Increased body fat mass explains the positive association between circulating estradiol and insulin resistance in postmenopausal women

Geneviève B Marchand[a,b], Anne-Marie Carreau[b,c], S John Weisnagel[d], Jean Bergeron[d], Fernand Labrie[e], Simone Lemieux[a,f], André Tchernof[a,b]

[a] *School of Nutrition, Laval University, Quebec City, Canada,* [b] *Quebec Heart and Lung Institute, Quebec City, Canada,* [c] *Department of Medicine, Sherbrooke University, Sherbrooke, Canada* [d] *CHU de Quebec-Université Laval Research Center, Quebec City, Canada,* [e] *Endoceutics Inc, Quebec City, Canada,* [f] *Institute of Nutrition and Functional Foods, Quebec City, Canada*

Email addresses: genevieve.marchand@criucpq.ulaval.ca; Anne-Marie.Carreau@USherbrooke.ca; john.weisnagel@crchudequebec.ulaval.ca; jbergeron_4@sympatico.ca; fernand.labrie@endoceutics.com; Simone.Lemieux@fsaa.ulaval.ca; andre.tchernof@criucpq.ulaval.ca

Running head: Estradiol and insulin resistance in postmenopausal women

Keywords: Estrogens, postmenopausal women, body composition, hyperinsulinemic-euglycemic clamp, computed tomography

Address for correspondence: Andre Tchernof, Ph.D.
Quebec Heart and Lung Institute - Laval University
2725 Chemin Sainte-Foy (Y-4323)
Québec, PQ
CANADA G1V 4G5
Tel: 418-656-8711
Email: andre.tchernof@criucpq.ulaval.ca

ABSTRACT

The relationship between circulating estrogen levels and cardiometabolic risk factors such as insulin resistance is unclear in postmenopausal women. High estradiol (E2) levels have been reported to predict increased risk of type 2 diabetes in this population. We aimed to examine associations among estrogen levels, adiposity measurements and cardiometabolic risk variables including insulin resistance in postmenopausal women. 101 healthy participants (mean±SD: age 57±4 years; BMI 27.9±4.8 kg/m^2) were included in the analysis. Fifteen plasma steroids or metabolites were measured by liquid chromatography-tandem mass spectrometry. Insulin sensitivity was assessed with a hyperinsulinemic-euglycemic clamp. Body composition and fat distribution were determined with hydrostatic weighing and computed tomography respectively. Blood lipids and circulating cytokines were also measured. Circulating E2 was positively correlated with all adiposity indices ($r=0.62$ to 0.42, $p<0.0001$) except waist-to-hip ratio. E2 was positively correlated with VLDL-cholesterol, plasma-, VLDL- and HDL-triglyceride levels ($r=0.31$ to 0.24, $p<0.02$) as well as with hs-CRP and IL-6 ($r=0.52$ and 0.29, $p<0.005$) and negatively with HDL-cholesterol, adiponectin and insulin sensitivity ($r=-0.36$ to -0.20, $p<0.02$). When adjusting for percent body fat, correlations between E2 and metabolic risk variables were no longer significant. Similar results were observed for circulating estrone (E1) and estrone-sulfate (E1-S) levels. In conclusion, circulating estrogen concentrations are proportional to adipose mass in postmenopausal women although they remain in the low range. Insulin resistance as well as altered blood lipids and cytokines are observed when circulating estrogen levels are high within that range, but these differences are explained by concomitant variation in total adiposity.

INTRODUCTION

Estrogens play important roles in the development and function of the reproductive system in women, but the presence of estrogen receptors in a variety of peripheral, non-reproductive tissues, notably in the liver, skeletal muscle, central nervous system and adipose tissue (16) suggests a crucial role of these steroids in metabolic homeostasis. The two most biologically important estrogens in women are estradiol (E2) and estrone (E1) (41), whereas E1-S is one of the major circulating estrogen metabolites (45). The menopause transition is characterized by important alterations in circulating hormones, including a substantial decrease in ovarian estrogen secretion (6). In postmenopausal women, positive correlations were reported between E2 or E1 concentrations and adiposity estimates such as the body mass index (BMI) or waist circumference (WC) (3,18). Conversely, intervention studies with estrogen therapy in postmenopausal women have shown either a decrease in (21) or no effect on (10,12) body fat mass. In the scant data available for E1, some studies show a decrease in plasma E1 concentration with weight loss (49) or a positive correlation between plasma E1 level and BMI (34,37). A study with E1-S reported similar results (34).

The association between plasma estrogen concentrations and cardiometabolic alterations, including insulin resistance, has also been investigated. Estrogens may improve insulin response or insulin sensitivity through various mechanisms such as increased insulin synthesis in Langerhans islets, higher GLUT4 expression in skeletal muscle or decreased hepatic gluconeogenesis and glycogenolysis (16,38), although these effects may be dependent on time since menopause (43). A recent meta-analysis coupled with a longitudinal study concluded that total circulating E2 was positively associated with the risk of type 2 diabetes in postmenopausal women independently of BMI (40). A commentary on the latter study proposed that aside from

the belief that estrogens impair carbohydrate metabolism, circulating estradiol in postmenopausal women may not be the driver of estrogen action, but the reflection of leakage from extragonadal synthesis sites including an expanded adipose tissue mass (36). This hypothesis has not been directly tested.

The relationship between circulating androgens and adipose accumulation in women is quite equivocal, likely because of methodological limitations, as we observed in a literature survey (11). A positive correlation is often reported between free testosterone (T) and abdominal fat accumulation in women without androgen excess (19,20,23,39), but we have reported a negative relationship between circulating dihydrotestosterone (DHT) levels and visceral fat accumulation (11).

In the present study, we examined the relationships among plasma estrogen levels, adiposity indices and cardiometabolic risk variables including insulin resistance in postmenopausal women. Other steroids were also examined. Based on available data, we tested the hypothesis that total body fat mass explains the positive association between circulating estrogen levels and measurements of insulin resistance in postmenopausal women.

SUBJECTS AND METHODS

Subjects

In this cross-sectional study, 131 postmenopausal women were recruited through the local newspapers of Quebec City, Canada. This is a secondary analysis of a study primarily aimed at estimating the relative contribution of visceral adiposity and insulin resistance to cardiometabolic risk (44). All subjects provided written, informed consent in accordance with the Ethics Committee of the CHU de Québec Medical Center-Université Laval. Eligibility criteria were the following: postmenopausal (absence of menses for at least one year), weight stability, no hormonal therapy or other medication for chronic diseases (coronary heart disease, dyslipidemia, diabetes, endocrine diseases). Women included in this study were generally healthy, and therefore, were not under treatment for coronary heart disease, diabetes, glucose intolerance, dyslipidemia or endocrine disorders, except hypothyroidism treated with a stable dose of thyroxine. Five women without a previous diabetes diagnosis presented hyperglycemia at the time of testing. Circulating follicle-stimulating hormone level was measured to confirm menopausal status (between 28 and 127 IU/L). Twelve participants were excluded from the present analysis because circulating hormone levels could not be measured. Moreover, despite excluding women using hormone therapy, a small number of participants had estrogen levels that were not consistent with a lack of hormone therapy. A total 14 participants were excluded from the study: 5 participants had high plasma estradiol levels (111.16 to 644.66 pg/mL); 8 participants were excluded based on a value of E2≥20 pg/mL, which corresponds to levels that are at least 2 fold higher than the values for normal postmenopausal levels (10 pg/mL) as determined by the Mayo Clinics criteria (1). A previous study has shown that the 95th percentile of E2 levels in postmenopausal women not taking hormone therapy is 9.3 pg/mL (29), consistent with the Mayo Clinics cutoff; 1 woman was excluded based on a value of E2≥12.4 pg/mL (upper

99% tolerance limit with 95% confidence) and serum DHEA ≤ 3.13 ng/mL (mean plus one SD) as determined in a previous study of postmenopausal women not using hormonal therapy (29). They were excluded from the analysis because they could mask or impact the association between endogenous estrogens and metabolic variables. Participants whose menopause resulted from hysterectomy with total oophorectomy (n=3) or hormonal therapy (n=1) were also excluded. A total of 101 postmenopausal women were included in this study.

Body composition and fat distribution

Anthropometric measurements (height, body weight, waist and hip circumferences) were obtained with standard procedures as described previously (42). Body composition measurements were obtained from body density measured by the hydrostatic weighing technique, as previously described (42). Briefly, body density was obtained with the mean of 6 measurements of the participant's body density by complete immersion in a hydrostatic tank. Pulmonary residual volume was measured with the helium dilution method before immersion. Percent body fat was calculated with the Siri equation and total body fat was obtained by multiplying percent body fat with body weight. Computed tomography (CT) was used for the measurement of abdominal and mid-thigh adipose tissue cross-sectional areas, as described (17,42). Patients were examined in the supine position with arms stretched above the head. A skeleton radiograph was used to establish the scanning position. Measurements at the L4-L5 vertebrae level and at half distance between iliac crest and knee were performed. Tissue with attenuation between -30 to -190 Hounsfield units was considered as fat. Fat area surrounded by the muscle wall in the abdominal cavity was considered as visceral fat. Subcutaneous fat was obtained by the subtraction of visceral fat from total abdominal fat area. All measurements were performed with a GE High Speed Advantage scanner (GE Medical Systems, Milwaukee, WI).

