Clinical Nutrition xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Associations of different body fat deposits with serum 25-hydroxyvitamin D concentrations

Rachida Rafiq [a, *], Floor Walschot [a], Paul Lips [a], Hildo J. Lamb [b], Albert de Roos [b], Frits R. Rosendaal [c], Martin den Heijer [a, c], Renate T. de Jongh [a], Renée de Mutsert [c]

[a] Department of Internal Medicine and Endocrinology, VU University Medical Center, Amsterdam Movement Sciences, Amsterdam, the Netherlands
[b] Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands
[c] Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands

ARTICLE INFO

Article history:
Received 30 October 2018
Accepted 13 December 2018

Keywords:
Vitamin D
Obesity
Total body fat
Subcutaneous adipose tissue
Visceral adipose tissue
Hepatic fat

SUMMARY

Background & aims: Obesity is a well-established risk factor of vitamin D deficiency. However, it is unclear which fat deposit is most strongly related to serum 25-hydroxyvitamin D (25(OH)D) concentrations. Our aim was to distinguish the specific contributions of total body fat (TBF), abdominal subcutaneous adipose tissue (aSAT), visceral adipose tissue (VAT) and hepatic fat on 25(OH)D concentrations.

Methods: We performed a cross-sectional analysis of the Netherlands Epidemiology of Obesity study, a population-based cohort study. We used linear regression analyses to examine associations of TBF, aSAT, VAT (n = 2441) and hepatic fat (n = 1980) with 25(OH)D concentrations. Standardized values were used to compare the different fat deposits.

Results: Mean (SD) age and 25(OH)D concentrations of the study population was 56 (6) years and 70.8 (24.2) nmol/L, respectively. TBF was inversely associated with 25(OH)D concentrations in women, but not in men. One percent higher TBF was associated with 0.40 nmol/L (95%CI:-0.67 to −0.13) lower 25(OH)D. aSAT was not associated with 25(OH)D concentrations. One cm^2 higher VAT was associated with 0.05 nmol/L (−0.09 to −0.02) lower 25(OH)D in men, and 0.06 nmol/L (−0.10 to −0.01) lower 25(OH)D in women. Hepatic fat was only associated with 25(OH)D in men. A tenfold increase in hepatic fat was associated with 6.21 nmol/L (−10.70 to −1.73) lower 25(OH)D. Regressions with standardized values showed VAT was most strongly related to 25(OH)D.

Conclusions: In women, TBF and VAT were inversely related to 25(OH)D concentrations. In men, VAT and hepatic fat were inversely related to 25(OH)D concentrations. In both groups, VAT was most strongly associated with 25(OH)D concentrations.

1. Introduction

Obesity and vitamin D deficiency both strike in pandemic proportions [1,2]. Clinical evidence shows that these two major public health issues are linked with each other [3]. Measures of adiposity such as body mass index (BMI) and percentage of total body fat are inversely related to concentrations of 25-hydroxyvitamin D (25(OH)D) [4–8]. In addition, the prevalence of vitamin D deficiency in individuals with obesity is 35% higher compared to individuals without obesity [9].

Several explanations for the inverse relationship between obesity and vitamin D concentrations have been proposed [3]. One potential explanation is that individuals with obesity have lower 25(OH)D concentrations because of less exposure to sunlight. This is a consequence of less involvement in outdoor activities and clothing habits in persons with obesity [10–12]. A second potential explanation for vitamin D deficiency in obesity is that sequestration and storage of vitamin D by adipose tissue in individuals with obesity is increased compared with lean individuals [3,13,14]. After exposure to ultraviolet radiation and oral supplementation of vitamin D, individuals with obesity show a relatively smaller increase in serum concentrations of vitamin D compared with

* Corresponding author. VU University Medical Center, Department of Internal Medicine and Endocrinology, PO Box 7057, 1007, MB, Amsterdam, the Netherlands.
E-mail address: ra.rafiq@vumc.nl (R. Rafiq).

https://doi.org/10.1016/j.clnu.2018.12.018
0261-5614/

individuals without obesity [15]. Third, it has been hypothesized that catabolism of 25(OH)D is higher with increasing amounts of adiposity [3]. This is due to the expression of 24-hydroxylase enzyme by adipose tissue which degrades $1,25(OH)_2D$ by further hydroxylation [3,16]. Fourth, it has been hypothesised that the clearance of vitamin D could be increased due to obesity-associated inflammation. Obesity is associated with a state of chronic low-grade inflammation and several studies have shown vitamin D to decrease during inflammation [17–19]. Finally, the hydroxylation on 25-hydroxyvitamin D in the liver might be decreased in obese individuals [20]. Obesity is closely related to non-alcoholic fatty liver disease (NAFLD), which covers disorders in a spectrum of increasing severity from simple hepatic steatosis (fatty liver) to hepatocellular carcinoma [21].

Despite the well-known link between obesity and vitamin D deficiency, it is yet unclear which fat deposit is most strongly related to serum 25(OH)D concentrations. More insight in the specific contributions of the different fat deposits on 25(OH)D concentrations may clarify mechanisms that underlie this relationship and show which individuals are at highest risk of vitamin D deficiency. Therefore, our aim for this study was to examine associations of total body fat, abdominal subcutaneous adipose tissue, visceral adipose tissue and hepatic fat with 25(OH)D concentrations.

