Novel insights into blood markers and cardiovascular disease: Results of the Netherlands Epidemiology of Obesity study

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CHAPTER 3

SEX DIFFERENCES IN BODY FAT DISTRIBUTION ARE RELATED TO SEX DIFFERENCES IN SERUM LEPTIN AND ADIPONECTIN CONCENTRATIONS

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Abstract

It is debated whether sex differences in fat-derived hormones adiponectin and leptin can be fully explained by sex differences in body fat distribution. In this analysis of the Netherlands Epidemiology of Obesity study, total body fat was assessed by bio-impedance (n=6,494), and visceral fat (VAT) by magnetic resonance imaging (n=2,516). Associations of measures of body fat and sex with serum adiponectin and leptin concentrations were examined using linear regression analysis. Sex differences were additionally adjusted for the measure of body fat that was most strongly associated with adiponectin or leptin concentrations. Median adiponectin concentrations in women and men were 10.5 mg/L (IQR, interquartile range: 7.7-13.9) and 6.1 mg/L (IQR: 4.5-8.2), the mean difference was 4.6 mg/L (95% CI: 4.3, 4.9). Median leptin concentrations in women and men were 19.2 µg/L (IQR: 11.5-30.0) and 7.1 (IQR: 4.6-11.1), the mean difference was 15.1 µg/L (95% CI: 14.4, 15.8). VAT was most strongly associated with adiponectin, while total body fat was most strongly associated with leptin. After adjustment for VAT, women had 3.8 mg/L (95% CI: 3.3, 4.3) higher adiponectin than men. After adjustment for total body fat, leptin concentrations in women were 0.4 µg/L lower than in men (95% CI: -1.2, 2.0). We observed that total body fat was strongly associated with leptin concentrations. Higher leptin concentrations in women than in men were completely explained by differences in total body fat. Visceral fat was modestly associated with adiponectin concentrations, and did not completely explain higher adiponectin concentrations in women than in men.

Introduction

The adipose tissue derived hormones adiponectin and leptin may mediate association between obesity and metabolic disease [32, 91-93]. Adiponectin is decreased in individuals with obesity, and may cause insulin resistance and diabetes [94, 95]. Leptin is associated with atherosclerosis via direct effects on endothelial function and inflammation [96].

Women have higher average leptin concentrations than men [97]. Individuals with overweight or obesity have elevated blood concentrations of leptin compared with individuals at normal weight, mainly due to increased depots of subcutaneous fat [93, 98]. Women also have higher adiponectin concentrations than men [97, 99]. Adiponectin is decreased in the presence of excess visceral fat [98]. It is unclear whether sex differences in fat distribution explain the differences in concentrations of leptin and adiponectin between sexes, as previous studies investigated non-specific measures of body fat, or did not take into account skewed distributions of adiponectin and leptin in the analyses [97, 100-103]. In the present study, we aimed to extensively investigate to what extent sex differences in adiponectin and leptin concentrations are explained by sex differences in body fat. Therefore, we investigated the associations of measures of body fat with adiponectin and leptin concentrations.

Materials and Methods

Study design and population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort study of 6,671 men and women aged between 45 and 65 years. The study design and population are described in detail elsewhere [59]. Men and women with a self-reported body mass index (BMI) of 27 kg/m^2 or higher and living in the greater area of Leiden, the Netherlands were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one municipality adjacent to Leiden (Leiderdorp, the Netherlands) were invited to participate irrespective of their BMI. Prior to the study visit, participants completed questionnaires at home with respect to demographic, lifestyle, and clinical information. Participants visited the NEO study centre after an overnight fast for an extensive physical examination including blood sampling. In a random subgroup of participants without contraindications (body circumference ≥ 170 cm, implanted metallic devices, or claustrophobia) magnetic resonance imaging (MRI) of abdominal fat was performed. Research nurses recorded current medication use by means of a medication inventory.

The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the protocol. All participants gave their written informed consent.

Data collection

Measures of body fat

Height was measured without shoes using a calibrated, vertically fixed tape measure. Body weight and percent total body fat were measured by the Tanita bio-impedance balance (TBF-310, Tanita International Division, UK) without shoes, one kilogram was subtracted to correct for the weight of clothing. Body mass index (BMI) was calculated by dividing body mass in kilograms by body height in meters squared. Total fat mass was calculated by multiplying total body fat with body weight. Waist circumference (WC) was measured halfway between the iliac crest and the lowest rib using a flexible steel tape measure.

