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ORIGINAL ARTICLE

Expression of vitamin D-metabolizing enzymes in human adipose tissue—the effect of obesity and diet-induced weight loss

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OBJECTIVE: Low vitamin D (VD) levels are common in obesity. We hypothesized that this may be due to metabolism of VD in adipose tissue (AT). Thus, we studied (1) whether the VD-metabolizing enzymes were expressed differently in AT of lean and obese individuals and in visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT), and (2) whether their expression was influenced by weight loss.

METHODS: Samples of SAT and VAT were analyzed for expression of the vitamin-D-25-hydroxylases CYP2R1, CYP2J2, CYP27A1 and CYP3A4, the 25-vitamin-D-1α-hydroxylase CYP27B1, the catabolic vitamin-D-24-hydroxylase CYP24A1, and the vitamin D receptor, using reverse transcriptase-PCR. Moreover, plasma 25-hydroxy-vitamin D (25OHD) level was measured and related to the expression of these enzymes. Samples of SAT and VAT from 20 lean women and 20 obese women, and samples of SAT from 17 obese subjects before and after a 10% weight loss were analyzed.

RESULTS: A plasma 25OHD level $<50\,\text{nmol}\,\text{l}^{-1}$ was highly prevalent in both lean (45%) and obese (90%) women ($P<0.01$). Plasma 25OHD increased by 27% after weight loss in the obese individuals ($P<0.05$). Expression levels of the 25-hydroxylase CYP2J2 and the 1α-hydroxylase CYP27B1 were decreased by 71% ($P<0.0001$) and 49% ($P<0.05$), respectively, in SAT of the obese. CYP24A1 did not differ between lean and obese women, but the expression was increased by 79% ($P<0.05$) after weight loss.

CONCLUSION: Obesity is characterized by a decreased expression of the 25-hydroxylase CYP2J2 and the 1α-hydroxylase CYP27B1 in SAT, whereas the catabolic CYP24A1 does not differ between lean and obese women. However, the expression of CYP24A1 is increased after weight loss. Accordingly, AT has the capacity to metabolize VD locally, and this can be dynamically altered during obesity and weight loss.

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Keywords: vitamin D; adipose tissue; weight loss; VDR; cytochrome P450; 1α-hydroxylase

INTRODUCTION

Low levels of circulating 25-hydroxy-vitamin D (25OHD) are associated with increased fat mass and body mass index,[1–5] but the underlying mechanism for this association is not fully elucidated.[6] Vitamin D (VD) is stored in adipose tissue (AT), and release of VD from AT is proposed to serve as an endogenous source of VD during the winter, when cutaneous production is low or absent in several parts of the world.[7] As low circulating levels of 25OHD are common in association with the obese state,[8] several studies have examined the effect of weight loss on circulating levels of 25OHD. However, results are inconsistent. After diet- and exercise-induced weight loss, both increased[9–11] and unaltered[12,13] circulating levels of 25OHD have been reported. Likewise, after major weight loss by bariatric surgery, both temporary[14–16] and long-term increases[16–18] in circulating levels of 25OHD have been reported, along with reports of no changes one year after surgery.[13,19]

Taken together, these findings indicate that body weight is an important factor for circulating 25OHD levels. Several other explanations for the low levels of 25OHD in obesity have been proposed: a decreased exposure to sun light,[20] or a decreased hepatic 25-hydroxylation of VD in obesity inhibited by elevated levels of 1,25-OH_2D and PTH[21] could contribute to the decreased levels of 25OHD in obesity. Wortsman *et al.*[22] showed an attenuated increase in plasma VD in obese subjects in response to a single dose of VD, and hypothesized that VD is accumulated and sequestered within AT and not readily available for the circulation. However, this hypothesis was recently challenged by Drincic *et al.*,[23] who showed that differences in 25OHD levels between lean and obese subjects could be explained by simple volumetric dilution. In their hyperbolic model, differences in body weight accounted for 13% of the variation of plasma 25OHD. We speculate that, in addition, metabolism of VD in AT could further contribute to the difference in 25OHD levels between obese and normal-weight individuals.

