1α,25-dihydroxyvitamin D_3 inhibits uncoupling protein 2 expression in human adipocytes[1]

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SPECIFIC AIMS

This study was conducted to determine the role of1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2$-D_3) in modulating human adipocyte uncoupling protein 2 (UCP2) expression via genomic action mediated by nuclear vitamin D receptor.

PRINCIPAL FINDINGS

1. 1α,25-$(OH)_2$-D_3 inhibits human adipocyte basal, isoproterenol, and fatty acid-stimulated UCP2 expression

Figure 1, top panel, shows that 48 h treatment of human adipocytes with 1 nM 1α,25-$(OH)_2$-D_3 caused a 40% decrease in UCP2 mRNA ($P<0.003$) whereas direct stimulation of Ca^{2+} influx with 10 mM KCl, a cell membrane depolarization agent, exerted no effect. Similar results were observed on UCP2 protein levels measured by Western blot ($P<0.002$, Fig. 1, bottom panel). 10 nM isoproterenol caused a ~twofold increase in adipocyte UCP2 mRNA ($P<0.002$), which was completely blocked by 1α,25-$(OH)_2$-D_3 but by only 20% by KCl. Although KCl inhibited isoproterenol-stimulated increases in UCP2 protein ($P<0.006$), 1α,25-$(OH)_2$-D_3 exerted a more potent effect, reducing UCP2 protein below basal levels. Free fatty acids (oleic acid, linoleic acid, and stearic acid mixture) caused a ~twofold increase in UCP2 mRNA that was completely prevented by 1α,25-$(OH)_2$-D_3, but not by KCl. These data indicate that 1α,25-$(OH)_2$-D_3 inhibition of UCP2 expression is largely independent of its effects on Ca^{2+} influx or fatty acid flux.

2. Membrane vitamin D receptor does not mediate the inhibitory effect of 1α,25-$(OH)_2$-D_3 on human adipocyte UCP2 expression

To study whether membrane vitamin D receptor (mVDR) mediates this inhibition of 1α,25-$(OH)_2$-D_3 on adipocyte UCP2 expression, 1α,25-dihydroxylumisterol$_3$ (1α,25-$(OH)_2$-lumisterol$_3$), a specific mVDR agonist, and 1β,25-dihydroxyvitamin D (1β,25-$(OH)_2$-D_3), a specific mVDR antagonist, were used to treat human adipocytes. 1α,25-$(OH)_2$-lumisterol$_3$ failed to exert an inhibitory effect on UCP2 mRNA, whereas 1β,25-$(OH)_2$-D_3 was unable to reverse 1α,25-$(OH)_2$-D_3-induced inhibition on UCP2 mRNA. Similarly, the mVDR agonist and antagonist exerted no effect on isoproterenol- and fatty acid-stimulated UCP2 expression. These data indicate that mVDR does not mediate the inhibitory effect of 1α,25-$(OH)_2$-D_3 on adipocyte UCP2 expression.

3. Nuclear vitamin D receptor mediates the inhibitory effect of 1α,25-$(OH)_2$-D_3 on human adipocyte UCP2 expression

We next investigated the role of the nuclear vitamin D receptor (nVDR) in mediating the inhibitory effect of 1α,25-$(OH)_2$-D_3 on adipocyte UCP2 expression. Using RT-PCR, we detected a 465 bp nVDR fragment in human adipocytes and preadipocytes. This was confirmed by Western blot analysis; using a nVDR monoclonal antibody, we detected a ~50 kDa protein. We then performed a transient transfection of antisense ODN to knock out the nVDR. A time course study shows that treatment with nVDR antisense ODN inhibited nVDR protein from 48 h through 96 h, whereas the mutant antisense ODN was without effect (**Fig. 2**, top panel). We then treated the nVDR knockout adipocytes with 1α,25-$(OH)_2$-D_3. Figure 2 (bar graph) shows that 1α,25-$(OH)_2$-D_3 inhibited UCP2 mRNA by 60% in either control adipocytes or adipocytes treated with mutant ODN. However, 1α,25-$(OH)_2$-D_3 was unable to exert the inhibitory effect in nVDR knockout adipocytes. Similar results were observed on UCP2 protein levels measured by Western blot (Fig. 2, bottom). These data indicate that this inhibitory effect of 1α,25-$(OH)_2$-D_3 on UCP2 expression is mediated by the nVDR.

