Department of Obstetrics and Gynecology, Helsinki University Hospital and
University of Helsinki

Doctoral Programme in Clinical Research, University of Helsinki,
Helsinki, Finland

Dissertationes Universitatis Helsingiensis 324/2024

Estrogen biosynthesis in subcutaneous and visceral adipose tissue in women

Natalia Hetemäki

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine of the University
of Helsinki, for public examination in the Seth Wichmann auditorium of
the Women's Hospital, Helsinki University Hospital
on December 13, 2024 at 12 noon.

Helsinki 2024

Supervisors:

Adjunct Professor Hanna Savolainen-Peltonen, MD, PhD
Department of Obstetrics and Gynecology, Helsinki University Hospital
Faculty of Medicine, University of Helsinki, Finland

Professor Tomi Mikkola, MD, PhD
Department of Obstetrics and Gynecology, Helsinki University Hospital
Faculty of Medicine, University of Helsinki, Finland

Professor Matti J. Tikkanen, MD, PhD
Heart and Lung Center, Helsinki University Hospital
Faculty of Medicine, University of Helsinki, Finland

Reviewers:

Associate Professor Eija Laakkonen, PhD
Gerontology Research Center
Faculty of Sport and Health Sciences, University of Jyväskylä, Finland

Professor Matti Poutanen, PhD
Institute of Biomedicine, University of Turku, Finland

Official opponent:

Professor Terhi Piltonen, MD, PhD
Department of Obstetrics and Gynecology, Oulu University Hospital
Faculty of Medicine, University of Oulu, Finland

The Faculty of Medicine uses the Ouriginal system (plagiarism recognition) to examine all doctoral dissertations.

Publisher: University of Helsinki
Series: Dissertationes Universitatis Helsingiensis 324/2024

ISBN 978-952-84-0438-5 (print)
ISBN 978-952-84-0437-8 (online)
ISSN 2954-2898 (print)
ISSN 2954-2952 (online)

PunaMusta, Joensuu 2024

Abstract

Adipose tissue is an important site of steroid hormone storage, production, and metabolism. Both the amount and distribution of adipose tissue in a female body change during a woman's life. Menopause is an important watershed, at which the relative amount of visceral compared to subcutaneous adipose tissue increases. This may lead to abdominal obesity and related metabolic comorbidities, such as cardiovascular disease and type 2 diabetes. Postmenopausal obesity is also a risk factor for estrogen-dependent cancers, including breast or endometrium cancer.

Whether the menopause-associated change in adipose tissue distribution is affected by the sex steroids produced and stored in subcutaneous and visceral adipose tissue has not been established. With menopause, estrogen production seizes in the ovaries and adipose tissue becomes the most important source of estrogens. Before menopause, the role of adipose tissue in estrogen production is less clear. Additionally, menopausal hormone therapy may mitigate the relative increase in visceral adipose tissue, but the effects of hormone therapy on adipose tissue sex steroid concentrations and metabolism have not been investigated previously.

The aim of this study was to explore possible changes in adipose tissue estrogen concentrations and production around the menopausal transition. We explored serum and subcutaneous and visceral adipose tissue estrogen concentrations and biosynthesis at the premenopausal (I) and postmenopausal (II) stages of the female reproductive lifespan. Moreover, we compared women with and without peroral menopausal hormone therapy (III). Estrogen (I-III), androgen, and progesterone (III) concentrations were quantitated using liquid chromatography-tandem mass spectrometry. The enzyme activities of estrone-producing steroid sulfatase and estradiol-producing 17β-hydroxysteroid dehydrogenases in adipose tissue were determined using isotopic precursors. mRNA expression of genes encoding sex steroid-metabolizing enzymes were analyzed with real-time reverse transcription quantitative polymerase chain reaction.

Whereas higher waist circumference before menopause was associated with increased production of estradiol in subcutaneous adipose tissue (I), in postmenopausal women it correlated with heightened estradiol production in visceral adipose tissue (II). In postmenopausal women, increasing body mass index was associated with higher visceral adipose tissue concentration of estrone (II). Estrone was the predominant estrogen in both adipose tissue depots already before menopause, and estrogen concentrations were higher in premenopausal than in postmenopausal adipose tissue (I-II). Active estrogen production in adipose tissue took place already before menopause (I). Use of menopausal hormone therapy was linked to 4- to 7-fold higher concentrations of estrogens in adipose tissue compared to women not using hormone therapy (III). Estrogen-to-androgen concentration ratios in adipose tissue were 4- to 12-fold higher in users compared to non-users of hormone therapy. In visceral adipose tissue, estrogen-to-androgen ratios increased with

higher doses of estradiol in the hormone therapy regimen. (III) The mRNA expression levels of the genes studied were mostly similar in pre- and postmenopausal women with and without hormone therapy (I-III).

These findings show that in postmenopausal women, the role of visceral fat in sex steroid biosynthesis was highlighted. The results also indicate that active estrogen production took place already in premenopausal adipose tissue, despite ongoing ovarian estrogen production. Menopausal hormone therapy was associated with increased estrogen-to-androgen ratios in adipose tissue, suggesting that, in addition to reflecting the effects of exogenous estrogen treatment, local adipose tissue sex steroid production could be altered. This could be a mechanism through which hormone therapy mitigates the menopause-associated relative increase in visceral adipose tissue.

Tiivistelmä

Rasvakudos varastoi ja muodostaa steroidihormoneja. Sekä rasvakudoksen määrä että jakautuminen kehon eri osissa muuttuvat naisen elämän aikana. Vaihdevuosissa sisäelinrasvakudoksen suhteellinen osuus ihonalaisrasvakudokseen verrattuna kasvaa. Muutos altistaa keskivartalolihavuuden ja siihen liittyvien aineenvaihdunnallisten sairauksien, kuten sydän- ja verisuonisairauksien ja tyypin 2 diabeteksen riskeille. Vaihdevuosien jälkeinen lihavuus lisää myös estrogeeniriippuvaisen rinta- ja kohtusyövän riskiä.

Ei tiedetä, ovatko muutokset rasvakudoksen jakautumisessa yhteydessä rasvakudoksen steroidihormonituotantoon. Vaihdevuosien myötä munasarjojen estrogeenituotanto lakkaa ja rasvakudoksesta tulee tärkein estrogeenin lähde. Ennen vaihdevuosia rasvakudoksessa tapahtuvan estrogeenituotannon rooli on epäselvä. Vaihdevuosioireiden hormonihoito saattaa vähentää vaihdevuosiin liittyvää sisäelinrasvan suhteellista lisääntymistä, mutta sen vaikutuksia rasvakudoksen steroidihormonipitoisuuksiin ja -aineenvaihduntaan ei ole aiemmin tutkittu.

Tutkimuksen tavoitteena oli selvittää mahdollisia muutoksia rasvakudoksen estrogeenipitoisuuksissa ja -tuotannossa ennen ja jälkeen menopaussin. Tutkimme seerumin ja ihonalais- ja sisäelinrasvakudoksen estrogeenipitoisuuksia ja -aineenvaihduntaan ennen (I) ja jälkeen vaihdevuosien (II). Lisäksi vertasimme vaihdevuosien hormonihoitoa käyttäviä naisia heihin, jotka eivät käyttäneet hormonihoitoa (III). Määritimme estrogeeni- (I-II), androgeeni- ja progesteronipitoisuudet (III) nestekromatografia-tandemmassaspektrometrian avulla. Tutkimme estronia tuottavan steroidisulfataasientsyymin sekä estradiolia tuottavien 17β-hydroksisteroididehydrogenaasientsyymien aktiivisuutta rasvakudoksessa. Estrogeeni- ja androgeenituotantoon osallistuvia entsyymejä koodaavien geenien ilmentymistä tukittiin nukleiinihappo-osoitusmenetelmällä (real-time reverse transcription quantitative polymerase chain reaction).

Vyötärönympäryksen kasvu ennen vaihdevuosia liittyi kiihtyvään estradiolituotantoon ihonalaisrasvakudoksessa (I), mutta vaihdevuosien jälkeen se yhdistyi estradiolituotannon kasvuun sisäelinrasvassa (II). Vaihdevuodet ohittaneilla naisilla korkeampi painoindeksi oli yhteydessä sisäelinrasvan suurempaan estronipitoisuuteen (II). Estroni oli vallitseva estrogeeni molemmissa rasvakudostyypeissä jo ennen vaihdevuosia, ja estronipitoisuudet rasvakudoksessa olivat korkeampia ennen vaihdevuosia kuin niiden jälkeen (I-II). Rasvakudos tuotti estrogeeneja jo ennen vaihdevuosia (I). Vaihdevuosien jälkeisen hormonihoidon käyttö liittyi 4-7 kertaa korkeampiin estrogeenipitoisuuksiin rasvakudoksessa verrattuna naisiin, jotka eivät käyttäneet hormonihoitoa (III). Estrogeeni- ja androgeenisuhteet olivat 4-12 kertaa korkeampia hormonihoitoa käyttävillä. Sisäelinrasvassa nämä suhdeluvut kasvoivat hormonihoidon estradiolimäärän myötä. (III)

Tutkitut geenien ekspressiotasot olivat pääosin samankaltaisia ennen ja jälkeen vaihdevuosien (I-III).

Tulokset osoittavat, että vaihdevuosien jälkeisessä sukuhormonien tuotannossa sisäelinrasvan merkitys korostui. Rasvakudos tuotti estrogeeneja aktiivisesti jo ennen vaihdevuosia huolimatta munasarjojen estrogeenituotannosta. Vaihdevuosien hormonihoitoon liittyivät korkeammat estrogeeni- ja androgeenisuhteet rasvakudoksessa, mikä voisi kuvastaa paitsi estrogeenihoidon vaikutusta, myös hormonihoidon seurauksena muuntunutta paikallista estrogeenituotantoa rasvakudoksessa. Rasvakudoksen estrogeenituotannon muuttuminen saattaisi vaikuttaa hormonihoidon käyttäjien sisäelinrasvan määrään vaihdevuosissa.

Contents

Abstract ..... **iv**

Tiivistelmä ..... **vi**

List of original publications ..... **xi**

Abbreviations ..... **xii**

Introduction **1**

Review of the literature **3**

Adipose tissue depots and distribution **3**

Adipose tissue composition and function **4**

Adipose tissue dysfunction in obesity **5**

Sex steroids in women **7**

Effects of sex steroids on adiposity and metabolic homeostasis **10**

Estrogens 10

Androgens 13

11-ketoandrogens 14

Progesterone 15

Overview of menopause physiology and metabolic changes **15**

Menopausal hormone therapy **16**

Peripheral estrogen and androgen biosynthesis and the concept of intracrinology **18**

STS……. 22

17β-HSD enzymes 23

Aromatase 24

Determination of sex steroid hormones **25**

Aims of the study **28**

Materials and Methods **29**

Study subjects **29**

Methods **30**

Blood and adipose tissue samples 30

Sex steroid analysis in serum and adipose tissue 31

Serum analyses of FSH, SHBG (I-III) and progesterone (I) 31

Serum E_1, E_2, and E_1S (I-III) 31
Serum A_4, T, DHEA, and progesterone (III) 32
Separation and purification of adipose tissue sex steroids and quantitation with LC/MS-MS 32
Adipose tissue E_1 and E_2 (I-III) 32
Adipose tissue A_4, T, DHEA, and progesterone (III) 33
STS activity assay in adipose tissue (I-III) 34
Inhibition of STS in adipose tissue (I) 34
Reductive 17β-HSD activity assay in adipose tissue (I-II) 34
Preparation of RNA and complementary DNA, and quantification of mRNA (I-III) 35
Other methods 35
Statistical analysis 35
Results 37
Estrogen concentrations in serum and in subcutaneous and visceral adipose tissue 37
Premenopausal women (I) 37
Postmenopausal women without HT (II-III) 37
Postmenopausal women with HT (III) 39
Sex steroid concentrations in serum and in subcutaneous and visceral adipose tissue in postmenopausal women with or without HT (III) 39
STS (I-III) and reductive 17β-HSD (I-II) activities 44
mRNA expression of genes encoding sex steroid hormone-converting enzymes (I-III) 46
Discussion 50
Estrogen metabolism in subcutaneous compared to visceral adipose tissue 50
Adipose tissue estrogen metabolism in women before and after menopause 51
Adipose tissue sex steroid metabolism in postmenopausal women with or without HT 53
Strengths and limitations 55
Conclusions and future perspective 57
Acknowledgements 59
References 61
Original publications 80

List of original publications

This thesis is based on the following publications:

I Hetemäki N, Mikkola TS, Tikkanen MJ, Wang F, Hämäläinen E, Turpeinen U, Haanpää M, Vihma V, Savolainen-Peltonen H. Adipose tissue estrogen production and metabolism in premenopausal women. *J Steroid Biochem Mol Biol*. 2021;209;105849.

II Hetemäki N, Savolainen-Peltonen H, Tikkanen MJ, Wang F, Paatela H, Hämäläinen E, Turpeinen U, Haanpää M, Vihma V, Mikkola TS. Estrogen Metabolism in Abdominal Subcutaneous and Visceral Adipose Tissue in Postmenopausal Women. *J Clin Endocrinol Metab*. 2017;102(12):4588-4595.

III Hetemäki N*, Robciuc A*, Vihma V, Haanpää M, Hämäläinen E, Tikkanen MJ, Mikkola TS, Savolainen-Peltonen H. Adipose Tissue Sex Steroids in Postmenopausal Women with and without Menopausal Hormone Therapy. *J Clin Endocrinol Metab*. Published online July 10, 2024. doi:10.1210/clinem/dgae458

The publications are referred to in the text by their Roman numerals (I-III). The original publications are reproduced with the permission of the copyright holders.

*Equal contribution to this work.

Abbreviations

17*β*-HSD	17*β*-hydroxysteroid dehydrogenase
AKR1C3	aldo-keto reductase 1C3
BMI	body mass index
cDNA	complementary DNA
CYP	cytochrome 450
CYP19A1	gene encoding aromatase
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
Dpm	disintegrations per minute
E_1	estrone
E_1S	estrone sulfate
E_2	17*β*-estradiol
ERα	estrogen receptor alpha (ESR1)
ERβ	estrogen receptor beta (ESR2)
FSH	follicle-stimulating hormone
GPR30	membrane-bound G protein-coupled estrogen receptor
HSD17B1	gene encoding 17*β*-hydroxysteroid dehydrogenase type 1
HSD17B2	gene encoding 17*β*-hydroxysteroid dehydrogenase type 2
HSD17B7	gene encoding 17*β*-hydroxysteroid dehydrogenase type 7
HSD17B12	gene encoding 17*β*-hydroxysteroid dehydrogenase type 12
HT	hormone therapy
HUSLAB	Helsinki University Hospital laboratory
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LNG-IUS	levonorgestrel-releasing intrauterine system
LOQ	limit of quantification
mRNA	messenger ribonucleic acid

NADP+/NADPH	nicotinamide adenine dinucleotide phosphate; oxidized/reduced forms
PCOS	polycystic ovary syndrome
RIA	radioimmunoassay
RT-qPCR	real-time reverse transcription quantitative polymerase chain reaction
SHBG	sex hormone-binding globulin
STS	steroid sulfatase
STS	gene encoding steroid sulfatase
STX64/667Coumate	specific STS enzyme inhibitor
v/v	volume-to-volume ratio

Introduction

Adipose tissue is more than a storage site for energy – it is a hormonally and immunologically active tissue involved in the production and metabolism of sex steroids (Kershaw and Flier, 2004; Li *et al*, 2015). After menopause, when the ovaries seize to produce estrogens, adipose tissue becomes the main source of estrogens (Nelson and Bulun, 2001; Labrie, 2015).

Sex steroids modify adipose tissue function and distribution (Tchernof and Després, 2013). Menopause is a transition point at which the protective effect of endogenous estrogens against visceral fat accrual and cardiovascular disease disappears (Morselli *et al*, 2017). During the menopausal transition, the gynoid distribution of adipose tissue with an emphasis on the gluteal and femoral regions shifts towards an android pattern, with a relative increase in visceral adipose tissue (Karvonen-Gutierrez and Kim, 2016). Visceral adipose tissue is metabolically deleterious and abdominal obesity carries an increased risk of comorbidities such as type 2 diabetes and cardiovascular disease (Carr, 2003). Obesity in postmenopause also increases the risk of estrogen-dependent breast and endometrium cancers (He *et al*, 2021).

The amount and distribution of adipose tissue in women before and after menopause may be associated with differences in circulating and local sex steroid concentrations in subcutaneous and visceral adipose tissue. The significance of local adipose tissue estrogen synthesis in women before menopause is less clear, and whether adipose tissue production of estrogens is suppressed when the ovaries produce estrogens is not known.

Systemic hormone therapy is used to treat bothersome menopausal symptoms (Davis and Baber, 2022). Hormone therapy increases circulating estrogen concentrations (Kim *et al*, 2021; Ensrud *et al*, 2022) and may mitigate the postmenopausal increase in relative visceral adiposity (Ambikairajah *et al*, 2019). It is unclear, however, whether hormone therapy affects local adipose tissue sex steroid concentrations and metabolism.

We studied estrogen production before and after menopause in subcutaneous and visceral adipose tissue, the two main adipose tissue depots. We also investigated the effect of menopausal hormone therapy on sex steroid concentrations and biosynthesis within adipose tissue. The aim was to characterize

possible differences between the adipose tissue depots at different stages of women's reproductive life.

