Circulating steroid levels as correlates of adipose tissue phenotype in premenopausal women

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ABSTRACT

Background: Obesity-related alterations in the circulating steroid hormone profile remain equivocal in women. Our objective was to identify circulating steroid levels that relate to increased adiposity and altered adipose phenotype in premenopausal women. **Materials and methods:** In a sample of 42 premenopausal women (age 46±3 years; BMI 27.1±4.2 kg/m^2), 19 plasma steroids were quantified by ESI-LC-MS/MS. Body composition and fat distribution were assessed by dual-energy X-ray absorptiometry and computed tomography, respectively. Markers of adipose tissue function including adipocyte size distributions, radiological attenuation, and macrophage infiltration were also analyzed in surgically obtained visceral and subcutaneous fat samples. **Results:** Many negative correlations were observed between adiposity measurements such as BMI, body fat percentage or total abdominal adipose tissue area and plasma levels of androstenedione ($r=-0.33$ to -0.39, $p\leq0.04$), androsterone ($r=-0.30$ to -0.38, $p\leq0.05$) and plasma levels of steroid precursor pregnenolone ($r=-0.36$ to -0.46, $p\leq0.02$). Visceral adipocyte hypertrophy was observed in patients with low pregnenolone concentrations ($p<0.05$). Visceral adipose tissue radiologic attenuation, a potential marker of adipocyte size, was also positively correlated with pregnenolone levels ($r=0.33$, $p<0.05$). Low levels of pregnenolone were related to increased number of macrophages infiltrating visceral and subcutaneous adipose tissue ($p<0.05$). **Conclusion:** Plasma levels of androgens and their precursors are lower in women with increased adiposity and visceral adipocyte hypertrophy. Low circulating pregnenolone concentration may represent a marker of adipose tissue dysfunction.

Keywords: adipocyte; androstanes; computed tomography

INTRODUCTION

Excess fat accumulation on anatomical structures such as the greater omentum or mesentery, termed visceral obesity, is associated with dyslipidemia, insulin resistance and a chronic, low-grade inflammatory state in both men and women [1]. Visceral obesity also closely relates to many markers of altered adipose tissue function including adipocyte hypertrophy [2], low x-ray attenuation [3] and macrophage infiltration [4].

The sex dimorphism in body fat distribution, with predominant visceral fat accumulation in men compared to women [1, 5-8] suggests an important role of sex steroid hormones. But how endogenous steroid hormone levels relate to body fatness, fat distribution and markers of adipose tissue dysfunction remains poorly understood, especially in women. A general assumption is that abdominal, visceral obesity in women is associated with high circulating levels of androgens [9-12], but this conclusion mainly reflects observations in women suffering from the polycystic ovary syndrome (PCOS), a condition that may not be comparable to that of women without androgen excess [13]. Studies in non-PCOS women with abdominal obesity have yielded widely conflicting results, as we critically reviewed in [14]. We concluded that methodological limitations as well as variation in study design make it impossible to draw clear conclusions regarding the relation between androgens and adiposity or body fat distribution in women [14]. Among the small number of studies available on dihydrotestosterone (DHT) and androstenedione ($\Delta 4$), the levels of these steroids were either negatively related or unrelated to measurements of abdominal adiposity [14]. Discrepancies in the literature were also observed regarding the relationship between total adiposity, often estimated with the body mass index (BMI), and circulating androgens in women. Some studies observed a positive relationship between testosterone (T) and BMI [15, 16] while others did not [17, 18]. No association was observed

between BMI and other androstanes, such as Δ4 and dehydroepiandrosterone sulfate (DHEAS) [16-18], except one study showing a positive relation with DHEAS [15]. In studies using more precise methods for adiposity estimation, namely dual-energy X-ray absorptiometry (DXA) [19] or bioimpedance [20] and LC-MS/MS for steroid measurements [21], no association was observed between total adiposity and levels of T [19, 21] or other androstanes [20]. Further studies are clearly needed to address discrepancies in the circulating steroid hormone profile of healthy women as a function of total or regional adiposity measurements.