Oral glucose tolerance test (OGTT)

On the morning of a 12h-fast, a 75g OGTT was performed and blood samples were collected through a venous catheter from an antecubital vein at -15, 0, 15, 30, 45, 60, 90, 120, 150 and 180 min in EDTA-containing tubes.

Hyperinsulinemic-euglycemic clamp

The hyperinsulinemic-euglycemic clamp, as developed by DeFronzo et al (13) was used to determine insulin sensitivity, as previously described (44). After a 12h fast, insulin (Humulin R 40; 40 mU/m^2/min) was perfused for 2 hours and plasma glucose levels were monitored every 5 min using glucometer (Bayer Corporation, inc. Tarrytown, NY) and later confirmed by enzymatic method (46). 20% dextrose IV perfusion was adjusted to achieve stable euglycemia. Steady-state was attained after 2 hours. Plasma insulin concentration was later documented by radioimmunoassay in blood samples taken at 10-minute intervals and stored at -20°C. Glucose disposal rate (GDR) was determined by dividing the glucose infusion rate during the last 30 minutes of the clamp by body weight in kilograms. Insulin sensitivity (MI) was calculated by dividing GDR by the mean insulin concentration during the last 30 minutes of the clamp.

Plasma measurements

During the OGTT, plasma glucose and insulin concentrations were measured at each time point with an enzymatic method (46) and a radioimmunoassay with polyethylene glycol separation (14), respectively. C-peptide was also measured with a polyclonal antibody and ethylene glycol precipitation by a modification of the Heding method (14,22). Free fatty acids (FFA) were measured spectrophotometrically in the fasting state (Wako ChemicalsGmbH, Neuss, Germany).

Blood samples were collected on the morning of the clamp. A total of 15 steroids and metabolites were measured in plasma by liquid chromatography − tandem mass spectrometry (LC-MS/MS) (24,25). These steroids and/or metabolites are androstenedione (Δ4), androstenediol (Δ5), DHEA, DHT, E1, E2, T, androstanediol-glucuronide (AD17G), androsterone-glucuronide (ADTG), ethiocholanolone-glucuronide (ETIOG), androsterone-sulfate (ADT-S), dehydroepiandrosterone-sulfate (DHEA-S), E1-S, allopregnanolone-sulfate (AlloP-S) and pregnenolone-sulfate (Preg-S). Samples were submitted to liquid-liquid or solid phase extraction before being injected into the LC-MS/MS system as described (24,25). Validation was performed according to FDA guidelines. Mean circulating steroid values of the participants were 469.75±190.71 pg/mL for Δ4, 307.95±141.75 pg/mL for Δ5, 2704.17±1419.57 pg/mL for DHEA, 45.79±24.15 pg/mL for DHT, 23.23±9.87 pg/mL for E1, 5.21±3.30 pg/mL for E2, 158.96±81.93 pg/mL for T, 623.82±404.09 ng/mL for AD17G, 15.59±9.47 pg/mL for ADTG, 19.40±9.08 pg/mL for ETIOG, 298.46±174.11 pg/mL for ADT-S, 248.17±215.93 pg/mL for E1-S, 1.10±0.94 pg/ml for AlloP-S and 22.34±13.26 pg/mL for Preg-S.

Triglycerides (TG) and total cholesterol (TC) measurements were performed as previously described (35) using enzymatic techniques (Randox Laboratories, Crumlin, U.K). Lipoprotein fractions including very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) were isolated by ultracentrifugation and precipitation (7). High-sensitivity C-reactive protein (hs-CRP) was measured in plasma using latex-enhanced high sensitive assay with Behring Nephelometer BN-100 (Behring Diagnostic, Westwood, MA). Tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and adiponectin concentrations were assessed by ELISA with the Quantikinine HS Immunoassay kit (R&D Systems Inc.,

Minneapolis, MN).

Statistical analyses

Incremental area under the curve (IAUC) of glucose, insulin and C-peptide responses during OGTT were calculated. To elucidate the relationship between circulating estrogens and insulin resistance, insulinogenic index (IGI) (Insulin 30 min – basal / Glucose 30 min - basal), disposition index (DI) (IGI / MI) and adipo-IR (baseline FFA x fasting insulin) were calculated. Pearson correlations were computed to quantify associations between adiposity indices or cardiometabolic risk variables and steroid levels. For visual representation, further analyses of estrogen levels were performed by stratifying the sample into tertiles of E2, E1 or E1-S. Differences in IAUC, MI, baseline FFA, circulating cytokine levels or the lipid profile among estrogen tertiles were determined by ANOVA and the Tukey HSD post-hoc test. Partial correlation analyses were performed to adjust for concomitant variation in percent body fat. Moreover, stepwise multivariate linear regression models were used to adjust associations between steroid levels and metabolic risk variables for adiposity indices. Box-Cox or log10 transformation was used for non-normal distributions, as determined with the Shapiro-Wilk test. P-values ≤ 0.05 were considered significant. All statistical analyses were performed with JMP and SAS software (SAS Institute, Cary, NC).

RESULTS

Population characteristics

Population characteristics are shown in **Table 1.** Mean age was 57±4 years (range: 48-68). Women were overweight with an average BMI of 27.9±4.8 kg/m^2. Average values for plasma lipid concentrations fell within the normal ranges. Mean fasting blood glucose and blood pressure were also in the normal range.

Adiposity, metabolic alterations and circulating estrogen concentrations

Pearson correlations of levels of steroids or metabolites with adiposity or metabolic parameters are presented in **Table 2**. Plasma E2 concentration was positively and significantly correlated with all adiposity measurements except for the waist-to-hip ratio. E2 levels were 3.26±1.24, 4.55±2.81 and 8.00±3.53 pg/mL in normal weight, overweight and obese women respectively ($p<0.0001$). In each of these BMI categories, mean adipose tissue mass was 19, 28 and 39 kg respectively. The ratios of E2 concentrations divided by adipose tissue mass were 0.18, 0.16 and 0.20 pg/mL per kg of body fat in lean, overweight and obese women respectively.

Positive correlations were also observed with metabolic parameters such as fasting FFA, blood lipids, pro-inflammatory cytokines and adipo-IR. Negative correlations were detected between E2 level and HDL-C as well as MI. Much like E2, E1 concentration was positively and significantly correlated with all adiposity indices, except the waist-to-hip ratio. Circulating E1 was also positively correlated to fasting FFA, some blood lipid indices, adipo-IR and pro-inflammatory cytokine concentrations. A negative correlation was found with MI. Plasma E1-S levels were also significantly and positively correlated with all adiposity measurements. Plasma E1-S levels were significantly and positively correlated with many metabolic parameters, namely,

fasting FFA, adipo-IR, pro-inflammatory cytokine levels, glucose IAUC during the OGTT, and blood lipids. E1-S was also negatively correlated with MI, HDL-C and adiponectin concentration.

Differences in metabolic parameters according to plasma E2 concentration tertiles are shown in **Figure 1**. Women in the upper E2 tertile were characterized by higher fasting FFA, lower MI, higher adipo-IR, an altered lipid profile and higher pro-inflammatory cytokines. For E1 (**Figure 2**), women in the upper tertile had significantly higher fasting FFA, HDL-TG, hs-CRP and lower MI. Participants in the upper E1S tertile (**Figure 3**) had higher glucose IAUC during the OGTT, higher fasting FFA, lower MI, adipo-IR, an altered lipid profile and higher levels of inflammatory cytokines.

Correlations between E2 and metabolic parameters were adjusted for percent body fat mass as shown in **Figure 4**. All correlations were no longer significant after this adjustment. After adjustment of correlations between E1 and metabolic parameters for total body fat percentage, only the associations between circulating E1 and hs-CRP or HDL-TG remained significant. Finally, the positive correlations between E1-S levels and concentrations of TG, VLDL-C and VLDL-TG also remained significant after the adjustment. Multivariate linear regression analyses shown in **Table 3** were consistent with these findings. Body fat percentage explained between 37% and 17% of the variance in metabolic parameters when this variable was included along with circulating E2 in the models. In the majority of the models, E2 concentration did not have a significant contribution to metabolic parameters, except for plasma levels of TG, VLDL-TG and hs-CRP. Similarly, percent body fat explained 37 to 17% of the variance in metabolic parameters when circulating E1 was included in the models. The contribution of E1 was significant for

plasma levels of VLDL-C, HDL-TG and hs-CRP. Regarding E1-S, percent body fat again explained 37 to 17% of the variance in metabolic parameters. E1-S had a significant contribution to the variance in fasting FFA, MI, Adipo-IR, HDL-C and IL-6.