2. Methods

2.1. Study design and study population

The present study is a cross-sectional analysis of baseline measurements of the Netherlands Epidemiology of Obesity (NEO) study, a population-based prospective cohort study in 6671 men and women aged between 45 and 65 years. The study population and study design have been described in detail previously [22]. Men and women who had a self-reported BMI of 27 kg/m^2 or higher, aged between 45 and 65 years and living in the greater area of Leiden (in the West of the Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants of one municipality (Leiderdorp) aged between 45 and 65 years were invited to respond irrespective of their BMI, allowing for a reference distribution of BMI. Participants visited the NEO study center of the Leiden University Medical Center (LUMC) after an overnight fast. Prior to this study visit, participants completed questionnaires to report demographic, lifestyle and clinical information. At the study center, the participants completed a screening form, asking about anything that might create a health risk or interfere with magnetic resonance imaging (MRI) (most notably metallic devices, claustrophobia, or a body circumference of more than 1.70 m). Of the participants who were eligible for MRI, approximately 35% were randomly selected to undergo MRI. All participants underwent other baseline measurements including anthropometry and blood sampling. For the present analysis, we included all participants with successful measurements of abdominal subcutaneous and visceral adipose tissue and hepatic fat, and excluded participants with missing data. The study was approved by the medical ethics committee of the LUMC (CME LUMC 058, N° of approval P08.109) and all participants gave written informed consent.

2.2. Measures of different body fat deposits

Total body fat (%) and body weight (kg) were measured by the Tanita bio impedance balance (total body fat-310, Tanita International Division, UK) without shoes and 1 kg was subtracted from the body weight to correct for the weight of clothes. Body mass index (BMI) (kg/m^2) was calculated by using measured body weight (kg) and height (cm). Abdominal subcutaneous and visceral adipose tissue were quantified by MRI using a turbo spin echo imaging protocol. At the level of the fifth lumbar vertebra three transverse images each with a slice thickness of 10 mm were obtained during a breath-hold. Abdominal subcutaneous and visceral adipose tissue were converted from the number of pixels to centimeters squared and the average of three slices was used in the analyses.

Hepatic triglyceride content was quantified by ^{1}H-MRS of the liver. An 8 ml voxel was positioned in the right lobe of the liver, avoiding gross vascular structures and adipose tissue depots. Sixty-four averages were collected with water suppression. Spectra were obtained with an echo time of 26 ms and a repetition time of 3000 ms. Data points were collected using a 1000 Hz spectral line. Without changing any parameters, spectra without water suppression, with a repetition time of 10 s, and with four averages were obtained as an internal reference. ^{1}H-MRS data were fitted using Java-based magnetic resonance user interface software (jMRUI version 2.2, Leuven, Belgium). HTGC relative to water was calculated as (signal amplitude of triglyceride)/(signal amplitude of water) x 100. All imaging was performed on a 1.5 T whole-body MR scanner, (Philips Medical Systems, Best, the Netherlands).

2.3. 25(OH)D concentrations

At the baseline study visit a fasting blood sample of venous blood was collected by venipuncture, and immediately sent to the central clinical laboratory of the LUMC for the assessment of serum 25-hydroxyvitamin D (25(OH)D) concentrations. During the inclusion period of the NEO study, quantification of the 25(OH)D concentration in the serum was done by three subsequent methods. From 1 September 2008 to 4 October 2010 the radioimmunoassay (RIA) method was used (Diasorin, Italy). From 5 October 2010 to 29 September 2011 the Chemiluminescent Immunoassay was used (iSYS analyzer, ImmunoDiagnostics Inc., Boldon, UK). Finally, from 30 September 2011 until the end of the study, the 2nd generation Electrochemiluminescence Immunoassay (ECLIA) (Modular Analytics E170 analyzers, Roche Diagnostics, Mannheim, Germany) was used. Two level commercial IQC samples were used in all three methods to monitor performance. Maximum overall CVa was <12%. All methods have stated specificity for both 25-hydroxyvitamin D_2 and D_3 ($25(OH)D_2$ and $25(OH)D_3$). Because three different immunoassays were used during the study period, serum 25(OH)D was calibrated towards the "golden standard" LC-MS/MS method (isotope dilution/online solid-phase extraction liquid chromatography/tandem mass spectrometry (ID-XLC-MS/MS) to minimize possible variations. These LC-MS/MS measurements were performed at the Endocrine Laboratory of the VU University Medical Center (Amsterdam, the Netherlands) as described before [23]. The limit of quantitation (LOQ) was 4.0 nmol/L; intra-assay CV was <6%, and inter-assay CV was <8% for concentrations between 25 and 180 nmol/L $25(OH)D_2$ and $25(OH)D_3$ were measured separately. From measurements of each of the three different 25(OH)D assays used, 50 samples were selected to determine serum 25(OH)D with LC/MS–MS. Previous studies have shown that 50 samples suffice to fit an equitation for comparison between the different assays [24]. Samples were selected according to tentiles of serum 25(OH)D within each of the methods used. Within each tentile 5 samples were selected on time slots of the total period during which the method was used. This time-dependent sampling was added to minimize the contribution of inter assay variation to the comparisons made between the different assays. Calibrated serum 25(OH)D concentrations were then calculated using linear regression formulas.