Abdominal subcutaneous adipose tissue (aSAT) and visceral adipose tissue (VAT) were quantified by MRI (1.5 Tesla MR imaging, Philips Medical Systems) using a turbo spin echo imaging protocol in a random subgroup. At the level of the fifth lumbar vertebra, three transverse images with a slice thickness of 10 mm were obtained during a breath-hold. The fat areas were quantified by converting the number of pixels to centimetres squared for all three slices. The mean of the three slices was used in the analyses.

Blood sampling and analysis

Glucose, high-density lipoprotein cholesterol, and total cholesterol concentrations were determined in the central clinical chemistry laboratory of the LUMC by using standard methods. Low-density lipoprotein cholesterol was calculated using the Friedewald equation.

Serum adiponectin concentrations were measured using a latex particle-enhanced turbidimetric immunoassay (Cat Nr A0299, Randox Laboratories Limited) on an automated analyzer (Roche Modular P800).

The concentration of leptin was measured in serum with a human leptin competitive RadioImmunoAssay (RIA) (Cat Nr HL-81HK, Merck Millipore, Darmstadt, Germany) . The concentration was counted using a gamma counter (Wizard 2 3470, Perkin Elmer, StatLia software). Coefficients of variation for leptin as determined with internal control materials were calculated based on 22 runs over 105 days and were 12-14% at concentrations between 19 and 55 µg/l.

DNA was extracted from blood and genotyping was performed by the Centre National de Génotypage (Paris, France), using the Illumina HumanCoreExome-24 BeadChip (Illumina Inc., San Diego, CA, USA). Subsequently, genotypes were imputed to the 1000 Genome Project reference panel (v3 2011) using IMPUTE (v2.2) software. [104, 105]

Population characteristics and other variables

Ethnicity was self-identified in the questionnaire and was regrouped into white (reference) and other. Highest completed level of education was reported in ten categories according to the Dutch education system and regrouped in two categories: low education (no education, primary education or lower vocational education) and high education (other). Participants reported the frequency and duration of their physical activity during leisure time using the Short Questionnaire to Assess Health-enhancing physical activity questionnaire and this was expressed in metabolic equivalents hours per week . Smoking status was self-reported. Menopausal state was categorized in pre-, and postmenopausal state according to information on ovariectomy, hysterectomy and self-reported state of menopause in the questionnaire. Carotid intima media thickness (cIMT) was used as a measure of subclinical atherosclerosis. cIMT was assessed by ultrasonography of the common carotid arteries, using a 7.5–10 MHz linear-array probe and the Art.Lab system in B-mode setting and using a wall-track system (ART.LAB version 2.1, Esaote, Maastricht, The Netherlands) [80].

Statistical analysis

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 [60]. 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 [16]. 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.

In the present analyses, we excluded participants with missing blood samples, as well as participants who used hormone replacement therapy. Analyses with MRI measures as exposure variable were restricted to the participants who underwent MRI.

Descriptive characteristics were summarized as mean (SD), median (25^{th}, 75^{th} percentiles), or as percentage, and stratified by sex. We made scatterplots of the different measures of body fat and adiponectin and leptin concentrations. We observed extreme high leptin concentrations in a small group of women, and we decided to investigate the background of these extreme concentrations further in post-hoc analyses which are described below.

For straightforward comparison, we standardised the values of BMI, total body fat (mass), waist circumference, VAT, and aSAT and calculated z-scores with a mean of zero with a standard deviation of one. Visual inspection of histograms of adiponectin and leptin concentrations indicated that adiponectin and leptin concentration distributions were skewed. Furthermore, scatterplots of adiponectin and leptin concentrations with measures of body fat showed non-linear relations with body fat. To be able to perform linear regression analysis, we transformed adiponectin and leptin concentrations to the natural logarithm.

First, linear regression analyses were performed to examine associations between the standardized measures of body fat and transformed concentrations of adiponectin and leptin. The results were back-transformed and can be interpreted as the relative change per standard deviation of the measure of body fat. We performed all analyses separately for men and women.