In this study, we examined the circulating steroid hormone profiles of premenopausal women to investigate their association with total adiposity, fat distribution and markers of visceral as well as subcutaneous adipose tissue dysfunction. Based on available data [14] and a previous investigation [22], we tested the hypothesis that body fatness and adipose tissue dysfunction markers relate negatively to circulating levels of steroid precursors as well as androgen and androgen metabolite levels.

SUBJECTS AND METHODS

Subjects

59 women scheduled for total hysterectomies, with or without salpingo-oophorectomy or cauterization of endometrial lesions were recruited. Women receiving oral contraceptives interrupted their treatment at least 4 weeks before blood sampling. Women suffering from cancer, Cushing syndrome, hyperthyroidism, diabetes, PCOS, or having important weight variation in the past year, and who were younger than 35 or older than 60 years, were excluded. All subjects provided written informed consent in accordance to the Ethics Committee of CHU de Québec Medical Center-Université Laval. Menopausal status was determined with a questionnaire and

plasma follicle stimulating hormone levels measured by enzyme-linked immunosorbent assay (ALPCO, Salem, USA). Postmenopausal women and women receiving gonadotropin-releasing hormone agonist treatment (n=17) were excluded, which allow for analyses in 42 premenopausal women.

Body composition and fat distribution

Body fat mass, fat percentage and lean body mass values were obtained by DXA using a Hologic QDR-4500A densitometer and Whole-body fan beam software V8.269:3 (Hologic, Bedford, MA). Computed tomography (CT) was used as previously described [23] for subcutaneous and visceral adipose tissue cross-sectional area measurements with a GE Light Speed 1.1 CT scanner (GE Medical Systems, Milwaukee, WI). An attenuation range of -190 to -30 Hounsfield units was used for quantification of adipose tissue. Mean adipose tissue attenuation was also assessed as a marker of adipose tissue radiological density [3].

Steroid hormone quantification

Plasma samples were used for the analysis of the following 19 steroids which were quantified using an extended version of the Absolute*IDQ*^{TM} Stero17 Kit and liquid chromatography with electrospray ionization tandem mass spectrometry (ESI-LC-MS/MS): aldosterone (A), Δ4, androsterone (ADT), corticosterone (CORT), cortisol (F), cortisone (E), 11-deoxycorticosterone (11-DOC), 11-deoxycortisol (11-deoxy), dehydroepiandrosterone (DHEA), DHEAS, DHT, estradiol, estrone, etiocholanolone (Et), 17α-hydroxyprogesterone (17 OH-P), progesterone, T, pregnenolone (PREG), pregnanediol. Detailed methods were previsouly described [24]. Assay validation was performed by the manufacturer according to FDA guidelines [25]. All steroid values were above the lower limit of quantification (LLOQ) except for the following: 14

participants for 11-DOC, 19 participant for Et and 10 participants for DHT. Estradiol, estrone, progesterone and pregnanediol were not examined due to potential menstrual cycle variation. Mean ± standard deviation (SD) plasma levels of circulating steroids in our sample were 2863±1451 nM for DHEAS, 10.0±7.5 nM for DHEA, 2.5±1.2 nM for Δ4, 0.7±0.2 nM for ADT, 0.3±0.3 nM for Et, 0.54±0.36 nM for T, 0.40±0.26 nM for DHT, 4.5±2.7 nM for PREG, 2.3±1.9 nM for 17-OH-P, 0.16±0.12 nM for 11-DOC, 0.82±0.59 nM for 11-deoxy, 13.1±12.0 nM for CORT; 0.61±0.36 nM for A; 585.9±219.8 nM for F and 64.7±15.9 nM for E.

Plasma cytokines

Plasma Interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) levels were measured with Quantikine HS ELISA kits (R&D, Minneapolis, MN, USA). Plasma leptin and adiponectin levels were analyzed with Human Leptin kit (EMD Millipore, St.Charles, Missouri, USA) and Human adiponectin ELISA kit (B-Bridge, Japan) respectively. Microplates were read with the Infinite M1000 (Tecan, Mauriceville, NC, USA).