Other steroids

Correlations between androgens, androgen/estrogen metabolites or precursors and adiposity or metabolic parameters are shown in **Table 2.** Levels of some androgens and their metabolites (Δ5, DHEA, ADTG) were negatively correlated with age. Moreover, positive correlations were observed between androgen precursors (Δ5, DHEA, DHEAS) as well as glucuronide conjugates (ETIOG, ADTG, AD17G) and fat-free mass. Even if some positive correlations were observed between levels of androgens, precursors and/or metabolites with adiposity or metabolic parameters, no specific pattern could be established when examining circulating levels of these steroids and adiposity indices or risk variables.

DISCUSSION

The present data show lower clamp-measured insulin sensitivity in women with higher plasma estrogen concentrations. Higher plasma estrogen concentrations were also linked to altered blood lipids and a proinflammatory cytokine profile. However, strong and positive correlations were concomitantly observed between circulating estrogens and all indices of overall adiposity. Most of the associations between high estrogen levels and markers of altered metabolic homeostasis were explained by concomitant variation in percent body fat, suggesting a statistical contribution of adiposity to both estrogen concentrations and metabolic variables. To our knowledge, this is the first study to precisely evaluate the link between estrogens, adiposity and insulin resistance in postmenopausal women.

A highly accurate method (hydrostatic weighing) was used to estimate body fat mass, which allows, for the first time, the possibility of estimating the amount of E2 generated in the circulation as a function of total adipose tissue mass. These ratios were, on average, fairly constant in lean, overweight and obese women, suggesting that circulating estrogen concentrations are, indeed, proportional to adipose mass. However, levels clearly remain in the very low range, as demonstrated before (28). This may explain previous discrepancies in studies using less sensitive methods for estrogen measurements and anthropometric assessments of adiposity levels (3,37,50). The impact at the tissue or cell level of hormones synthetized locally through intracrine mechanisms remains of great interest for continuing research on intra-adipose synthesis of active hormones. Interestingly, the activity of aromatase is upregulated with increasing age (9) but in this study, no correlation was found between the circulating E2-to-fat-mass ratio and age. Moreover, associations between estrogens and adiposity were systematically stronger with indicators of total adiposity compared to indicators of abdominal fat accumulation,

suggesting that circulating estrogens was more closely associated with total, rather than visceral body fat mass. Our group already reported that aromatase mRNA expression is not higher (and even tends to be lower) in visceral adipose tissue than in the subcutaneous fat compartment (4).

In our study, circulating estrogen levels were positively correlated with insulin resistance. However, these associations were mostly absent when adjusting for percent body fat. These results suggest that total fat mass is the main factor in relation with insulin resistance and circulating estrogens in postmenopausal women. Many studies have reported a positive correlation between body fat mass and plasma E2 concentrations in women (2,3,8,18,37). Moreover, a recent meta-analysis has shown a positive relation between circulating E2 and type 2 diabetes in postmenopausal women (40). In that study, the relation was still significant when adjusting for BMI. In our study, women were tested with more precise methods for body composition and adipose tissue distribution, as well as insulin sensitivity and hormone concentrations. Discrepancies in the literature could also partly be explained by the assay method for plasma E2. A consensus statement by the Council of The Endocrine Society proposed that conclusions drawn from epidemiological studies are limited by the variability and quality of the assays used (47). E2 measurement is especially challenging for individuals with very low E2 concentrations such as patients taking aromatase inhibitors, children, men or postmenopausal women (47). Gas chromatography coupled with mass spectrometry is recognized as an accurate method (26), but it has been largely replaced by tandem mass spectrometry coupled with liquid chromatography (24,30,31).

Although most associations between plasma estrogen levels and metabolic parameters were dependent on total body fat mass, some significant correlations persisted between blood lipids

and E1-S concentrations in our sample. One previous study observed a positive correlation between blood TG and E1-S in African-American premenopausal women, but not in Caucasians after adjusting for BMI (32). Postmenopausal women treated with 0.625 mg of E1-S per day for 12 days had significantly higher blood TG and VLDL-C levels (33). More studies are obviously needed to assess the impact of E1-S on the lipid profile. Presumably, these effects are less dependent on fat mass both from the statistical and physiological standpoints than those of E2 and E1. One possible explanation is the longer half-life of E1-S (48) possibly providing a more representative portrait of long-term estrogen exposure. In our study, we observed that correlations with metabolic parameters remained significant for E1-S, but not with the two other estrogens, suggesting that the association among metabolic alterations, adiposity and estrogens could differ slightly from an estrogen to another. This could explain prior discrepancies in the literature on estrogens and insulin resistance or glucose tolerance.

In the present sample, plasma levels of many androstanes were positively correlated with some adipose tissue markers, but results were inconsistent and not generalized to all androgens and androgen precursors or metabolites. In a literature survey, we concluded that discrepancies in the current literature prevent any firm conclusion regarding a positive relationship between androgens and abdominal fat accumulation in healthy, non-hyperandrogenic women (11). Our group already reported a negative relation between circulating DHT and visceral fat accumulation (11), but this result was not observed in the present study. The most consistent observation regarding androgens was the positive correlation between androgen precursors or glucuronide metabolites with fat-free mass. One interventional study reported an increase in thigh muscle mass with a 12-month DHEA therapy in post-menopausal women (15). It was also reported that ADTG was correlated with fat-free mass gain (5). Moreover, we observed a negative correlation

between levels of many steroids and age or time since menopause. Decreases in circulating C19 steroids with increasing age hava been described (27). The importance of androgen metabolism in relation with body composition and body fat distribution needs to be re-examined in healthy women.

Limitations of the study should be acknowledged. The cross-sectional design does not allow us to conclude on causality effects in the estrogen-adiposity-insulin sensitivity relationship. Considering the fact that our sample included Caucasian women, results cannot be extended to other populations. Moreover, specific criteria were used to exclude women possibly taking some form of hormone replacement therapy. While this has the advantage of excluding exogenous sources of estrogens, it may also slightly underestimate the amount of estrogens measured in each BMI category as well as the correlations with adiposity and metabolic parameters. The precise methods used to measure adiposity, circulating hormones and insulin sensitivity are significant strengths of the study.

In conclusion, circulating estrogen levels are related to reduced insulin sensitivity, altered blood lipids and a pro-inflammatory cytokine profile in postmenopausal women. However, these associations are mainly explained by concomitant differences in total adiposity. Circulating estrogen concentrations are proportional to adipose mass, but they remain clearly in the very low range.

ACKNOWLEDGEMENTS

We would like to thank Sofia Laforest and Serge Simard for their contributions to this study.

FUNDING

Supported in part by funds from the Canadian Institutes of Health Research and the Heart and Stroke Foundation of Canada. GBM was funded by the Natural Sciences and Engineering Research Council of Canada, *Diabète Québec* and the Canadian Institutes of Health Research. AMC was funded by *Fond de recherche du Québec – Santé* and Canadian Diabetes Association.

DISCLOSURES

Labrie F is President of Endoceutics Inc. A.T. receives research grant support from Johnson & Johnson Medical Companies for studies unrelated to this manuscript.

Abbreviations

Δ4: androstenedione; Δ5: androstenediol; AD17G: androstanediol-glucuronide; ADTG: androsterone-glucuronide; ADTS: androsterone-sulfate; AlloP-S: allopregnanolone-sulfate; BMI: body mass index; DHEA: dehydroepiandrosterone; DHEAS: dehydroepiandrosterone-sulfate; DHT: dihydrotestosterone; DI: disposition index; E1: estrone; E1-S: estrone-sulfate; E2: estradiol; ER: estrogens receptor; ETIOG: ethiocholanolone-glucuronide; FFA: free fatty acids; GDR: glucose disposal rate; HDL: high-density lipoproteins; hs-CRP: high sensitive C-reactive protein; IAUC: incremental area under the curve; IGI: insulinogenic index; IL-6: interleukin-6; LC-MS/MS: liquid chromatography and tandem mass spectrometry; LDL: low-density lipoproteins; MI: Insulin sensitivity; OGTT: oral glucose tolerance test; PCOS: polycystic ovary syndrome; Preg-S: pregnenolone-sulfate; T: testosterone; TC: total cholesterol; TG: Triglycerides; TNF-α: tumor necrosis factor alpha; VLDL: very-low-density lipoproteins; WC: waist circumference.