In AT, VD is primarily stored not only in the form of native VD (vitamin D_2 and vitamin D_3), but also as 25OHD.[24–27] 25-Hydroxy-vitamin D is the dominant circulating form of VD, and the best indicator of VD status.[8] The formation, activation and catabolism of 25OHD is a complex process, which involves the mitochondrial and microsomal cytochrome P450 enzymes. Synthesis of 25OHD is initiated by conversion of 7-dehydrocholesterol to cholecalciferol (vitamin D_3) by UVB irradiation in the skin. Moreover, VD can be obtained through the diet. VD is then hydroxylated twice to be fully active. First, VD is converted by the 25-hydroxylases to 25OHD. Second, the 1α-hydroxylase converts the 25OHD into the active form of VD, 1,25-dihydroxy-vitamin D (1,25$(OH)_2$D), which is a potent activator of the vitamin D receptor (VDR). In humans, four

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cytochrome P450 enzymes, CYP2R1,[28,29] CYP3A4,[30] CYP27A1[ref. 31] and CYP2J2,[32] possess 25-hydroxylase activity, with CYP2R1 being the most specific.[28] Hydroxylation in the 1α-position is effected by the mitochondrial CYP27B1.[33] This process was classically located to the kidney,[34] but recently, extra-renal 1α-hydroxylase activity has been described in several other tissues.[35] Finally, $1,25(OH)_2D$ stimulates its own degradation by induction of the 24-hydroxylase (CYP24A1), which catabolizes 25OHD and $1,25(OH)_2D$ to calcitroic acid and other inactive metabolites.[36,37] Some of the VD-metabolizing enzymes, the CYP2R1,[38] CYP3A4,[38] CYP27B1[ref. 39] and CYP24A1,[39] have been sporadically reported to be expressed in AT, but a systematic assessment of the VD-metabolizing enzymes in AT has not yet been investigated.

$1,25(OH)_2D$ interacts with its nuclear VDR to regulate transcription of a large number of genes involved in the regulation of cell proliferation and differentiation, immune function and metabolism, in addition to calcium homeostasis in various cell types.[40] VDR is also expressed in AT,[41,42] and taken together this could imply that AT is not merely an organ of VD storage, but also a target organ for $1,25(OH)_2D$, and that local metabolism of VD may occur in the AT.

We hypothesize that AT expresses many of the VD-metabolizing enzymes, and therefore an enlarged AT mass as seen in obesity might have the possibility to influence the level of circulating 25OHD. Thus, we investigated (1) whether the expression levels of the VD-metabolizing enzymes and the VDR differ among subcutaneous and visceral AT of lean and obese women; (2) whether their expressions are influenced by weight loss; and (3) whether expression of these metabolizing enzymes in AT correlated with the circulating levels of 25OHD.

MATERIALS AND METHODS

Subjects

The current study comprises three study populations:

Study 1: a cross-sectional study of lean and obese women. Blood samples and paired samples of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were analyzed. Samples were obtained during abdominal surgery after an overnight fast. VAT samples were taken from the omental depot and SAT samples were taken at the umbilical level at the beginning of the operation. Samples were taken from 20 obese women undergoing bariatric surgery for obesity and from 20 lean women undergoing gynecological surgery (body mass index (BMI): 46.1 (44.0–48.2) vs 23.4 (22.3–24.5) $kg\,m^{-2}$; $P<0.0001$ and age: 47.9 (44.2–51.6) vs 47.4 (44.3–50.4) years; $P=0.81$), as previously reported elsewhere.[43]

Study 2: This was an intervention study with focus on the effects of weight loss in obese individuals as previously described.[44] Briefly, the obese subjects completed an 8-week intervention with a very low calorie diet (800 kcal per day), which was followed by a 4-week weight maintenance program. The diet formula contained 8 μg of VD per day. Seventeen healthy obese subjects (nine males and eight females, age: 35.0 (31.5–38.5) years, BMI: 35.6 (33.6–37.6) $kg\,m^{-2}$) were investigated, and mean weight loss was 12.5 (10.2–14.8) kg or 11.4% (9.5–13.4)% of initial body weight after 12 weeks. Blood samples and subcutaneous abdominal fat biopsies collected at baseline and at week 12 were analyzed in this study.

Study 3: a cross-sectional study in which AT was obtained by liposuction for cosmetic purposes from the SAT depot of six lean women (BMI: 24.9 (20.4–29.4) $kg\,m^{-2}$, age: 32.8 (25.9–40.0) years). The AT was separated in stroma-vascular cells and in isolated mature adipocytes in order to investigate the expression of the various VD-metabolizing enzymes in these fractions.

All subjects in the studies were healthy and receiving no medication known to affect VD metabolism.