Adipose tissue sampling, cell sizing and macrophage infiltration measurements

Subcutaneous fat samples were collected in the lower abdomen at the surgical incision site. Visceral fat samples were taken from the distal portion of the greater omentum. Adipocyte diameters were determined in a subsample (n=28) by histological analysis [26]. Briefly, 50 to 100 mg of the tissue was fixed in formalin, paraffin-embedded and mounted on histological slides and hematoxylin/eosin-stained or used for macrophage detection with immunohistochemical detection (see section below). The slides were scanned at 20X magnification using a NanoZoomer Hamamatsu scanner (Hamamatsu Photonics, KK, Systems Division). The smallest

and the largest diameters of 250 adipocytes per sample were manually measured using CaloPix software (Tribvn, Chatillon, France) and the mean of theses two values was used for analyses.

Total macrophage number (defined by the number of CD68+ cells) was assessed on a population subsample (n=28). Immunohistochemical detection was performed with the avidin-biotin peroxidase method (ABCYS Biospa, Milan, Italy) [27] and staining visualization with diaminobenzidine (Dako Cytokation). Macrophage infiltration was expressed as the number of macrophages per 100 adipocytes. Details of the measurement method were previously published [28, 29].

Statistical analyses

Participants were subdivided in subgroups of BMI [lean (<25 kg/m^2), overweight (25-29.9 kg/m^2) or obese (≥30 kg/m^2)]. Comparison of steroid levels among these subgroups was performed with ANOVA and Tukey HSD post-hoc test. Pairwise correlation coefficients were computed to assess associations between plasma hormone levels and adiposity measurements. For steroids that were significantly related to multiple adiposity measurements, the sample was subdivided in 2 subgroups based on median of the distribution of each steroid. The Kolmogorov-Smirnov test was used to investigate the differences in adipocyte diameter distributions between the low and high steroid subgroups. Student's t tests were used to assess the difference between these subgroups for cytokines and macrophage infiltration. Variables with a non-normal distribution as determined by a Shapiro-Wilk test were log10- or Box Cox-transformed. Distributions of attenuation measurements could not be normalized. Spearman rank correlations were computed to investigate the relationship between these variables and plasma steroid levels. Statistical analyses were performed with JMP and SAS software (SAS Institute, Cary, NC**).**

RESULTS

Study population characteristics and BMI-related differences

Anthropometric and metabolic characteristics of the participants are shown in **Table 1**. A large range of BMI and other adiposity values was observed, but women were slightly overweight on average. Plasma levels of selected steroids in BMI subgroups are shown in **Figure 1**. Several steroids tended to be reduced with increasing BMI, and significance was reached for Δ4 and PREG. A trend was observed for T. Other steroids were different as a function of BMI categories (not shown).

Adiposity measurements and androgen or androgen precursor/metabolite levels

Correlations observed between plasma steroid concentrations and total or regional adiposity measurements and adipocyte mean diameters are shown in **Tables 2A** and **2B**. Several negative correlations were found between adiposity measurements and plasma levels of Δ4. DHEA and ADT were negatively correlated with waist circumference and ADT level was also negatively correlated with abdominal subcutaneous fat area. DHT was negatively correlated with percent body fat and a trend was observed with BMI. Trends for negative correlations were observed between DHEAS, DHEA or T and some adiposity measurements. Interestingly, visceral adipocyte cell size was negatively correlated with plasma levels of Δ4 and T while no correlation was observed with subcutaneous adipocyte cell size. A trend for a negative correlation was also observed between ADT or DHT level and visceral adipocyte diameter.

Adiposity measurements and circulating steroid precursors, mineralocorticoids or glucocorticoids

Circulating levels of steroid precursor PREG were significantly and negatively correlated with all adiposity measurements, including visceral and subcutaneous fat area. 17-OH-P showed no significant correlation with adiposity indices, but a trend for a negative correlation was found with BMI. Trends for negative associations were also observed between plasma levels of mineralocorticoid precursor 11-DOC and BMI or abdominal subcutaneous fat area. Mean adipocyte diameter was not associated with these steroids, except for a trend for a negative correlation between visceral adipocyte size and PREG level. No significant correlation was observed between anthropometric parameters or mean adipocyte diameters and C21 steroid levels.