Please cite this article as: Rafiq R et al., Associations of different body fat deposits with serum 25-hydroxyvitamin D concentrations, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2018.12.018

2.4. Potential confounding variables

Potential confounders were age, ethnicity, educational level, chronic diseases, smoking, alcohol use, physical activity and sex. The participants reported ethnicity by self-identification in eight categories, which were grouped into 'white' and 'other'. Educational level was reported on the questionnaire and grouped into 'high' and 'low'. Low education was defined as no education, only primary school or lower vocational education. Chronic diseases were defined as a self-reported medical history of diabetes, osteoarthritis and cardiovascular diseases (defined as myocardial infarction, angina, congestive heart failure, stroke and peripheral vascular disease). Smoking was categorized into never smoked, former smoker and current smoker. Physical activity during leisure time was reported by the participants in the Short Questionnaire to Asses Health-enhancing physical activity (SQUASH) and expressed in metabolic equivalents hours per week (MET-hours/week) [25,26]. MET is calculated by multiplying the average amount of time of participation in specified physical activities with the specific MET value of each activity [26]. Alcohol consumption was assessed using the Frequency Food Questionnaire, and categorized into 4 categories: 0 g per day, ≤1 g per day, 1–5 g per day and ≥5 g per day.

2.5. Statistical analysis

Data were analysed using STATA version 13 (StataCorp LP, College Station, TX, USA). In the NEO study individuals with a BMI of 27 kg/m^2 or higher were oversampled. To correctly represent baseline associations in the general population, adjustments for the oversampling of individuals with a BMI ≥27 kg/m^2 were made [27]. This was done by weighting all participants towards the BMI distribution of participants from the Leiderdorp municipality, whose BMI distribution was similar to the BMI distribution of the general Dutch population [28,29]. All results were based on weighted analyses. Consequently, the results apply to a population-based study without oversampling of individuals with a BMI ≥27 kg/m^2. Baseline characteristics of the study population were stratified by sex. Pearson's correlation coefficients were calculated between total body fat, abdominal subcutaneous adipose tissue, visceral adipose tissue, and hepatic fat.

Linear regression analyses were performed to examine relationships of the different fat deposits with 25(OH)D concentrations. We also calculated population-based Z-scores and standardized the values of total body fat, abdominal subcutaneous adipose tissue, visceral adipose tissue, and hepatic fat to compare the contributions of the different fat deposits with each other. Regression analyses were performed for men and women separately. The variable hepatic fat was highly skewed and therefore log transformed for the analyses. The regression coefficient of the analyses with hepatic fat can be interpreted as the difference in 25(OH)D concentration associated with a 10-fold increase in hepatic fat. First, we adjusted the crude model for age. The second model was additionally adjusted for ethnicity, educational level, chronic diseases, alcohol use, smoking, physical activity and season. Finally, to study the specific effects of the fat deposits, the models of abdominal subcutaneous and visceral adipose tissue were additionally adjusted for total body fat, and the models of hepatic fat were additionally adjusted for total body fat and visceral adipose tissue. Because serum 25(OH)D concentrations follow a sinusoidal pattern throughout the year, adjustment for season was performed using a cosinor model [30]. In this model the variable *t* date of visit (month, day, year) was transformed as 1) Sin [(2π *t)/365.25] and 2) Cos [(2π *t)/365.25] and added as covariate to the regression models. All confounding variables were tested for collinearity before entering them in the model. This was done by calculating variance inflation factors (VIFs). VIF values were considered appropriate if they were below 10 in all models.

3. Results

3.1. Baseline characteristics

From the total of 6671 participants in the NEO study, 2580 participants underwent an MRI of the abdomen. Abdominal subcutaneous and visceral adipose tissue was available for 2569 participants. Hepatic fat was available for 2083 participants. Participants with missing data for total body fat (n = 4), 25(OH)D concentrations (n = 12) and potential confounders (n = 112) were excluded. This resulted in the inclusion of 2441 participants in our analyses for total body fat, abdominal subcutaneous and visceral adipose tissue, and 1980 for hepatic fat. Table 1 presents the characteristics of the study population. Mean (SD) age and serum 25(OH)D concentrations of the study population was 56 [6] years and 70.8 nmol/L (24.2), respectively. Overall, women had higher 25(OH)D concentrations, a higher amount of total body fat and more abdominal subcutaneous adipose tissue compared to men. Men had a higher amount of visceral adipose tissue and hepatic fat compared to women.

3.2. Correlations between measures of different body fat deposits

We calculated Pearson's correlation coefficients between measures of different body fat deposits for men and women separately (Table 2). Total body fat was strongly correlated with both abdominal subcutaneous and visceral adipose tissue in both men and women, in women stronger than in men. In addition, visceral adipose tissue and abdominal subcutaneous adipose tissue were correlated in women. Hepatic fat was most strongly correlated with visceral adipose tissue.

3.3. Associations of different fat deposits with 25(OH)D concentrations

The associations of total body fat, abdominal subcutaneous adipose tissue, visceral adipose tissue and hepatic fat with serum 25(OH)D concentrations are shown separately for men and women in Table 3. Total body fat was inversely associated with 25(OH)D concentrations in women, but not in men. After adjustment for confounders, one percent higher total body fat was associated with −0.40 nmol/L (95%CI: −0.67 to −0.13) lower 25(0H)D in women. Abdominal subcutaneous adipose tissue was not associated with 25(OH)D after adjustment for total body fat. Visceral adipose tissue was inversely associated with serum 25(OH)D concentrations in both men and women. After adjustment for total body fat, one cm^2 higher visceral adipose tissue was associated with 0.05 nmol/L (−0.09 to −0.02) lower 25(OH)D in men, and 0.06 nmol/L (−0.10 to −0.01) lower 25(OH)D in women. After adjustment for total body fat and visceral adipose tissue, hepatic fat was only associated with 25(OH)D in men. A tenfold increase in hepatic fat was associated with 6.21 nmol/L (−10.70 to −1.73) lower 25(OH)D.