REFERENCES

1. Estradiol, Serum. Mayo Medical Laboratories [Online]. 2017;. Accessed 2 nov. 2017 https://www.mayomedicallaboratories.com/test-catalog/Overview/81816.
2. Barbosa JC, Shultz TD, Filley SJ, Nieman DC. The relationship among adiposity, diet, and hormone concentrations in vegetarian and nonvegetarian postmenopausal women. *Am J Clin Nutr.* 1990;51:798-803.
3. Bezemer ID, Rinaldi S, Dossus L, et al. C-peptide, IGF-I, sex-steroid hormones and adiposity: a cross-sectional study in healthy women within the European Prospective Investigation into Cancer and Nutrition (EPIC). *Cancer Causes Control.* 2005;16:561-572.
4. Blouin K, Veilleux A, Luu-The V, Tchernof A. Androgen metabolism in adipose tissue: recent advances. *Molecular and cellular endocrinology.* 2008;301:97-103.
5. Bouchard C, Tchernof A, Tremblay A. Predictors of body composition and body energy changes in response to chronic overfeeding. *International journal of obesity (2005).* 2014;38:236-242.
6. Burger HG. The menopause: when it is all over or is it? *Aust N Z J Obstet Gynaecol.* 1994;34:293-295.
7. Burstein M, Samaille J. [On a rapid determination of the cholesterol bound to the serum alpha- and beta-lipoproteins]. *Clin Chim Acta.* 1960;5:609.
8. Castracane VD, Kraemer GR, Ogden BW, Kraemer RR. Interrelationships of serum estradiol, estrone, and estrone sulfate, adiposity, biochemical bone markers, and leptin in post-menopausal women. *Maturitas.* 2006;53:217-225.
9. Cleland WH, Mendelson CR, Simpson ER. Effects of aging and obesity on aromatase activity of human adipose cells. *The Journal of clinical endocrinology and metabolism.* 1985;60:174-177.
10. Cooper BC, Burger NZ, Toth MJ, Cushman M, Sites CK. Insulin resistance with hormone replacement therapy: associations with markers of inflammation and adiposity. *Am J Obstet Gynecol.* 2007;196:123 e121-127.
11. Côté J, Lessard J, Mailloux J, Laberge P, Rhéaume C, Tchernof A. Circulating 5a-dihydrotestosterone and abdominal adipocyte characteristics in healthy women. *Horm Mol Biol Clin Investig.* 2012;12:391-400.
12. Dedeoglu EN, Erenus M, Yoruk P. Effects of hormone therapy and tibolone on body composition and serum leptin levels in postmenopausal women. *Fertility and sterility.* 2009;91:425-431.
13. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol.* 1979;237:E214-223.
14. Desbuquois B, Aurbach GD. Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *The Journal of clinical endocrinology and metabolism.* 1971;33:732-738.
15. Diamond P, Cusan L, Gomez JL, Belanger A, Labrie F. Metabolic effects of 12-month percutaneous dehydroepiandrosterone replacement therapy in postmenopausal women. *The Journal of endocrinology.* 1996;150 Suppl:S43-50.
16. Faulds MH, Zhao C, Dahlman-Wright K, Gustafsson JA. The diversity of sex steroid action: regulation of metabolism by estrogen signaling. *The Journal of endocrinology.* 2012;212:3-12.

17. Ferland M, Despres JP, Tremblay A, et al. Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. *The British journal of nutrition.* 1989;61:139-148.
18. Freeman EW, Sammel MD, Lin H, Gracia CR. Obesity and reproductive hormone levels in the transition to menopause. *Menopause.* 2010;17:718-726.
19. Goss AM, Darnell BE, Brown MA, Oster RA, Gower BA. Longitudinal associations of the endocrine environment on fat partitioning in postmenopausal women. *Obesity (Silver Spring, Md.).* 2012;20:939-944.
20. Guthrie JR, Dennerstein L, Taffe JR, et al. Central abdominal fat and endogenous hormones during the menopausal transition. *Fertility and sterility.* 2003;79:1335-1340.
21. Hansen RD, Raja C, Baber RJ, Lieberman D, Allen BJ. Effects of 20-mg oestradiol implant therapy on bone mineral density, fat distribution and muscle mass in postmenopausal women. *Acta Diabetol.* 2003;40 Suppl 1:S191-195.
22. Heding LG. Radioimmunological determination of human C-peptide in serum. *Diabetologia.* 1975;11:541-548.
23. Janssen I, Powell LH, Kazlauskaite R, Dugan SA. Testosterone and visceral fat in midlife women: the Study of Women's Health Across the Nation (SWAN) fat patterning study. *Obesity (Silver Spring, Md.).* 2010;18:604-610.
24. Ke Y, Bertin J, Gonthier R, Simard JN, Labrie F. A sensitive, simple and robust LC-MS/MS method for the simultaneous quantification of seven androgen- and estrogen-related steroids in postmenopausal serum. *The Journal of steroid biochemistry and molecular biology.* 2014;144 Pt B:523-534.
25. Ke Y, Gonthier R, Isabelle M, et al. A rapid and sensitive UPLC-MS/MS method for the simultaneous quantification of serum androsterone glucuronide, etiocholanolone glucuronide, and androstan-3alpha, 17beta diol 17-glucuronide in postmenopausal women. *The Journal of steroid biochemistry and molecular biology.* 2015;149:146-152.
26. Labrie F, Belanger A, Belanger P, et al. Androgen glucuronides, instead of testosterone, as the new markers of androgenic activity in women. *The Journal of steroid biochemistry and molecular biology.* 2006;99:182-188.
27. Labrie F, Belanger A, Cusan L, Gomez JL, Candas B. Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. *The Journal of clinical endocrinology and metabolism.* 1997;82:2396-2402.
28. Labrie F, Belanger A, Pelletier G, Martel C, Archer DF, Utian WH. Science of intracrinology in postmenopausal women. *Menopause.* 2017;24:702-712.
29. Labrie F, Cusan L, Gomez JL, et al. Effect of intravaginal DHEA on serum DHEA and eleven of its metabolites in postmenopausal women. *The Journal of steroid biochemistry and molecular biology.* 2008;111:178-194.
30. Labrie F, Ke Y, Gonthier R, Belanger A. Letter to the Editor: Superior Mass Spectrometry-Based Estrogen Assays Should Replace Immunoassays. *The Journal of clinical endocrinology and metabolism.* 2015;100:L86-87.
31. Labrie F, Ke Y, Gonthier R, Belanger A. Why both LC-MS/MS and FDA-compliant validation are essential for accurate estrogen assays? *The Journal of steroid biochemistry and molecular biology.* 2015;149:89-91.

32. Lamon-Fava S, Barnett JB, Woods MN, et al. Differences in serum sex hormone and plasma lipid levels in Caucasian and African-American premenopausal women. *The Journal of clinical endocrinology and metabolism.* 2005;90:4516-4520.
33. Luciano AA, Miller BE, Schoenenfeld MJ, Schaser RJ, Ogen/Provera Study G. Effects of estrone sulfate alone or with medroxyprogesterone acetate on serum lipoprotein levels in postmenopausal women. *Obstet Gynecol.* 2001;97:101-108.
34. Mahabir S, Baer DJ, Johnson LL, et al. Usefulness of body mass index as a sufficient adiposity measurement for sex hormone concentration associations in postmenopausal women. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2006;15:2502-2507.
35. Major GC, Piche ME, Bergeron J, Weisnagel SJ, Nadeau A, Lemieux S. Energy expenditure from physical activity and the metabolic risk profile at menopause. *Med Sci Sports Exerc.* 2005;37:204-212.
36. Mauvais-Jarvis F. Is Estradiol a Biomarker of Type 2 Diabetes Risk in Postmenopausal Women? *Diabetes.* 2017;66:568-570.
37. McTiernan A, Wu L, Chen C, et al. Relation of BMI and physical activity to sex hormones in postmenopausal women. *Obesity (Silver Spring, Md.).* 2006;14:1662-1677.
38. Meyer MR, Clegg DJ, Prossnitz ER, Barton M. Obesity, insulin resistance and diabetes: sex differences and role of oestrogen receptors. *Acta Physiol (Oxf).* 2011;203:259-269.
39. Mongraw-Chaffin ML, Anderson CA, Allison MA, et al. Association between sex hormones and adiposity: qualitative differences in women and men in the multi-ethnic study of atherosclerosis. *The Journal of clinical endocrinology and metabolism.* 2015;100:E596-600.
40. Muka T, Nano J, Jaspers L, et al. Associations of Steroid Sex Hormones and Sex Hormone-Binding Globulin With the Risk of Type 2 Diabetes in Women: A Population-Based Cohort Study and Meta-analysis. *Diabetes.* 2017;66:577-586.
41. Nelson LR, Bulun SE. Estrogen production and action. *J Am Acad Dermatol.* 2001;45:S116-124.
42. Pascot A, Despres JP, Lemieux I, et al. Contribution of visceral obesity to the deterioration of the metabolic risk profile in men with impaired glucose tolerance. *Diabetologia.* 2000;43:1126-1135.
43. Pereira RI, Casey BA, Swibas TA, Erickson CB, Wolfe P, Van Pelt RE. Timing of Estradiol Treatment After Menopause May Determine Benefit or Harm to Insulin Action. *The Journal of clinical endocrinology and metabolism.* 2015;100:4456-4462.
44. Piche ME, Weisnagel SJ, Corneau L, Nadeau A, Bergeron J, Lemieux S. Contribution of abdominal visceral obesity and insulin resistance to the cardiovascular risk profile of postmenopausal women. *Diabetes.* 2005;54:770-777.
45. Purdy RH, Engel LL, Oncley JL. The characterization of estrone sulfate from human plasma. *J Biol Chem.* 1961;236:1043-1050.
46. Richterich R, Kuffer H, Lorenz E, Colombo JP. [The determination of glucose in plasma and serum (hexokinase-glucose-6-phosphate dehydrogenase method) with the Greiner electronic selective analyzer GSA II (author's transl)]. *Z Klin Chem Klin Biochem.* 1974;12:5-13.