Markers of adipose tissue function

Additional significant associations between levels of androstanes and markers of adipose tissue dysfunction were observed. A trend was observed in visceral adipocyte size distribution for T and DHT, where the adipocyte size distribution seemed shifted to the right in the low T and DHT subgroups ($p<0.10$, data not shown). This suggests a tendency for adipocyte hypertrophy in the visceral fat compartment with low circulating T and DHT. A significantly higher macrophage infiltration in visceral fat was also detected for the lower circulating ADT subgroup ($p<0.05$). None of these results were observed in the subcutaneous fat depot. Plasma leptin concentration was significantly higher in the low DHT and low $\Delta 4$ subgroup ($p<0.05$, data not shown). No other trend or significant association was detected for androstanes as a function of adipose tissue function markers.

Regarding the low- and high-PREG subgroups (**Figure 3**) a shift to the right in visceral adipocyte size distribution was observed in the low PREG level subgroup. A significant positive correlation

between PREG and visceral adipose tissue attenuation was also found, consistent with the previous finding that low radiologic attenuation is a marker of adipocyte hypertrophy. Even if no significant difference was observed in circulating leptin, macrophage infiltration in visceral and subcutaneous adipose tissue was higher in participants with lower circulating PREG levels. None of these results were observed in subcutaneous adipose tissue.

DISCUSSION

We assessed plasma steroid profile alterations in relation to obesity, body fat distribution and markers of adipose tissue function in premenopausal women. We hypothesized that that body fatness and adipose tissue dysfunction markers relate negatively to circulating levels of steroid precursors as well as androgen and androgen metabolite levels. Our findings partly confirmed our initial hypothesis. We found that increased total adiposity was associated with lower levels of androgens, androgen precursors or metabolites, including Δ4 and ADT as well as adrenal steroid precursor PREG. In general, this circulating steroid profile was more closely related to markers of total body fat accumulation rather than specific abdominal or visceral fat accumulation. Yet, the association of low PREG levels with markers of adipose tissue dysfunction was more pronounced in visceral fat, even if higher macrophage infiltration was also detected in subcutaneous fat tissue in the low-PREG subgroup.

Available literature shows that circulating androgen levels are low in obese or abdominally obese men (reviewed in [14]). This association is much more equivocal in women [14]. Negative relationships between circulating DHT levels in women and adiposity indices have been reported [14], including in one women sample from our group [30]. Even if the DHT levels were negatively associated with only on adiposity measurements (percent body fat), some adipose

tissue function markers were still negatively related to DHT, namely leptin level and visceral adipocyte diameter distribution. The current analysis was done in a sample of women that was different from those previously studied, and hormone level measurements were performed in a different center with a distinct methodological approach, thereby excluding technical issues related to steroid hormone level measurements. We also report here that total T levels were not increased with visceral obesity. Considering our clear failure to find increased circulating androgen levels in abdominally obese women from two completely distinct samples, the generalized assumption that high androgen levels are associated with an android body fat distribution pattern should be critically re-examined, at least in women without androgen excess.

Despite significantly higher adiposity in women with lower androgens and precursors, not all adipose tissue function markers (notably cytokine plasma concentrations) were significant correlates of steroids in the present study. Yet, adipocyte diameter frequency distribution, a marker of adipose tissue function [31], was shifted toward adipocyte hypertrophy with low circulating levels of PREG. PREG level was also positively correlated with visceral adipose tissue radiologic attenuation. Our group previously reported a negative correlation between attenuation and adipocyte size, suggesting that attenuation could actually reflect adipocyte size [3]. We also observed lower macrophage numbers in visceral and subcutaneous adipose tissue in women with high PREG levels. The physiological mechanism underlying these associations remains unknown. Leptin has been shown to inhibit Steroidogenic Acute Regulatory (StAR) Protein both at the mRNA and protein levels in primary rat adrenocortical cells without affecting the expression of the P450 side-chain cleavage enzyme [32], which would be consistent with the present findings. In addition, leptin deficient mice have fatty ovaries with low StAR protein also suggesting impaired ovarian steroidogenesis in the obese state [33]. However, findings are not

unanimous. Adipocyte-conditioned medium as well as IL-1β, the latter which is frequently increased in the obese state, were found to stimulate StAR and/or steroidogenesis in adrenocortical cells [34, 35]. More studies are needed to clarify the mechanistic basis of the associations observed in the present study.