Review of the literature

Adipose tissue depots and distribution

Adipose tissue makes up approximately 30% of body weight in women with normal BMI (Maniyadath *et al*, 2023). The two main depots of adipose tissue are subcutaneous and visceral adipose tissue (Ghaben and Scherer, 2019). Subcutaneous adipose tissue is located under the skin both in the upper and lower parts of the body (Kwok *et al*, 2016) and comprises about 80% of total body fat (Karastergiou and Fried, 2017; Ibrahim, 2010). Subcutaneous adipose tissue grows both through hyperplasia, an increase in the number of adipocytes, and hypertrophy, the growth of individual adipocytes in size (Drolet *et al*, 2008). Scarpa's fascia separates superficial and deep subcutaneous adipose tissue in the abdominal area of the body (Sakers *et al*, 2022).

Visceral adipose tissue, comprising fat in the peritoneal cavity including the omental and mesenteric regions (Wajchenberg, 2000; Tchkonia *et al*, 2013; Cypess, 2022), typically makes up about 5-20% of all body fat (Palmer and Clegg, 2015; Ibrahim, 2010). Visceral adipose tissue grows principally through hypertrophy (Hwang and Kim, 2019). This depot is in close contact with the portal vasculature and hence its effects on the liver may be especially prominent (Brown and Scherer, 2023).

In fertile-aged women, fat is predominantly stored in the subcutaneous compared to the visceral compartment (Camhi *et al*, 2011; Tchernof and Després, 2013). This gynoid adipose tissue distribution, concentrated mainly in the gluteo-femoral region, is considered beneficial in terms of protection against metabolic dysfunction like cardiometabolic disease and type 2 diabetes (Karpe and Pinnick, 2015; Palmer and Clegg, 2015; Pi-Sunyer, 2004). Visceral adipose tissue, especially in the context of obesity, is prone to metabolic dysfunction stemming from increased inflammation, impaired insulin balance, and fibrosis (Schleinitz *et al*, 2020). In visceral obesity, ectopic fat can build up outside of the conventional adipose tissue depots, for example in the liver, pancreas, skeletal muscle, and the heart (Heilbronn *et al*, 2004; Brown and Scherer, 2023; Valenzuela *et al*, 2023).

Whole-body imaging, including dual X-ray absorptiometry, computer tomography and magnetic resonance imaging, may be used to quantify distinct

adipose tissue depots (Neeland *et al*, 2021). While these techniques are precise and can provide data for evaluating risks of obesity-related comorbidities (Valenzuela *et al*, 2023), availability is restricted and thus anthropometric parameters are often used as surrogates for estimating adiposity. While body mass index (BMI) cut-offs are used to define obesity, BMI does not describe body fat distribution (Bray, 2023). Waist circumference is considered a better clinical predictor of abdominal visceral obesity (Rankinen *et al*, 1999). Furthermore, body adiposity index (Li *et al*, 2021) describes body fat percentage better than BMI (Fedewa *et al*, 2019), and waist to hip ratio seems to be a better predictor of excess mortality in both normal weight and overweight individuals (Bowman *et al*, 2017).

Adipose tissue composition and function

White adipose tissue, the major adipose tissue type in humans (Zwick *et al*, 2018) is a specialized loose connective tissue responsible for storage of energy (Saely *et al,* 2012). It also has various important functions relating to the control of appetite and satiety, insulin sensitivity, inflammation, and cardiovascular health (Ali *et al*, 2013; Scheja and Heeren, 2019). Humans also have a small amount (0.2–3.0% of total adipose tissue in adults) of functionally distinct brown adipose tissue (Cypess, 2022), considered primarily to be involved in thermogenesis (Rosen and Spiegelman, 2014).

Adipocytes are lipid-storing cells which make up about 90% of adipose tissue volume but only 20-40% of the cellular composition (Kanneganti and Dixit, 2012). Adipocytes can grow significantly in size as a result of lipid storage (Goossens *et al*, 2021), and roughly 10% of adipocytes are replaced per year (Spalding *et al*, 2008). It has also been suggested that there are different subpopulations of mature adipocytes with differing functions and differing sensitivity to insulin and risk for type 2 diabetes (Duerre and Galmozzi, 2022; Maniyadath *et al*, 2023; Bäckdahl *et al*, 2021; Emont *et al*, 2022).

The non-adipocyte part of adipose tissue forms the stromal-vascular fraction, composed of preadipocytes (15 to 50% of adipose tissue cells) (Tchkonia *et al*, 2013), resident immune cells (roughly 30% of adipose tissue cells) (Vijay *et al*, 2020), fibroblasts, smooth muscle cells, and endothelial cells (Kanneganti and Dixit, 2012; Duerre and Galmozzi, 2022). Adipogenesis, the formation of new adipocytes through differentiation and development from preadipocytes, is influenced by growth and transcriptional factors. These include insulin-like growth factor and glucocorticoids, and tissue conditions such as the composition of the surrounding extracellular protein matrix (Cristancho and Lazar, 2011; Tang and

Lane, 2012). In addition to structural support, the extracellular matrix surrounding adipose tissue cells can affect cell to cell crosstalk and function (Gliniak *et al*, 2023).

Adipose tissue produces and secretes adipokines. These include cytokines and other adipose tissue-produced biologically active peptides, such as leptin, adiponectin, resistin, interleukin-6, and tumor necrosis factor-α. Adipokines regulate insulin sensitivity, appetite, and modulate the local and systemic inflammatory environment (Fasshauer and Blüber, 2015; Kershaw and Flier, 2004; Ouchi *et al*, 2011). The adipokine expression and secretion of each depot of adipose tissue is different, affecting the local functional milieu (Kershaw and Flier, 2004; Samaras *et al*, 2010).

Lipogenesis is the synthesis of fatty acids and formation of triacylglycerides, mainly in the liver and adipose tissue (Kersten, 2001). Lipolysis in adipose tissue is the hydrolysis of triacylglycerides to fatty acids and glycerol, and their subsequent release into the circulation for use as energy (Duncan *et al*, 2007). In a healthy state, a dynamic balance of these two processes contributes to metabolic homeostasis (Frayn, 2002). Sensitivity to insulin after feeding is necessary for adipocyte function in the uptake of glucose and fatty acids from the circulation and lipogenesis (Maniyadath *et al*, 2023). Insulin plays a key role in promoting storage of excess energy in adipose tissue by stimulating the uptake of glucose from the circulation, *de novo* lipogenesis, and curbing lipolysis (Sakers *et al*, 2022). Insulin-like growth factor-1 can also bind to its receptor and inhibit lipolysis (Grabner *et al*, 2021).

Adipose tissue dysfunction in obesity

Obesity is defined as a BMI equal to or exceeding 30 kg/m^2, while overweight begins at BMI 25 (National Institutes of Health, 1998; World Health Organization, 2000). Obesity prevalence in adult women is greater compared to men, with a peak between the ages of 60 to 64 years (GBD 2015 Obesity Collaborators, 2017). In 2017, 28% of Finnish women were overweight, and over half of women over 65 years presented with abdominal obesity (waist circumference over 90 cm) (Finnish National Institutes of Health, 2017). Globally, obesity has increased nearly three-fold since 1975 (World Health Organization, 2022), and mortality rates related to obesity and its comorbidities including type 2 diabetes remain high (Chew *et al*, 2023).

Obesity involves an excessive accumulation of dysfunctional adipose tissue. In obesity, the capacity of subcutaneous adipose tissue to grow through both hyperplasia and hypertrophy is exceeded, and excess fat accumulation takes place in the visceral adipose tissue through hypertrophy (Gustafson *et al*, 2019; Valenzuela *et al*, 2023).

In normally functioning adipose tissue, the limited size of adipocytes is thought to ensure adequate vascularization and thus inflow of oxygen and nutrients to adipose tissue cells (Gu and Xu, 2013). With visceral adipocyte hypertrophy, angiogenesis is insufficient to provide nutrients to adipose tissue cells, resulting in local tissue hypoxia and cell death (Herold and Kalucka, 2021). Adipose tissue is infiltrated with immune cells, especially macrophages, causing both local inflammation and a proinflammatory adipokine profile (Valenzuela, 2023). The extracellular matrix surrounding adipose tissue cells accumulates, leading to adipose tissue fibrosis, hindering tissue function and plasticity (Gliniak *et al*, 2023). Hypertrophic adipocytes feed fatty acids into the circulation, predisposing to insulin resistance (Duncan *et al*, 2007; Morigny *et al*, 2016). The excess fat may build up as ectopic adipose tissue, further exacerbating insulin resistance and inflammation (Després and Lemieux, 2006; Valenzuela *et al*, 2023).

Excessive visceral adiposity (Figure 1) with adipose tissue dysfunction increases the risk of metabolic syndrome (Neeland *et al*, 2019). It is associated with increased risks of hyperglycemia, insulin resistance and type 2 diabetes, elevated triglycerides and high low-density lipoprotein cholesterol, hypertension, and cardiovascular disease (Valenzuela *et al*, 2023; Lee *et al*, 2013; Grundy *et al*, 2004). Obesity also increases the risk of several types of cancer through metabolic dysfunction and altered adipokine secretion (Brown and Scherer, 2023).

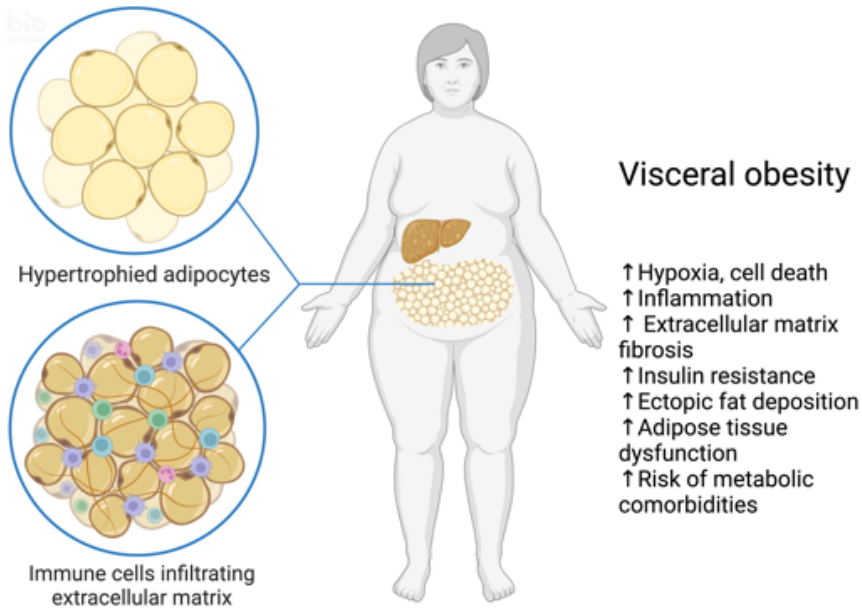


Figure 1 Features of visceral obesity. Adapted from "Metabolically Unhealthy Obesity" by BioRender.com. Retrieved from https://app.biorender.com/biorender-templates (accessed on 24 Feb 2024).

Sex steroids in women

Sex steroids are steroid hormones that interact with specific sex steroid hormone receptors. Sex steroids include estrogens (mainly 17β-estradiol (E_2), estrone (E_1), and estriol, in the order of highest to lowest biological activity) (Poutanen, 2012; Gruber *et al*, 2002), androgens (active androgens testosterone (T) and dihydrotestosterone, and their derivatives 11-ketotestosterone and 11-ketodihydrotestosterone, and androgen precursors androstenedione (A_4), dehydroepiandrostenedione (DHEA)) (Miller and Auchus, 2011; Poutanen *et al*, 2023; Storbeck and O'Reilly, 2023), and progesterone (Kolatorova *et al*, 2022).

Cholesterol is the starting point for steroidogenesis, the synthesis of sex steroids (Miller and Auchus, 2011). Cytochrome P450 (CYP) enzymes, including CYP11A1, which initiates the de novo sex steroid synthesis (Payne and Hales, 2004), and CYP19A1 or aromatase, as well as hydroxysteroid dehydrogenase (HSD) enzymes, are integral for the formation and interconversion of sex steroids (Payne and Hales, 2004; Li *et al*, 2015). Key pathways in sex steroid biosynthesis are presented in Figure 2.

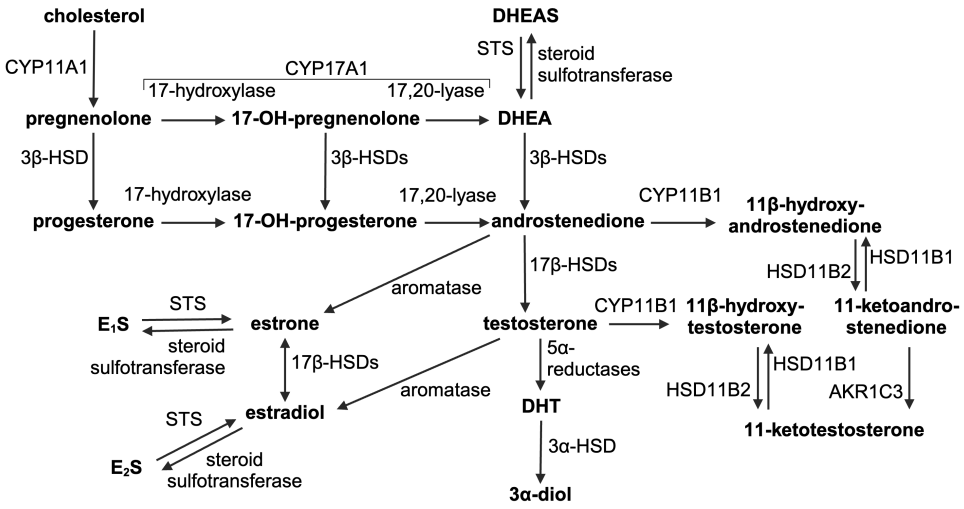


Figure 2 Key pathways of sex steroid biosynthesis in humans. Adapted from Miller and Auchus, 2011. Created using BioRender.com. Abbreviations: CYP, cytochrome P450; HSD, hydroxysteroid dehydrogenase; DHEA, dehydroepiandrostenedione; DHEAS, dehydroepiandrostenedione sulfate; DHT, dihydrotestosterone; STS, steroid sulfatase; E_1S, estrone sulfate; E_2S, estradiol sulfate; 17-OH-progesterone, 17-hydroxyprogesterone; 17-OH-pregnenolone, 17-hydroxypregnenolone.

The main functions of sex steroids in women are within reproduction, sexual function, and maintenance of female characteristics (Nelson and Bulun, 2001; Schiffer *et al*, 2018). In fertile-aged women, the production of sex steroids is regulated by the hypothalamic-pituitary-gonadal axis. Ovarian follicular cells produce large amounts of estrogens, mainly E_2, and progesterone depending on the stage of the menstrual cycle (Miller and Auchus, 2011) (Table 1). The ovaries also produce and secrete some A_4 and T (Miller and Auchus, 2011). T and its precursors, mainly A_4 and DHEA, are also produced in the adrenal glands and in peripheral tissues (Skiba *et al*, 2019; Piltonen *et al*, 2002). During pregnancy, the placenta also produces large amounts of E_2 and estriol (Vuppaladhadiam *et al*, 2021).

Progesterone is produced by the ovaries and the placenta, and to a lesser extent, the adrenal glands, and its function mainly relates to the menstrual cycle and reproduction. In the luteal phase of the menstrual cycle, the corpus luteum secretes progesterone for the maintenance of the endometrial lining in preparation for implantation. Progesterone is also important in the maintenance of pregnancy. (Nagy *et al*, 2021)

The traditional action of sex steroids is endocrine – production in the gonads and transport in plasma to their target tissues where they exert their actions through sex steroid receptors (Rubinow, 2018). Sex steroids are also produced in several peripheral tissues, mainly for local use (Simpson, 2000; Simpson *et al*, 2003; Labrie *et al*, 1998). Estrogens are produced in tissues including the breast, musculature, bone, the brain (Simpson *et al*, 2005), and in peripheral blood leukocytes and macrophages (Rubinow, 2018), and also in adipose tissue. Adipose tissue is the main source of estrogens after menopause through local production of estrogens from circulating precursor steroids (Nelson and Bulun, 2001; Labrie, 2015). As opposed to women in the fertile age when ovarian E_2 is the main circulating estrogen, postmenopausal women exhibit higher concentrations of E_1 compared to E_2 in the circulation (Simpson and Davis, 2001) (Table 1).

Circulating concentrations of androgens decline with age with no apparent major decline at menopause (Davison *et al*, 2005). Postmenopausal ovaries continue to produce and secrete T into the circulation, but the majority is formed locally in peripheral tissues from circulating androgen precursors (Arlt, 2006; Simpson, 2003). A_4 and DHEA from the adrenals and ovaries, and DHEAS of adrenal origin, are important precursors for the peripheral formation of active androgens and estrogens (Li J *et al*, 2015; Schiffer *et al*, 2018).

Sex steroids can circulate in the bloodstream either freely or bound to other molecules, mainly proteins such as albumin and sex hormone-binding globulin (SHBG) (Hammond, 2016). Only a few percent of androgens (Schiffer *et al*, 2018) and estrogens (Wu *et al*, 1976) circulate in their free form, and subsequently, the concentrations of sex steroid-binding molecules regulate the bioavailability of sex

steroids to target tissues. Bioavailable sex steroids circulate freely or are bound to albumin, as albumin binds sex steroids with lower affinities than SHBG (Schiffer *et al*, 2018). SHBG concentrations in blood are lower in women with obesity (Grow, 2002) and diabetes (Goldman *et al*, 2017), as well as in hypothyroidism and polycystic ovary syndrome (PCOS). On the other hand, hyperthyroidism and increased estrogen concentrations during pregnancy or with peroral exogenous estrogen use are associated with higher SHBG levels (Goldman *et al*, 2017).

