demonstrated that calcitriol stimulated the expression of macrophage inhibitory factor (MIF) and macrophage surface-specific protein CD14, two key factors in regulating macrophage function and survival, as well as IL-6 and IL-8 in differentiated human adipocytes. In addition, calcitriol upregulated macrophage colony-stimulating factor (M-CSF), macrophage inflammatory protein (MIP), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1) expression in mouse adipocytes; it also stimulated tumor necrosis factor alpha (TNF-α) and IL-6 expression in mouse macrophages. Moreover, coculture of adipocytes with macrophages significantly increased the expression and production of multiple inflammatory cytokines in response to calcitriol in both cell types. Consistent with this, in vivo suppression of calcitriol using high-calcium diets inhibited the expression of pro-inflammatory factors (TNF-α and IL-6) but stimulated the expression of the anti-inflammatory factors (IL-15 and adiponectin) in adipose tissue. Although such alterations in the balance of pro-inflammatory and anti-inflammatory cytokines may contribute to the pathogenesis of obesity-associated metabolic syndrome, these molecules can also play a key role in regulating energy partitioning in the tissue-tissue cross-talk. For example, TNF-α, which is expressed and produced by adipocytes and increases in obesity, has been show to induce muscle wasting via multiple mechanisms. On the other hand, leptin and adiponectin, which are also synthesized and secreted by adipose tissue, alter lipid partitioning in skeletal muscle by increasing fat oxidation and decreasing fatty acid incorporation into triacylglycerols. Moreover, adipocytes and muscle both produce IL-6 and in vitro studies suggest that IL-6 may stimulate adipocyte fatty acid release and muscle fatty acid oxidation, although its in vivo effect remains unclear. Notably, IL-15, a cytokine highly expressed in skeletal muscle, decreases fat deposition in adipose tissue but increases skeletal muscle fiber growth. Thus, a reciprocal regulation between adipose tissue and skeletal muscle may exist and may control adiposity by regulating the synthesis of fat and protein in adipose tissue and skeletal muscle, respectively.

We have recently employed an adipocyte-skeletal myocyte coculture system to investigate the effects of calcitriol on energy partitioning between adipose tissue and skeletal muscle. We found calcitriol to coordinately decrease muscle cell fatty acid oxidation and increase adipocyte FAS gene expression, and these effects were partially reversed by calcium channel antagonism with nifedipine. Calcitriol also suppressed adiponectin production, while this effect was reversed with nifedipine. Notably, recent studies from our laboratory also suggested that calcitriol regulates both adipocyte and macrophage production of inflammatory factors via calcium-dependent and mitochondrial uncoupling-dependent mechanisms; moreover, these effects are

![Figure 3](image_url) **Figure 3** Schematic summary of the role of calcitriol in the regulation of energy metabolism in adipocytes and the potential effect of dietary calcium in modulating calcitriol.
amplified with coculture of both cell types, indicating that calcitriol may contribute to local and circulating cytokine production. Although the role of these cytokines in the modulation of energy metabolism is not yet fully clear, they appear to be key mediators in communication between different tissues in response to various types of energy and stress status, and calcitriol appears to play an important role in this process (Figure 2).

CONCLUSION

As summarized in Figure 3, emerging data from the past decade provide a clear mechanistic framework for calcitriol modulation of energy metabolism. Initial studies of the mechanism and actions of agouti in mouse yellow obesity syndrome revealed an important role of intracellular cytokine signaling in the regulation of lipid metabolism. Calcitriol, a calcitrophic hormone that can be suppressed by high dietary calcium, induces a rapid calcium influx by binding to its membrane receptor 1,25D,MARRS and consequently favors fatty acid synthesis and inhibits lipolysis. Calcitriol also suppresses UCP2 expression via its nVDR and thereby increases energy efficiency. Interestingly, calcitriol appears to exert a dose-dependent impact on adipocyte apoptosis, with physiological low-dose inhibiting apoptosis and pharmacological doses stimulating apoptosis. In addition, calcitriol regulates adipose tissue fat depot location and expansion by stimulating 11β-HSD expression in human adipocytes and promoting glucocorticoid production and release. More recent data suggest a pivotal role of calcitriol in modulating ROS and cytokine production in adipocytes, macrophages, and skeletal muscle; this effect is mediated via both the genomic and non-genomic effects of calcitriol. Further, calcitriol modulates production of cytokines which mediate the cross-talk between adipocytes and macrophages and consequently contribute to the local and circulating inflammation. Calcitriol also participate in the modulation of adipocyte-skeletal muscle cross-talk in energy homeostasis by coordinately promoting adipocyte lipid storage and inhibiting skeletal muscle fatty acid oxidation. This effect appears attributable, in part, to calcitriol modulation of adiponectin, IL-16, IL-15, and possibly other cytokines as well. Accordingly, strategies for reducing circulating calcitriol levels, such as increasing dietary calcium, may promote a favorable shift in energy metabolism, resulting in reduced lipid storage in adipose tissue and increased skeletal muscle lipid utilization.

REFERENCES