47. Rosner W, Hankinson SE, Sluss PM, Vesper HW, Wierman ME. Challenges to the measurement of estradiol: an endocrine society position statement. *The Journal of clinical endocrinology and metabolism.* 2013;98:1376-1387.
48. Ruder HJ, Loriaux L, Lipsett MB. Estrone sulfate: production rate and metabolism in man. *The Journal of clinical investigation.* 1972;51:1020-1033.
49. Stolzenberg-Solomon RZ, Falk RT, Stanczyk F, et al. Sex hormone changes during weight loss and maintenance in overweight and obese postmenopausal African-American and non-African-American women. *Breast Cancer Res.* 2012;14:R141.
50. Tworoger SS, Eliassen AH, Missmer SA, et al. Birthweight and body size throughout life in relation to sex hormones and prolactin concentrations in premenopausal women. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2006;15:2494-2501.

559 **Table 1.** Study sample characteristics (n=101)

	Mean ± SD	Range (min-max)
Age (years)	57 ± 4	48 - 68
Time since menopause (years)	8.6 ± 6.9	0.3 - 32
Anthropometric variables		
Weight (kg)	71 ± 13	48 - 122
Waist circumference (cm) [a]	89 ± 12	66 - 119
BMI (kg/m^2)	27.9 ± 4.8	19.0 - 48.2
Metabolic parameters		
Cholesterol (mmol/L)	5.53 ± 0.90	3.46 - 7.48
LDL cholesterol (mmol/L)	3.63 ± 0.81	1.47 - 5.64
HDL cholesterol (mmol/L)	1.41 ± 0.36	0.88 - 2.69
Triglyceride (mmol/L)	1.29 ± 0.65	0.51 - 3.50
Systolic blood pressure (mmHg) [b]	130 ± 15	105 - 170
Diastolic blood pressure (mmHg) [b]	82 ± 7	60 -100
Insulin (at the OGTT) (pmol/L) [a]	73.9 ± 33.2	0.0 -185.0
Fasting glucose (at the OGTT) (mmol/L)	5.57 ± 0.79	3.85 - 9.50
Estrogen concentrations		
Estradiol (pg/mL)	5.21 ± 3.30	1.41 - 17.14
Estrone (pg/mL)	23.23 ± 9.87	7.59 - 66.48
Estrone-sulfate (pg/mL)	248.17 ± 215.93	32.82 - 1385.46

560 [a] n=97, [b] n=95
561
562

563 **Table 2.** Pairwise correlations between steroids or steroid metabolites and adiposity measurements as well as metabolic parameters (n=101)

	E2	E1	E1-S	Δ4	Δ5	DHEA	DHEAS	T	DHT	ETIOG	ADTG	AD17G	ADTS
Age (years)	0.10	0.24*	0.16	-0.07	-0.23*	-0.22*	-0.15	0.12	-0.09	-0.18	-0.21*	-0.14	-0.06
Time since menopause (years)[a]	0.02	0.04	-0.04	-0.16	-0.08	-0.15	-0.10	-0.06	-0.11	-0.13	-0.10	-0.04	-0.19
Anthropometric parameters and body composition													
BMI (kg/m^2)	0.62***	0.54***	0.57***	0.06	0.12	0.05	0.11	0.02	-0.12	-0.10	0.13	-0.02	0.21*
Waist Circumference (cm)[a]	0.49***	0.41***	0.56***	0.02	0.15	0.12	0.15	-0.03	-0.11	-0.02	0.19	0.05	0.23*
Waist-to-hip ratio	0.13	0.07	0.33**	-0.10	0.02	0.06	0.09	-0.16	-0.12	-0.10	0.01	-0.02	0.15
Fat-free mass (kg)[b]	0.16	0.19	0.23*	0.12	0.28*	0.31*	0.29*	-0.07	0.08	0.24*	0.28*	0.22*	0.19
Fat mass (kg)[b]	0.53***	0.50***	0.49***	0.10	0.18	0.09	0.11	0.05	-0.07	-0.01	0.17	0.06	0.17
Body fat percentage (%)[b]	0.56***	0.51***	0.48***	0.08	0.10	0.01	0.03	0.08	-0.10	-0.08	0.10	-0.0002	0.13
Adipose tissue area (cm^2)													
Subcutaneous[c]	0.57***	0.50***	0.49***	0.11	0.14	0.08	0.13	0.08	-0.05	0.05	0.25*	0.09	0.26*
Visceral[c]	0.42***	0.30*	0.47***	0.01	0.07	0.07	0.08	-0.02	-0.11	-0.06	0.08	0.02	0.15
Mid-thigh[d]	0.60***	0.56***	0.42***	0.19	0.18	0.12	0.16	0.21*	0.09	0.04	0.26*	0.13	0.30*
Metabolic parameters													
IAUC glucose (mM x min)	0.17	0.17	0.34**	-0.12	0.004	-0.04	0.09	-0.13	-0.06	-0.11	0.05	0.06	0.15
IAUC insulin (mM x min)[e]	0.07	0.002	0.10	-0.09	-0.05	-0.03	-0.04	-0.05	-0.06	-0.21*	-0.14	-0.07	0.03
IAUC c-peptid (mM x min)	0.02	-0.04	0.04	-0.04	-0.03	-0.01	0.03	-0.12	-0.02	-0.11	0.02	0.03	0.09
Fasting free fatty acid (mM)[a]	0.25*	0.31*	0.20*	-0.02	-0.04	-0.16	-0.11	0.08	-0.10	-0.17	0.02	-0.06	-0.13
Insulin sensitivity (MI)[f]	-0.36**	-0.29*	-0.38***	0.005	-0.04	0.02	-0.05	-0.02	0.10	0.14	-0.04	-0.09	-0.16
Insulinogenic index (IGI)[c]	-0.09	-0.13	-0.14	0.01	-0.05	-0.004	-0.10	0.04	-0.04	-0.13	-0.11	-0.11	-0.05
Disposition Index (DI)[b]	0.12	0.06	0.11	-0.01	-0.05	-0.04	-0.05	0.03	-0.10	-0.17	-0.04	-0.03	0.04
Adipo-IR[c]	0.28*	0.24*	0.29*	0.002	-0.07	-0.07	-0.06	0.02	-0.12	-0.13	0.06	-0.01	0.01
Triglycerides (nM)	0.31*	0.27*	0.36**	0.09	0.07	0.08	0.08	-0.01	-0.15	0.04	0.07	0.05	0.06
VLDL-Cholesterol (nM)	0.30*	0.26*	0.33**	0.13	0.13	0.13	0.11	0.03	-0.08	0.05	0.11	0.08	0.10
VLDL-Triglycerides (nM)	0.31*	0.27*	0.36**	0.09	0.09	0.08	0.09	0.002	-0.14	0.02	0.08	0.07	0.08
LDL-Cholesterol (nM)	-0.10	-0.15	-0.13	-0.15	-0.16	-0.17	-0.19	-0.12	-0.22*	-0.06	-0.11	-0.18	-0.21*
LDL-Triglycerides (nM)	0.14	0.12	0.22*	-0.002	-0.02	0.003	0.05	-0.16	-0.16	0.03	0.001	-0.06	-0.03
HDL-Cholesterol (nM)	-0.23*	-0.11	-0.22*	-0.04	-0.13	-0.13	-0.14	0.12	0.002	-0.0006	-0.15	-0.11	-0.09
HDL-Triglycerides (nM)	0.24*	0.30*	0.25*	0.14	-0.05	0.003	-0.08	0.03	-0.18	0.02	0.02	-0.10	-0.04
hs-CRP (mg/L)[c]	0.52***	0.46***	0.40***	0.13	0.12	0.10	0.11	0.07	-0.02	-0.11	0.08	0.07	0.06
IL-6 (pg/mL)[a]	0.29*	0.25*	0.32*	0.01	0.04	0.002	-0.05	0.03	-0.11	-0.18	-0.03	-0.15	-0.06
TNF-α (pg/mL)	0.13	0.15	0.22*	-0.003	-0.06	-0.09	0.02	0.09	-0.15	-0.07	-0.08	-0.13	0.02
Adiponectin (µg/mL)	-0.20*	-0.19	-0.35**	-0.13	-0.22*	-0.27*	-0.26*	0.07	0.03	-0.07	-0.16	-0.14	-0.19