Sex steroids also circulate in their sulfonated form, which makes them more soluble in water and prolongs their half-life (Kuhl, 2005). Freely circulating hormones are lipophilic and can diffuse freely across cell membranes (Poutanen, 2012), whereas the sulfonated and other conjugated forms of sex steroids need active transport by carrier proteins between tissues and the circulation (Mueller *et al*, 2015; Schiffer *et al*, 2019). Examples of conjugated sex steroids are estrone sulfate (E_1S) and DHEA sulfate (DHEAS), which circulate in the plasma at much higher concentrations than free sex steroids (Labrie, 1998; Mueller *et al*, 2015). As tissues can either sulfonate or desulfate sex steroids, the sulfonated forms in the circulation represent both metabolites as well as precursors for active sex steroid hormones (Kuhl, 2005).

At their target tissue, sex steroids bind to specific nuclear steroid receptors (O'Malley, 1971). After dimerization of the activated receptors, they regulate the expression and transcription of target genes, either through transcription factors associated with DNA or by directly binding to sex steroid-responsive elements in target gene promoters (Morselli *et al*, 2017; O'Lone *et al*, 2004). Non-genomic signaling, with faster effects compared with the classical genomic pathways, includes actions through membrane-bound sex steroid receptors. Effects generated through this rapid cellular signaling can take place in minutes (Roepke *et al*, 2007).

E_2 is the strongest activator of the estrogen receptors, which include the nuclear estrogen receptors alpha (ERα) and beta (ERβ), and the membrane-bound G protein-coupled estrogen receptor (GPR30) (Olde and Leeb-Lundberg, 2009). ERα and ERβ are encoded by the *ESR1* and *ESR2* genes, respectively (Edwards, 2005). With regards to the reproductive effects of estrogens, most are brought about through the classical nuclear estrogen receptors (Mauvais-Jarvis *et al*, 2013).

Dihydrotestosterone and T have the highest affinities for the androgen receptor (Rizk *et al*, 2023), encoded by the human X-chromosomal *AR* gene (Poutanen *et al*, 2023). Also adrenal-derived 11-oxygenated androgens, such as 11-ketotestosterone and 11-ketodihydrotestosterone, are now known to form an essential part of the human androgen pool (Storbeck and O'Reilly, 2023). As with the estrogen receptors, androgens also initiate rapid intracellular non-genomic signaling events through membrane-associated androgen receptors within the cytoplasm of cells (Rizk *et al*, 2023).

The progesterone receptors are encoded by the *PR* gene (Vázquez-Martínez *et al*, 2014). The receptor has two isoforms, progesterone-receptor A (PR-A) and B (PR-B), which in some tissues may bring about opposing actions (Liu, 2021).

Effects of sex steroids on adiposity and metabolic homeostasis

Estrogens

In addition to reproduction, endogenous estrogens have a broader role in the regulation of metabolic health (Wise *et al*, 1996). In fertile-aged women, E_2 controls appetite and satiety and improves expenditure of energy, protecting against the accrual of excess adipose tissue (Palmer and Clegg, 2015). Circulating estrogens help maintain a gynoid adipose tissue distribution by curbing hypertrophic growth of visceral adipose tissue. E_2 also has beneficial effects on insulin sensitivity and glucose balance and improves the function of pancreatic β-cells. (Palmer and Clegg, 2015; Goossens *et al*, 2021) Beneficial cardiovascular effects, including peripheral vasodilation and antiatherogenic properties, are attributed to endogenous estrogens (Morselli *et al*, 2017).

Estrogens influence the distribution of adipose tissue between different regions of the body (Tchernof and Després, 2013). Men present with less total body fat than women, but with a tendency for abdominal obesity (Mauvais-Jarvis *et al*, 2013). A turn towards an android adipose tissue distribution takes place in women at menopause with the decline of circulating estrogens (Bracht *et al*, 2020; Palmer and Clegg, 2015; Goossens *et al*, 2021). Estrogens influence adipose tissue function and structure by regulating the size and number of adipocytes. In cultured subcutaneous and visceral adipose tissue preadipocytes, E_2 increased the proliferation of adipocyte precursor cells in pre- and postmenopausal women (Anderson *et al*, 2001). When postmenopausal women were given transdermal E_2, femoral adipose tissue samples showed increased preadipocyte proliferation without changes in ERα or ERβ expression (Cox-York *et al*, 2017). In femoral area adiposity, women present with adipocyte hyperplasia and men with hypertrophic adipocytes (Tchoukalova *et al*, 2008), even though in *in vitro* conditions, preadipocytes from the same adipose tissue depots in women and men proliferate and differentiate similarly (Tchoukalova *et al*, 2010). This seems to support sex steroid-dependent modulation of adipose tissue structure and expansion.

Table 1 Sex steroid concentrations in serum

Hormone	Premenopausal				Postmenopausal		Measurement method
(pmol/l)	Overall	Follicular phase	Midcycle	Luteal phase	HT-	HT+	
E_1		144-487[1]	260-913[1]	200-663[1]	132-357[1]		RIA
	63-740[2]				26-148[2]		LC-MS/MS
						396 (179)[3]	RIA
					1405 (1239-1594)[4]	5126 (4427-5933)[4]	RIA
		464 (162)[5]	650 (134)[5]	496 (36)[5]			LC-MS/MS
					23-130[6]		GC-MS
					89-348[7]		LC-MS/MS
	87-518[8]				29-147[8]		LC-MS/MS
					52-255[9]		RIA
						372 (309-449)[10]	LC-MS/MS
E_2		80-920[1]	140-2380[1]	80-1150[1]	<100[1]		ICMA
	55-1287[2]		>1100[2]		<37[2]		LC-MS/MS
						106 (33)[3]	RIA
					40 (35-48)[4]	115 (99-133)[4]	RIA
		250 (68)[5]	360 (70)[5]	392 (30)[5]			LC-MS/MS
					4-39[6]		GC-MS
		31-1742[11]	275-2864[11]	39-1941[11]	<26[11]		LC-MS/MS
	57-1021[8]				<36-72[8]		LC-MS/MS
					11-99[9]		RIA
						84 (70-102)[10]	LC-MS/MS
Free E_2	2.2-26[2]				2.2-26[2]		LC-MS/MS
					27 (22-33)[4]	51 (43-59)[4]	RIA
E_1S						110721 ± 31961[12]	RIA
	885-9988[13]				114-1684[13]		LC-MS/MS
					107-1772[6]		LC-MS/MS
	761-7634[14]				179-1144[14]		LC-MS/MS

T	400-2000[1]						LC-MS/MS
	277-2080[2]						LC-MS/MS
	208-1075[15]				208-902[15]		GC-MS
						794 (381)[3]	RIA
		534 (56)[5]	787 (59)[5]	648 (52)[5]			LC-MS/MS
					173-1089[6]		GC-MS
					170-830[7]		LC-MS/MS
	380-1570[8]				250-1230[8]		LC-MS/MS
					284-1893[9]		RIA
Free T	9-30[1]						LC-MS/MS
	28-347[2]						LC-MS/MS
					5-36[9]		RIA
A_4	300-10300[1]						LC-MS/MS
	1047-6983[2]						LC-MS/MS
	1571-5726[15]				594-2479[15]		GC-MS
		3100 (1800-6910)[16]		4190 (1260-12810)[16]	2340 (1130-9460)[16]		LC-MS/MS
DHEA	9000-38000[1]						LC-MS/MS
	27736- 45071[2]				17335-20802[2]		LC-MS/MS
	5305-31689[15]				1942-13833[15]		GC-MS
		15800 (3500-41300)[16]		13800 (4000-58600)[16]	6700 (1400-24000)[16]		LC-MS/MS
Prog		200-2800[1]		5800-76000[1]	<700[1]		ChLIA
		≤2830[2]	≤38160[2]	5724-76321[2]	≤636[2]		ECLIA
					239 (206-276)[4]	197 (174-224)[4]	RIA
		560 (60-18640)[16]		13230 (70-83000)[16]	250 (30-11420)[16]		LC-MS/MS
SHBG	12-137[1]						ChLIA
	18-136[2]				17-125[2]		IA
					36 (32-40)[4]	91 (77-107)[4]	ChLIA
		85 (9)[5]	99 (9)[5]	103 (11)[5]			FIA
					16-115[9]		ChLIA

Serum concentrations in premenopausal women (overall / according to stage of menstrual cycle), postmenopausal women without (HT-) and with hormone therapy (HT+). E_1, estrone; E_2, estradiol; E_1S, estrone sulfate; T, testosterone; free T, free testosterone; A_4, androstenedione; DHEA, dehydroepiandrosterone; SHBG, sex steroid hormone-binding globulin. RIA, radioimmunoassay; ICMA, immunochemiluminometric assay; LC-MS/MS, liquid chromatography–tandem mass spectrometry; GC-MS, gas chromatography-mass spectrometry; ChLIA, chemiluminescent immunoassay; ECLIA, electrochemiluminescence immunoassay; IA, immunoenzymatic assay; FIA, fluoroimmunometric assay. Specifications for reference values / units, HT regimens are listed below.

[1]Reference values from a Finnish laboratory (HUSLAB) 2/2024. DHEA (all women); A_4 (all women).
[2]Reference values from a US laboratory (Mayo Clinic Laboratory) 2/2024. Free E_2 (adult females); T (women ≥19 years); free T (women 20-50 years); DHEA (women 19-50 years / women ≥51 years); A_4 (all women).
[3]Shifren *et al*, 2007. Postmenopausal women using 0.625 mg conjugated equine estrogen + oral micronized progesterone 100 mg daily. Mean (standard deviation).
[4]Edlefsen *et al* 2010. Postmenopausal women using 0.625 mg conjugated equine estrogen + 2.5 mg medroxyprogesterone acetate daily. Mean (95% confidence interval).
[5]Rothman *et al*, 2011. Mean (standard error of the mean).
[6]Ke *et al*, 2018.
[7]Davis *et al*, 2019. 10th-90th percentile.
[8]Mezzullo *et al*, 2020.
[9]Stanczyk *et al*, 2022.
[10]Ensrud *et al*, 2022. Postmenopausal women using 0.5 mg E_2 daily. Mean (95% confidence interval).
[11]Verdonk *et al*, 2019.
[12]Slater *et al*, 2001. Postmenopausal women using 1 mg oral micronized E_2 daily. Mean ± standard error of the mean.
[13]Labrie *et al*, 2008. 5th–95th percentiles.
[14]Kleider *et al*, 2022. Range.
[15]Labrie *et al*, 2006. 5th–95th centiles.
[16]Eisenhofer *et al*, 2017.

Estrogens may also regulate the composition of adipose tissue extracellular matrix by increasing fibrosis through an estrogen receptor-mediated mechanism in human cultured fibroblasts (Soldano *et al*, 2010). E_1 and E_2 concentrations in subcutaneous adipose tissue of severely obese women were associated with increased pericellular fibrosis (Ostinelli *et al*, 2022).

E_2 enhances the antilipolytic effect of insulin and inhibits catecholamine-stimulated lipolysis in subcutaneous adipose tissue of postmenopausal women (Van Pelt *et al*, 2003). Subcutaneous adipose cells in the gluteal area have shown lower rates of basal lipolysis and higher lipoprotein lipase activity in early postmenopausal compared to perimenopausal women in some studies (Ferrara *et al*, 2002), while in other studies no significant differences between pre- and postmenopausal women have been found (Raison *et al*, 1988). The effects of estrogens on lipogenesis and lipolysis may be different depending on adipose tissue depot and menopausal status, and the exact mechanism by which this modulation occurs is not known (Newell-Fugate, 2017).

In human subcutaneous and visceral adipose tissue, ERα seems to be metabolically beneficial. It enhances insulin signaling and glucose uptake and downregulates the proliferation of preadipocytes and size of adipocytes. ERβ seems to bring about the opposite effects. (Foryst-Ludwig and Kintscher, 2010; Rodrigo *et al*, 2011). The messenger ribonucleic acid (mRNA) expression of *ESR1* in subcutaneous adipose tissue in obese women are lower than in normal weight women (Nilsson *et al*, 2007), and it has been suggested that ERα is more important than ERβ in regulating the accumulation of adiposity (Mauvais-Jarvis *et al*, 2013).

Androgens

In men, low plasma androgen concentrations tend to correlate with central or abdominal obesity, while in women the inverse has traditionally been considered to be the case (Tchernof *et al*, 2018; Schiffer *et al*, 2019). In premenopausal women, increased total and abdominal adiposity are associated with lower concentrations of circulating androgen precursors. Moreover, lower concentrations of A_4 and T correlated with visceral adipocyte hypertrophy. (Marchand *et al*, 2018)

Hyperandrogenism in women, for example with PCOS, has been associated with abdominal obesity, elevated blood pressure, defective endothelial function, type 2 diabetes, and consequently an increased cardiovascular disease risk (Hirschberg, 2023; Yanes Cardozo *et al*, 2017). Androgen synthesis within subcutaneous adipose tissue in women PCOS is elevated (Schiffer *et al*, 2019).

A decline in SHBG concentrations at menopause is the result of decreased ovarian production of estrogens, and it results in an increase in bioavailable circulating T (Hirschberg, 2023). Similarly to total T, a higher concentration of bioavailable T is associated with visceral adiposity at menopause (Janssen *et al*, 2015). A decrease in the ratio of circulating E_2 to T may be related to an unfavorable lipid profile that increases the risk of coronary artery disease in postmenopausal women (Dai *et al*, 2012). However, the definitive impact of T on cardiovascular disease in postmenopausal women is unclear (Spoletini *et al*, 2014).

The androgen receptor is expressed in human adipose tissue (Dieudonné *et al*, 1998) and may be more abundant in visceral compared to subcutaneous adipose tissue (Joyner *et al*, 2002). Androgens blunt adipogenesis and preadipocyte differentiation (Chazenbalk *et al*, 2013; Rizk *et al*, 2023). T and dihydrotestosterone impair differentiation of preadipocytes and adipogenesis in subcutaneous and visceral adipose tissue from both women and men, and dihydrotestosterone decrease lipolysis in cultured visceral adipocytes from women (Blouin *et al*, 2010). The tendency of androgens to enhance lipogenesis and hinder lipolysis in women can increase visceral adiposity (Newell-Fugate, 2017).

11-ketoandrogens

In recent years, in addition to classical androgens, 11-ketoandrogens have emerged as markers of adrenal androgen excess (Schiffer *et al,* 2023). Adrenal-derived 11-oxygenated androgen precursors can be converted to potent 11-ketoandrogens in peripheral tissues. Adipose tissue androgen activating enzyme aldo-keto reductase 1C3 (AKR1C3) can produce 11-ketotestosterone from circulating 11-ketoandrostenedione. In women, the concentration of 11-ketotestosterone has been reported to be similar to that of testosterone, and the two also possess similar levels of androgenic activity. (Storbeck and O'Reilly, 2023) 11-ketotesterone concentration does not appear to decrease with age (Nanba *et al*, 2019) and has been shown to increase with BMI (Schiffer *et al*, 2023), highlighting the importance of adipose tissue in the production of 11-ketoandrogens.

Progesterone

The progesterone receptors are expressed in adipose tissue (O'Brien *et al*, 1998). Adipose tissue cells can metabolize progesterone (Zhang *et al*, 2009), but the effects of progesterone on adipose tissue function remain unestablished (Wawrzkiewicz-Jałowiecka *et al*, 2021).

Overview of menopause physiology and metabolic changes

Natural menopause, the date of the final menstrual period, occurs at age 45-55 on average (Davis and Baber, 2022). The years after menopause cover over a third of a woman's lifespan, as life expectancy in developed countries for women is around 81 years (Murray *et al*, 2015). The date of menopause can be defined at 12 months after the final menstrual period and signals the end of reproductive function in women (Hall, 2015).

The preceding years before menopause, the menopausal transition, include a decline in ovarian follicle reserve and cyclic ovarian function (Harlow *et al*, 2012; Hall, 2015; Santoro *et al*, 2021). E_2 and progesterone concentrations decrease, followed by inhibin B (Broekmans *et al*, 2009), leading to increasing follicle-stimulating hormone (FSH) and luteinizing hormone (LH) concentrations (Santoro *et al*, 2021; Sherman *et al*, 1976). The cessation of ovarian function leads to hypoestrogenism (Hall, 2015; Davis ad Baber, 2022).

Menopause confers short-term and long-term effects. In the short term, hypoestrogenism can lead to disturbing menopausal symptoms, including vasomotor symptoms (night sweating, hot flushes), sleep disorders, mood changes, and genitourinary symptoms (Davis and Baber, 2022). Around 75% of women face menopause-related vasomotor symptoms (Gartoulla *et al*, 2015) and 50% experience genitourinary symptoms like vulvovaginal atrophy (Sturdee and Panay, 2010).

In the long term, the diminished estrogen levels predispose women to bone loss and osteoporosis (Charatcharoenwitthaya *et al*, 2007) resulting from increased bone resorption compared to bone formation (Davis and Baber, 2022). Endogenous estrogens have been shown to protect from cardiovascular diseases and consequently after menopause, cardiovascular disease risk increases (Menazza and Murphy, 2016; Barton and Meyer, 2009). Postmenopausal obesity in particular is associated with an unfavorable lipid profile (Sites *et al*, 2000) including high levels of low-density lipoprotein cholesterol and triglycerides (El Khoudary *et al*, 2019). A

deleterious adipokine and cytokine profile secreted by visceral adipose tissue further contributes to the increased risk of cardiovascular disease in postmenopausal women (Lee *et al*, 2009). Moreover, changes in hepatic metabolism increases lipid accumulation and a risk for non-alcoholic fatty liver disease in postmenopausal women (Cherubini *et al*, 2023). The genitourinary syndrome of menopause (Stuenkel *et al*, 2015) with urogenital atrophy and vaginal dryness and irritation, as well as urinary symptoms, can persist in the long term (Talaulikar, 2022).