3.4. Associations of standardized measures of different body fat deposits with 25(OH)D concentrations

Figure 1 shows the results of the linear regression analyses with standardized values of total body fat, abdominal subcutaneous adipose tissue, visceral adipose tissue and hepatic fat for men and women separately. In women, total body fat and visceral adipose

Please cite this article as: Rafiq R et al., Associations of different body fat deposits with serum 25-hydroxyvitamin D concentrations, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2018.12.018

Table 1
Characteristics of participants in the Netherlands Epidemiology of Obesity study aged between 45 and 65 years stratified by sex.

	All	Men (47%)	Women (53%)
Age (years)	56 (6)	56 (6)	55 (6)
BMI (kg/m^2)	25.9 (4.0)	26.7 (3.6)	25.3 (4.1)
Ethnicity (% white)	96	96	95
Education level (% low)	53	50	57
Tobacco smoking (%)			
Never	40	37	43
Former	45	47	44
Current	14	16	13
Physical activity (MET-hours/week)	30.0 [16.0–51.5]	31.0 [15.8–53.0]	29.5 [16.5–49.7]
Alcohol consumption (%)			
Abstainer	1	0	1
0–1 units per day	49	35	61
1–4 units per day	47	58	38
≥5 units per day	3	6	0
Chronic disease (%)	31	29	34
Measures of different body fat deposits			
Total body fat (%)	30.8 (8.3)	24.7 (5.8)	36.2 (6.1)
aSAT (cm^2)	235.7 (96.6)	210.3 (84.8)	258.5 (98.5)
VAT (cm^2)	89.8 (56.2)	115.7 (61.1)	66.6 (40.8)
Hepatic fat (%)	2.7 [1.4–6.3]	3.8 [2.0–8.7]	1.8 [1.1–4.5]
25(OH)D (nmol/L)	70.8 (24.2)	68.6 (24.8)	72.8 (23.4)

Results are based on analyses weighted towards the BMI distribution of the general population (n = 2441; Hepatic fat n = 1980). Data are shown as mean (SD), median [interquartile range] or percentage.
MET: Metabolic equivalent; aSAT: abdominal subcutaneous adipose tissue; VAT: visceral adipose tissue; 25(OH)D: 25-hydroxyvitamin D.

Table 2
Pearson correlation coefficients between measures of different body fat deposits in men (left lower corner) and women (right upper corner) aged between 45 and 65 years participating in the NEO study.

	Total body fat	aSAT	VAT	Hepatic fat	
Total body fat		0.84	0.71	0.54	**Women**
aSAT	0.77		0.62	0.46	
VAT	0.65	0.46		0.63	
Hepatic fat[a]	0.48	0.33	0.50		
	Men				

Results are based on analyses weighted towards the BMI distribution of the general population (n = 2441; Hepatic fat n = 1980).
aSAT: abdominal subcutaneous adipose tissue; VAT: visceral adipose tissue.
[a] 10 log transformation.

tissue were similarly associated with serum 25(OH)D concentrations. One standard deviation higher total body fat (8.3%) was associated with 3.35 nmol/L (−5.58 to −1.11) lower 25(OH)D. One standard deviation higher visceral adipose tissue (56.2 cm^2) was associated with 3.20 nmol/L (−5.90 to −0.50) lower 25(OH)D. In men visceral adipose tissue was the most contributing factor. One standard deviation higher visceral adipose tissue (56.2 cm^2) was associated with 3.08 nmol/L (−4.87 to −1.28) lower 25(OH)D.

4. Discussion

In this population-based study of middle-aged men and women, we observed that the relationship between different body fat deposits and 25(OH)D concentrations was different for men and women. In women, total body fat and visceral adipose tissue were inversely related to 25(OH)D concentrations. In men, visceral adipose tissue and hepatic fat were related to 25(OH)D concentrations. In both men and women, visceral adipose tissue was most strongly associated with 25(OH)D concentrations.

Our results are in line with previous studies that showed an inverse association of total body fat with 25(OH)D concentrations in women [6,31,32]. In the National Health and Nutrition Examination Survey (NHANES) III, the relationship between total body fat and 25(OH)D concentrations in women was studied in different age

Table 3
Associations of total body fat, abdominal subcutaneous adipose tissue, visceral adipose tissue and hepatic fat with serum 25(OH)D concentrations of men and women participating in the Netherlands Epidemiology of Obesity study aged between 45 and 65 years.