Conflicting results in the literature may arise from methodological issues. Immunoassays used in the majority of studies are less reliable to measure androgens in women than GC- or LC-MS/MS [36]. Furthermore, the populations examined may be difficult to compare. Some studies included hyperandrogenic women while others did not. Obesity level and menopausal status of the samples may also have led to conflicting results [37, 38]. The effect of age *per se* may also influence findings owing to age-related decreases in DHEA, Δ4 and T, which are concomitant with increased central fat accumulation, supporting the possibility that low androgen levels would be observed in women with high adiposity [39, 40]. Our sample had a mean age of 45.8±3.4 years (i.e. late premenopausal age range), which may explain some discrepancies with studies in younger premenopausal women [38, 41]. Moreover, most studies described the relation between T or free T with adiposity, but few studies examined other circulating androgens [14]. In the scant data available, other androgens do not seem to be positively linked to increased adiposity [14]. The frequent observation that free T is positively correlated with adiposity in women could be partly explained by the well-known inverse relation between sex-hormone binding globulin (SHBG) and abdominal adiposity [14], this transporter often being used to estimate the unbound T fraction. Our study detected no association between T and adiposity indices, except for a negative trend with visceral adiposity. It cannot be excluded that previous studies reporting no association between T and adiposity could have yielded results similar to ours, had they assessed a full steroid profile as opposed to T levels alone.

Low Δ4 concentration was also related to higher macrophage number in visceral adipose tissue. Limited power may have impaired our ability to detect significant differences in macrophage infiltration in stratifications based on other steroids. Published data on the association between circulating androgens and adipocyte size are scarce. Two studies did not find any association between total T, Δ4, DHEAS and adipocyte size in women [42, 43], whereas one study observed a positive correlation between percent free T and fat cell volume [43]. More studies are needed to evaluate the functional impact of androgens on adipose tissue function.

Circulating androgen metabolites are interesting measures of androgenicty or overall androgen exposure. It has been observed that levels of 5α-androstane-3α,17β-diol-glucuronide, a metabolite of DHT, are positively associated with visceral fat accumulation in men [44]. Moreover, consistent with the notion of an origin in adipose tissue, levels of this metabolite increase with weight gain [45], and decrease with weight loss [46]. Unfortunately, 3α-reduced metabolites could not be examined in the present study. Further investigation will be needed to address this question in women.

This study has some limitations. To gain access to intra-abdominal adipose tissue, women were recruited through a gynecological surgery schedule and were not all cycling normally. However, apart from their gynecological condition, they were generally healthy and showed no sign of PCOS. Abnormal menstrual cycling was then assumed to result from gynecological anomalies. The small sample also represents a limitation of this study. The limited number of participants in the obese class II and III categories also limits the conclusion regarding this specific population. However, considering the important difficulties of accessing human adipocytes, we suggest that

our study brings a significant contribution to the scant literature on circulating androgens and adipose tissue function markers in women. Trends were observed between some steroids and adiposity measurements, which may be related to sample size. These near-significant results support the main finding of the study that increased overall adiposity, rather visceral fat accumulation, is related to lower levels of androgens and adrenal steroid precursors in premenopausal women. This study is based on precise measurement techniques in a non-PCOS population and emphasizes the need to critically revise the general assumption that higher circulating androgen concentrations are found in conjunction with abdominal obesity in women.

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FOOTNOTE

Abbreviations

Δ4: Androstenedione; A: Aldosterone; ADT: Androsterone; 11-DOC: 11-deoxycorticosterone; 11-deoxy: 11-deoxycortisone; 17 OH-P: 17α-hydroxyprogesterone; BMI: Body mass index; CORT: Corticosterone; CT: Computed tomography; ELISA: Enzyme-linked immunosorbent assay; DHEA: Dehydroepiandrosterone; DHEAS: Dehydroepiandrosterone sulphate; DHT: Dihydrotestosterone; DXA: Dual-energy X-ray absorptiometry; E: Cosrtisone; ESI-LC-MS/MS: Liquid chromatography with electrospray ionization tandem mass spectrometry; Et: Etiocholanolone; F: Cortisol; IL-6: Interleukin 6; PCOS: Polycystic ovary syndrome; PREG: Pregnenolone; SD: Standard deviation; T: Testosterone; TNF-α: Tumor necrosis factor alpha