Tissue samples

The AT samples were transported to the laboratory in a sterile container. The AT samples were immediately washed with isotonic saline and then frozen in liquid nitrogen and kept at −80 °C for later RNA extraction. To isolate adipocytes from the stroma-vascular fraction (SVF), adipocytes were isolated by collagenase digestion as previously described.[45] Briefly, the AT fragments were treated with collagenase (0.15 $mg\,g^{-1}$ AT) in 10 $mmol\,l^{-1}$ HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer. The isolated adipocytes were washed three times in buffer containing 5% albumin and then snap-frozen in liquid nitrogen. After the initial collagenase digestion, the remaining SV fraction was washed three times in buffer, and cells were collected by centrifugation at 6300 × *g* for 15 min and then snap-frozen in liquid nitrogen. Both fractions were kept at −80 °C for later RNA extraction. Thorough separation of the fractions was confirmed by measuring the messenger RNA (mRNA) levels of adipocyte-specific leptin and macrophage-specific CD14 and CD68, as previously reported by our group.[45]

Isolation of RNA

Total RNA was isolated from the AT fragments using TriZol reagent (Gibco BRL, Life Technologies, Roskilde, Denmark); RNA was quantified by measuring absorbance at 260 and 280 nm, and the inclusion criterion was a ratio ⩾1.8. Finally, the integrity of the RNA was checked by visual inspection of the two ribosomal RNAs, 18S and 28S, on an agarose gel.

Real-time reverse transcriptase-PCR for mRNA analysis

For real-time reverse transcriptase PCR, complementary DNA was constructed using random hexamer primers as described by the manufacturer (Verso cDNA kit, VWR, Herlev, Denmark). PCR-master mix, containing the specific primers, was added and real-time quantification of genes was performed by SYBR-green real-time reverse transcription PCR assay (KAPA SYBR Fast Universal kit, Ken-En-Tec, Taastrup, Denmark) using an ICycler from Bio-Rad (Bio-Rad Laboratories, Hercules, CA, USA). The following primer pairs were used: CYP2R1: sense primer 5′-TGGCATCCT GCCTTTTGGAAA-3′ and antisense primer 5′-GCTGAGGTAGCTGAGGCTTT-3′; CYP2J2: sense primer 5′-TCCATCCTCGGACTCTCCTAC-3′ and antisense primer 5′-GCGCCGTCTTTTGAGAAAGT-3′; CYP3A4: sense primer 5′-AGTC GCCTCGAAGATACAC-3′ and antisense primer 5′-GTGAGTGGCCAGTTCAT ACAT-3′; CYP27A1: sense primer 5′-CGGCAACGGAGCTTAGAGG-3′ and antisense primer 5′-GGCATAGCCTTGAACGAACAG-3′; CYP27B1: sense primer 5′-GGAACCCTGAACGTAGTC-3′ and antisense primer 5′-AGTCCGA ACTTGTAAAATTCCCC-3′. The housekeeping gene, GADPH, was amplified using sense primer 5′-AAGGTGAAGGTCGGAGTCAA-3′ and antisense primer 5′-AATGAAGGGGTCATTGATGG-3′. All primers were from DNA Technology (Risskov, Denmark). cDNA with specific primers was amplified in separate tubes, and the increase in fluorescence was measured in real time. The threshold cycle was calculated, and the relative gene expression was calculated essentially as described in the User Bulletin No-2, 1997 from Perkin Elmer (Perkin Elmer Cetus, Norwalk, CT, USA) using the formula: $k \times 2^{-(CT-\text{target}\,-\,CT-\text{housekeeping}),}$ where *k* is a constant, set to 1. All samples were amplified in duplicate. A similar set-up was used for negative controls, except that the reverse transcriptase was omitted and no PCR products were detected under these conditions.

Assays

Plasma 25OHD levels were analyzed by isotope dilution liquid chromatography–tandem mass spectrometry (LC–MS/MS), using a method adapted from Maunsell *et al.*[46] The method quantifies $25OHD_2$ and $25OHD_3$, including the 3-epimer form, which is not separated from $25OHD_3$. Calibrators traceable to NIST SRM 972 (Chromsystems, GmbH, Munich, Germany) were used. Commutability was confirmed directly to NIST SRM 972 level 1–4 (the sum of 25OHD3 and its epimer was compared). Mean coefficients of variation for $25OHD_3$ were 6.4% and 9.1% at levels of 66.5 and 21.1 $nmol\,l^{-1}$, respectively, and for $25OHD_2$ the CV values were 8.8% and 9.4% at levels of 41.2 and 25.3 $nmol\,l^{-1}$, respectively.[47]

Statistical analysis

Data were logarithmically transformed to achieve normality when appropriate. Normal distribution of data was determined by visual inspection of QQ-plots. Paired samples were analyzed using paired *t*-test. Groups were compared using Student's *t*-test. χ^2-test was used for categorical outcomes. A *P* value <0.05 was considered as significant; however, for the comparison of VAT and SAT in obese and lean individuals, the Bonferroni correction for multiple comparisons was applied and a *P* value <0.0125 was considered significant. The statistical software package Stata version 11.2 (StataCorp. LP, College Station, TX, USA) for Windows was used for the statistical analysis.