		Model 1	Model 2	Model 3
		Difference in 25(OH)D concentrations (nmol/L) with 95% CI		
Total body fat (%)	Men	−0.03 (−0.36 to 0.30)	−0.08 (−0.42 to 0.26)	
	Women	−0.45 (−0.73 to −0.16)	−0.40 (−0.67 to −0.13)	
aSAT (cm^2)	Men	−0.02 (−0.04 to 0.00)	−0.01 (−0.03 to 0.01)	−0.02 (−0.05 to 0.00)
	Women	−0.03 (−0.04 to −0.01)	−0.02 (−0.04 to −0.01)	−0.01 (−0.03 to 0.02)
VAT (cm^2)	Men	−0.04 (−0.07 to −0.01)	−0.04 (−0.07 to −0.01)	−0.05 (−0.09 to −0.02)
	Women	−0.09 (−0.12 to −0.05)	−0.07 (−0.11 to −0.04)	−0.06 (−0.10 to −0.01)
Hepatic fat[a] (%)	Men	−4.26 (−9.20 to 0.68)	−6.36 (−10.54 to −2.18)	−6.21 (−10.70 to −1.73)
	Women	−5.92 (−10.31 to −1.53)	−5.73 (−9.61 to −1.86)	−2.46 (−7.38 to 2.45)

Results are based on analyses weighted towards the BMI distribution of the general population (n = 2441; Hepatic fat n = 1980) and were derived from regression coefficients with 95% confidence intervals from linear regression analyses and expressed as differences in serum 25(OH)D concentrations per unit total body fat, abdominal subcutaneous adipose tissue, visceral adipose tissue and hepatic fat. Model 1 shows the results of the regression analyses adjusted for age. Model 2 is similar to Model 1 with addition of confounders: ethnicity, educational level, chronic diseases, smoking, alcohol use, physical activity and season. Model 3 is similar to Model 2 with addition of total body fat for abdominal subcutaneous and visceral adipose tissue, and addition of total body fat and visceral adipose tissue for hepatic fat.
aSAT: abdominal subcutaneous adipose tissue; VAT: visceral adipose tissue; 25(OH)D: 25-hydroxyvitamin D.
[a] 10 Log transformation: regression coefficient can be interpreted as the difference in 25(OH)D concentration associated with a 10-fold increase in hepatic fat.

Please cite this article as: Rafiq R et al., Associations of different body fat deposits with serum 25-hydroxyvitamin D concentrations, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2018.12.018

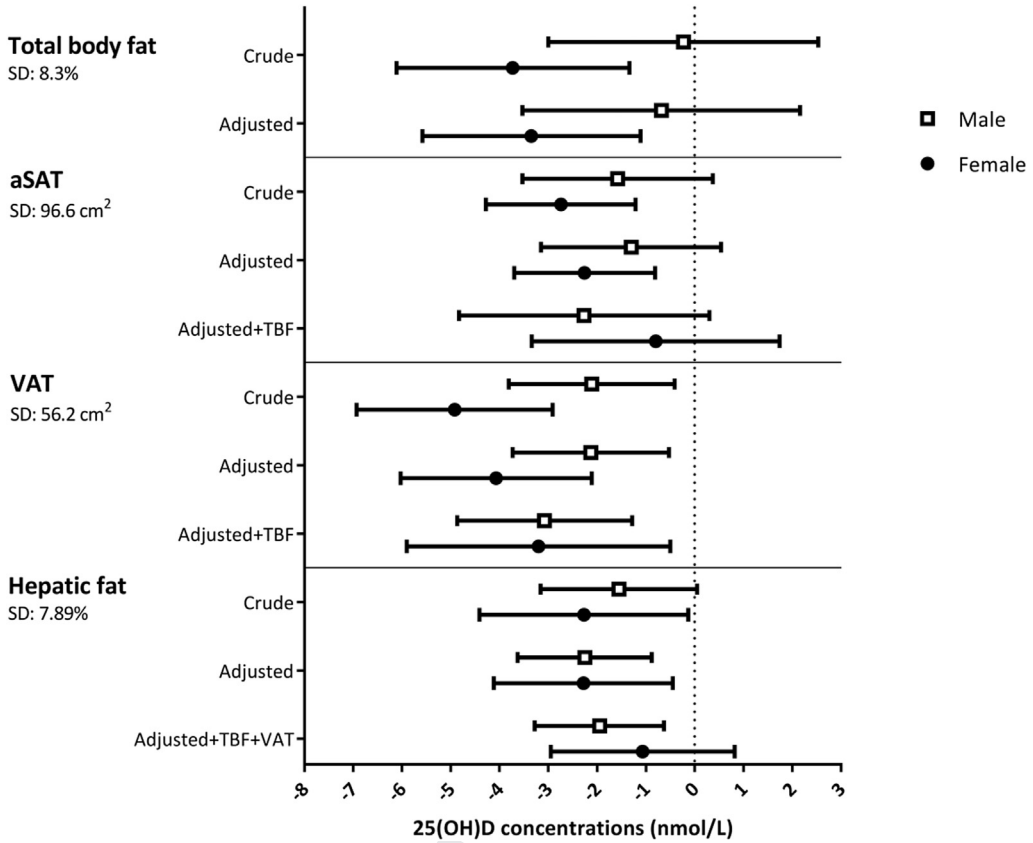

Fig. 1. Associations of standardized total body fat, abdominal subcutaneous adipose tissue, visceral adipose tissue and hepatic fat with serum 25(OH)D concentrations of men and women participating in the Netherlands Epidemiology of Obesity study aged between 45 and 65 years. Results are based on analyses weighted towards the BMI distribution of the general population (n = 2441; Hepatic fat n = 1980) and were derived from regression coefficients with 95% confidence intervals from linear regression analyses and expressed as differences in serum 25(OH)D concentrations per standard deviation of total body fat, abdominal subcutaneous and visceral adipose tissue, and hepatic fat. All analyses were adjusted for age, ethnicity, educational level, chronic diseases, smoking, alcohol use and physical activity. Analyses of abdominal subcutaneous and visceral adipose tissue were additionally adjusted for total body fat. Analyses of hepatic fat were additionally adjusted for total body fat and visceral adipose tissue. aSAT: abdominal subcutaneous adipose tissue; VAT: visceral adipose tissue; TBF: total body fat; 25(OH)D: 25-hydroxyvitamin D; SD: standard deviation.

groups and races. Although the strength of the relationship varied with race and age group, total body fat was inversely related to serum 25(OH)D concentrations in all groups [31]. This inverse association was confirmed by two other studies in women using dual energy x-ray absorptiometry (DXA) to measure total body fat [6,32]. In a cohort of older persons, total body fat was associated with 25(OH)D concentrations in both men and women [5].