Second, we performed linear regression analyses between sex and non-transformed adiponectin and leptin concentrations to assess the absolute difference in adiponectin and leptin concentrations between men and women. Subsequently, to investigate to what extent these absolute sex differences in leptin and

adiponectin concentrations are explained by differences in body fat, we adjusted these absolute differences for the measure of body fat that was most strongly associated with either adiponectin or leptin concentrations.

All crude analyses were adjusted for age, ethnicity, education, smoking status, physical activity, menopausal status, and serum C-reactive protein concentrations. Because abdominal fat is strongly related to total body fat, for the study of specific effects of abdominal fat we additionally adjusted models of VAT for total body fat, and vice versa [54]. Analyses were performed with STATA Statistical Software (Statacorp, College Station, TX, USA), version 14.

Post-hoc analyses in women with high leptin concentrations

Several women were found to have leptin concentrations exceeding 100 µg/l, which have seldom been observed in previous studies [106]. These women all had a body fat percentage in excess of 44%. In an attempt to uncover why these women had such high leptin concentrations, without complaints or clinical symptoms, we performed various post-hoc analyses on this specific group of women. These analyses were not weighted towards a normal BMI distribution. First, we compared demographic and clinical characteristics between women with and without extreme leptin concentrations, further stratified for total body fat. Second, genetic variants may explain extreme leptin concentrations [63, 107]. Therefore, we performed a candidate gene study of the genes coding for leptin (*LEP*), and leptin receptor (*LEPR*), and leptin-associated genes in a recent genome wide association study (*GCKR, CCNL1, SLC32A1, COBLL,* and *FTO*). Because the phenotype was only observed in women, and sex hormones may play a role in leptin expression, we additionally targeted the estrogen and androgen receptor genes *ESR1* and *AR1* [100]. Single nucleotide polymorphisms (SNPs) in the target genes, within 50 000 base pairs up- or downstream of the target genes or in a quantitative trait locus (QTL) according to the GTEx V6p database were indexed [108]. We calculated odds ratios with 95 percent confidence intervals for SNPs in high leptin (≥100 µg/l) compared with normal leptin concentrations (<60 µg/l). *P*-values lower than $5*10^{-5}$ were considered indicative for a suggestive signal. Genetic analyses were performed in white women to avoid admixture, and we additionally excluded women with intermediate leptin concentrations (60-100 µg/l), women with less than 44.5% total body fat, and women with insufficient genotyping quality, or indications of relatedness [109].

Results

After exclusion of 177 participants that did not meet inclusion criteria, 6,494 participants (56% women, of whom 60% were postmenopausal) were included in the present study, with a mean age of 56 (SD: 6) years, and a mean BMI of 26.3 (SD: 4.4) kg/m^2 (Table 1). Of the participants that underwent MRI examination, 52% were women. Women had more total body fat than men, while men had more visceral adipose tissue than women. The median adiponectin concentration in women was 10.5 (IQR: 7.7-13.9) mg/l, in men this was 6.1 (IQR: 4.5-8.2) mg/l. Women had a median leptin concentration of 19.2 (IQR: 11.5-30.0) µg/l, while in men this was 7.1 (IQR: 4.6-11.1) µg/l.

Table 1 – Characteristics of participants in the Netherlands Epidemiology of Obesity (NEO) study (n=6,494), stratified by sex.

	Total population	Men (44 %)	Women (56 %)
Age (y)	56 (6)	56 (6)	55 (6)
BMI (kg/m^2)	26 (4)	27 (4)	26 (5)
Total body fat (%)	32 (9)	25 (6)	37 (7)
Total body fat (kg)	25 (10)	23 (9)	28 (10)
Waist circumference (cm)	92 (13)	98 (11)	87 (13)
Abdominal subcutaneous adipose tissue (cm^2)	235 (97)	209 (81)	259 (104)
Visceral adipose tissue (cm2)	90 (56)	115 (58)	67 (43)
Menopausal status (%)	n.a.	n.a.	60
Diabetes (%)	6	7	4
Cardiovascular disease (%)	6	8	4
Fasting blood concentrations			
LDL cholesterol (mmol/L)	3.5 (1.0)	3.5 (1.0)	3.5 (1.0)
Glucose (mmol/L)	5.5 (1.0)	5.7 (1.1)	5.3 (0.8)
Leptin (µg/L)	12.1 (6.7-22.6)	7.1 (4.6-11.1)	19.2 (11.5-30.0)
Adiponectin (mg/L)	8.3 (5.6-11.9)	6.1 (4.5-8.2)	10.5 (7.7-13.9)

Values are represented as mean (SD), median (25th -75th percentile) or percentage. Results were based on analyses weighted towards a normal BMI distribution (n = 6,494).