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FIGURE HEADINGS

Figure 1: Plasma steroid concentrations as a function of BMI categories. BMI units in kg/m^2

*p≤0.1 **p≤0.05 ***p≤0.01

Figure 2: Visceral adipocyte diameter distributions in low-versus-high PREG subgroups (A), correlations between PREG and visceral attenuation (B) and markers of adipose tissue function in the low-versus-high PREG subgroups (C). Visceral adipocyte diameter and macrophages: n=13 in each group; Leptin: n=21 in each group; *p≤0.1 **p≤0.05 ***p≤0.01.

Figure 1

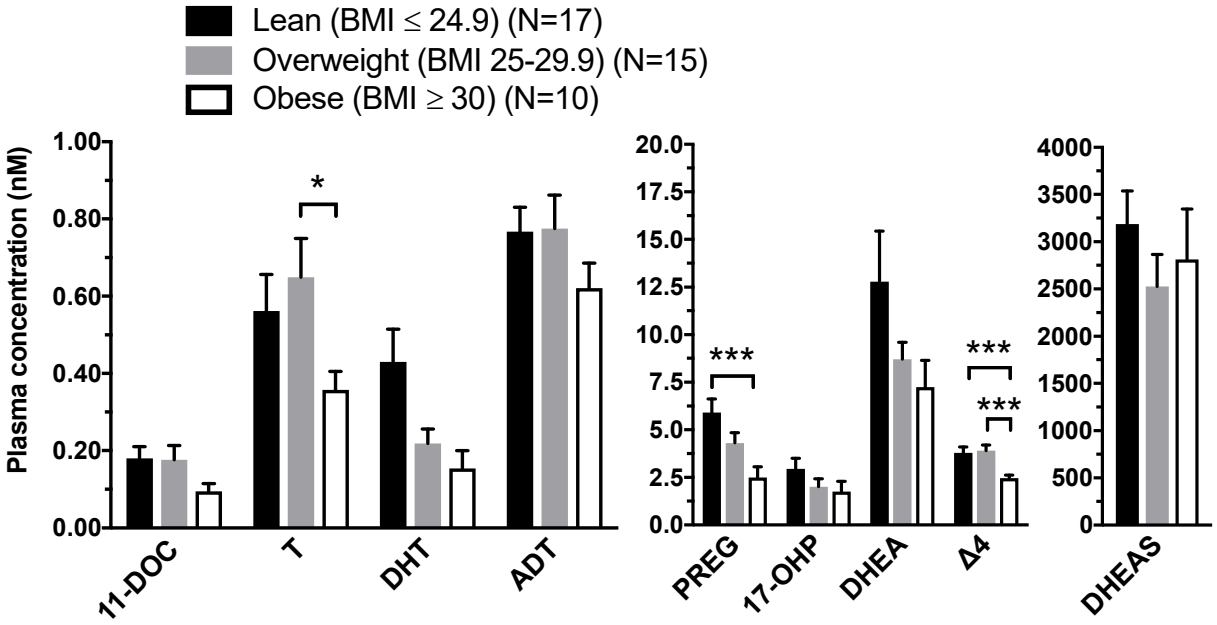
Lean (BMI ≤ 24.9) (N=17)
Overweight (BMI 25-29.9) (N=15)
Obese (BMI ≥ 30) (N=10)
Plasma concentration (nM)
1.00
0.80
0.60
0.40
0.20
0.00
*
11-DOC
T
DHT
ADT
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
***
***
***
PREG
17-OHP
DHEA
Δ4
4000
3500
3000
2500
2000
1500
1000
500
0
DHEAS