In our study we found that visceral adipose tissue, and not abdominal subcutaneous adipose tissue, was associated with serum 25(OH)D in both men and women. Because total body fat was available, we could adjust for overall adiposity and study the specific contribution of the different fat deposits. Our finding is consistent with previous studies [26,32–34]. A study conducted in prediabetic individuals showed that, after correction for total body fat, visceral adipose tissue was associated with 25(OH)D concentrations, while subcutaneous adipose tissue was not associated with 25(OH)D concentrations [26]. In the Framingham study, abdominal subcutaneous and visceral adipose tissue were inversely associated with 25(OH)D concentrations [13]. Visceral adipose tissue was more strongly related to 25(OH)D concentrations compared to abdominal subcutaneous adipose tissue. However, while the relationships studied were stratified for different BMI categories, no adjustments were made for the amount of total body fat or BMI. This might explain why abdominal subcutaneous adipose tissue was related to 25(OH)D concentrations in this study. Similarly, a study in Hispanic and African-Americans showed abdominal subcutaneous and visceral adipose tissue were inversely related to 25(OH)D [34]. This study, however, did not adjust for measures of total body fat.

Finally, we also investigated the association of hepatic fat with 25(OH)D concentrations. Our study is in line with previous literature showing an inverse association of hepatic fat content with 25(OH)D concentrations [21,35]. The Changfeng Study observed a negative association between liver fat content and serum 25(OH)D concentrations in men but not in women [35]. In this study, ultrasonography was used to measure liver fat. In a study with NAFLD patients, serum 25(OH)D concentrations were inversely related to disease severity [21].

The differences between men and women found in our study might be caused by differences in body fat distribution [36]. In general, women have a higher percentage of total body fat compared to men, which could potentially explain why only women showed associations of total body fat with 25(OH)D concentrations. Additionally, men store more fat in the visceral region while women typically store more fat in the gluteal-femoral region [37]. As men generally have higher amounts of visceral adipose tissue and hepatic fat, there is a potential influence of abdominal adiposity on lower 25(OH)D concentrations [37,38].

There are a number of potential explanations for the association between obesity and decreased 25(OH)D concentrations. One hypothesis is that the fat-soluble vitamin D is sequestered in adipose tissue, which decreases its bioavailability. This suggests that 25(OH)

Please cite this article as: Rafiq R et al., Associations of different body fat deposits with serum 25-hydroxyvitamin D concentrations, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2018.12.018

D concentrations should correlate closely with subcutaneous adipose tissue measures, as it is the largest volume fat deposit in the body and on average represents 85% of total body fat [13,15,39]. This is not in line with our finding that abdominal subcutaneous adipose tissue was not associated with 25(OH)D. Other studies suggest that visceral adipose tissue attributes the most to decreased serum 25(OH)D concentrations in obesity [13,40]. In our study, linear regressions with standardized estimates showed that visceral adipose tissue was most strongly related to 25(OH)D concentrations. This might be due to the metabolic activity of visceral adipose tissue. In contrast to subcutaneous adipose tissue, visceral adipose tissue secretes more pro-inflammatory and less anti-inflammatory adipokines. It has been shown that vitamin D concentrations are inversely related to inflammatory markers [41]. This hypothesis is in line with our finding that visceral adipose tissue, and not subcutaneous adipose tissue is related to 25(OH)D concentrations [3,26]. In addition, it is suggested that inflammation may lower 25(OH)D concentrations by potential effects on vitamin D binding proteins [26].

While we did not study the prevalence of vitamin D deficiency in our study population, our study suggests that individuals with a larger amount of visceral adipose tissue are at a higher risk of lower vitamin D concentrations. As vitamin D deficiency is highly prevalent in individuals with obesity, specific attention to persons with a large amount of visceral adiposity should be given. Future studies on the effects of vitamin D supplementation should specifically target persons with obesity and study specific effects in groups with visceral and hepatic fat.

The strengths of our study include the large study population, the availability of MRI of abdominal subcutaneous and visceral adipose tissue, and proton-MRS of hepatic fat. This enabled us to investigate the individual contributions of the different fat deposits. In addition, total body fat was available which allowed us to adjust for total body fat and to investigate the specific effects of the different fat deposits on 25(OH)D concentrations. Our study cohort is very well phenotyped, which allowed adjustment for potential confounding factors. This study also has some limitations. First, due to the observational nature of this study, causal inference is not possible, as we cannot exclude the presence of residual confounding and reverse causation. While we studied the effects of different fat deposits on 25(OH)D concentrations, several studies have hypothesized that vitamin D might play a causal role in adipogenesis [3,40]. However, a systematic review of randomized controlled trials showed no effect of vitamin D supplementation on weight reduction [42]. In addition, lower serum 25(OH)D concentrations and obesity might be related as they are both associated with a sedentary lifestyle. We have tried to correct for this by adjusting for lifestyle factors such as physical activity, smoking and alcohol use. Second, we used cross-sectional images at the level of the fifth lumbar vertebra of visceral adipose tissue and abdominal subcutaneous adipose tissue and therefore did not have total subcutaneous and visceral adipose tissue. However, earlier studies have shown that such cross-sectional images are highly correlated to total volumes (correlation coefficients around 0.8) and can therefore validly represent abdominal subcutaneous and visceral adipose tissue [43]. Third, during the study period, serum 25(OH)D measurements were performed by three different assays, which could led to variations in the measurements. To minimize these possible variations, we calibrated our serum 25(OH)D measurements to the golden standard LC-MS/MS. Lastly, our study population primarily consists of white individuals. Previous studies have shown that the relationship between adiposity measures and 25(OH)D concentrations differ between ethnicities [44,45].