BMI, Body mass index; LDL, Low density lipoprotein; SD, standard deviation

Measures of body fat with adiponectin concentrations in men and women

High waist circumference and visceral adipose tissue were associated with reduced adiponectin concentrations, while we observed no association for measures of overall body fat and adiponectin concentrations (Table 2). The strongest association was observed between VAT and adiponectin concentrations, one SD of VAT (56 cm^2) was associated with 0.77-fold reduced adiponectin concentrations (95% CI: 0.75, 0.79). In women, one SD increased VAT was associated with 0.80-fold (95% CI: 0.75, 0.85) reduced adiponectin concentrations, while in men, one SD increased VAT was associated with 0.94-fold (95% CI: 0.90, 0.98) reduced adiponectin concentrations.

Women had 6.1 mg/l (95% CI: 5.6, 6.6) higher serum concentrations of adiponectin than men. After additional adjustment for VAT, the association attenuated but remained 4.4 mg/l (95% CI: 3.5, 5.4) higher adiponectin concentrations than in men (Table 3).

Measures of body fat with leptin concentrations in men and women

All measures of body fat were associated in linear regression analysis with higher leptin concentrations (Table 2). Total body fat was most strongly associated with leptin: per SD increased total body fat (9%), leptin concentrations were 1.89-fold increased (95% CI: 1.79, 1.99). The associations between total body fat and leptin concentrations were similar in men and women.

Mean leptin concentrations in women were 18.6 µg/l (95% CI: 17.6, 19.7) higher than in men, while after adjustment for total body fat this attenuated (0.4 µg/l; 95% CI: -1.2, 2.0) (Table 3).

Table 2 – Regression coefficients of linear regression analysis between measures of body fat and adiponectin and leptin concentrations in the total population (n=6,494), between MRI-determined aSAT and VAT, and adiponectin and leptin concentrations (n=2,516), and for men and women separately.

		Adiponectin (%)			Leptin (%)		
		Relative change	Men (44%)	Women (56%)	Relative change	Men (44%)	Women (56%)
BMI (kg/m2)	Crude	0.86 (0.84, 0.87)	0.88 (0.85, 0.90)	0.89 (0.87, 0.90)	1.65 (1.61, 1.70)	1.92 (1.84, 2.01)	1.69 (1.65, 1.73)
SD: 4 kg/m2	Adjusted	0.97 (0.94, 1.00)	0.98 (0.92, 1.05)	0.98 (0.94, 1.01)	1.17 (1.12, 1.22)	1.24 (1.12, 1.38)	1.16 (1.11, 1.21)
Waist circumference (cm)	Crude	0.80 (0.79, 0.81)	0.89 (0.86, 0.91)	0.86 (0.85, 0.88)	1.31 (1.28, 1.34)	1.87 (1.80, 1.94)	1.68 (1.64, 1.72)
SD: 13 cm	Adjusted	0.91 (0.88, 0.94)	0.98 (0.93, 1.04)	0.88 (0.85, 0.91)	1.17 (1.12, 1.21)	1.34 (1.24, 1.44)	1.08 (1.03, 1.13)
Total body fat (%)	Crude	1.12 (1.10, 1.14)	0.86 (0.83, 0.89)	0.86 (0.83, 0.88)	2.12 (2.07, 2.16)	2.22 (2.09, 2.35)	2.14 (2.07, 2.22)
SD: 9%	Adjusted	0.96 (0.92, 1.00)	0.95 (0.90, 1.01)	1.01 (0.95, 1.07)	1.89 (1.79, 1.99)	1.94 (1.76, 2.14)	1.85 (1.73, 1.98)
Total fat mass (kg)	Crude	0.96 (0.94, 0.98)	0.88 (0.86, 0.91)	0.88 (0.86, 0.90)	2.04 (1.98, 2.09)	1.94 (1.85, 2.03)	1.80 (1.75, 1.85)
SD: 10 kg	Adjusted	0.99 (0.95, 1.02)	0.97 (0.92, 1.02)	1.04 (0.99, 1.10)	1.67 (1.60, 1.74)	1.76 (1.64, 1.89)	1.61 (1.51, 1.71)
aSAT (cm2)	Crude	0.99 (0.96, 1.02)	0.92 (0.88, 0.97)	0.90 (0.87, 0.93)	2.06 (1.98, 2.15)	2.02 (1.88, 2.17)	1.80 (1.73, 1.87)
SD: 97 cm2	Adjusted	1.01 (0.98, 1.04)	1.03 (0.98, 1.08)	1.04 (1.00, 1.08)	1.61 (1.56, 1.66)	1.70 (1.61, 1.80)	1.57 (1.51, 1.64)
VAT (cm2)	Crude	0.77 (0.75, 0.79)	0.89 (0.86, 0.92)	0.79 (0.76, 0.82)	1.24 (1.19, 1.30)	1.61 (1.52, 1.71)	1.90 (1.79, 2.02)
56 cm2	Adjusted	0.89 (0.86, 0.93)	0.94 (0.90, 0.98)	0.80 (0.75, 0.85)	1.18 (1.13, 1.23)	1.18 (1.12, 1.24)	1.17 (1.09, 1.25)