Figure 2

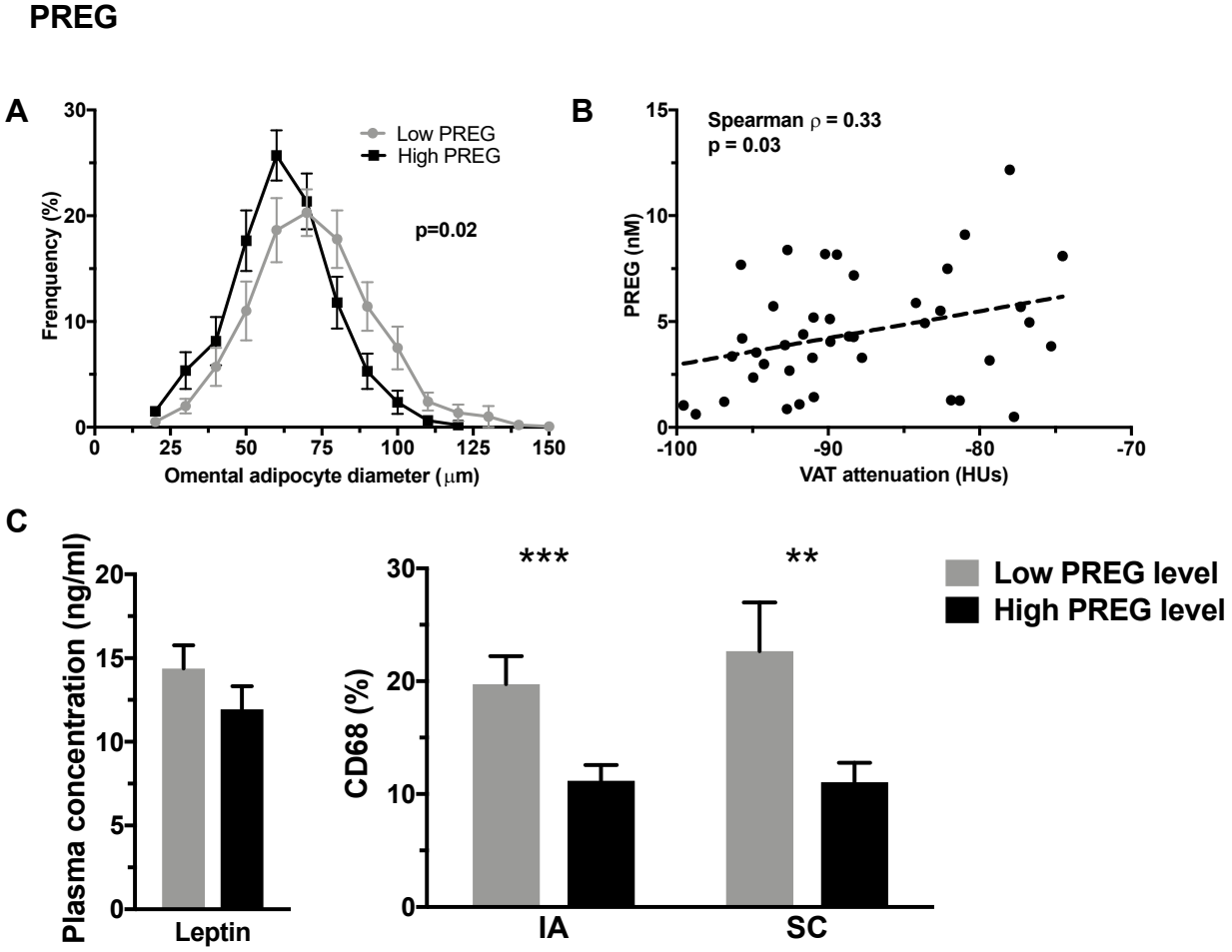
PREG
A
Low PREG
High PREG
p=0.02
Frenquency (%)
Omental adipocyte diameter (μm)
B
Spearman ρ = 0.33
p = 0.03
PREG (nM)
VAT attenuation (HUs)
C
Plasma concentration (ng/ml)
Leptin
CD68 (%)
***
**
IA
SC
Low PREG level
High PREG level

Table 1. Population characteristics (n=42)

	Mean	±	SD	Range (min-max)
Age (years)	45.8	±	3.4	38.2 – 52.3
Anthropometrics and body composition				
Waist circumference (cm)	92.7	±	11.3	72.5 – 123.5
BMI (kg/m^2)	27.1	±	4.2	20.2 – 37.0
Fat mass (kg)	25.5	±	7.3	12.4 – 43.6
Lean body mass (kg)	43.2	±	4.9	35.6 – 55.3
Total body fat percentage (%)	35.3	±	5.0	22.7 – 43.9

Abbreviations: BMI, body mass index; SD: standard deviation.