In conclusion, this study showed that in women total body fat and visceral adipose tissue were inversely associated with lower 25(OH)D concentrations. In men, visceral adipose tissue and hepatic were inversely related to 25(OH)D concentrations. Compared with the other body fat deposits, visceral adipose tissue was most strongly related to 25(OH)D concentrations in both men and women. This implies that specific attention for vitamin D deficiency should be given to individuals with a high amount of visceral adipose tissue. Further research should elucidate which mechanisms underlie the contribution of visceral adipose tissue to low 25(OH)D concentrations.

Declaration of interest

The authors declared no conflict of interest.

Funding

The NEO study is supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Center, and by the Leiden University, Research Profile Area 'Vascular and Regenerative Medicine'.

Acknowledgements

We express our gratitude to all participants of the Netherlands Epidemiology in Obesity study, in addition to all participating general practitioners. We furthermore thank P.R. van Beelen and all research nurses for collecting the data and P.J. Noordijk and her team for sample handling and storage and I. de Jonge for data management.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2018.12.018.

References

[1] Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr 2008;87(4):1080S–6S.
[2] Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000;894(i-xii):1–253.
[3] Earthman CP, Beckman LM, Masodkar K, Sibley SD. The link between obesity and low circulating 25-hydroxyvitamin D concentrations: considerations and implications. Int J Obes (Lond). 2012;36(3):387–96.
[4] Rajakumar K, de las Heras J, Chen TC, Lee S, Holick MF, Arslanian SA. Vitamin D status, adiposity, and lipids in black American and Caucasian children. J Clin Endocrinol Metab 2011;96(5):1560–7.
[5] Snijder MB, van Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM, et al. Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. J Clin Endocrinol Metab 2005;90(7):4119–23.
[6] Arunabh S, Pollack S, Yeh J, Aloia JF. Body fat content and 25-hydroxyvitamin D levels in healthy women. J Clin Endocrinol Metab 2003;88(1):157–61.
[7] McGill AT, Stewart JM, Lithander FE, Strik CM, Poppitt SD. Relationships of low serum vitamin D3 with anthropometry and markers of the metabolic syndrome and diabetes in overweight and obesity. Nutr J 2008;7:4.
[8] Vilarrasa N, Maravall J, Estepa A, Sanchez R, Masdevall C, Navarro MA, et al. Low 25-hydroxyvitamin D concentrations in obese women: their clinical significance and relationship with anthropometric and body composition variables. J Endocrinol Invest 2007;30(8):653–8.
[9] Pereira-Santos M, Costa PR, Assis AM, Santos CA, Santos DB. Obesity and vitamin D deficiency: a systematic review and meta-analysis. Obes Rev 2015;16(4):341–9.
[10] Kull M, Kallikorm R, Lember M. Body mass index determines sunbathing habits: implications on vitamin D levels. Intern Med J 2009;39(4):256–8.
[11] Pourshahidi LK. Vitamin D and obesity: current perspectives and future directions. Proc Nutr Soc 2015;74(2):115–24.
[12] Savastano S, Barrea L, Savanelli MC, Nappi F, Di Somma C, Orio F, et al. Low vitamin D status and obesity: role of nutritionist. Rev Endocr Metab Disord 2017.
[13] Cheng S, Massaro JM, Fox CS, Larson MG, Keyes MJ, McCabe EL, et al. Adiposity, cardiometabolic risk, and vitamin D status: the Framingham heart study. Diabetes 2010;59(1):242–8.