Normal weight and moderately obese women gain increased body fat by around 5% at menopause, while at the same time, muscle and lean body mass decrease (Leeners *et al*, 2017; Juppi *et al*, 2020). Fat oxidation and energy expenditure during rest and exercise seem to be lower in postmenopausal compared to premenopausal women (Abildgaard *et al*, 2013; Lovejoy *et al*, 2008). Moreover, the effects of estrogens on satiety are reduced at menopause and may increase food intake (Leeners *et al*, 2017). Weight gain in later life does not directly result from menopause but is associated with aging (Ambikairajah *et al*, 2019).

Menopausal hormone therapy

Menopausal hormone therapy (HT) is prescribed to alleviate symptoms associated with the loss of estrogens around menopause. Systemic HT typically consists of E_2 (typically, micronized E_2, or E_2 valerate or E_2 hemihydrate) or conjugated equine estrogens alone for women with previous hysterectomy, or the estrogen component combined with a progestin to prevent endometrial hyperplasia (Davis and Baber, 2022). The estrogen component is typically administered perorally or transdermally in the form of a gel, patch, or spray. The progestin is taken orally, in a combination patch with E_2, or through a levonorgestrel-releasing intrauterine system (LNG-IUS) (Depypere and Inki, 2015).

Circulating concentrations of estrogens are increased with systemic HT (Table 1). With peroral HT, exogenous E_2 is largely metabolized into E_1 and E_1S, and SHBG production is increased in the liver (Davis and Baber, 2022). Oral E_2 treatment may to lead to a relative androgen deficiency, likely through increased SHBG production and possibly through decreased androgen production in the ovaries and adrenal glands (Simon, 2002). With transdermal E_2 administration, liver first pass metabolism is avoided and serum E_2 and E_1 concentrations are similar (Kuhl, 2005; Davis and Baber, 2022). Increases in SHBG concentrations are smaller with transdermal compared to oral E_2 treatment (Shifren *et al*, 2008). Another benefit of transdermal administration is that does not stimulate the production of

inflammatory and coagulation factors in the liver (Vigneswaran and Hamoda, 2022).

Systemic estrogen is efficient in alleviating vasomotor symptoms (Vigneswaran and Hamoda, 2022; Davis and Baber, 2022). Vulvovaginal atrophy can be treated with local topical estrogen (Santen, 2015). HT also protects against vertebral and non-vertebral fractures, both in women with and without osteopenia or osteoporosis (de Villiers *et al*, 2012; Zhu *et al*, 2016). HT use is associated with decreased insulin resistance and decreased risk of type 2 diabetes (Salpeter *et al*, 2006), as well as improved glucose control in women with diabetes (Szmuilowicz *et al*, 2009; Xu *et al*, 2014). It may protect to some extent against the development of non-alcoholic fatty liver disease (DiStefano, 2020). HT is also protective against colorectal cancer (Nelson *et al*, 2002). While endogenous estrogens protect women against hypertension and cardiovascular disease, exogenous estrogens do not affect blood pressure levels (Manrique-Acevedo *et al*, 2020). The "window of opportunity" hypothesis postulates that HT is beneficial for the prevention of cardiovascular disease when started within 10 years after menopause or before 60 years of age (Mikkola *et al*, 2015; Savolainen-Peltonen *et al*, 2016; Schierbeck *et al*, 2012; Hodis *et al*, 2016; El Khoudary *et al*, 2020). Prescribing HT solely for the primary prevention of cardiovascular disease is however currently not recommended (Stuenkel *et al*, 2015; "The 2022 Hormone Therapy Position Statement of The North American Menopause Society" Advisory Panel", 2022).

Moreover, HT confers benefits in counteracting the accrual of visceral adipose tissue associated with menopause, while it does not influence the total fat mass (Haarbo *et al*, 1991; Sites *et al*, 2001; Ambikairajah *et al*, 2019). To the best of our knowledge, the effects of HT on the production of sex steroids and their concentrations in adipose tissue have not been characterized.

The use of peroral HT is associated with an increased risk of venous thrombosis/thromboembolism and stroke (Boardman *et al*, 2015), and it seems to be related both to the type and dose of estrogen and type of progestin (Vigneswaran and Hamoda, 2022). The risk of venous thrombosis or stroke is not increased with transdermal estrogen (Scarabin, 2018; Vinogradova *et al*, 2019), although there are no head-to-head data comparing the two administration routes ("The 2022 Hormone Therapy Position Statement of The North American Menopause Society" Advisory Panel, 2022). The risk of breast cancer, the most common type of female cancer (Sung *et al*, 2021), is increased with the use of estrogen combined with progestin (Fournier *et al*, 2008). A lower breast cancer risk has been associated with estrogen-only therapy (Vigneswaran and Hamoda, 2022). Long-term HT use may be associated with a slightly increased risk for the development of Alzheimer's disease (Savolainen-Peltonen *et al*, 2019).

Postmenopausal androgen treatment for women remains out of the mainstream clinical practice. Currently, the prescription of transdermal T for the treatment of

hypoactive sexual desire disorder can be considered, if concentrations of T in the circulation do not exceed physiological levels (Davis *et al*, 2019). Non-estrogen treatment with vaginal DHEA and T may be effective in alleviating symptoms of menopause-related genitourinary atrophy (Casiano Evans *et al*, 2023) and dyspareunia (Davis and Baber, 2022). DHEA but not T or A_4 administration increases serum E_1 and E_2 concentrations (Marina *et al*, 2020).

Peripheral estrogen and androgen biosynthesis and the concept of intracrinology

The local production and metabolism of sex steroids, as opposed to endocrinology, is referred to as tissue intracrinology and paracrinology, whereby individual cells are capable of producing and metabolizing the sex steroids they need for local use within a cell or tissue, independently of circulating concentrations (Labrie, 1991; Rubinow, 2018) (Figure 3).

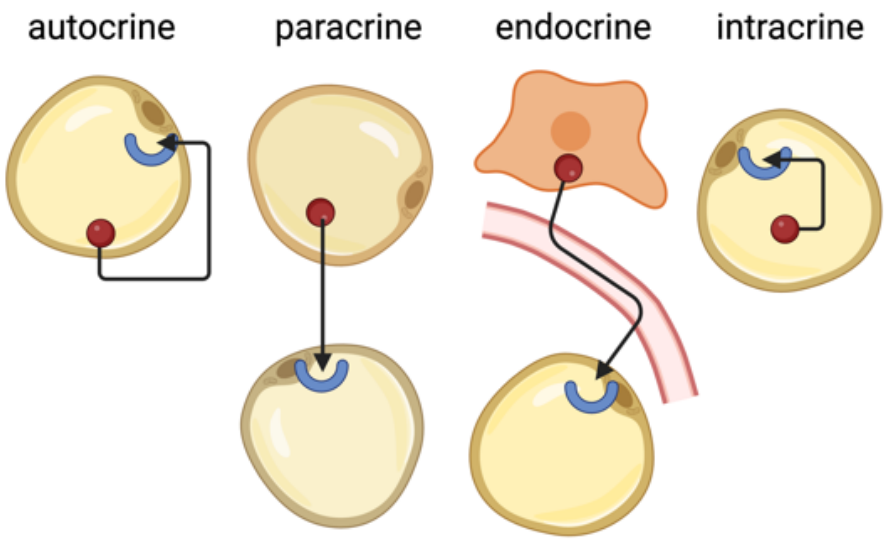


Figure 3 Ways of cell signaling. Autocrine – a product by a cell is secreted outside the cell and binds to a receptor on the surface of the secreting cell. Paracrine – a product by a cell is secreted outside the cell to induce effects in nearby cells. Endocrine – a product secreted by a cell enters the circulation and is transported to a target tissue. Intracrine – a product by a cell induces effects within the same cell. Created using Biorender.com.

Much of the study on female sex steroids has focused on sex steroid endocrinology and reproductive functions. Intracrine production and interconversions of sex steroids on peripheral tissue level have received less attention, and data on the local concentrations and balance between estrogens and androgens in individual tissues, such as adipose tissue, is scarce.

In the classical endocrine context of sex steroid action, peripheral tissues are targets of the sex steroids produced and secreted by the gonads and adrenals and circulating concentrations of these sex steroids dictate the extent to which peripheral tissues are exposed to sex steroid action (Rubinow, 2018; Labrie *et al*, 2017). Peripheral tissues herein refer to extragonadal tissues where active production and metabolism of sex steroids takes place, as a wide range of tissues express genes encoding sex steroid-metabolizing enzymes (Schiffer *et al*, 2018; Simpson, 2003).

The concept of intracrinology postulates that each sex steroid is produced in a cell, within which the sex steroid acts, and where afterwards it can be inactivated (Labrie *et al*, 1998). Intracrine sex steroid metabolism, thus, refers to metabolism both before and after biological effects at the steroid receptor level (Schiffer *et al*, 2019). The local production and biological action of estrogens and androgens is dependent on 1) their precursor reaching a tissue and being able to enter a cell, which 2) possesses the enzyme machinery for sex steroid metabolism (activation, interconversion, inactivation), and 3) the expression of estrogen and androgen receptors (Stocco, 2012). Cells within a tissue may also exert autocrine and paracrine effects on themselves or on neighboring cells. This is exemplified in the development of breast cancer, where crosstalk between tumor cells and breast adipose tissue directly influences the development and growth of breast cancer tumors (Brown and Scherer, 2023).

Intracrine sex steroids are produced according to specific local need. This allows for autonomous regulation of local sex steroid metabolism and subsequent concentrations of sex steroids available for receptor activation (Rubinow, 2018). The dominant intracrine sex steroid pathways and their balance vary depending on the type of peripheral tissue (Labrie *et al*, 2017; Mueller *et al*, 2015). Local tissue sex steroid metabolism is comprised of dynamic networks (Figure 3) rather than parallel pathways of substrates and metabolites, as tissues are composed of various cell types, each expressing their own set sex steroid-metabolizing enzyme machinery. The balance of sex steroids within a tissue is affected by the metabolism in individual cell types within that tissue (Tchernof *et al*, 2015; Li *et al*, 2015). Each enzyme involved in sex steroid metabolism works, for the most part, in a single direction, and an increase of a product does not result in feedback mechanism back to the substrate (Miller and Auchus, 2011). Intratissue concentrations of a specific sex steroid reflect the balance of several metabolic pathways which are intertwined,

as the same steroid can be produced by different enzymes (Forney *et al*, 1981) (Figure 2).

The complexity of peripheral sex steroid metabolism is exemplified by the inactivation of a sex steroid hormone in one tissue and subsequent activation in another. Estrogens can be inactivated through sulfonation for example in the liver, be released into the bloodstream as estrogen sulfates such as E_1S for excretion through the kidneys, but they may also be taken up by another peripheral tissue such as adipose tissue as a substrate for conversion back to the active steroid (Rubinow, 2018). DHEAS and E_1S are key sex steroid precursors which can be hydrolyzed to DHEA and E_1 through the action of the steroid sulfatase (STS) enzyme in tissues (Mueller *et al*, 2015). Catalysis of DHEAS to DHEA in adipose tissue has previously been shown to take place in adipose tissue (Paatela *et al*, 2016).

The sex steroids with the highest local concentrations in subcutaneous and visceral adipose tissue in both pre- and postmenopausal women are DHEA and A_4 (Table 2). Concentrations of E_1 exceed those of E_2 in subcutaneous and visceral adipose tissue of premenopausal (Kinoshita *et al*, 2014; Ostinelli *et al*, 2022), and in subcutaneous, visceral, and breast adipose tissue of postmenopausal women (Vihma *et al*, 2016; Kinoshita *et al*, 2014; Savolainen-Peltonen *et al*, 2014; Yamatani *et al*, 2013), and E_1 concentrations are many times higher than in the circulation. The same applies to most androgen concentrations (Kinoshita *et al*, 2014; Ostinelli *et al*, 2022). Circulating concentrations of sex steroids do not always correlate with local tissue concentrations of the corresponding steroids (Ostinelli *et al*, 2022) and concentrations measured in the plasma are therefore not a sufficient index or determinant of the sex steroid production and metabolism taking place in peripheral tissues.

While local sex steroid production in peripheral tissues primarily serves the purpose of catering to local tissues needs, spillover of these sex steroids may also contribute to circulating concentrations (Simpson, 2003; Siiteri, 1987). Serum concentrations of E_2 and E_1 increase with higher BMI in women after menopause (McTiernan *et al*, 2006; Penell *et al*, 2021). Adipose tissue production of sex steroids may influence circulating concentrations directly or through indirect effects on other tissues by adipose tissue-derived adipokines (Kershaw and Flier, 2004; Ronconi *et al*, 2008; Campos *et al*, 2008).

Table 2 Sex steroid concentrations in adipose tissue in women

Hormone (pmol/kg)		Premenopausal	Postmenopausal	Method
E_1	sc	1487 ± 1174[1]	962 ± 854[1]	LC-MS/MS
		1274 (225)[2]	414 (78)[2]	LC-MS/MS
		5375 ± 3923[3]		LC-MS/MS
	visc	2116 ± 1675[1]	1587 ± 1801[1]	LC-MS/MS
		2161 (387)[2]	556 (236)[2]	LC-MS/MS
		5382 ± 3518[3]		LC-MS/MS
	breast sc		893 (333-3130)[6]	LC-MS/MS
		2214 (1896-2825)[8]		LC-MS/MS
E_2	sc	379 ± 378[1]	84 ± 48[1]	LC-MS/MS
		235 (75)[2]	57 (35)[2]	LC-MS/MS
		3134 ± 2360[3]		LC-MS/MS
		422 (117-1514)[5a]		FIA
		609 (1751673)[5b]		
	visc	402 ± 480[1]	110 ± 103[1]	LC-MS/MS
		194 (64)[2]	46 (21)[2]	LC-MS/MS
		3270 ± 2535[3]		LC-MS/MS
		398 (102-1649)[5a]		FIA
		586 (203-1156)[5b]		
	breast sc		62 (35-88)[7]	LC-MS/MS
		692 (503-841)[8]		LC-MS/MS
T	sc	624 ± 520[1]	589 ± 520[1]	LC-MS/MS
		2545 ± 3371[3]		LC-MS/MS
	visc	763 ± 520[1]	555 ± 416[1]	LC-MS/MS
		2045 ± 2068[3]		LC-MS/MS
A_4	sc	19099 ± 12325[1]	15643 ± 9672[1]	LC-MS/MS
		26592 ± 65736[3]		LC-MS/MS
	visc	37744 ± 29818[1]	20321 ± 16969[1]	LC-MS/MS
		57212 ± 57338[3]		LC-MS/MS
DHEA	sc	115834 ± 138751[1]	57657 ± 45280[1]	LC-MS/MS
		75792 ± 144541[3]		LC-MS/MS
	visc	105849 ± 74195[1]	65700 ± 56755[1]	LC-MS/MS
		108663 ± 112359[3]		LC-MS/MS
		32200-101000[4]	99700-162000[4]	LC-MS/MS

E_1, estrone; E_2, estradiol; T, testosterone; A_4, androstenedione; DHEA, dehydroepiandrosterone; Sc, subcutaneous adipose tissue; visc; visceral adipose tissue; breast sc, breast subcutaneous adipose tissue. Measurement methods: FIA, fluoroimmunoassay; LC-MS/MS, liquid chromatography–tandem mass spectrometry.

[1]Kinoshita *et al*, 2014. Premenopausal women, n=12; postmenopausal, n=4. Mean ± ?

[2]Yamatani *et al*, 2013. Premenopausal and postmenopausal, n=8 each. Mean (standard error of the mean).

[3]Ostinelli *et al*, 2022. Premenopausal, n=31; BMI > 40. Median (interquartile range).

[4]Wang *et al*, 2011. Premenopausal, n=5; postmenopausal, n=2. Range.

[5]Wang *et al*, 2013. [5a] Premenopausal, obese (BMI >40), n=19-22; [5b] premenopausal, control (BMI 28), n=8. Median (range).

[6]Vihma *et al*, 2016. Postmenopausal women without breast cancer, n=11. Median (range).

[7]Savolainen-Peltonen *et al*, 2014. Postmenopausal women without breast cancer, n=14. Adjusted mean (95% confidence interval).

[8]Savolainen-Peltonen *et al*, 2018. Premenopausal women without breast cancer, n=17. Mean (95% confidence interval).

Sex steroid metabolism in tissues involves multiple interlinked pathways and interconversions between androgens and estrogens (Figure 2). It is therefore important to evaluate ratios of estrogens to androgens, instead of or in addition to each sex steroid group separately to understand the roles that sex steroids have in the modulation of tissue metabolism (Bélanger *et al*, 2002; Ostinelli *et al*, 2022). Key enzymes involved in adipose tissue sex steroid metabolism are presented below.

STS

The STS enzyme is membrane-bound and acts within the lumen of the endoplasmic reticulum (Thomas and Potter, 2013) in virtually all cells of the body (Reed *et al*, 2005). In steroid metabolism, this enzyme catalyzes the removal of the sulfate group of sulfated steroids such as DHEAS and E_1S, with a stronger affinity for the latter (Hanson *et al*, 2004). Other steroid substrates for the enzyme include E_2 sulfate, cholesterol sulfate, and pregnenolone sulfate (Reed *et al*, 2005). STS, consequently, is involved in the production and regeneration of a number of active steroids from inactivate steroid metabolites.