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[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.02–0255fje; to cite this article, use *FASEB J.* (September 5, 2002) 10.1096/fj.02–0255fje

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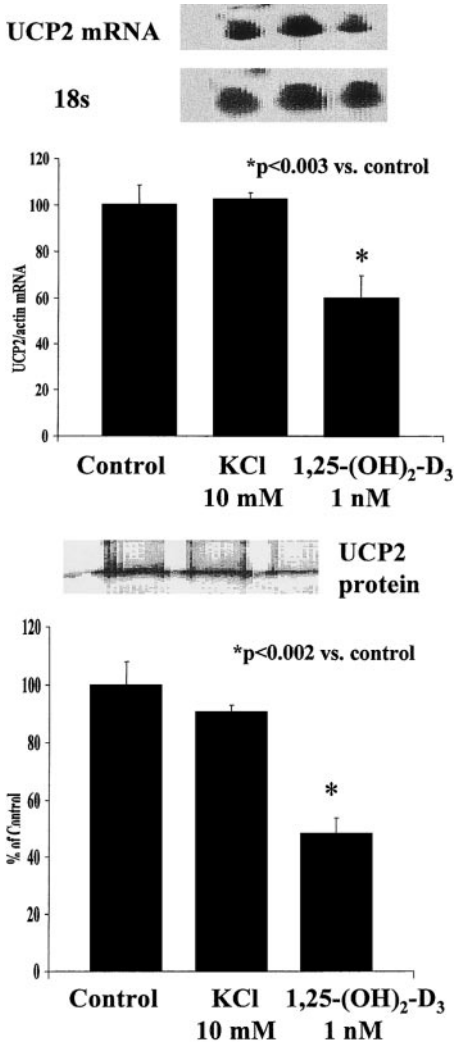

Figure 1. The effect of 1α,25-$(OH)_2$-D_3 on basal UCP2 mRNA (top panel) and protein level (bottom) in human adipocytes. Human adipocytes were treated with 1α,25-$(OH)_2$-D_3 (1 nM) or KCl (10 mM). UCP2 mRNA and protein level were measured by Northern blot and Western blot analysis, respectively. Upper panel: blot is representative of 4 similar experiments. $*P < 0.003$ vs. control; lower panel: blot is representative of 3 similar experiments. $*P < 0.002$ vs. control; data are expressed as mean ± SE.

CONCLUSIONS

It is now well recognized that 1α,25-$(OH)_2$-D_3 generates biological responses via both genomic and nongenomic pathways. 1α,25-$(OH)_2$-D_3 generates genomic actions via binding to a specific nuclear hormone receptor, nVDR. Moreover, 1α,25-$(OH)_2$-D_3 generates rapid, nongenomic signal transduction, including modulation of calcium channels, via a putative membrane mVDR in a wide variety of cells.

Our previous and present data extend these observations by demonstrating that 1α,25-$(OH)_2$-D_3 elicits genomic and nongenomic action in adipocytes. We previously reported that 1α,25-$(OH)_2$-D_3 stimulates adipocyte $[Ca^{2+}]i$, promotes lipogenesis, and inhibits lipolysis via a rapid nongenomic action. Data presented here further demonstrate that 1α,25-$(OH)_2$-D_3 exerts an inhibitory effect on adipocyte UCP2 expression via a genomic action. Therefore, 1α,25-$(OH)_2$-D_3 appears to play an important role in modulating adipocyte lipid metabolism and energy homeostasis.