Adjusted: Adjusted for age, sex, total body fat, smoking status, physical activity, type II diabetes, fasting glucose, C-reactive protein concentrations, use of glucose lowering medication

Results were based on weighted analyses (n=6,494 for BMI, waist circumference, total body fat, and total fat mass; n=2,516 for aSAT and VAT)

aSAT, abdominal subcutaneous adipose tissue; BMI, body mass index; VAT, visceral adipose tissue; WC, waist circumference

Table 3. Absolute difference (95% confidence interval) in leptin and adiponectin concentrations between men and women, and adjusted for visceral fat area (adiponectin) or total body fat (leptin).

	Difference in adiponectin concentration (mg/l)		Difference in leptin concentration (µg/l)	
	Adjusted	+ VAT	Adjusted	+ TBF
Men versus women (ref)	6.1 (5.6, 6.6)	4.4 (3.5, 5.4)	18.6 (17.6, 19.7)	0.4 (-1.2, 2.0)

Adjusted: age, total body fat, smoking status, physical activity, type II diabetes, fasting glucose, C-reactive protein concentrations, use of glucose lowering medication and VAT (adiponectin) or TBF (leptin)

Results were based on weighted analyses (n=6,494 for BMI, waist circumference, total body fat, and total fat mass; n=2,516 for aSAT and VAT)

SD, standard deviation; TBF, total body fat

Post-hoc analysis of women with high leptin concentrations

Descriptive characteristics

Forty-four women had leptin concentrations of ≥100 µg/l, combined with total body fat was over 44.5 % (Figure 1). Table 4 shows the characteristics of women stratified by leptin concentrations and total body fat.

Women in the extreme leptin group used more thyroid hormone medication, glucose-lowering drugs, and lipid-lowering medication, and had higher fasting concentrations of glucose, insulin, LDL-cholesterol, and CRP than women in both other groups (Table 4).

Table 4 – Characteristics of 44 female participants in the NEO study with leptin concentrations ≥ 100 µg/l, compared with 3,319 female participants with lower leptin concentrations, stratified by total body fat. A, B, and C correspond with groups plotted in Figure 1.

	A	B	C
	TBF <44.5%	TBF ≥44.5%	TBF ≥44.5%
	Leptin <100 µg/l	Leptin <100 µg/l	Leptin ≥100 µg/l
	n=2,122	n=1,197	n=44
Age (years)	56 (6)	56 (6)	56 (6)
BMI (kg/m2)	28 (4)	35 (5)	42 (7)
Height (cm)	166 (6)	168 (6)	165 (6)
Thyroid hormone use (%)	5	9	16
Glucose lowering drug use (%)	3	7	9
Lipid lowering drug use (%)	9	15	20
Weight at age 20 (kg)	60 (55-65)	65 (60-74)	67 (60-79)
Total body fat (%)	39 (5)	48 (2)	51 (3)
Total fat mass (kg)	30 (7)	47 (9)	59 (15)
VAT (cm2)	78 (41)	136 (52)	136 (25)
Fasting glucose (mmol/l)	5.4 (0.9)	5.9 (1.1)	6.2 (1.5)
Fasting insulin (mmol/l)	8.1 (5.4-11.8)	13.1 (9.4-19.2)	21.8 (14.2-30.3)
Adiponectin (mg/l)	10.0 (7.3-13.3)	8.6 (6.2-11.5)	8.3 (6.2-10.2)
CRP (mg/l)	1.5 (0.8-2.8)	3.3 (1.8-5.9)	4.8 (3.0-8.7)
Leptin (µg/l)	25.2 (16.2-34.9)	45.8 (35.0-58.9)	136.5 (116.5-171.4)
cIMT (µm)	612 (86)	632 (84)	645 (90)

CRP, C-reactive protein; cIMT, carotid intima media thickness; IQR, interquartile range; SD, standard deviation; TBF, total body fat; VAT, visceral adipose tissue.