STS mRNA expression and STS protein, as well as its catalytic activity in converting DHEAS to DHEA, have been documented in human subcutaneous and visceral adipose tissue (Dalla Valle *et al*, 2006; Paatela *et al*, 2016). The activity was higher in visceral than in subcutaneous adipose tissue in premenopausal women (Paatela *et al*, 2016). The gene mRNA expression of *STS* is enhanced by preadipocyte differentiation in cell culture (Blouin *et al*, 2009). Posttranscriptional modification of *STS* expression and STS activity is poorly characterized mechanistically but is likely to be affected by growth factors and cytokines (Wang J *et al*, 2024). STS is upregulated in many hormone-dependent cancers and,

showcasing the role of inflammation in regulating STS activity, in breast tumor cells, tumor necrosis factor α and interleukin-6 synergistically enhanced STS activity (Purohit *et al*, 1996; Purohit *et al*, 2011). In vitro, inflammatory nuclear factor-κB activation induced human *STS* expression in hepatocytes (Jiang *et al*, 2016).

The steroid sulfotransferase enzyme is responsible for the inactivation of sex steroids by catalyzing the addition of a sulfate molecule to the steroid. This prohibits binding to the estrogen or androgen receptor and the enhanced water solubility provided by the sulfate group prepares for urinary excretion (Strott, 1996). Some (Ihunnah *et al*, 2014) but not all (Dalla Valle *et al*, 2006; Paatela *et al*, 2016) studies indicate that sulfotransferase is expressed in human adipose tissue.

Whereas originally sulfated steroids were considered inactivated metabolites, it has become clear that both DHEAS and E_1S form considerable circulating reservoirs for intracrine production of active estrogens and androgens. This is particularly important in the context of malignant tumors of the breast (Santner *et al*, 1984) where both *STS* mRNA expression and enzyme activity were increased. This has led to the development of STS inhibitors such as Irosustat (STX64, 667Coumate, BN83495) which has been investigated in phase I/II trials against breast and endometrial cancer (Foster, 2021).

17β-HSD enzymes

17β-HSD enzymes activate sex steroid hormones in the last steps of sex steroid biosynthesis. They can perform either oxidative or reductive reactions and are dependent on the presence of nicotinamide adenine dinucleotide phosphate (NADP+/NADPH; the oxidized/reduced forms) as cofactors (Moeller and Adamski, 2009). Apart from sex steroids, the enzymes in the family regulate the metabolism of fatty and bile acids, and retinoids (Tsachaki and Odermatt, 2019). Some 17β-HSDs are membrane-bound and located in the endoplasmic reticulum, some within mitochondria, peroxisomes, cytosol, and within lipid droplets (Tsachaki and Odermatt, 2019).

17β-HSD enzymes are expressed widely in human tissues, both in steroidogenic tissues such as placenta and the gonads, but also in tissues in the periphery, such as adipose tissue and breast (Saloniemi *et al*, 2012; Poutanen and Penning, 2019). The enzymes are all encoded by different genes and their substrate specificities vary (Moeller and Adamski, 2006). Gene mRNA expression levels of at least types 1, 2, 3, 7, 8, and 12 of 17βHSDs have been detected in adipose tissue (Blouin *et al*, 2009). Within adipose tissue, the cellular localization depends on the type of 17β-HSD. The mRNA expression of reductive, E_2-producing 17β-HSD types 1, 7, and 12 have been reported in preadipocytes and differentiated adipocytes, and the conversion of E_1

to E_2 increased with adipocyte differentiation (Bellemare *et al*, 2009). On the other hand, oxidative, E_1-producing 17β-HSD type 2 seems to be located in the vascular structures of adipose tissue, and its activity is higher in visceral compared to subcutaneous adipose tissue (Fouad Mansour *et al*, 2023).

17β-HSD12 has been considered an important producer of premenopausal E_2 with high expression and activity levels in the ovaries and placenta (Luu-The *et al*, 2006). In addition, it has been implicated to participate strongly in fatty acid and prostaglandin metabolism (Moeller and Adamski, 2009). 17β-HSD7 seems to be more important in cholesterol metabolism than sex steroid conversion (Moeller and Adamski, 2009; Saloniemi *et al*, 2012). Currently, 17β-HSD1 is most strongly associated with the production of E_2 from E_1 (Heinosalo *et al*, 2019).

The family of 17β-HSDs includes both activators and deactivators of sex steroids and thus, the functional balance of each of the 17β-HSDs modulates the bioavailability of sex steroids for interaction with sex steroid hormone receptors. Their roles in sex steroid hormone-dependent disease states such as breast cancer, endometriosis, and osteoporosis have been extensively studied and considered as therapeutic targets (Marchais-Oberwinkler *et al*, 2011), and much has been established through the preclinical study of mouse models (Saloniemi *et al*, 2012). The in vivo contribution of individual 17β-HSDs to estrogen and androgen metabolism is complex to assess and depend on tissue and location (Labrie *et al*, 2000; Moeller and Adamski, 2006).

Aromatase

The P450 aromatase, encoded by the gene *CYP19A1*, converts androgens to estrogens and is a key enzyme in the regulation of estrogen concentrations in adipose tissue (Stocco, 2012). Aromatase catalyzes the conversion of A_4 to E_1, and T to E_2, respectively (Figure 2), and in addition to adipose tissue, it is expressed in steroidogenic tissues such as the gonads, and non-steroidogenic tissues such as the breast and bone (Simpson, 1994; Simpson, 2003; Tchernof *et al*, 2015).

In adipose tissue, aromatase is localized mainly in the non-adipocyte stromal vascular fraction of adipose tissue, including in preadipocytes (Ackerman *et al*, 1981; Price *et al*, 1992). The basal transcription of the aromatase-encoding gene in adipose tissue is influenced by proinflammatory cytokines including interleukin-6 and tumor necrosis factor α (Brown and Scherer, 2023; Simpson, 1997), as well as glucocorticoids (Zhao *et al*, 1995). Epigenetic control may also play a role as in adipocytes, increased DNA methylation may upregulate the mRNA expression of *CYP19A1* (Lewis *et al*, 2013). The effect of adipogenesis on *CYP19A1* mRNA expression and activity is unclear. It may be that expression is increased after preadipocytes are induced to differentiate (Blouin *et al*, 2009).

Aromatase expression and activity in adipose tissue seem to be higher with increasing age (Bulun *et al*, 1994; Misso *et al*, 2005; Cleland *et al*, 1985). There also seem to be adipose tissue depot-specific differences in the expression of the aromatase encoding-gene and aromatase activity. *CYP19A1* mRNA expression is higher in subcutaneous compared to visceral adipose tissue in premenopausal women (Wang *et al*, 2013; Paatela *et al*, 2016), and the production of E_1 from A_4 in femoral and gluteal adipose tissue is higher than in subcutaneous abdominal adipose tissue (Killinger *et al*, 1990). In both pre- and postmenopausal women, plasma conversion of A_4 to E_1 was correlated with body weight (Edman *et al*, 1978; MacDonald *et al*, 1978). In turn, treatment with an aromatase inhibitor was associated with an increase in the ratio of visceral compared to subcutaneous adipose tissue (Battisti *et al*, 2014), and aromatase-knockout mice exhibit increased visceral obesity (Jones *et al*, 2001).

Determination of sex steroid hormones

Traditionally, estrogens and androgens have been quantitated with immunoassays (Kushnir, 2011). In a radioimmunoassay (RIA), an analyte (antigen) and a radiolabeled antigen compete for binding to a fixed amount of a specific antibody or receptor binding sites (Wudy *et al*, 2018). Radio- or direct immunoassays typically measure one analyte at a time. Sex steroid measurements are often prone to involve problems with analyte specificity, as sex steroids are small molecules and biologically different sex steroids structurally resemble each other. This may cause cross-reactivity, leading to falsely inflated analyte concentrations with immunoassays. (Wudy *et al*, 2018) This is especially problematic with direct immunoassays at low analyte concentrations, such as estrogens in postmenopausal women or in men, or in non-gonadal tissues (Rosner *et al*, 2013). The immunoanalysis of a substrate may also be confounded by matrix effects on the measurement assay, including interfering binding proteins and unknown matrix components. Direct immunoassays work within a limited dynamic range (Hoofnagle and Wener, 2009). To some extent, problems with specificity and accuracy can be overcome with preceding purification steps of samples (Stanczyk *et al*, 2010), and direct immunoassays are still performed, because of their affordability and wide availability, as well as relative technical simplicity (Taylor *et al*, 2015).

Mass spectrometric assays are highly specific for certain substrates and allow for the parallel measurement of several analytes (Wudy *et al*, 2018). Mass spectrometric analysis separates multiple ions from a sample according to their mass to charge ratios in an electromagnetic field and quantifies their amounts. The

development of liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods for precise, sensitive, and specific quantitation of sex steroids in a wide dynamic range, especially in subjects with low physiological concentrations of analytes, has greatly increased the quality and realiability of sex steroid determination. It also allows for the determination of multiple analytes simultaneously. (Kushnir, 2011; Wudy *et al*, 2018; Laforest *et al*, 2019) The determination of sex steroids using LC-MS/MS analytical methods is considered the gold standard in medical literature (Handelsman and Wartofsky, 2013).

Adipose tissue is an analytically challenging matrix for reliable extraction and quantification of sex steroids owing to the presence of multiple co-resembling lipidic compounds (Laforest *et al*, 2019). Careful extraction and purification of analytes before LC-MS/MS analysis is required (Laforest *et al*, 2019) to avoid coelution of analyte peaks due to interference from matrix components, especially in samples where analyte concentrations are low (Payne and Hales, 2004). LC-MS/MS method validation includes testing for linearity, precision, sensitivity, accuracy, stability, specificity, recovery, matrix effects, limits of detection and quantification, and reproducibility (Honour, 2011). Our research group has previously developed sensitive methods for the accurate quantification of DHEA (Wang *et al*, 2011), E_2 (Wang *et al*, 2012; Wang *et al*, 2013) and E_1 (Vihma *et al*, 2016) in adipose tissue. The use of $^{13}C_3$-labeled internal standards has improved analytical accuracy due to their high label stability and their close chemical resemblance to the respective analytes, and their use has erased the need to use radioactive labels (Wudy *et al*, 2018).

Aims of the study

The present study was designed to investigate the roles of subcutaneous and visceral adipose tissue depots and the menopausal transition in adipose tissue estrogen biosynthesis and metabolism. The specific aims were to study and compare:

1. Estrogen concentrations and metabolism between subcutaneous and visceral adipose tissue in women (I-III)

2. Estrogen concentrations and metabolism in adipose tissue in premenopausal and postmenopausal women (I-II)

3. The effects of menopausal hormone therapy on estrogen, androgen, and progesterone concentrations and sex steroid metabolism in adipose tissue (III).

Materials and Methods

Study subjects

Abdominal subcutaneous and visceral adipose tissue samples were collected during laparoscopic or abdominal gynecological surgery for nonmalignant reasons at the Helsinki University Hospital, Department of Obstetrics and Gynecology. The ethics committee of the Helsinki University Hospital accepted the study protocol (permission numbers Dnro 27/13/03/04/2009 and 286/13/03/03/2011), and the women included in the study gave informed consent to participate. The indications for surgery are described in Studies I-III.

Study I included 28 premenopausal women, and **Study II** 37 postmenopausal women. Women using oral contraceptives, menopausal HT, gonadotrophin-releasing hormone agonists 4 weeks or less before surgery, or women with endometriosis, were excluded.

In Study I, 12 women using the LNG-IUS (Mirena©, Bayer AG, Leverkusen, Germany) were included as the absorption of levonorgestrel into the bloodstream was considered low (Seeber *et al*, 2012; Reinecke *et al*, 2018). Information regarding the menstrual cycle was recorded, and serum concentrations of FSH and progesterone were measured. Date of last menstrual period, concentration of serum progesterone and serum E_2, and information on the pathologic-anatomic diagnosis of the endometrium, where available, were used to define the stage of menstrual cycle of the participants (follicular, n=14; periovulatory, n=5; luteal, n=7; not determined, n=2). Premenopausal status was determined with FSH ≤26.6 IU/L (HUSLAB) and with data on the menstrual cycle.

Study III participants included 63 postmenopausal women, with 50 women using peroral HT (HT+) and 13 non-users of HT (HT-). The HT+ group included 17 users of estrogen only (E_2 conjugates: E_2 valerate or E_2 hemihydrate), and 33 users of estrogen-progestin therapy (E_2 + 52 mg LNG-IUS; Mirena©; n=5); E_2 + norethisterone acetate (n=7); E_2 + medroxyprogesterone acetate (n=11); or E_2 + dydrogesterone (n=10). The daily dose of E_2 in the HT regimen was between 0.5

and 2 mg, the majority (78%) using 1 mg daily. Between-group comparisons were carried out between users and non-users of HT.

Anthropometric parameters for estimation of body adiposity (BMI, waist/hip ratio, waist circumference) were measured. In addition, in Study III, hip circumference, height, and body adiposity index (BAI) were calculated. BAI was calculated using the formula: BAI=hip circumference (cm)/[height (m) x SQRT(height)] – 18 (Li *et al*, 2021). The clinical characteristics of study subjects in Studies I through III are presented in Table 3.

Table 3 Clinical characteristics of subjects in Studies I-III.

	Study I Premenopausal n=28	**Study II Post HT- n=37**	**Study III Post HT- n=13**	**Study III Post HT+ n=50**
Age (years)	46 (30-52)	61 (46-84)	62 (55-80)	62 (51-81)
BMI (kg/m^2)	25 (17-40)	26 (21-37)	28 (22-36)	26 (20-34)
Waist circumference (cm)	88 (65-115)	92 (73-114)	97 (81-120)	90 (70-115)
Waist/hip ratio	0.83 (0.71-0.99)	0.87 (0.74-1.05)	0.89 (0.79-1.02)	0.87 (0.74-1.05)
Serum FSH (IU/l)	6 (2-22)	64 (34-188)	50 (25-71)	38 (1-110)
Serum SHBG (nmol/l)	62 (18-183)	56 (13-97)	51 (16-89)	72 (28-176)

Data are presented as median (range). BMI, body mass index; FSH, follicle-stimulating hormone; SHBG, sex hormone−binding globulin. Premenopausal, premenopausal women; Post HT-, postmenopausal women without menopausal hormone therapy; Post HT+, postmenopausal women with menopausal hormone therapy.

Methods

Blood and adipose tissue samples

Blood samples were drawn prior to surgery. Centrifugation was performed within 1 hour for separation of serum, and the samples were stored at −80°C. During surgery, the operating gynecologist obtained adipose tissue samples ranging from 50 mg to 8 g each. These were immediately snap-frozen in liquid nitrogen and stored at −80°C until analysis. Samples of subcutaneous adipose tissue were obtained from adipose tissue adjacent to the surgical wound, and visceral adipose tissue from the omentum.

Sex steroid analysis in serum and adipose tissue

Together with collaborators at Helsinki University Hospital laboratory (HUSLAB), our research group has over the span of over two decades developed and refined methodology for the quantification of sex steroids in both adipose tissue and serum. The extraction and purification of sex steroid analytes before LC-MS/MS, as well as improvements to the LC-MS/MS methods, are processes that have advanced in several consecutive steps and have also evolved during Studies I-III. Original methods and changes to previous methods have been published in several journals and are quoted and described both in the following sections and in more detail in Studies I-III.

Serum analyses of FSH, SHBG (I-III) and progesterone (I)

Serum FSH and SHBG were measured using accredited methods at the HUSLAB. FSH was determined using a competitive electrochemiluminescence immunoassay (Roche Modular Analytics E170 system, Roche Diagnostics, Indianapolis, IN). For quantitation of serum SHBG, an Immulite 2000 Xpi analyzer with a chemiluminescent enzyme immunoassay (Siemens Healthcare Diagnostics, Siemens Healthineers, Tarrytown, NY) was used.

Serum progesterone in Study I was quantitated using LC–MS/MS as described previously (Savolainen-Peltonen *et al*, 2018). Briefly, $^{13}C_3$-progesterone (IsoSciences LLC, King of Prussia, PA) was added to serum as an internal standard, after which serum samples were extracted with diethylether. An LC-MS/MS system with an API 3000 triple quadrupole mass spectrometer (PE Sciex, Foster City, CA), an Agilent series 1200 HPLC system with a binary pump (Waldbronn, Germany) and a SunFire C18 column (2.1 x 50 mm; Waters, Milford, MA) was used to study calibrators (Riedel-deHaën, Buchs, Switzerland) and serum extracts. The limit of quantification (LOQ) was 0.1 nmol/l.

Serum E_1, E_2, and E_1S (I-III)

In **Study I**, the methods of quantification of E_1 and E_2 in serum were developed from **Study II** to allow for simultaneous quantification of the two analytes in one run with LC-MS/MS. Briefly, assay calibrators of 10–1000 pmol/l E_1 (Vetranal, Sigma–Aldrich) and 13–1275 pmol/l of E_2 (Sigma–Aldrich) were prepared in methanol:water (1:1, v/v). $^{13}C_3$-E_1 (IsoSciences) and $^{13}C_3$-E_2 (IsoSciences) were added as internal standards to samples and calibrators before analysis with LC-MS/MS. An LC–MS/MS with an Agilent 1200 HPLC and an AB Sciex Triple Quad

5500 mass spectrometer was used for simultaneous quantification of serum E_1 and E_2 as described in detail in Study I. The LOQ for both analytes in serum was 10 pmol/l.

In **Study II**, serum E_1 was quantitated using $^{13}C_3$-E_1 (IsoSciences LLC) as an internal standard, and 0.25 ml of serum was purified and analyzed using LC-MS/MS as described previously in detail (Vihma *et al*, 2016). The LOQ for E_1 in serum was 15 pmol/l. Serum E_2 was quantitated using $^{13}C_3$-E_2 (E-073 Cerilliant; Sigma-Aldrich, St Louis, MO) as an internal standard, and 0.25 ml was prepared for analysis of E_2 with LC-MS/MS as described before (Paatela *et al*, 2016). The LOQ for E_2 in serum was 15 pmol/l.