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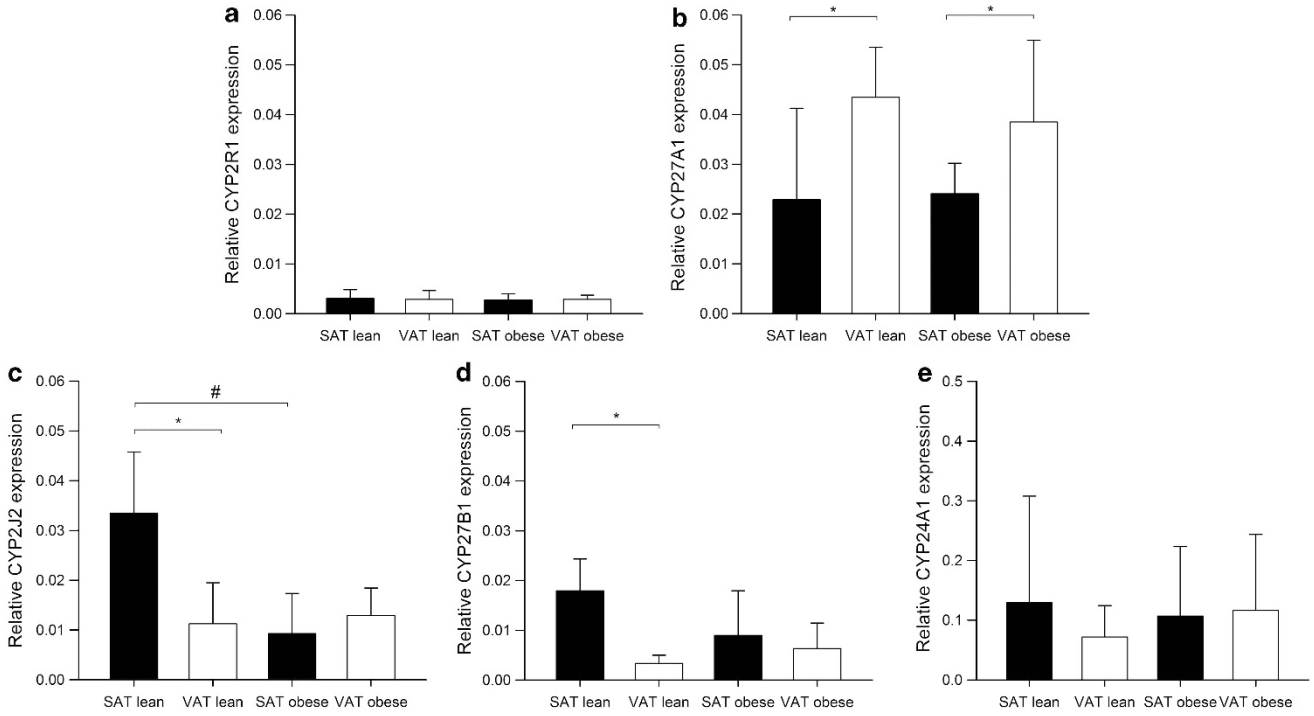

Figure 1. Relative gene expression of VD-metabolizing enzymes in SAT (black columns) and VAT (blank columns) of 20 lean and 20 obese women. (**a**) Vitamin D-25-hydroxylase CYP2R1. (**b**) Vitamin D-25-hydroxylase CYP27A1. (**c**) Vitamin D-25-hydroxylase CYP2J2. (**d**) 25-Hydroxy-vitamin D-1α-hydroxylase CYP27B1. (**e**) 1,25-Dihydroxy-vitamin D-24-hydroxylase CYP24A1. Data are given as median with interquartile range (IQR). $^{*}P<0.0125$ for paired comparison of SAT vs VAT. $^{\#}P<0.0125$ for group wise comparison of lean vs obese (Bonferroni-corrected).

RESULTS

VD and VD-metabolizing enzymes in AT: effect of fat depots and body weight (study 1)

Plasma 25-OHD in obese and lean women. Total plasma 25OHD was (median and interquartile range (IQR)) 54 nmol l^{-1} (31–67 nmol l^{-1}) in lean women and 29 nmol l^{-1} (22–40 nmol l^{-1}) in obese women ($P=0.006$). Plasma 25OHD correlated negatively with BMI ($r=-0.38$; $P=0.017$). 25-OH-vitamin D_2 was not detectable in plasma in any of the women (data not shown). Low VD levels (defined as 25OHD <50 nmol l^{-1}) were found in 45% and 90% of lean and obese women, respectively (χ^2, $P=0.002$).