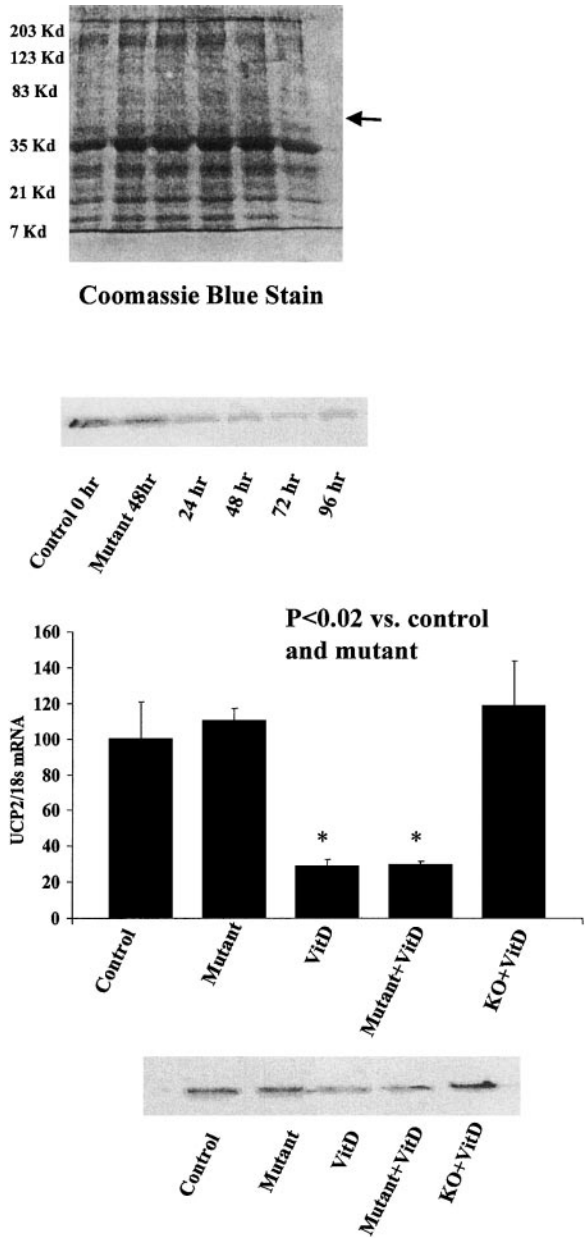

Figure 2. Nuclear vitamin D receptor (nVDR) knockout by antisense oligodeoxynucleotide (ODN) prevented the inhibitory effect of 1α,25-$(OH)_2$-D_3 on adipocyte UCP2 expression. A time course of nVDR knockout by antisense ODN; an equal amount of protein loading was achieved by sample DNA measurement and confirmed by SDS-PAGE visualized with Coomassie blue stain (top panel). Adipocytes were transfected with nVDR antisense ODN or mutant ODN as indicated. Lower panels: nVDR knockout by antisense ODN prevented the inhibitory effect of 1α,25-$(OH)_2$-D_3 on adipocyte UCP2 mRNA (bar graph) and protein (bottom panel). nVDR knockout adipocytes or adipocytes transfected with mutant ODN were treated with or without 1α,25-$(OH)_2$-D_3. UCP2 mRNA and protein were measured by quantitative real-time RT-PCR and Western blot, respectively.