In **Study III**, serum E_1 and E_2 were quantitated simultaneously in one run using LC-MS/MS as described in Study I, with minor methodological enhancements as described in Study III. The LOQ for E_1 and E_2 in serum were 5 pmol/l.

A novel method for quantifying serum E_1S was developed (Study II). Briefly, D_4-E_1S (C/D/N Isotopes Inc., Pointe-Claire, QC, Canada) was added as an internal standard to calibrators and serum samples. Serum samples went through solid phase extraction (Oasis® HLB 1 cc, 30 mg; Waters, Ireland) and after drying under vacuum, were collected with 0.5 ml of methanol. The residue, after evaporation, was dissolved in 0.15 ml water:methanol (1:1, v/v). Sample extracts and calibrators were analyzed for E_1S with LC-MS/MS. The same method for quantifying serum E_1S was also used in Studies I and III. The LOQ for E_1S in serum was 100 pmol/l.

Serum A_4, T, DHEA, and progesterone (III)

Serum A_4, T, DHEA, and progesterone were quantitated using LC-MS/MS as described (Robciuc *et al*, 2022) with minor modifications described in Study III. For A_4, T, and progesterone, the LOQ were 10 pmol/l, and 100 pmol/l for DHEA.

Separation and purification of adipose tissue sex steroids and quantitation with LC/MS-MS

Adipose tissue E_1 and E_2 (I-III)

Paired subcutaneous and visceral adipose tissue samples were analyzed within the same series to minimize the effects of interassay variation. In **Studies I-II**, adipose tissue E_1 was extracted and purified, and the concentration determined using LC/MS-MS (Vihma *et al*, 2016). In short, 200 mg of adipose tissue was homogenized in water and tritiated E_1 was added as an internal standard. Pooled male serum with 180 and 540 pmol/l of added exogenous E_1 (Sigma-Aldrich) were

used as control. Diethyl ether/ethyl acetate (1:1, v/v) was used to extract samples. After evaporation to dryness and addition of chloroform/hexane (1:2, v/v) the samples were transferred to a Sephadex LH-20 column. After washing the column with the same solvent, E_1 was eluted using methanol and evaporated to dryness. Another Sephadex LH-20 column chromatography in 9% methanol in toluene was performed and E_1 was eluted with the same solvent. Extraction recovery was calculated by determining tritiated E_1 internal standard and an aliquot was processed further for quantitation of E_1 with LC-MS/MS. Prior to analysis of E_1 with LC-MS/MS, $^{13}C_3$-E_1 was added as an internal standard. The LOQ for E_1 in adipose tissue was 15 pmol/l.

Adipose tissue E_1 determination was further developed by including $^{13}C_3$-E_1 (IsoSciences) as an internal standard after adipose tissue homogenization, before sample extraction and purification (**Study III**). The LOQ for E_1 in adipose tissue was 10 pmol/l.

The method previously used in our laboratory for extraction, purification, and determination with LC-MS/MS of adipose tissue E_2 was developed further by including $^{13}C_3$-E_2 (E-073 Cerilliant; Sigma-Aldrich) as an internal standard before adipose tissue sample extraction and purification as described previously (Wang F *et al*, 2012; Wang F *et al*, 2013). Briefly, 200 mg of adipose tissue was homogenized in water and exctracted four times with diethyl ether/ethyl acetate (1:1, v/v) and evaporated to dryness. Chloroform/hexane (1:1, v/v) was added and the sample was applied on a Sephadex LH-20 column, which was washed with chloroform/hexane and the E_2 fraction was eluted using methanol. Further processing and quantification of E_2 with LC-MS/MS as well as method validation are described in Study I. Data on the method validation are described in detail in **Study II**. The LOQ for E_2 in adipose tissue was 20 pmol/l (I-II) and 10 pmol/l (III).

Adipose tissue A_4, T, DHEA, and progesterone (III)

Samples of adipose tissue for quantitation of A_4, T, DHEA, and progesterone were extracted and purified as described for DHEA in Wang *et al*, 2011 with slight modifications. Briefly, $^{13}C_3$-A_4, $^{13}C_3$-T, $^{13}C_3$-DHEA, and $^{13}C_3$-progesterone (IsoSciences) were added as internal standards before extraction and purification of adipose tissue samples. The samples were analyzed for A_4, T, DHEA, and progesterone with LC-MS/MS as described (Robciuc *et al*, 2022). Details regarding the validation of the methods are described in Study III. The LOQ were 10 pmol/l for A_4, T, and progesterone, and 100 pmol/l for DHEA.

STS activity assay in adipose tissue (I-III)

The assay for steroid STS activity in converting E_1S to E_1 in adipose tissue is described in Study II and was modified from the STS activity assay in converting DHEAS to DHEA (Paatela *et al*, 2016). Briefly, purified tritium-labeled E_1S was added to 200 mg adipose tissue homogenates (a weighted mean of 5.2×10^5 disintegrations per minute (dpm), corresponding to 5.8 pmol per 200 mg adipose tissues in Study I; 5.3×10^5 dpm / 6.0 pmol per 200 mg adipose tissue in Study II; and 5.3×10^5 dpm / 6.0 pmol per 200 mg adipose tissue for women using HT; and 5.6×10^5 dpm / 6.3 pmol per 200 mg adipose tissue for women not using HT in Study III. Incubation of tritium-labeled E_1S in Tris-HCl was used as control. The adipose tissue homogenates and control samples were incubated for 3 hours at +37°C, after which they were extracted with diethyl ether/ethyl acetate (1:1, v/v), the tritium-labeled E_1S–containing water phase was quick-frozen for separation from the organic fraction. After evaporation, dry residues were dissolved in hexane/chloroform (2:1, v/v) and applied to a Sephadex LH-20 chromatography column which was washed with the same solvent to remove the lipophilic fraction. Tritium-labeled E_1 was eluted with methanol and the radioactivity was determined by liquid-scintillation counting (Rackbeta; Wallac, Turku, Finland). STS activity was expressed as tritium-labeled E_1 in nmol formed from tritium-labeled E_1S per mass of adipose tissue in kg per incubation hours.

Inhibition of STS in adipose tissue (I)

To verify the activity of STS in converting E_1S to E_1 in adipose tissue, the inhibition of STS with a specific inhibitor STX64 (Irosustat 667COUMATE; kindly supplied by Professor Barry Potter, University of Oxford, United Kingdom) was studied as described in Study I.

Reductive 17β-HSD activity assay in adipose tissue (I-II)

We developed an assay to study the activity of reductive 17β-HSD enzymes in converting E_1 to E_2 in subcutaneous and visceral adipose tissue. In short, 200 mg samples of adipose tissue were homogenized in potassium phosphate buffer at pH 7.4. $^{13}C_3$-E_1 (IsoSciences LLC), 150 nmol/l E_1 [1,3,5(10)-estratrien-3-OL-17-one; Steraloids, Newport, RI], and 3.7 mmol/l NADPH (Roche Diagnostics) were added, and the mixture was incubated for 3 hours at +37°C. Incubation of the mixture of reagents without adipose tissue and incubation of buffer alone served as controls.

Deuterated E_2 was added as an internal standard and aliquots of samples were analyzed for $^{13}C_3$-E_2 by LC-MS/MS. The enzyme activity was expressed as $^{13}C_3$-E_2 in nmol formed from $^{13}C_3$-E_1 per mass of adipose tissue in kg per time of incubation in h. The LOQ for detection of $^{13}C_3$-E_2 in adipose tissue was 10 pmol/l.

Preparation of RNA and complementary DNA, and quantification of mRNA (I-III)

The isolation, purification, and reverse-transcription of mRNA in adipose tissue was performed as described previously (Wang F *et al*, 2013). Briefly, 150 to 200 mg of frozen adipose tissue was homogenized in TRIzol (Thermo Fisher Scientific, Waltham, MA, USA), and RNeasy lipid tissue mini kit (QIAGEN, Hilden, Germany) was used to isolate and purify total RNA. SuperScript VILO cDNA synthesis kit (Invitrogen by Life Technologies, Carlsbad, CA, USA) was used for reverse-transcribing RNA into cDNA. Real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used to quantitate the relative mRNA expression levels of *STS, CYP19A1*, *HSD17B2*, *HSD17B7*, and *HSD17B12* (Studies I-III), and and *HSD17B1*, *ESR1, ESR2, AR, PRb* and *PRa/b (*Study III*)*. Normalization was calculated using the geometric mean of reference genes *importin 8*, *lysine-specific demethylase 2B,* and *glyceraldehyde-3-phosphate dehydrogenase (I-II), and importin 8, glyceraldehyde-3-phospate dehydrogenase*, and *14-3-3 protein zeta/delta* (III).

Other methods

Circulating free E_2 was calculated using the formula:
Free E_2 = $[10^{(-0.003 \times SHBG + 0.389)} / 100] \times E_2$, where E_2 is the concentration of E_2 in serum (nmol/l) and SHBG is expressed in nmol/l (Moore *et al*, 1982) (Studies II-III).

Serum free T concentration was calculated using Anderson's formula (Vihma *et al*, 2017; Anderson *et al*, 1975), where free T [pmol/l] = total T [nmol/l] × [2.28 – 1.38 × log(SHBG [nmol/l]/10)] × 10 (Study III).

Statistical analysis

We used IBM SPSS Statistics 22.0 software (Studies I-II) and SPSS Statistics 28.0 software (Study III). Normality of variables was assessed using the Shapiro-Wilk test. The Wilcoxon signed rank test was used for pairwise comparisons, and

between-group differences were evaluated using the independent-samples Mann-Whitney *U* test and the independent-samples Kruskall-Wallis test for nonparametric variables. For parametric variables, between-group differences were examined using the Student's *t* test. Data are expressed as median (interquartile range) or median (range) unless otherwise stated. Spearman nonparametric correlation was used for correlation analyses. The level of statistical significance was $P<0.05$.

In Study III, the HT+ and HT- groups differed in terms of BMI in their clinical characteristics. To study the possible effect of this difference, each HT- group subject (n=13) was matched with two subjects (n=26) from the HT+ group and between-group analyses were redone for comparison.

Results

Estrogen concentrations in serum and in subcutaneous and visceral adipose tissue

Premenopausal women (I)

E_1 was the predominant estrogen in adipose tissue before menopause with a higher concentration in visceral compared to subcutaneous adipose tissue (Table 4). Concentrations of E_2 were similar in the two adipose tissue depots. The median concentrations of E_1 in adipose tissue were five to ten times higher than in serum (P<0.001 for both subcutaneous and visceral adipose tissue; n=17-25). Visceral adipose tissue E_2 concentration was 1.3 times higher than in the circulation (P<0.01; n=22). No statistically significant difference was observed in E_2 concentrations between subcutaneous adipose tissue and serum (P=0.14; n=13). The E_2/E_1 ratio in subcutaneous adipose tissue was higher than in visceral adipose tissue (0.45 vs 0.21; P=0.01, n=14).

In premenopausal women, estrogen concentrations in serum or subcutaneous or visceral adipose tissue did not correlate with BMI or waist circumference.

Postmenopausal women without HT (II-III)

In postmenopausal women, E_1 was the most abundant unconjugated estrogen in both serum and adipose tissue (Table 4). E_1 concentration in visceral adipose tissue was higher than in subcutaneous adipose tissue, while E_2 concentrations in the two depots were similar (II). There was a positive correlation between BMI and E_1 concentration in visceral but not subcutaneous adipose tissue (r= 0.46; P=0.01; n=30 for visceral adipose tissue). The E_2/E_1 ratio was higher in subcutaneous than in visceral AT (0.08 vs 0.06; P<0.01; n=14).

Subcutaneous and visceral adipose tissue E_1 concentrations were 8 to 11 times higher compared to serum concentrations (P<0.001; n=25-30) and subcutaneous and visceral adipose tissue E_2 concentrations were three times higher than in the circulation (P<0.001; n=21-22) (II). Serum E_1S concentration was positively

correlated with visceral adipose tissue E_1 (r=0.70, P<0.001, n=24) and E_2 (r=0.46, P<0.005, n=22) concentrations. Serum and subcutaneous and visceral adipose tissue concentrations of E_1 and E_2 were positively correlated (Table 2 in Study II).

Postmenopausal women without HT had higher concentrations of E_1 compared to E_2 in both subcutaneous and visceral adipose tissue (III). The difference between subcutaneous and visceral adipose tissue E_1 concentrations did not reach statistical significance. Median serum concentrations of E_1 and E_2 were low in this group. E_1 concentrations in subcutaneous and visceral adipose tissue were 21 and 42 times higher than the serum E_1 concentration (P<0.01; n=12 and P=0.001; n=13, respectively), and E_2 concentrations in subcutaneous and visceral adipose tissue were 11 and 9 times higher than in serum (P=0.01; n=11 and P<0.01; n=13).

Table 4 Serum and adipose tissue estrogen concentrations in premenopausal women and in postmenopausal women with and without menopausal hormone therapy.

		Study I Premenopausal	**Study II Post HT-**	**Study III Post HT-**	**Study III Post HT+**
SERUM, pmol/l					
E_1S		4360 (2715-12575)[a]	650 (400-1000)[b]	1200 (600-1750)[c]	14600 (4625-27300)[d]
E_1		279 (172-427)[a]	87 (61-115)[e]	39 (27-70)[c]	427 (306-1095)[f]
E_2		432 (230-861)[a]	19 (12-32)[g]	6 (1-18)[c]	94 (55-168)[f]
ADIPOSE TISSUE, pmol/kg					
E_1	sc	1459 (906-2707)[h]	706 (481-876)[i]	814 (586-1636)[c]	5989 (2192-9807)[j]
	visc	2657 (1625-4756)[j]	928 (622-1170)[k]	1629 (525-1748)[c]	6731 (2950-10192)[l]
	P-value	**<0.01** (n=17)	**<0.01** (n=24)	0.18 (n=12)	**<0.01** (n=23)
E_2	sc	651 (295-1286)[c]	57 (41-92)[m]	63 (5-144)[n]	241 (118-578)[k]
	visc	558 (459-1450)[m]	52 (29-144)[o]	51 (7-86)[c]	259 (175-390)[p]
	P-value	0.06 (n=14)	0.89 (n=15)	0.24 (n=11)	0.11 (n=27)

Data are presented as median (interquartile range). *P*-values, sc versus visc adipose tissue (Wilcoxon signed rank test). Statistical significance is denoted in bold. E_1S, estrone sulfate; E_1, estrone; E_2, estradiol; Sc, subcutaneous adipose tissue; Visc, visceral adipose tissue. Premenopausal, premenopausal women; Post HT-, postmenopausal women without menopausal hormone therapy; Post HT+, postmenopausal women with menopausal hormone therapy. [a] n=27; [b] n=28; [c] n=13; [d] n=48; [e] n=31; [f] n=50; [g] n=29; [h] n=17; [i] n=25; [j] n=24; [k] n=30; [l] n=42; [m] n=22; [n] n=11; [o] n=23; [p] n=44.

Postmenopausal women with HT (III)

E_1 concentrations exceeded those of E_2 in subcutaneous and visceral adipose tissue (Table 4). The concentration of E_1 was higher in visceral compared to subcutaneous adipose tissue. The E_2/E_1 ratio was higher in subcutaneous compared to visceral adipose tissue (Table 6).

Subcutaneous and visceral adipose tissue E_1 concentrations were 14 to 16 times higher than in serum ($P<0.001$, n=24; and $P<0.001$; n=42) and subcutaneous and visceral adipose tissue E_2 concentrations were three times higher than in the circulation ($P<0.001$; n=30 and $P<0.001$; n=44, respectively) (Table 4).

Sex steroid concentrations in serum and in subcutaneous and visceral adipose tissue in postmenopausal women with or without HT (III)

Users of HT had serum concentrations of E_1S, E_1, and E_2 that were an order of magnitude greater compared to non-users of HT (Table 4). In subcutaneous and visceral adipose tissue, E_1 and E_2 concentrations were 4 to 7 times higher in users than in non-users of HT. Consequently, in both users and non-users of HT the adipose tissue to serum ratios of E_1 and E_2 concentrations remained high, as shown in Table 4.

Whereas the concentrations of T in serum did not differ between users and non-users of HT, the concentration of free T was 40% lower in users compared to non-users of HT (Table 5). T in visceral adipose tissue was also roughly 30% lower in HT users than in non-users. When comparing BMI-matched subjects from the HT- to HT+ groups, concentrations of T in subcutaneous adipose tissue were similar (627 vs 502 pmol/kg; $P=0.08$).

Progesterone, A_4, and DHEA concentrations in serum and subcutaneous and visceral adipose tissue did not differ between users and non-users of HT (Table 5). In users or non-users of HT, serum or adipose tissue progesterone, T, A_4, or DHEA did not correlate with parameters describing adiposity.

While the E_2/E_1 ratios were comparable, ratios of estrogens to androgens in users of HT were 9 to 12 times higher in subcutaneous and 4 to 6 times higher in visceral adipose tissue than in non-users (Table 6).

Table 5 Concentrations of serum and adipose tissue progesterone and androgens in postmenopausal women with and without menopausal hormone therapy (Study III).