VD-metabolizing enzymes in AT. AT from both lean and obese women expressed both the 25-hydroxylase enzymes CYP2R1, CYP2J2 and CYP27A1, as well as the 1α-hydroxylase enzyme CYP27B1 and the degrading enzyme 24-hydroxylase CYP24A1 (Figure 1) in both the subcutaneous and the VAT depot. We were unable to detect expression of CYP3A4 in any of the depots, which, however, was highly expressed in a control sample of liver cells (data not shown). Expression of CYP27A1 were 39% and 36% lower in SAT than in VAT in lean ($P=0.004$) and obese ($P=0.003$) women, respectively (Figure 1b). Interestingly, expression of CYP2J2 was decreased by 71% in SAT of obese women compared with lean women ($P<0.0001$). In lean women, CYP2J2 expression was 2.8-fold higher in SAT as compared with VAT ($P<0.0001$) (Figure 1c). Expression of the 1α-hydroxylase CYP27B1 was decreased by 49% in SAT of obese women compared with lean women ($P=0.03$, Figure 1d). However, this was not significant after Bonferroni correction. In lean women, the expression of CYP27B1 was sixfold higher in SAT than in VAT ($P<0.0001$). There were no differences in the expression of CYP2R1 and CYP24A1 between AT depots (Figures 1a and e, respectively).

In VAT, but not in SAT, plasma 25OHD correlated positively with the 25-hydroxylase CYP27A1 ($r=0.32$; $P=0.048$, Figure 2a) and negatively with the 1α-hydroxylase CYP27B1 ($r=-0.36$; $P=0.036$, Figure 2b).

VDR expression in AT. VDR was expressed in all of the investigated fat depots (Figure 3). In lean women, VDR expression in SAT was twofold higher than in VAT ($P=0.0002$). In obese women, VDR expression did not differ significantly between VAT and SAT ($P=0.47$). In VAT, VDR expression was 33% higher in obese women than in lean women ($P=0.01$). In SAT, no differences in VDR expression ($P=0.35$) between lean and obese women were found.

Effect of weight loss on 25OHD levels and AT expression patterns (study 2)

The weight loss study group consisted of nine obese males and eight obese females, who completed 8 weeks of the very low calorie diet treatment and 4 weeks of weight maintenance. Mean weight loss was $\sim$11 kg after 12 weeks, as previously reported.[44] There were no differences in BMI, 25OHD or VDR expression between genders at either time point. Therefore, data from both genders were analyzed as one group. The median plasma 25OHD was 59 nmol l^{-1} (36–74 nmol l^{-1}) at baseline and 72 nmol l^{-1} (43–86 nmol l^{-1}) after the 12 weeks of weight loss corresponding to a mean relative increase of 27% ($P=0.03$). The relative change in plasma 25OHD correlated with relative weight loss ($r=0.67$; $P=0.005$, Figure 4) and correlated negatively with change in BMI ($r=-0.67$; $P=0.005$).

Concerning the expression of the various VD-metabolizing enzymes in AT, there were no differences between genders in the expression (data not shown). Therefore, data from both genders were analyzed together. The expressions of the 25-hydroxylases

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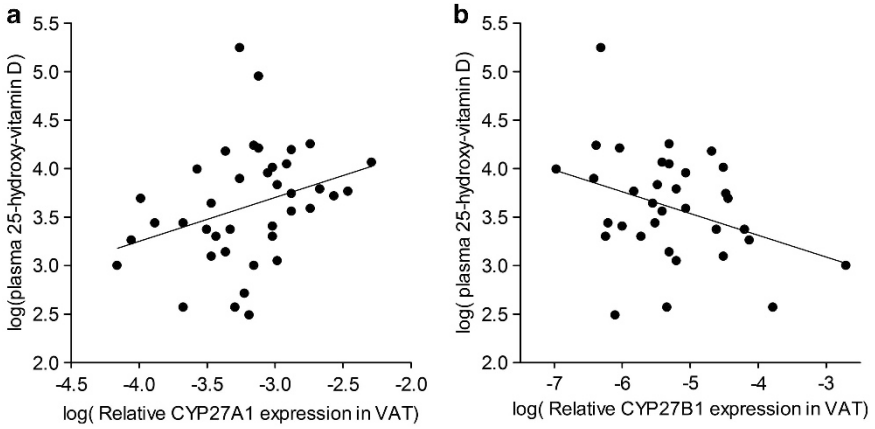

Figure 2. Correlations of plasma 25OHD and relative gene expression of VD-metabolizing enzymes. (**a**) Correlation of plasma 25OHD and vitamin D-25-hydroxylase CYP27A1 in VAT ($r = 0.32$; $P = 0.048$). (**b**) Correlation of plasma 25OHD and 25-hydroxy-vitaminD-1α-hydroxylase CYP27B1 in VAT ($r = -0.36$; $P = 0.036$).