Values are represented as mean (standard deviation), or median (interquartile range)

Candidate gene study

After exclusion of men (n=3,131), women with leptin concentrations 60-100 µg/l, less than 44.5% total body fat, or who did not meet genotyping criteria (n=2,631), a total of 830 women were analysed in the candidate gene study, of whom 41 had leptin concentrations ≥100 µg/l and 789 had leptin concentrations <60 µg/l. In total, 23,076 SNPs in and in close proximity to the *LEP*, *LEPR*, *GCKR*, *CCNL1*, *SLC32A1*, *COBLL*, *FTO*, *ER* and *AR* genes and 6 cis- and trans-QTL genes

of leptin were indexed. Of the women with leptin concentrations ≥100 µg/l, 45% were heterozygous, and 10% were homozygous for the risk allele of a single genetic variant (rs4731420-G), while in the women with leptin concentrations <100 µg/l, 27% were heterozygous, and 2% were homozygous for the risk allele. This corresponded to an odds ratio of 2.8 (95% CI: 1.7, 4.6, p= $1.70*10^{-5}$) for high leptin concentrations in women (Table 5). The rs4731420 SNP, with a minor allele frequency of 0.16, is located upstream of the *LEP* gene, annotated as *LOC101928423* and is in close linkage with a known SNP that increases the risk of type 2 diabetes (rs791595; D'=1.0). In a further linear regression analysis including all men and women, one risk allele of this SNP was associated with 3 µg/l higher leptin concentrations in women (95% CI: 1, 4), but not in men (-0 µg/l per allele, 95% CI: -1, 1). No other SNPs in the *LEP*, *LEPR*, *ER*, and *AR* genes were associated with leptin concentrations.

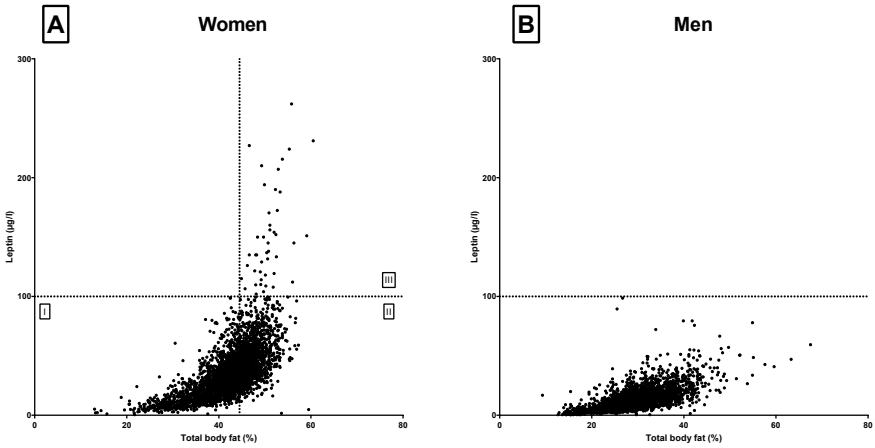

Figure 1 – Scatter plot of total body fat percentage and leptin concentrations in 3379 women (panel A) and 3115 men (panel B) in the NEO study, area III indicates a group of 44 women with leptin concentrations in excess of 100 µg/L, who were further compared with women in areas I and II in Table 4.

Table 5 – Odds ratio of the risk of leptin concentrations ≥100 µg/l in women as compared with leptin concentrations <60 µg/l (reference), related to a genetic variant in proximity to the LEP gene (n=830).