564 *** $p \leq 0.0001$, ** $p \leq 0.001$, * $p \leq 0.05$. [a] n=100, [b] n=98, [c] n=96, [d] n=93, [e] n=97, [f] n=99.Abbreviations: E2, estradiol; E1, estrone; E1-S, estrone-sulfate; Δ4, androstenedione; Δ5, androstenediol; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone- sulfate; T: testosterone; DHT, dihydrotestosterone; ETIOG, ethiocholanolone-glucuronide ; ADTG, androsterone-glucuronide; AD17G; androstenediol-glucuronide; ADTS, androsterone-sulfate; BMI, Body mass index; IAUC: incremental area under the curve; VLDL: very-low-density lipoproteins; LDL: low-density lipoproteins; HDL: high-density lipoproteins; hs-CRP: high sensitive C-reactive protein; IL-6: interleukin-6; TNF-α: tumor necrosis factor alpha. All metabolic variables except age, waist circumference, body fat percentage, adipose tissue areas, free fatty acids and LDL-cholesterol were transformed. Time since menopause, IAUC, MI, Adipo-IR, triglycerides, IL-6 and TNF-α were transformed with the Box Cox formula while the others were log-transformed. All steroid levels were log-transformed, except E2, T,, DHEAS and ADTS, which were transformed with the Box Cox formula.

Table 3. Multivariate regression models establishing the contribution of each estrogen and body fat percentage to the variance in metabolic parameters. In each model, the metabolic parameter was used as the dependent variables. Percent body fat and the estrogen level was used as the independent variables. In model 13, the dependent variable was the estrogen level and the independent variables were L4-L5 visceral fat area and percent body fat.

	E2		E1		E1-S	
	r^2	p-value	r^2	p-value	r^2	p-value
Model 1 – IAUC glucose						
Estrogen	-	-	-	-	0.11	0.001
Percent fat	-	-	-	-	0.01	0.25
Model 2 - fasting FFA						
Estrogen	0.0006	0.79	0.02	0.18	0.0004	0.84
Percent fat	0.17	≤ 0.0001	0.17	≤ 0.0001	0.17	≤ 0.0001
Model 3 - MI						
Estrogen	0.01	0.25	0.003	0.57	0.03	0.05
Percent fat	0.19	≤ 0.0001	0.19	≤ 0.0001	0.19	≤ 0.0001
Model 4 - Adipo-IR						
Estrogen	0.0006	0.79	0.002	0.63	0.0007	0.78
Percent fat	0.22	≤ 0.0001	0.22	≤ 0.0001	0.22	≤ 0.0001
Model 5 - TG						
Estrogen	0.10	0.002	0.02	0.17	0.15	0.0001
Percent fat	0.02	0.16	0.08	0.004	0.01	0.20
Model 6 - VLDL-C						
Estrogen	0.09	0.002	0.07	0.01	0.12	0.0004
Percent fat	0.01	0.30	0.02	0.14	0.01	0.31
Model 7 - VLDL-TG						
Estrogen	0.10	0.002	0.02	0.18	0.14	0.0001
Percent fat	0.02	0.18	0.08	0.004	0.01	0.21
Model 8 - LDL-TG						
Estrogen	-	-	-	-	0.07	0.01
Percent fat	-	-	-	-	0.0008	0.78
Model 9 - HDL-C						
Estrogen	0.006	0.43	-	-	0.009	0.33
Percent fat	0.07	0.01	-	-	0.07	0.01
Model 10 - HDL-TG						
Estrogen	0.07	0.008	0.08	0.001	0.08	0.004
Percent fat	0.01	0.30	0.007	0.41	0.01	0.25
Model 11 - hs-CRP						
Estrogen	0.04	0.02	0.03	0.02	0.01	0.15
Percent fat	0.37	≤ 0.0001	0.37	≤ 0.0001	0.37	≤ 0.0001
Model 12 - IL-6						
estrogen	0	0.97	0	0.95	0.009	0.29
Percent fat	0.25	≤ 0.0001	0.25	≤ 0.0001	0.25	≤ 0.0001
Model 13 – Estrogen						
L4-L5 visceral fat area	0.0001	0.91	0.01	0.24	0.02	0.13
Percent fat	0.30	≤ 0.0001	0.24	≤ 0.0001	0.23	≤ 0.0001

Models were not computed if the univariate correlations were not significant

FIGURE HEADINGS

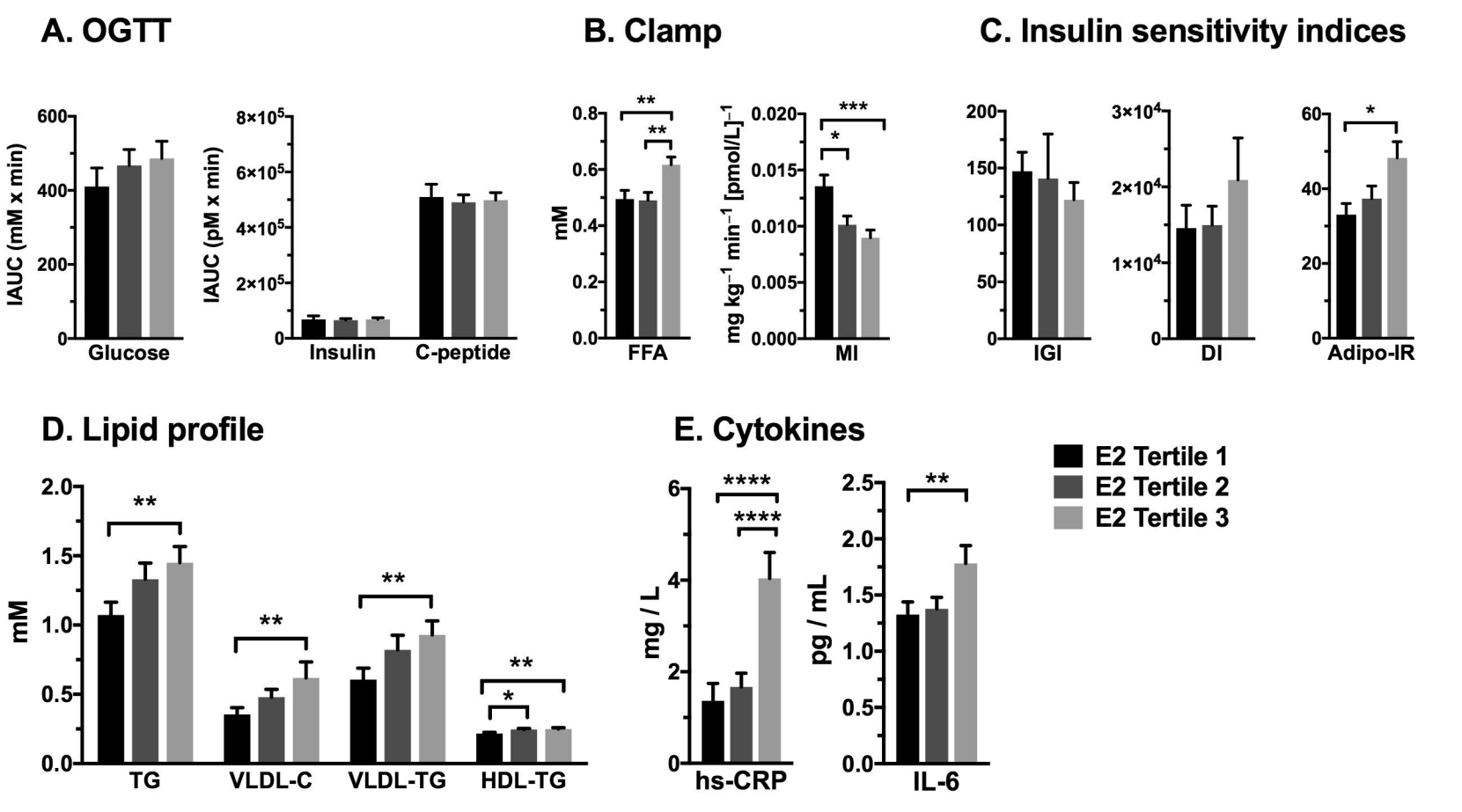
Figure 1: Markers of glucose tolerance, insulin sensitivity and metabolic homeostasis as a function of circulating E2 concentration tertiles. (A) Oral glucose tolerance test (OGTT); (B) hyperinsulinemic-euglycemic clamp; (C) Insulin sensitivity index; (D) blood lipids; and (E) circulating adipokines. Basic characteristics for tertiles 1, 2 and 3 respectively are: (mean±SD) 57±4, 58±4 and 57±5 years of age; 62.5±8.3, 71.1±9.2 and 79.2±13.5 kg total body weight; 82.9±10.5, 90.0±8.4 and 96.4±12.1 cm waist circumference; 24.8±3.0, 27.4±3.4 and 31.5±5.1 kg/m^2 BMI. FFA: free fatty acids; MI: insulin sensitivity as measured during the clamp; IGI: insulinogenic index; DI: disposition index; Adipo-IR: adipose tissue insulin resistance; TG: triglycerides; VLDL-C: very low density lipoprotein cholesterol; VLDL-TG: very low density lipoprotein triglycerides; HDL-TG: high density lipoprotein triglycerides; hs-CRP: high sensitivity C-reactive protein; IL-6: interleukin 6 *$p \leq 0.05$ **$p \leq 0.01$ ***$p \leq 0.001$ ****$p \leq 0.0001$