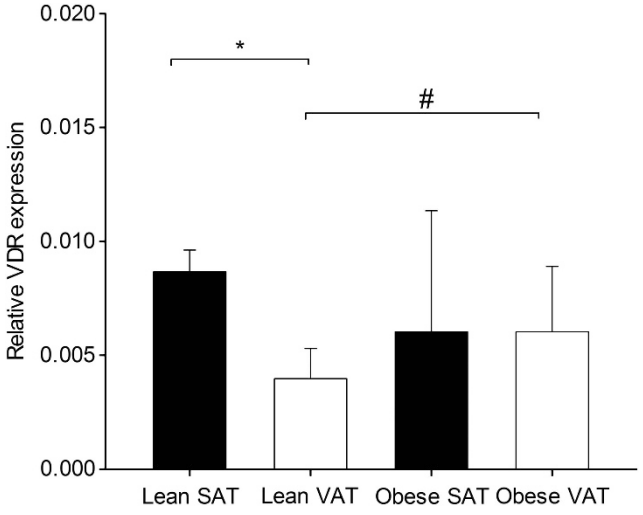

Figure 3. Relative mRNA expression of the VDR in SAT (black columns) and VAT (blank columns) of 20 lean and 20 obese women. Data are given as median ± IQR. *$P < 0.0125$ for paired comparison of SAT vs VAT. #$P < 0.0125$ for group wise comparison of lean vs obese (Bonferroni-corrected).

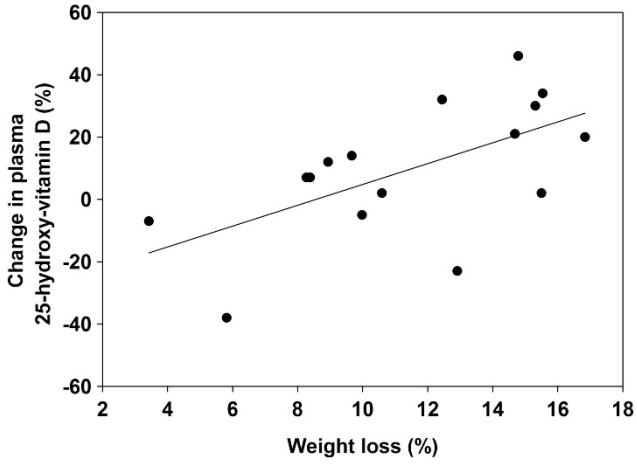

Figure 4. Change in plasma 25OHD after weight loss in 17 obese subjects after 8 weeks on VLCD followed by 4 weeks of weight maintenance. Correlation of relative change in plasma 25OHD and relative weight loss ($r = -0.67$; $P = 0.005$).

CYP2R1, CYP2J2 and CYP27A1 did not change during weight loss ($P = 0.54$, $P = 0.17$ and $P = 0.11$, respectively), neither did the expression of the 1α-hydroxylase CYP27B1 ($P = 0.16$). Interestingly, the expression of the catabolizing enzyme, CYP24A1, increased by 79% ($P = 0.01$) compared with baseline (Figure 5).

VDR expression did not change during weight loss ($P = 0.40$).

Expression of VD-metabolizing enzymes and VDR in AT subfractions

Samples of subcutaneous AT taken from lean women by liposuction were separated in the SVF (containing cell types such as endothelial cells, immune cells and fibroblasts) and isolated mature adipocytes. The expression of VDR, CYP2R1, CYP2J2 and CYP24A1 did not differ between the stroma-vascular cell fraction and that in the isolated adipocytes ($P = 0.98$, $P = 0.17$, $P = 0.17$ and $P = 0.13$, respectively). Interestingly, the CYP27A1 had a 2.7-fold higher expression ($P = 0.005$) and the CYP27B1 had a 1.2-fold higher expression ($P = 0.01$) in the SVF than in the isolated mature adipocytes (Figure 6).