Table 2A. Pairwise correlation coefficients between circulating levels of androgens, androgen precursors or metabolites and adiposity measurements

	DHEAS	DHEA[1]	Δ4	ADT	Et[4]	T	DHT[3]
Anthropometrics and body composition							
BMI	-0.17	-0.30 [c]	-0.38 [b]	-0.23	-0.11	-0.10	-0.32[c]
Waist circumference	-0.24	-0.36 [b]	-0.38 [b]	-0.38 [b]	-0.10	-0.15	-0.23
Percent body fat	-0.29 [c]	-0.28 [c]	-0.39 [b]	-0.28 [c]	-0.16	-0.06	-0.37[a]
Computed tomography abdominal adipose tissue areas							
Subcutaneous[1]	-0.20	-0.28 [c]	-0.33 [b]	-0.30 [b]	-0.13	-0.08	-0.27
Visceral[1]	-0.05	-0.17	-0.35 [b]	-0.26 [c]	-0.10	-0.26 [c]	-0.16
Adipocyte mean diameters							
Visceral[2]	-0.21	-0.24	-0.45 [b]	-0.34 [c]	-0.10	-0.48 [a]	-0.36[c]
Subcutaneous[3]	-0.20	-0.13	-0.30	-0.28	-0.04	-0.29	-0.15

[a] $p \leq 0.01$ [b] $p \leq 0.05$ [c] $p \leq 0.1$. [1]n=41 [2]n=28 [3]n=36 [4]n=38. Abbreviations: DHEAS, dehydroepiandrosterone sulfate; DHEA, dehydroepiandrosterone; Δ4, androstenedione; ADT: androsterone; Et: etiocholanolone; T: testosterone; DHT, dihydrotestosterone. BMI and visceral abdominal tissue area were log-transformed. All steroid levels were log-transformed, except DHT which was transformed with the Box Cox formula.

Table 2B. Pairwise correlation coefficients between circulating levels of adrenal steroid precursors, mineralocorticoids or glucocorticoids and adiposity measurements

	PREG	17-OH-P	11-DOC	11-deoxy	CORT	A^2	F	E
Anthropometric parameters								
BMI	-0.43 [a]	-0.26 [c]	-0.26 [c]	-0.19	-0.21	-0.02	-0.07	-0.05
Waist circumference	-0.46 [a]	-0.24	-0.22	-0.17	-0.14	-0.12	-0.11	-0-16
Percent body fat	-0.36 [b]	-0.19	-0.21	-0.24	-0.07	-0.12	-0.18	-0.17
Abdominal tissue area								
Subcutaneous[1]	-0.41 [a]	-0.21	-0.26 [c]	-0.24	-0.14	-0.06	-0.18	-0.19
Visceral[1]	-0.38 [b]	-0.19	-0.19	-0.17	-0.20	0.02	0.01	-0.11
Adipocyte mean diameters								
Visceral[2]	-0.36 [c]	-0.32	-0.24	-0.16	-0.10	0.14	0.10	-0.22
Subcutaneous[3]	-0.27	-0.20	-0.18	-0.05	-0.01	0.15	-0.06	-0.25

[a] $p \leq 0.01$ [b] $p \leq 0.05$ [c] $p \leq 0.1$. [1]n=41 [2]n=28 [3]n=26. Abbreviations: PREG: pregnenolone; 17-OH-P: 17-OH-progesterone; 11-DOC: 11-deoxycorticosterone; 11-deoxy: 11-deoxycortisone; CORT: corticosterone; A: aldosterone; F: cortisol; E: cortisone. BMI and visceral abdominal tissue area were log-transformed. All steroids were log-transformed, except pregnenolone which was transformed with the Box Cox formula