		HT- n=10-13	HT+ n=24-50	*P*-value*
SERUM, pmol/l				
Progesterone		120 (100-210)	130 (80-160)	0.79
T		835 (510-1333)	600 (400-880)	0.17
Free T		10.8 (7.7-17.3)	6.1 (4.6-9.6)	**<0.01**
Free T, %		1.3 (1.2-1.5)	1.1 (0.9-1.2)	**<0.001**
A_4		2500 (1500-3100)	2300 (1500-3100)	0.96
DHEA		7500 (4800-12300)	7200 (5100-11800)	0.96
ADIPOSE TISSUE, pmol/kg				
Progesterone	sc	1910 (1139-2883)	1200 (1078-2190)	0.35
	visc	4482 (2167-8886)	2878 (1855-4204)	0.15
	P-value#	*P*=0.07 (n=9)	***P*=0.02** (n=8)	
T	sc	627 (541-936)	407 (316-629)	**0.02**
	visc	658 (623-1292)	440 (164-853)	**<0.01**
	P-value#	*P*=0.55 (n=9)	*P*=0.78 (n=8)	
A_4	sc	16246 (12369-22667)	12561 (10985-14691)	0.15
	visc	22390 (20414-29517)	18529 (11749-27750)	0.16
	P-value#	***P*=0.03** (n=9)	***P*=0.02** (n=8)	
DHEA	sc	41340 (35970-66337)	35010 (28614-44368)	0.20
	visc	45358 (36878-79301)	40113 (23133-65455)	0.29
	P-value#	*P*=0.17 (n=9)	*P*=0.34 (n=8)	

Data are presented as median (interquartile range). *P*-value*, HT- vs HT+ (Mann-Whitney *U* Test); *P*-value#, subcutaneous vs visceral adipose tissue (Wilcoxon signed rank test). Statistical significance is shown in bold. HT-, non-users of menopausal hormone therapy; HT+, users of menopausal hormone therapy; T, testosterone; A_4, androstenedione; DHEA, dehydroepiandrosterone; sc, subcutaneous adipose tissue; visc, visceral adipose tissue.

Table 6 Ratios of estrogens to androgens in postmenopausal women with or without menopausal hormone therapy (Study III).

RATIO		HT- n=10-13	HT+ n=8-38	*P*-value*
E_2/E_1	sc	0.07 (0.01-0.09)	0.06 (0.05-0.08)	0.57
	visc	0.04 (0.02-0.07)	0.04 (0.03-0.05)	0.58
	P-value#	0.08 (n=11)	**0.02** (n=18)	
E_2/T	sc	0.11 (0.02-0.18)	0.99 (0.30-1.85)	**<0.001**
	visc	0.08 (0.01-0.13)	0.48 (0.30-1.45)	**<0.001**
	P-value#	0.17 (n=9)	0.40 (n=7)	
E_1/A_4	sc	0.06 (0.06-0.08)	0.67 (0.24-1.00)	**<0.001**
	visc	0.06 (0.04-0.08)	0.26 (0.14-0.57)	**<0.001**
	P-value#	0.29 (n=9)	0.13 (n=7)	
$E_1+E_2/T+A_4$	sc	0.06 (0.06-0.11)	0.70 (0.26-1.03)	**<0.001**
	visc	0.06 (0.04-0.08)	0.27 (0.13-0.69)	**<0.001**
	P-value	0.40 (n=8)	0.23 (n=5)	

Data are shown as median (interquartile range). *P*-value*, HT- vs HT+ (Independent-samples Mann-Whitney *U* Test); *P*-value#, subcutaneous vs visceral adipose tissue (Wilcoxon signed rank test). HT-, non-users of menopausal hormone therapy; HT+, users of menopausal hormone therapy; E_2, estradiol; E_1, estrone; T, testosterone; A_4, androstenedione; Sc, subcutaneous adipose tissue; Visc, visceral adipose tissue.

In the HT+ group, serum concentrations of E_1S, E_1, and E_2, as well as adipose tissue concentrations of E_1 (Figure 4) and E_2 (Figure 5), were generally higher, and the concentrations of T in serum and adipose tissue lower (Figure 6), with increasing dose of estradiol in the HT regimen. The strongest correlation was detected for visceral adipose tissue E_1 (r=0.46, P<0.01, n=41) and E_2 (r=0.45, P<0.01, n=43). In addition, the ratios of estrogens to androgens (E_2 to T, E_1 to A_4, and E_1+E_2 to $T+A_4$) in visceral adipose tissue were positively correlated with the E_2 dose of the HT regimen (r=0.49, P=0.02, n=22; r=0.56, P<0.01, n=24; and r=0.57, P<0.01, n=22, respectively).

In the HT- but not the HT+ group, the estrogen to androgen ratios, as represented by $E_1+E_2/T+A_4$, in both subcutaneous and visceral adipose tissue were positively correlated with body adiposity index (r=0.62, P<0.05, n=11; r=0.70, P<0.05, n=9, respectively).

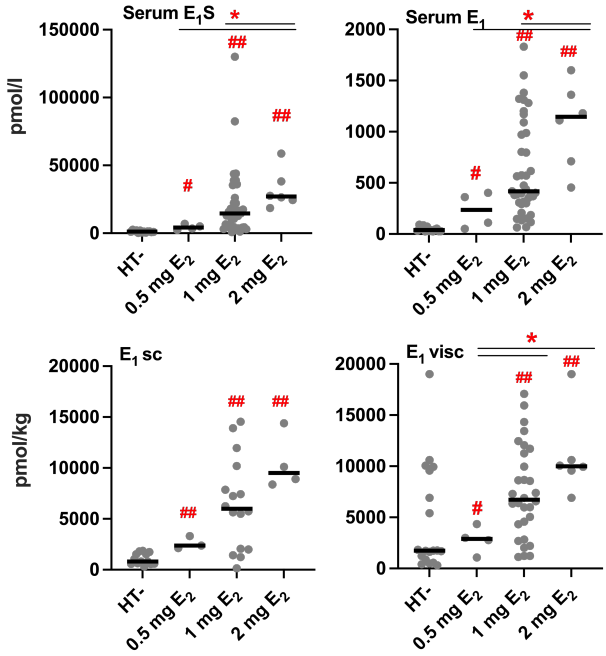


Figure 4 Serum estrone sulfate and estrone, and adipose tissue estrone concentrations according to dose of estradiol in the menopausal hormone therapy regimen (Study III). Median is denoted by the black line. #, $P<0.05$; ##, $P<0.001$, compared to HT; *, $P<0.05$, compared to 1 mg or 2 mg E_2 (Mann-Whitney *U* Test). E_1S, estrone sulfate; E_1, estrone; E_2, estradiol; HT-, non-users of menopausal hormone therapy; Sc, subcutaneous adipose tissue; Visc, visceral adipose tissue.

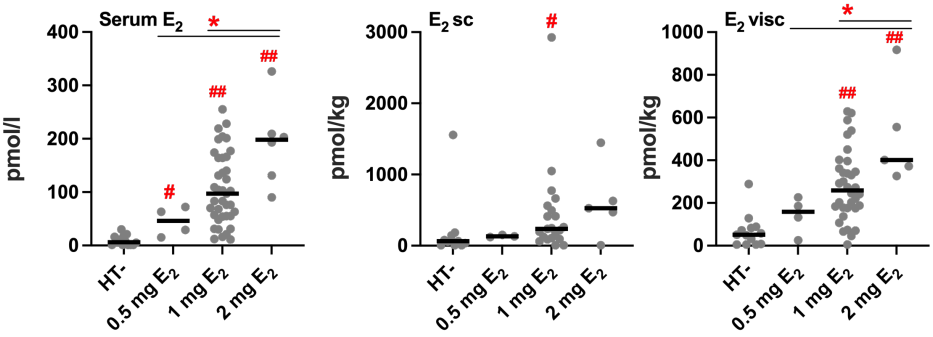


Figure 5 Serum and adipose tissue estradiol concentrations according to dose of estradiol in the menopausal hormone therapy regimen (Study III). Median is denoted by the black line. #, $P<0.05$; ##, $P<0.001$, compared to HT; *, $P<0.05$, compared to 1 mg or 2 mg E_2 (Mann-Whitney *U* Test). E_2, estradiol; HT-, non-users of menopausal hormone therapy; Sc, subcutaneous adipose tissue; Visc, visceral adipose tissue.

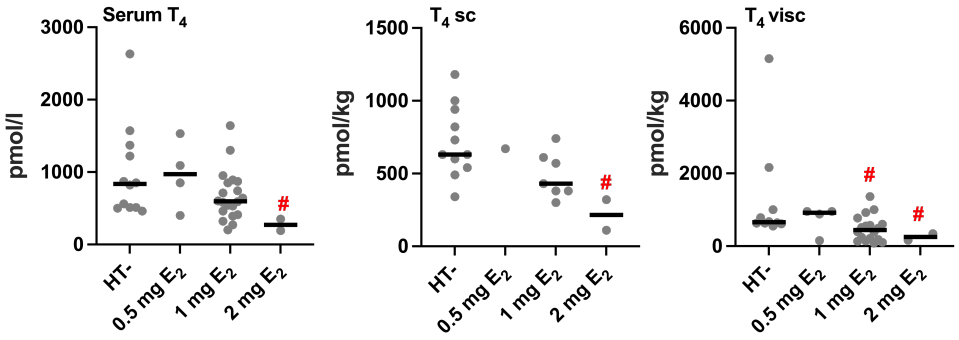


Figure 6 Serum and adipose tissue testosterone concentrations according to dose of estradiol in the menopausal hormone therapy regimen (Study III). Median is denoted by the black line. #, $P<0.05$, compared to HT (Mann-Whitney *U* Test). T, testosterone; E_2, estradiol; HT-, non-users of menopausal hormone therapy; Sc, subcutaneous adipose tissue; Visc, visceral adipose tissue.

Overall, DHEA was the most abundant sex steroid in subcutaneous and visceral adipose tissue, followed by A_4 (Table 5). In both users and non-users of HT, concentrations of T and DHEA were similar between subcutaneous and visceral adipose tissue. Visceral adipose tissue A_4 concentrations exceeded those in subcutaneous adipose tissue. In users but not in non-users of HT, progesterone concentration was higher in visceral compared to subcutaneous adipose tissue.

In non-users of HT, concentrations of T in subcutaneous and visceral adipose tissue were similar to the serum T concentration (P=0.25; n=11, and P=0.45; n=10, respectively). Progesterone concentrations in subcutaneous and visceral adipose tissue were 16 and 37 times higher than in the circulation (P=0.003; n=11, and P=0.005; n=10, respectively) (Table 5). A_4 concentrations in subcutaneous and visceral adipose tissue were 6.5 and 9 times higher than in the circulation (P=0.003; n=11, and P=0.005; n=10, respectively), and DHEA concentrations in subcutaneous and visceral adipose tissue were 5.5 and 6 times higher than in the circulation (P=0.003; n=11, and P=0.005; n=10, respectively).

In users of HT, concentration of T in serum was roughly 30% lower compared to the concentrations in subcutaneous and visceral adipose tissue (P=0.01; n=10, and P=0.03; n=24, respectively). Progesterone_concentrations in subcutaneous and visceral adipose tissue were 9 and 22 times higher than in the circulation (P=0.005; n=10, and $P<0.001$; n=24, respectively). A_4 concentrations in subcutaneous and visceral adipose tissue were 5.5 and 8 times higher than in the circulation (P=0.005; n=10, and P=0.001; n=24, respectively), and DHEA concentrations in subcutaneous and visceral adipose tissue were 5 and 6 times higher than in the circulation (P=0.005; n=10, and $P<0.001$; n=24, respectively).

STS (I-III) and reductive 17β-HSD (I-II) activities

STS activity in converting E_1S to E_1 was similar between subcutaneous and visceral adipose tissue in both premenopausal (subcutaneous, 5.2 (4.8-5.4) vs visceral, 5.0 (4.8-5.4) nmol/kg adipose tissue/hour; median (interquartile range); *P*=0.47; n=15) and postmenopausal women (subcutaneous, 4.7 (4.5-5.2) vs visceral, 5.1 (4.7-5.8) nmol/kg adipose tissue/hour; *P*=0.10; n=17) women (Figure 7).

In postmenopausal women, STS activity in subcutaneous adipose tissue was positively correlated with subcutaneous adipose tissue E_2 (*r*=0.78; *P*=0.002; n=13) but not E_1 concentration (*r*=0.23; *P*=0.39; n=17) (Study II).

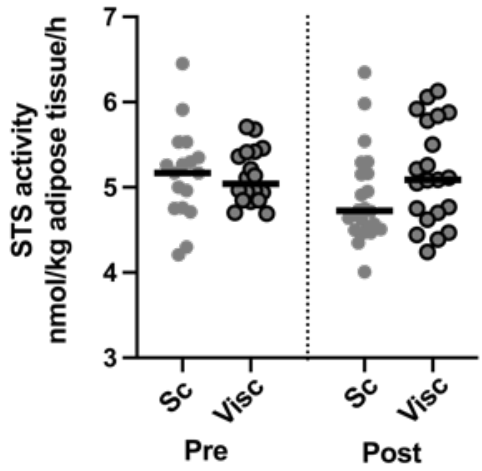


Figure 7 STS activity in the formation of [^{3}H]-E_1 from [^{3}H]-E_1S in subcutaneous (Sc) and visceral (Visc) adipose tissue in premenopausal (Pre) and postmenopausal (Post) women (Studies I-II). Activity of STS is expressed as the amount of [^{3}H]-E_1 (nmol) formed from [^{3}H]-E_1S per mass of adipose tissue (kg) per time of incubation (h). Median is represented by the black line. Sc, subcutaneous; Visc, visceral adipose tissue.

Between BMI-matched postmenopausal HT users and non-users, STS activity in converting E_1S to E_1 was similar in subcutaneous [7.9 (7.2–8.5) vs 6.8 (6.5–7.7) nmol/kg AT/hour; median (interquartile range); *P*=0.07] and visceral [7.3 (6.9–8.6) vs 7.1 (6.6–7.6) nmol/kg AT/hour; *P*=0.09;] adipose tissue (Figure 8).

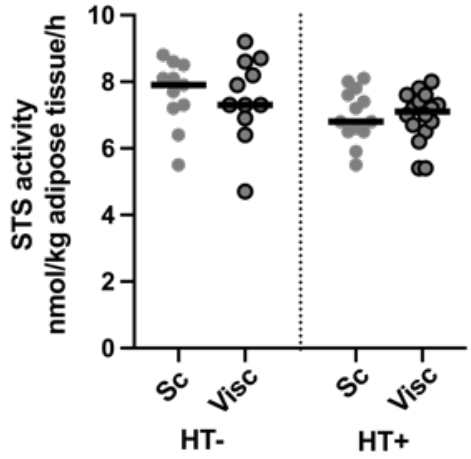


Figure 8 STS activity in the formation of [^{3}H]-E_1 from [^{3}H]-E_1S in subcutaneous (Sc) and visceral (Visc) adipose tissue in BMI-matched HT- and HT+ subjects (Study III). Activity of STS is expressed as the amount of [^{3}H]-E_1 (nmol) formed from [^{3}H]-E_1S per mass of adipose tissue (kg) per time of incubation (h). Median is represented by the black line. Sc, subcutaneous and Visc, visceral adipose tissue; HT-, non-users of menopausal hormone therapy; HT+, users of menopausal hormone therapy.

The activity of STS in subcutaneous and visceral adipose tissue was ascertained by the use of the specific STS inhibitor STX64, which reduced the enzyme activity in 90% ja 92%, respectively, in the two adipose tissue depots (Figure 2 in Study I).

In premenopausal women, the production of E_2 from E_1 by reductive 17β-HSD enzymes was similar between subcutaneous and visceral adipose tissue [median rate of $^{13}C_3$-E_2 production, 0.56 (0.45-0.87) vs 0.60 (0.28-0.82) nmol/kg adipose tissue/h; median (interquartile range); P=0.66; n=12] (Figure 9). In postmenopausal women, the activity of reductive 17β-HSD enzymes in converting E_1 to E_2 was higher in subcutaneous compared to visceral adipose tissue [median rate of $^{13}C_3$-E_2 production, 1.02 (0.67-1.34) vs 0.57 nmol/kg adipose tissue/h; P=0.004; n=12]. Activity was higher in postmenopausal compared to premenopausal subcutaneous adipose tissue [median rate of $^{13}C_3$-E_2 production, 1.02 (0.67-1.34) vs 0.56 (0.45-0.87) nmol/kg adipose tissue/h; P=0.006].

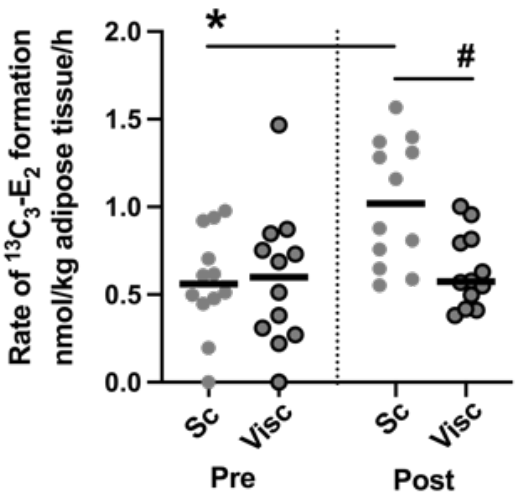


Figure 9 Activity of reductive 17β-HSD enzymes in converting estrone to estradiol, shown as rate of production of $^{13}C_3$-E_2 (nmol) formed from $^{13}C_3$-E_1 per mass of adipose tissue (kg) per time of incubation (h) in subcutaneous (Sc) and visceral (Visc) adipose tissue in premenopausal (Pre) and postmenopausal (Post) patients (Studies I-II). Median is represented by the black line. $^{*}P$=0.006, subcutaneous adipose tissue in premenopausal vs postmenopausal women (Mann-Whitney *U* test); $^{\#}P$=0.004, subcutaneous vs visceral adipose tissue in postmenopausal women (n=12, Wilcoxon signed rank test).