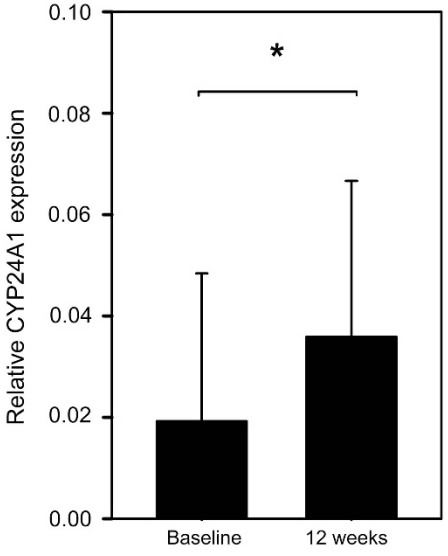

Figure 5. Relative mRNA levels of 1,25-dihydroxy-vitamin-D-24-hydroxylase CYP24A1 in SAT during weight loss. Data are given as median ± IQR. *denotes $P < 0.05$ for pairwise comparison.

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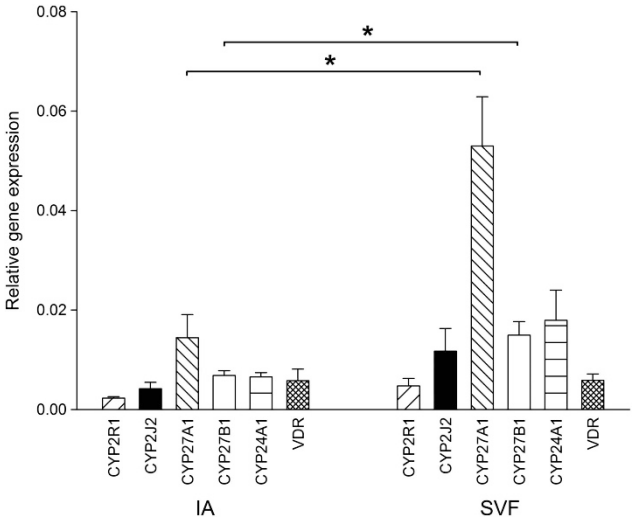

Figure 6. Relative gene expression of VD-metabolizing enzymes and the VDR in isolated adipocytes (IA) ($n = 6$) and in the stroma-vascular fraction (SVF) ($n = 5$) of SAT of lean women. Data are given as mean ± s.e. $^*P < 0.05$, groupwise comparison of isolated adipocytes vs SVF.

DISCUSSION

The prevalence of low VD levels (25OHD < 50 nmol l^{-1}) in our participants was similar to the findings in previous studies, that is, ~50% in lean women and as high as 90% in obese subjects.[4,5,19,48–52] The high prevalence of low VD levels in obesity is proposed to be due to volumetric dilution in obesity, as the volume of distribution of VD increases with increased body weight.[23]

Our study aimed to elucidate whether low plasma 25OHD levels in obesity could be further explained by an altered VD metabolism. We showed major differences between SAT and VAT in the expression of VD-metabolizing enzymes, with a differential distribution between lean and obese women. According to our findings, the expression of CYP27A1 is more pronounced in VAT compared with SAT, with no differences between lean and obese women, whereas the expression of CYP2J2 is more pronounced in SAT than in VAT in lean, but not in obese women. Accordingly, our findings point toward a compromised 25-hydroxylation in SAT in obese women owing to a lower expression of the CYP2J2. In general, VD is considered to be stored in AT as cholecalciferol, which is in equilibrium with the concentration in plasma.[25] Whether 25OHD synthesized in AT contributes to circulating levels is currently unknown. Our data do not exclude that AT may contribute to circulating levels of 25OHD, as we showed a positive correlation between the expression of CYP27A1 in VAT and plasma 25OHD concentrations. However, this does not explain the low 25OHD levels in obesity, as the expression of CYP27A1 according to our findings is not affected by obesity. Accordingly, our data do not support a decreased synthesis of 25OHD in AT as an explanation for low 25OHD levels in obesity. Moreover, our study did not show differences between lean and obese women in the expression of the CYP24A1, but as obese individuals have several fold more AT than lean subjects, it is plausible that they degrade more VD in their AT via the CYP24A1 enzyme, so our data could support the hypothesis of an increased catabolism of VD in obesity.

We found a low, basal expression of CYP2R1 in both lean and obese women. CYP2R1 is regarded as the major vitamin D-25-hydroxylase in the liver, but whether this applies to AT as well is unknown. However, when the amount of AT is considered, the CYP2R1 in AT could be biologically relevant. The expression of the 1α-hydroxylase CYP27B1 was decreased in SAT of obese women. In VAT, the expression correlated negatively with plasma 25OHD. CYP27B1 mRNA has also been detected in murine AT, and the enzyme was shown to be functional in cultures of 3T3-L1 adipocytes.[39] In human marrow stromal cells, a high expression of CYP27B1 corresponded to a high *in vitro* production of $1,25(OH)_2D$.[53] Given that CYP27B1 is equally functional in human AT, this would imply a smaller capacity to synthesize $1,25(OH)_2D$ in the SAT of the obese and in VAT.[23] Most likely, the hydroxylation of VD in AT is of importance to AT itself, as it allows for autocrine/paracrine actions of VD.[54,55] Our findings of differential expression of the VDR and CYP27B1 in lean and obese women warrants further investigations on the autocrine/paracrine actions of VD in different compartments of AT.