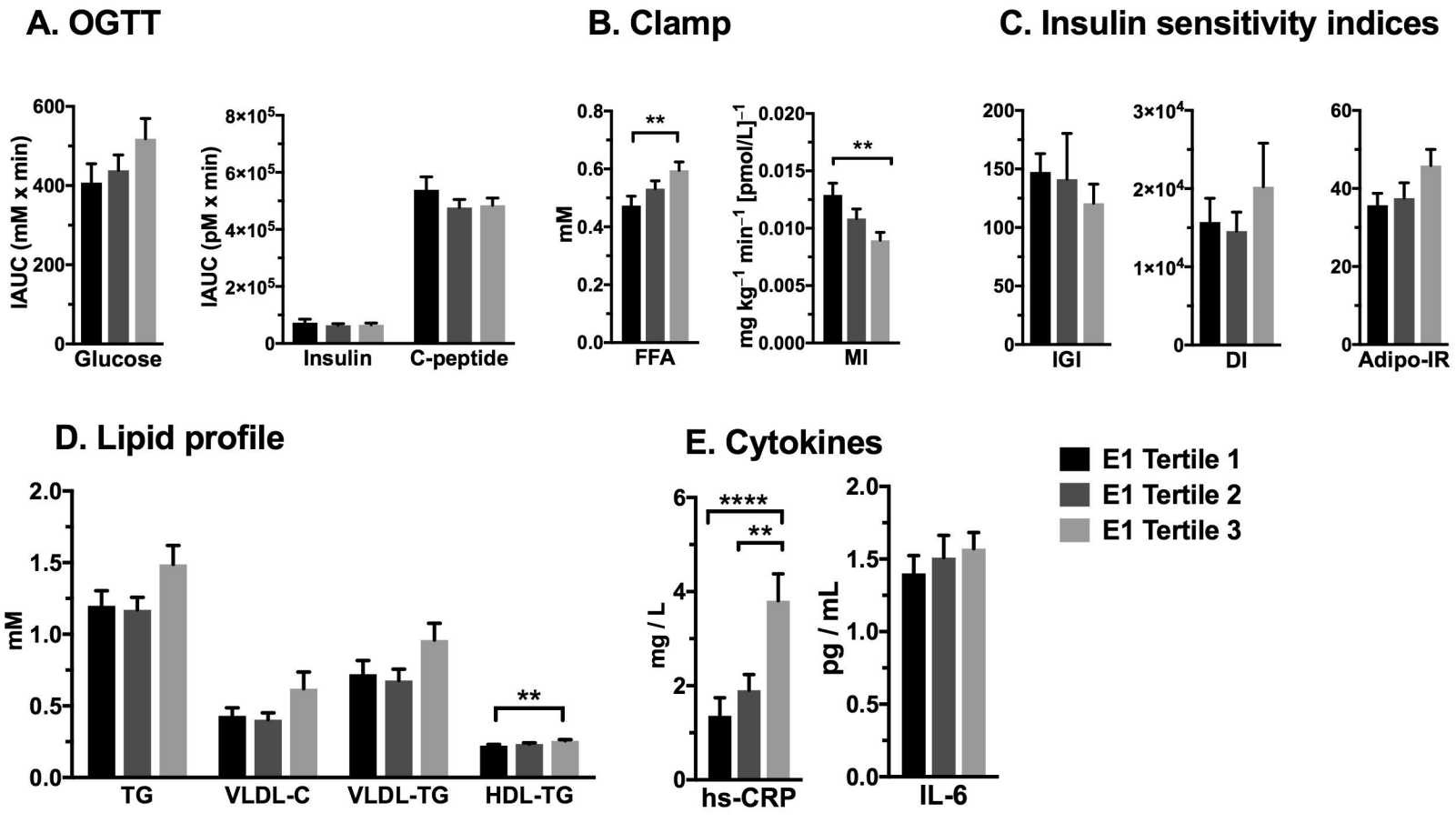
Figure 2: Markers of glucose tolerance, insulin sensitivity and metabolic homeostasis as a function of circulating E1 concentration tertiles. (A) Oral glucose tolerance test (OGTT); (B) hyperinsulinemic-euglycemic clamp; (C) Insulin sensitivity index; (D) blood lipids; and (E) circulating adipokines. Basic characteristics for tertiles 1, 2 and 3 respectively are: (mean±SD) 56±4, 57±5 and 58±4 years of age; 63.8±8.9, 71.2±10.9 and 77.9±13.4 kg total body weight; 84.0±10.9, 89.5±11.1 and 94.8±10.8 cm waist circumference; 24.9±3.1, 27.9±4.1 and 30.9±5.0 kg/m^2 BMI. FFA: free fatty acids; MI: insulin sensitivity as measured during the clamp; IGI: insulinogenic index; DI: disposition index; Adipo-IR: adipose tissue insulin resistance; TG: triglycerides; VLDL-C: very low density lipoprotein cholesterol; VLDL-TG: very low density lipoprotein triglycerides; HDL-TG: high density lipoprotein triglycerides; hs-CRP: high sensitivity

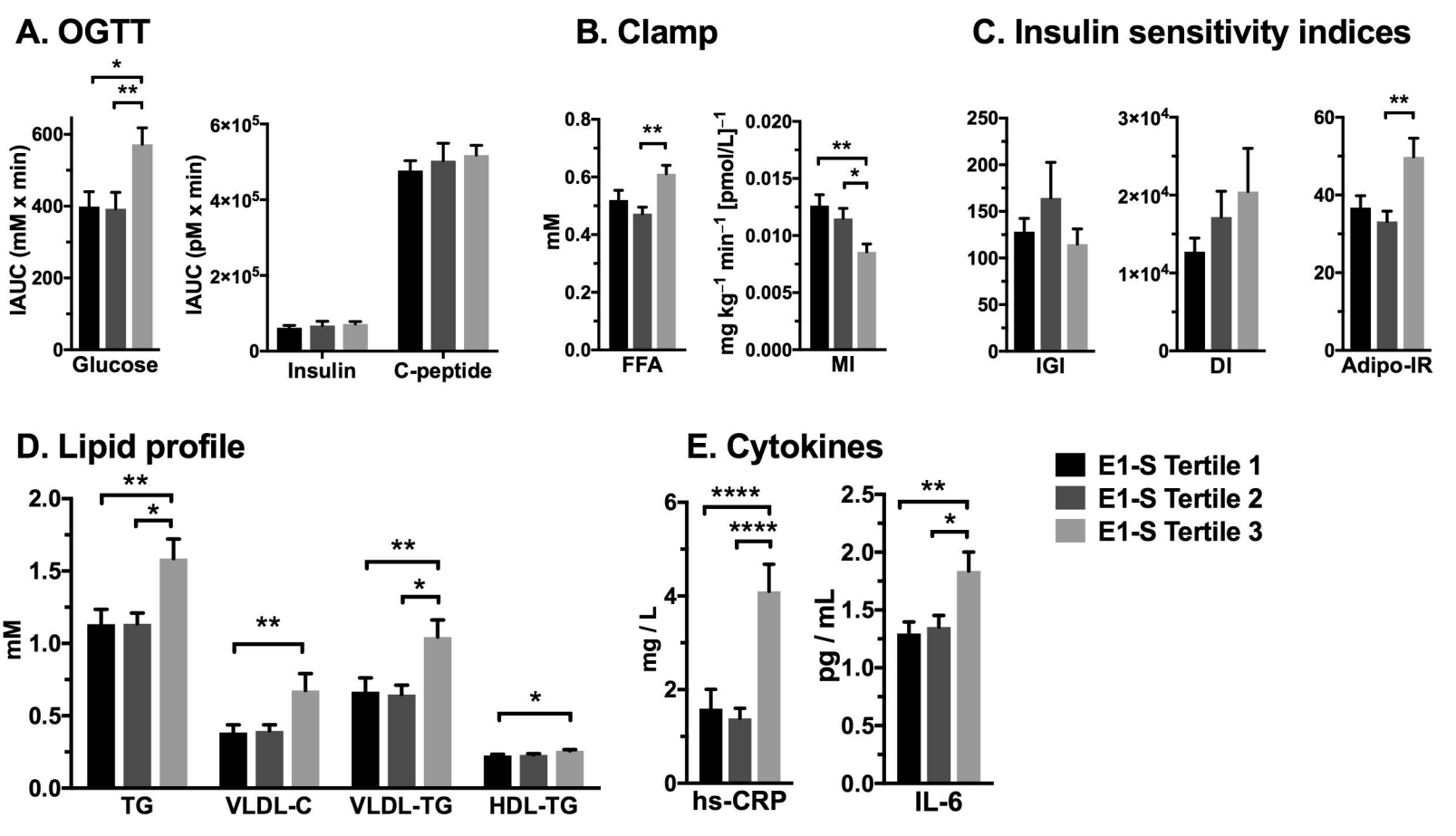
C-reactive protein; IL-6: interleukin 6 *p≤0.05 **p≤0.01 ***p≤0.001 ****p≤0.0001