[14] Pelczynska M, Grzelak T, Walczak M, Czyzewska K. Hypovitaminosis D and adipose tissue - cause and effect relationships in obesity. Ann Agric Environ Med 2016;23(3):403–9.
[15] Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;72(3):690–3.
[16] Li J, Byrne ME, Chang E, Jiang Y, Donkin SS, Buhman KK, et al. 1alpha,25-Dihydroxyvitamin D hydroxylase in adipocytes. J Steroid Biochem Mol Biol 2008;112(1–3):122–6.
[17] Compher C, Badellino KO. Obesity and inflammation: lessons from bariatric surgery. JPEN - J Parenter Enter Nutr 2008;32(6):645–7.
[18] Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. Diabetes 2007;56(4):1010–3.
[19] Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nat Clin Pract Endocrinol Metab 2008;4(2):80–90.
[20] Targher G, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G, et al. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis 2007;17(7):517–24.
[21] Dasarathy J, Periyalwar P, Allampati S, Bhinder V, Hawkins C, Brandt P, et al. Hypovitaminosis D is associated with increased whole body fat mass and greater severity of non-alcoholic fatty liver disease. Liver Int 2014;34(6):e118–27.
[22] de Mutsert R, den Heijer M, Rabelink TJ, Smit JW, Romijn JA, Jukema JW, et al. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. Eur J Epidemiol 2013;28(6):513–23.
[23] Dirks NF, Vesper HW, van Herwaarden AE, van den Ouweland JM, Kema IP, Krabbe JG, et al. Various calibration procedures result in optimal standardization of routinely used 25(OH)D ID-LC-MS/MS methods. Clin Chim Acta 2016;462:49–54.
[24] Heijboer AC, Blankenstein MA, Kema IP, Buijs MM. Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin D binding protein concentration. Clin Chem 2012;58(3):543–8.
[25] Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. J Clin Epidemiol 2003;56(12):1163–9.
[26] Ceglia L, Nelson J, Ware J, Alysandratos KD, Bray GA, Garganta C, et al. Association between body weight and composition and plasma 25-hydroxyvitamin D level in the diabetes prevention program. Eur J Nutr 2017;56(1):161–70.
[27] Korn EL, Graubard BI. Epidemiologic studies utilizing surveys: accounting for the sampling design. Am J Public Health 1991;81(9):1166–73.
[28] Lumley T. Analysis of complex Survey samples http://www.jstatsoft.org/v09/i08/paper2004.
[29] VWS Mv. Hoeveel mensen hebben overgewicht?. 2015 [Web page], http://www.rivm.nl/nldemaat.
[30] Sachs MC, Shoben A, Levin GP, Robinson-Cohen C, Hoofnagle AN, Swords-Jenny N, et al. Estimating mean annual 25-hydroxyvitamin D concentrations from single measurements: the multi-ethnic study of atherosclerosis. Am J Clin Nutr 2013;97(6):1243–51.
[31] Looker AC. Body fat and vitamin D status in black versus white women. J Clin Endocrinol Metab 2005;90(2):635–40.
[32] Kremer R, Campbell PP, Reinhardt T, Gilsanz V. Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. J Clin Endocrinol Metab 2009;94(1):67–73.
[33] Caron-Jobin M, Morisset AS, Tremblay A, Huot C, Legare D, Tchernof A. Elevated serum 25(OH)D concentrations, vitamin D, and calcium intakes are associated with reduced adipocyte size in women. Obesity (Silver Spring) 2011;19(7):1335–41.
[34] Young KA, Engelman CD, Langefeld CD, Hairston KG, Haffner SM, Bryer-Ash M, et al. Association of plasma vitamin D levels with adiposity in Hispanic and African Americans. J Clin Endocrinol Metab 2009;94(9):3306–13.
[35] Wang D, Lin H, Xia M, Aleteng Q, Li X, Ma H, et al. Vitamin D levels are inversely associated with liver fat content and risk of non-alcoholic fatty liver disease in a Chinese middle-aged and elderly population: the shanghai changfeng study. PLoS One 2016;11(6):e0157515.
[36] Lemieux S, Prud'homme D, Bouchard C, Tremblay A, Despres JP. Sex differences in the relation of visceral adipose tissue accumulation to total body fatness. Am J Clin Nutr 1993;58(4):463–7.
[37] Blaak E. Gender differences in fat metabolism. Curr Opin Clin Nutr Metab Care 2001;4(6):499–502.
[38] Johnson LK, Hofso D, Aasheim ET, Tanbo T, Holven KB, Andersen LF, et al. Impact of gender on vitamin D deficiency in morbidly obese patients: a cross-sectional study. Eur J Clin Nutr 2012;66(1):83–90.
[39] Gast KB, den Heijer M, Smit JW, Widya RL, Lamb HJ, de Roos A, et al. Individual contributions of visceral fat and total body fat to subclinical atherosclerosis: the NEO study. Atherosclerosis 2015;241(2):547–54.
[40] Hao Y, Ma X, Shen Y, Ni J, Luo Y, Xiao Y, et al. Associations of serum 25-hydroxyvitamin D3 levels with visceral adipose tissue in Chinese men with normal glucose tolerance. PLoS One 2014;9(1):e86773.
[41] Calton EK, Keane KN, Newsholme P, Soares MJ. The impact of Vitamin D levels on inflammatory status: a systematic review of immune cell studies. PLoS One 2015;10(11):e0141770.
[42] Rejnmark L, Bislev LS, Cashman KD, Eiriksdottir G, Gaksch M, Grubler M, et al. Non-skeletal health effects of vitamin D supplementation: a systematic review on findings from meta-analyses summarizing trial data. PLoS One 2017;12(7):e0180512.
[43] Shen W, Chen J, Gantz M, Velasquez G, Punyanitya M, Heymsfield SB. A single MRI slice does not accurately predict visceral and subcutaneous adipose tissue changes during weight loss. Obesity (Silver Spring) 2012;20(12):2458–63.
[44] Carroll JF, Chiapa AL, Rodriquez M, Phelps DR, Cardarelli KM, Vishwanatha JK, et al. Visceral fat, waist circumference, and BMI: impact of race/ethnicity. Obesity (Silver Spring) 2008;16(3):600–7.
[45] Freedman BI, Register TC. Effect of race and genetics on vitamin D metabolism, bone and vascular health. Nat Rev Nephrol 2012;8(8):459–66.

Please cite this article as: Rafiq R et al., Associations of different body fat deposits with serum 25-hydroxyvitamin D concentrations, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2018.12.018