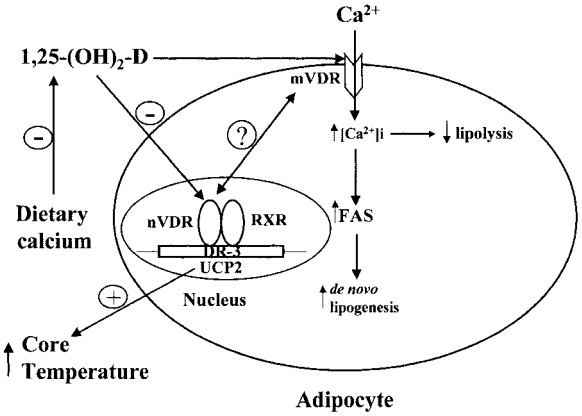

Figure 3. 1α,25-$(OH)_2$-D_3 plays an important role in modulating adipocyte lipid metabolism and energy homeostasis via genomic and nongenomic actions. 1α,25-$(OH)_2$-D_3 exerts an inhibitory effect on adipocyte UCP2 expression via a genomic action mediated by nVDR. On the other hand, 1α,25-$(OH)_2$-D_3 stimulates adipocyte $[Ca^{2+}]$i via a putative mVDR and, subsequently, stimulates lipogenesis and inhibits lipolysis via nongenomic action. Accordingly, dietary calcium suppression of 1α,25-$(OH)_2$-D_3 decreases adipocyte $[Ca^{2+}]$i, inhibits lipogenesis, stimulates lipolysis, and increases UCP2 expression, thereby reducing adiposity.

UCP2, a homologue of UCP1, is ubiquitously expressed, with the highest level in white adipose tissue. UCP2 has been shown to stimulate mitochondrial proton leak and therefore to exhibit a potential role in thermogenesis, energy metabolism, and obesity. Functional characterization of UCP2 promoter region has demonstrated several potent *cis*-acting regulatory elements, including multiple PPARγ and thyroid hormone responsive elements. However, little is known regarding negative regulatory factors of UCP2 expression. Here we report that 1α,25-$(OH)_2$-D_3 exerts an inhibitory effect on UCP2 expression. The mechanism of this nVDR-mediated inhibition of UCP2 is not known. However, human and mouse UCP2 promoters do contain several *cis*-acting negative regulatory elements that strongly repress promoter activity, although it is not clear whether nVDR acts on one of these silencers. Using a promoter analysis program (http://www.lsi.upc.es/cgi-bin/user/alggen/promo/promo/dynmat.cgi), we analyzed the human (Genbank accession no. AF208500) and mouse (Genbank accession no. AF115319) UCP2 promoters, which showed that multiple putative nVDR binding sites may exist on UCP2 promoter regions; at least one is located on the silencer regions. Alternatively, nVDR may also compete with other positive transcriptional factors containing similar DNA binding domains on the responsive element binding or a similar protein–protein interaction domain (such as PPARγ or TR) on heterodimerization with the same transcriptional factor (RXR). This has been evidenced by studies demonstrating that up-regulation or activation of nVDR by 1α,25-$(OH)_2$-D_3 antagonizes the effects of PPARγ or TR on adipocyte differentiation. However, further studies are required to address the mechanism whereby 1α,25-$(OH)_2$-D_3 inhibits UCP2 expression.

Regulation of adipocyte metabolism via 1α,25-$(OH)_2$-D_3 signaling pathways may play an important role in the development of obesity in vivo. Several lines of evidence demonstrate that the circulating 1α,25-$(OH)_2$-D_3 level is elevated in obese humans. Since increasing dietary calcium suppresses 1α,25-$(OH)_2$-D_3 levels, we used this strategy to demonstrate that suppression of 1α,25-$(OH)_2$-D_3 by increasing dietary calcium decreases adipocyte intracellular Ca^{2+}, stimulates lipolysis, inhibits lipogenesis, and increases adipocyte UCP2 expression and core temperature in aP2-agouti transgenic mice, thereby reducing body weight and fat mass in these animals. Recent data demonstrate comparable effects in humans.

In summary, these data indicate that 1α,25-$(OH)_2$-D_3 exerts an inhibitory effect on white adipocyte basal, isoproterenol, and fatty acid-stimulated UCP2 expression and that this effect is mediated via a genomic action. Thus, suppression of 1α,25-$(OH)_2$-D_3 and consequent up-regulation of UCP2 may contribute to our earlier observation of increased thermogenesis in mice fed a high-calcium diet. This effect, coupled with decreased lipogenesis and increased lipolysis secondary to decreased $[Ca^{2+}]_i$ mediated by nongenomic action, may contribute to an anti-obesity effect of dietary calcium. FJ