CYP3A4 is a major hepatic microsomal enzyme involved in the metabolism of a large number of therapeutic drugs. CYP3A4 25-hydroxylates vitamin D_2, but not vitamin D_3.[30] CYP3A4 was expressed in small amounts in SAT and VAT in the study by Ellero *et al.*,[38] but was not detectable in our samples. As a control we tested the expression in liver cells and found a high level of expression, and therefore, we are confident that no CYP3A4 is expressed in our AT samples. As 25-OH-vitamin D_2 was not detected in any of the plasma samples, it is likely that no substrate for the CYP3A4 was available in the AT of our patients.

To address whether the observed gene expression pattern was located in the adipocytes or in other cells present in the AT, we conducted an analysis on isolated adipocytes and the SVF. We found equal amounts of CYP2R1, CYP2J2, CYP24A1 and VDR mRNA in the two fractions of AT. The CYP27A1 and CYP27B1 were higher expressed in SVF as compared with the adipocyte fraction. The CYP27A1 was primarily expressed in VAT, and was especially located to the SVF. Whether CYP27A1 is related to macrophage infiltration, which is more pronounced in VAT and in obesity,[45] or to another component in the SVF remains to be clarified.

In the second substudy, we explored the components of VD metabolism during weight loss. Plasma 25OHD increased after weight loss, and the changes in 25OHD levels correlated with relative weight loss and with changes in BMI. This increase in 25OHD seems to be related to the magnitude of the weight loss, and does not seem attributable to the ingested VD, as all subjects ingested the same small amount of VD in the very low calorie diet. Similar findings were reported by others after diet and/or exercise-induced weight loss.[9,10,12] These findings are in agreement with the hypothesis that VD is being stored in the AT[24,26] and confirm previous data on how plasma 25OHD depends on the volume of distribution.[23] The increase in 25OHD fits very well with the observed increased expression of the degrading enzyme, the 24-hydroxylase CYP24A1, as CYP24A1 expression is induced by $1,25\text{-}OH_2D$.[36,37] As CYP24A1 degrades both 25OHD and $1,25(OH)_2D$, these findings suggest a higher turnover of both 25OHD and $1,25(OH)_2D$ following weight loss. After weight loss the subjects were still obese, which may explain why we did not find similar expression of the hydroxylases as we did in the lean subjects in the first study. However, we cannot rule out the possibility that the small amount of VD in the diet might also influence the expression of the hydroxylases in the AT.

The strength of our study is the collection of a relatively large sample of paired SAT and VAT samples from lean and obese women, and the thorough investigation of all known VD-converting enzymes coupled with measurement of 25OHD levels and the level of VDR expression. Furthermore, the collection of samples of SAT both before and after a significant weight loss allows us to explore longitudinal changes in VD status. Unfortunately, samples of VAT were not available from the weight loss trial, which could have provided additional information. The limitation of our study is the fact that we do not have any data regarding the activity of the enzymes; neither do we know the concentration of their substrates, VD and 25OHD, in the AT.

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Others have, however, found that protein levels of CYP27B1 and VDR measured by western blotting were consistent with mRNA levels.[53,56]

In conclusion, we found a high prevalence of low VD levels in obese women scheduled for bariatric surgery. We found that AT expresses the enzymes forboth the formation of 25OHD and of the active form, 1,25$(OH)_2$D, and for degradation of VD. As both VD-activating steps are co-located with the VDR in AT, these findings support a paracrine or autocrine effect of 1,25$(OH)_2$D in AT.

Furthermore, we showed that in the SAT, the obese women had a lower expression of one of the enzymes responsible for 25-hydroxylation of VD (CYP2J2), as well as a tendency toward a decreased expression of the 1α-hydroxylase, whereas there were no differences in the degrading enzyme CYP24A1. Accordingly, our data suggest that both 25-hydroxylation and the 1α-hydroxylation in SAT are impaired in obesity. Additionally, we showed a relative increase in plasma 25OHD after weight loss with an increased expression of degrading CYP24A1 in SAT. Based on our findings, we will propose the hypothesis that AT not only passively stores VD, but seems also to dynamically change its capacity for activation and deactivation of VD in obesity and during weight loss.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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