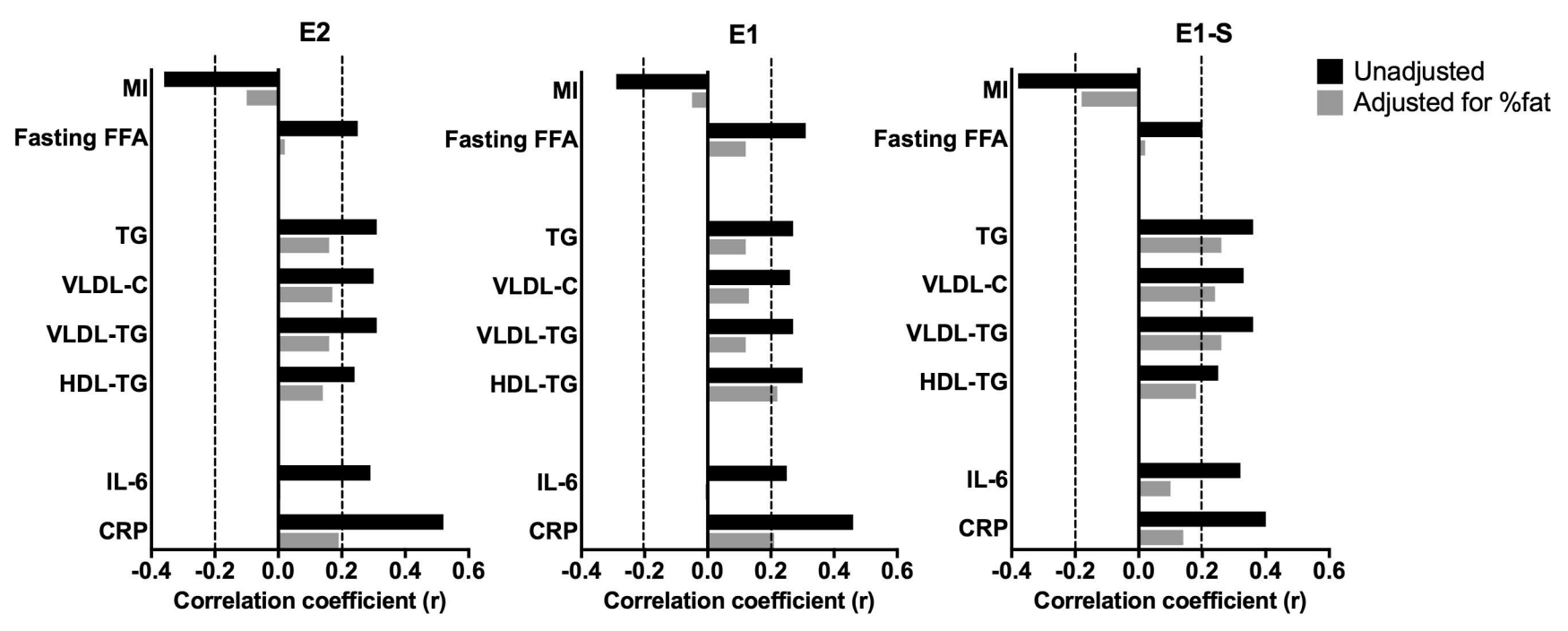
Figure 3: Markers of glucose tolerance, insulin sensitivity and metabolic homeostasis as a function of circulating E1-S concentration tertiles. (A) Oral glucose tolerance test (OGTT); (B) hyperinsulinemic-euglycemic clamp; (C) Insulin sensitivity index; (D) blood lipids; and (E) circulating adipokines. Basic characteristics for tertiles 1, 2 and 3 respectively are: (mean±SD) 57±4, 57±5 and 57±4 years of age; 64.1±10.4, 69.7±9.8, 79.2±12.5 kg total body weight; 82.7±10.4, 87.7±9.4 and 97.9±10.1 cm waist circumference; 25.0±3.6, 27.3±3.4 and 31.4±4.8 kg/m^2 BMI. FFA: free fatty acids; MI: insulin sensitivity as measured during the clamp; IGI: insulinogenic index; DI: disposition index; Adipo-IR: adipose tissue insulin resistance; TG: triglycerides; VLDL-C: very low density lipoprotein cholesterol; VLDL-TG: very low density lipoprotein triglycerides; HDL-TG: high density lipoprotein triglycerides; hs-CRP: high sensitivity C-reactive protein; IL-6: interleukin 6 *p≤0.05 **p≤0.01 ***p≤0.001 ****p≤0.0001

Figure 4: Pairwise correlations between plasma E2, E1 as well as E1-S and metabolic parameters, before and after statistical adjustment for body fat mass percentage. Vertical dotted line represent significance thresholds. MI: insulin sensitivity; FFA: free fatty acids; TG: triglycerides; VLDL-C: very low-density lipoprotein cholesterol; VLDL-TG: very low-density lipoprotein triglycerides; HDL-TG: high-density lipoprotein triglycerides; IL-6: interleukin 6; hs-CRP: high sensitivity C-reactive protein.


A. OGTT
B. Clamp
C. Insulin sensitivity indices
IAUC (mM x min)
IAUC (pM x min)
Glucose
Insulin
C-peptide
mM
FFA
mg kg⁻¹ min⁻¹ [pmol/L]⁻¹
MI
IGI
DI
Adipo-IR
D. Lipid profile
E. Cytokines
E2 Tertile 1
E2 Tertile 2
E2 Tertile 3
TG
VLDL-C
VLDL-TG
HDL-TG
mg / L
hs-CRP
pg / mL
IL-6


A. OGTT
B. Clamp
C. Insulin sensitivity indices
IAUC (mM x min)
600
400
200
0
Glucose
IAUC (pM x min)
8×10^5
6×10^5
4×10^5
2×10^5
0
Insulin
C-peptide
mM
0.8
0.6
0.4
0.2
0.0
**
FFA
mg kg^-1 min^-1 [pmol/L]^-1
0.020
0.015
0.010
0.005
0.000
**
MI
200
150
100
50
0
IGI
3×10^4
2×10^4
1×10^4
0
DI
60
40
20
0
Adipo-IR
D. Lipid profile
E. Cytokines
E1 Tertile 1
E1 Tertile 2
E1 Tertile 3
mM
2.0
1.5
1.0
0.5
0.0
**
TG
VLDL-C
VLDL-TG
HDL-TG
mg / L
6
4
2
0
****
**
hs-CRP
pg / mL
2.0
1.5
1.0
0.5
0.0
IL-6


A. OGTT
B. Clamp
C. Insulin sensitivity indices
D. Lipid profile
E. Cytokines
E1-S Tertile 1
E1-S Tertile 2
E1-S Tertile 3
IAUC (mM x min)
Glucose
IAUC (pM x min)
Insulin
C-peptide
mM
FFA
mg kg⁻¹ min⁻¹ [pmol/L]⁻¹
MI
IGI
DI
Adipo-IR
mM
TG
VLDL-C
VLDL-TG
HDL-TG
mg / L
hs-CRP
pg / mL
IL-6


E2
E1
E1-S
Unadjusted
Adjusted for %fat
MI
Fasting FFA
TG
VLDL-C
VLDL-TG
HDL-TG
IL-6
CRP
-0.4
-0.2
0.0
0.2
0.4
0.6
Correlation coefficient (r)