SNP	location	location relative to LEP	p-value	MAF	OR (95% CI)
rs4731420:G	7:127,863,295	-377,906 bp	1.70×10-5	0.161	2.8 (1.7, 4.7)

Analysis performed in 41 women with leptin concentrations ≥100 µg/l and 789 with leptin concentrations <60 µg/l.
GAS, genetic association study; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism

Discussion

In the present study, we confirmed that total body fat was strongly associated with leptin concentrations. We also confirmed that women had higher leptin concentrations than men, and showed that this sex difference was fully explained by differences in total body fat. Furthermore, we showed that visceral fat was most strongly associated with adiponectin concentrations. We confirmed that women had higher adiponectin concentrations than men, and we also found that this sex difference was not fully explained by differences in visceral adipose tissue. Finally, we observed remarkably high leptin concentrations in 44 women (1.3%) without clinical symptoms, but with high total body fat. A genetic variant in proximity to the *LEP* gene was associated with this phenotype only in women.

Our findings that VAT was more strongly negatively related to adiponectin concentration in women than in men were in line with findings of previous studies showing stronger correlation coefficients between visceral fat and adiponectin in women than in men [97, 99]. This sex difference in adiponectin concentrations may be due to a higher adiponectin mRNA expression in ectopic fat in women than in men [110]. However, this study also found that subcutaneous adipose tissue transcribed more mRNA than ectopic fat tissue, which seems in contrast to existing evidence that the main producer of adiponectin is visceral fat. A potential explanation could be that posttranscriptional regulation plays a major role in the secretion of adiponectin. This posttranscriptional regulation may be affected by androgens or inflammatory cytokines [111-113]. Previous studies also suggested that subcutaneous fat may modulate production of adiponectin by visceral fat [26, 102, 114], but the sex difference in our study remained after adjustment for total body fat. Further research could focus on inflammatory cytokines as a regulatory mechanism for adiponectin production in visceral fat.

In contrast with previous reports, we did not observe a sex difference in the association between total body fat and leptin concentrations [97, 101, 115]. This may in part be due to the inclusion of younger participants in previous studies than in ours, in which a sex difference in the association between total body fat and leptin concentrations may be more notable. Otherwise, the difference may be due to different methods to analyse the sex difference. Most notably, previous studies did not transform leptin concentrations in order to achieve a normal distribution, or used correlation analyses instead of multivariate regression analyses. It remains unclear which method would fit the natural relations most optimally. Sex hormones may affect leptin concentrations, which has been suggested in studies on exogenous sex hormone administration in transgender persons [116, 117].

Our result suggest that a genetic variant is associated with leptin concentrations only in women. To our knowledge, this sex-specific effect has not been described previously. This may indicate that the regulation of leptin expression is to some extent different in men and women. The SNP is located upstream of the *LEP* gene, which may have a regulatory function. A linked SNP, rs791595, has previously been linked to an increased risk of type 2 diabetes [118], suggesting a role for leptin in the development of type 2 diabetes. However, other studies suggest that leptin has a protective effect on the development of type 2 diabetes [119]. Further research is needed to unravel the interrelations between body fat, leptin concentrations, and type 2 diabetes.

The major strength of this study is the direct assessment of visceral fat using MRI, as previous literature related adiponectin specifically with visceral adipose tissue. Further strengths of the present study are the large number of participants with extensive phenotyping of potential confounding factors and leptin and adiponectin concentrations, as well as genotyping.

The present study also has several limitations that need to be considered. First, inherent to the observational cross-sectional design, we are not able to draw conclusions regarding the directionality or causality of the relations between body composition and adiponectin and leptin concentrations. Second, the present study included mainly participants of European ancestry. The associations may be different in people with other ethnic backgrounds. Last, due to the non-normal distribution of adiponectin and leptin concentrations in the study population, concentrations were log-transformed. Interactions between sex and measures of body fat may depend on appropriate transformation. Other studies have used log-transformation [120], quadratic transformation [106], or no transformation [121] in their statistical models, which may explain the difference in conclusions between different studies. However, log-transformation of biomarker data is often appropriate [122].

Conclusion

This study shows that higher concentrations of adiponectin in women than in men may not be completely explained by differences in visceral fat, while the sex dimorphism in leptin was completely explained by the difference in total body fat between women and men. Furthermore, we showed that within a sample of the general population, there are middle-aged women with high total body fat who have apparently asymptomatic extreme leptin concentrations. Our results suggest a role for other factors related to sex, most likely sex hormones, in the regulation of adiponectin and leptin concentrations.