In subcutaneous adipose tissue of premenopausal women, E_2 production was higher in women with higher waist circumference (r=0.60; P=0.04; n=12). In contrast, in postmenopausal women, increased waist circumference was correlated with a higher reductive 17β-HSD activity in visceral adipose tissue (r=0.65; P=0.02; n=12).

mRNA expression of genes encoding sex steroid hormone-converting enzymes (I-III)

Overall, the mRNA expression patterns for the measured enzymes in pre- and postmenopausal adipose tissue were similar (I-II). The mRNA expression levels of *STS* were higher in subcutaneous compared to visceral adipose tissue, and *HSD17B2* and *HSD17B7* were higher in visceral compared to subcutaneous adipose tissue (Figures 10 and 11). In postmenopausal women, *CYP19A1* mRNA expression was higher in subcutaneous compared to visceral adipose tissue (Figure 11).

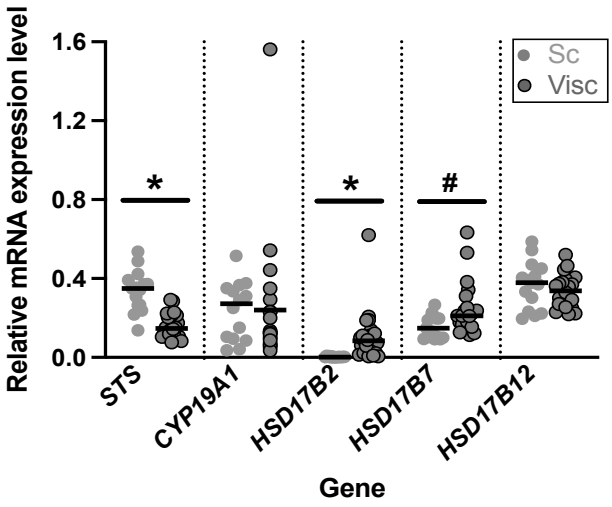


Figure 10 Gene mRNA expression levels in Sc, subcutaneous and Visc, visceral adipose tissue in premenopausal women (Study I). Data are expressed as relative mRNA expression levels. The median is represented by the black line. *, $P<0.001$ and #, $P<0.05$, subcutaneous vs visceral adipose tissue (Wilcoxon signed rank test, n=14-16). *STS*, steroid sulfatase; *CYP19A1*, aromatase; *HSD17B2, HSD17B7,* and *HSD17B12*, hydroxysteroid 17-β dehydrogenase 2, 7, and 12.

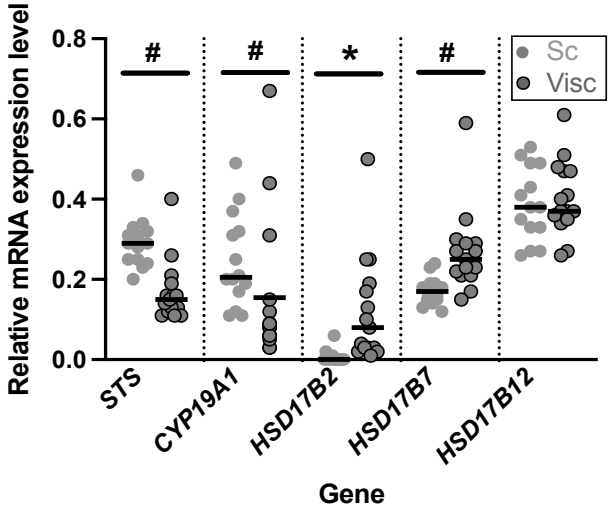


Figure 11 Gene mRNA expression levels in Sc, subcutaneous and Visc, visceral adipose tissue in postmenopausal women (Study II). Data are expressed as relative mRNA expression levels. The median is represented by the black line. *, $P<0.01$ and #, $P<0.05$, subcutaneous vs visceral adipose tissue (Wilcoxon signed rank test, n=12). *STS*, steroid sulfatase; *CYP19A1*, aromatase; *HSD17B2, HSD17B7,* and *HSD17B12*, hydroxysteroid 17β-dehydrogenase 2, 7, and 12.

In premenopausal (I) but not postmenopausal (II) women, *STS* mRNA expression in visceral adipose tissue increased with higher BMI (r=0.50, P=0.02, n=22) and waist circumference (r=0.56, P <0.01, n=22). In premenopausal women, subcutaneous adipose tissue *CYP19A1* mRNA expression correlated positively with BMI and waist circumference (r=0.81, P<0.001, n=14 for both). The E_1 concentration in visceral but not subcutaneous adipose tissue in premenopausal women correlated positively with mRNA expression of *CYP19A1* (r=0.65, P=0.003, n=19).

In postmenopausal women, the patterns of mRNA expression for genes encoding key estrogen- and androgen-metabolizing enzymes (Figure 12), and genes encoding sex steroid receptors (Figure 3 in Study III), did not markedly differ between BMI-matched users and non-users of HT (III).

In the HT+ group, mRNA expression levels did not correlate with adiposity parameters. In contrast, in the HT- group visceral adipose tissue *STS* mRNA expression level showed a positive correlation with BMI (r=0.62, P<0.05, n=13), waist circumference (r=0.65, P<0.01, n=13), and body adiposity index (r=0.77, P<0.05, n=13) (III). Also, the gene mRNA expression level of *CYP19A1* in subcutaneous adipose tissue correlated positively with BMI (r=0.67, P=0.01, n=13) and body adiposity index (r=0.71, P<0.01, n=13).

CYP19A1 mRNA expression did not correlate with estrogen to androgen concentration ratios in adipose tissue (III). *STS* mRNA expression level did not correlate with STS activity in subcutaneous or visceral adipose tissue (I-III).

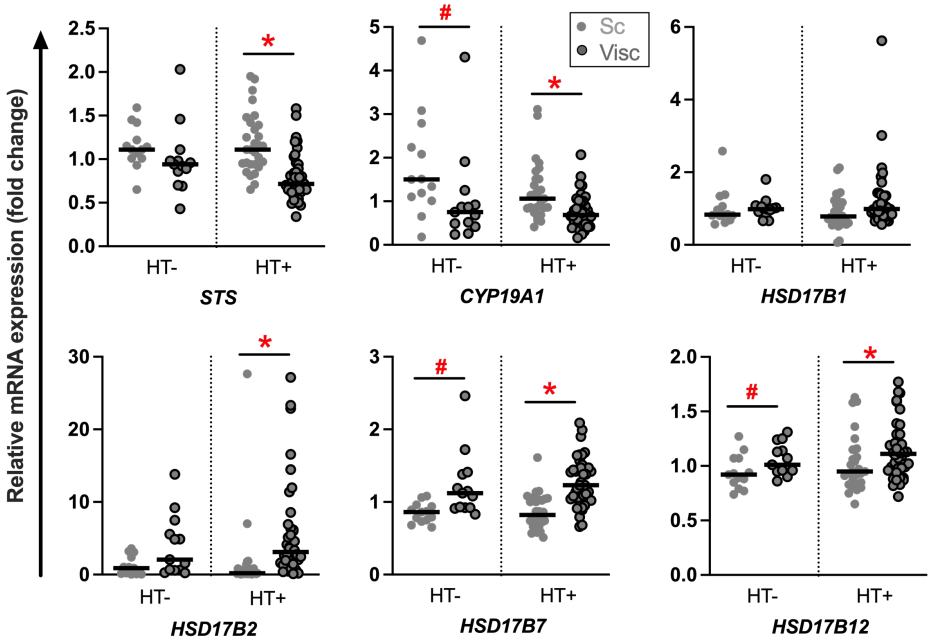


Figure 12 Gene mRNA expression levels in Sc, subcutaneous and Visc, visceral adipose tissue in postmenopausal women with or without menopausal hormone therapy (Study III). Data are presented as fold change (2^{-ddT}). The median is represented by the black line. *, $P<0.01$ and #, $P<0.05$, subcutaneous vs visceral adipose tissue (Wilcoxon signed rank test, n=12). *STS*, steroid sulfatase; *CYP19A1*, aromatase; *HSD17B1, HSD17B2, HSD17B7,* and *HSD17B12*, hydroxysteroid 17-β dehydrogenase 1, 2, 7, and 12; HT-, non-users of menopausal hormone therapy; HT+, users of menopausal hormone therapy.

Discussion

Adipose tissue is the main non-gonadal tissue involved in estrogen production in women, particularly after menopause (Nelson and Bulun, 2001; Labrie, 2015; Simpson, 2003). Menopause is associated with a relative increase in visceral adipose tissue, and postmenopausal women are at an increased risk for metabolic complications related to abdominal adiposity (Tchernof and Després, 2013). Menopausal HT may mitigate these changes (Haarbo *et al*, 1991; Sites *et al*, 2001; Ambikairajah *et al*, 2019; Salpeter *et al*, 2006). Our aim was to study local sex steroid composition and metabolism in subcutaneous and visceral adipose tissue; in women before and after menopause; and in women with or without HT.

Estrogen metabolism in subcutaneous compared to visceral adipose tissue

In fertile-aged women, the main estrogen is E_2 secreted by the ovaries into the circulation (Mauvais-Jarvis *et al*, 2013). Interpreting local conditions within tissues based on circulating concentrations of estrogens does not, however, take into account the local or intracrine and paracrine metabolism of estrogens (Ostinelli *et al*, 2022). Adipose tissue possesses the necessary enzymatic machinery to produce and metabolize active estrogens (Tchernof *et al*, 2015) also in premenopausal women (Kinoshita *et al*, 2014; Ostinelli *et al*, 2022). In contrast with the circulation, the predominant estrogen in premenopausal adipose tissue is E_1, not E_2 (Study I). The concentration of E_1 is higher in visceral compared to subcutaneous adipose tissue, and concentrations of E_1 are much higher in both fat depots than in serum. Adipose tissue to serum concentration ratios were in line with previous reports (Kinoshita *et al*, 2014; Wang *et al*, 2013; Vihma *et al*, 2016; Ostinelli *et al*, 2022).

Estrogenic effects may depend on the adipose tissue depot (Newell-Fugate, 2017), which is why understanding differences in the local adipose tissue estrogen environment is important. In premenopausal women, higher waist circumference was associated with increasing production of E_2 by reductive 17β-HSD enzymes in subcutaneous adipose tissue (Study I). This, together with the considerable local production of both E_1 and E_2 as represented by high local adipose tissue estrogen concentrations, suggest that intracrine estrogen production may play a role in

generating and maintaining the typical gynoid adipose tissue distribution in women before menopause.

In postmenopausal women, higher waist circumference was related to higher generation of E_2 by reductive 17β-HSD enzymes in visceral but not in subcutaneous adipose tissue. The concentration of E_1 in visceral adipose tissue increased with adiposity only in postmenopausal women without HT (Studies II and III). These results highlight the relevance of the visceral adipose tissue depot in postmenopausal women, where its excess is a known risk factor the metabolic syndrome (Neeland *et al*, 2019). While the role of estrogens produced in adipose tissue is considered mainly local (Labrie 2015), there is possible spillover into the circulation (Siiteri, 1987; Kershaw and Flier; 2004; Simpson, 2003). Our results highlight the importance of visceral compared to subcutaneous adipose tissue in postmenopausal women and suggest that visceral adipose tissue may play a role in modulating the overall exposure to estrogens in postmenopausal women, especially in abdominal obesity.

Adipose tissue estrogen metabolism in women before and after menopause

While the importance of local estrogen production in extragonadal tissues such as adipose tissue is well recognized in postmenopausal women (Tchernof *et al*, 2015; Simpson, 2003; Nelson and Bulun, 2001; Labrie, 2015), the extent and importance of adipose tissue production of estrogens in premenopausal women is less clear. One might expect that adipose tissue production of estrogens could be attenuated in fertile-aged women due to ovarian estrogen production (Simpson, 2003). However, high local concentrations of adipose tissue estrogens in premenopausal women have previously been reported in the breast (Savolainen-Peltonen *et al*, 2018) and in subcutaneous and visceral adipose tissue (Kinoshita *et al*, 2014; Ostinelli *et al*, 2022). In line with this are our results showing active production of E_1 and E_2 by reductive 17β-HSD enzymes in both subcutaneous and visceral adipose tissue (Study I) with much higher adipose tissue estrogen concentrations even compared to postmenopausal women (Studies II and III). This suggests that local adipose tissue concentrations of estrogens in fertile-aged women are not merely a reflection of circulating estrogen concentrations, but that active local production and metabolism of estrogen takes place already prior to menopause.

After menopause, some estrogens produced in fat could leak into the circulation (Simpson, 2001). Consequently, increasing adiposity could affect both circulating (Toth *et al*, 2000; Lovejoy *et al*, 2008; Baglietto *et al*, 2009) and local concentrations to a greater extent after menopause than before. This is in line with

our findings that adiposity especially in the visceral depot was associated with an increased local E_1 concentration and production of E_2 in postmenopausal women without HT (Studies II and III). Also in postmenopausal women without HT, serum E_1, E_2, and E_1S concentrations were higher with higher body adiposity index (Study III), while in premenopausal women, estrogen concentrations in serum or adipose tissue were not associated with measures of adiposity (Study I). Our results in postmenopausal women showing strong positive correlations between adipose tissue and serum concentrations of E_1 and E_2 (Study II) also support the notion that estrogens produced by postmenopausal adipose tissue may leak into the circulation and influence systemic exposure to estrogens.

The conversion rate of E_1S to E_1 by STS in adipose tissue was similar in pre- and postmenopausal women (Studies I and II). We were the first to confirm that this reaction takes place in subcutaneous and visceral adipose tissue (Study II). This was further verified using a specific STS inhibitor (Study I). As E_1S is a key circulating estrogen precursor (Martel *et al*, 2016), with concentrations vastly exceeding those of free E_1 and E_2 (Labrie, 1998; Mueller *et al*, 2015), confirmation of its conversion into active E_1 in adipose tissue suggests that STS may be a contributor to the high local concentrations of E_1. The strong correlations between serum E_1S and local concentration of E_1 in visceral adipose tissue suggests a significant role of this fat depot in estrogen metabolism of postmenopausal women (Study II). STS activity in converting DHEAS to DHEA has previously been shown to take place in adipose tissue (Paatela *et al*, 2016; Dalla Valle *et al*, 2016), with STS activity being higher in postmenopausal compared to premenopausal adipose tissue (Paatela *et al*, 2016).

E_1 is also produced by the aromatization of A_4, and reductive 17β-HSD enzymes convert E_1 to the more biologically potent E_2 (Moeller and Adamski, 2009). This conversion was shown to occur in both pre- and postmenopausal adipose tissue (Studies I and II). Postmenopausal women showed a higher rate of E_2 production in subcutaneous adipose tissue compared to premenopausal women. Subtypes of 17β-HSD enzymes also catalyze the opposite, oxidative reaction of converting E_2 to E_1 (Moeller and Adamski, 2009), and the balance between these reactions, as well as the rate of aromatization of androgens, regulate the concentrations of E_2 locally (Martel *et al*, 1992).

STS, 17β-HSD enzymes, and aromatase are key enzymes in regulating the local sex steroid milieu by modulating the bioavailable concentrations of active estrogens (Mueller *et al*, 2015; Tchernof *et al*, 2015; Stocco, 2012). Studying enzymatic reactions directly or indirectly via sex steroid concentration ratios, such as in Study III for the activity of aromatase, contribute to the understanding of adipose tissue intracrinology. Our data indicating high tissue concentrations of estrogens and local enzyme activity in producing E_1 and E_2 already before menopause (Study I) may suggest that the gynoid adipose tissue distribution in the body is influenced, in addition to circulating estrogens, also by local adipose tissue estrogen metabolism.

The mRNA expression profiles of genes encoding estrogen-metabolizing enzymes between subcutaneous and visceral adipose tissue were mostly similar in premenopausal (Study I) and postmenopausal women without (Study II) or with HT (Study III). This indicates that changes in adipose tissue sex steroid milieu may not be due to differences in the gene expression patterns. These patterns were also similar to the mRNA expression levels described earlier for the same genes (Paatela *et al*, 2016; Wang *et al*, 2013). mRNA expression levels of STS were not correlated with the corresponding STS enzyme activities in subcutaneous or visceral adipose tissue in pre- or postmenopausal women (Studies I-III). Furthermore, CYP19A1 mRNA expression did not correlate with the estrogen to androgen concentration ratios in adipose tissue (Study III). The mRNA expression levels of a gene do not equate to protein levels or dictate enzyme activity. Posttranscriptional and posttranslational modification, including effects caused by adipokines and local inflammatory markers (Kershaw and Flier, 2004; Purohit *et al*, 2001; Bracht *et al*, 2020) may affect local enzyme activities. This would contribute to the lack of correlation between gene mRNA expression levels and corresponding enzyme activity.

Adipose tissue sex steroid metabolism in postmenopausal women with or without HT

High local concentrations of E_1 and E_2 were determined in subcutaneous and visceral adipose tissue in women using menopausal HT, by far exceeding those without HT (Study III). Adipose tissue E_1 concentrations in HT users also exceeded the corresponding concentrations in premenopausal women (Study I). The adipose tissue to serum ratios of estrogen concentrations remained high in women using HT. This could reflect effects of peroral HT on adipose tissue estrogens, as is the case for serum concentrations (Kim *et al*, 2021; Ensrud *et al*, 2022). However, since the concentrations of E_1 were 14 to 16 times higher in adipose tissue than in serum, this concentration gradient could suggest the possibility of induced local production of estrogens in adipose tissue.

The activity of STS as well as the mRNA expression levels of STS were similar between women with and without HT (Study III), suggesting that STS may not be a key factor in explaining the differences in local estrone concentrations between the two groups. As described above, ratios of estrogens to androgens in adipose tissue have been considered as indirect estimates of local activity of aromatase (Ostinelli *et al*, 2022), a key regulator of adipose tissue estrogen to androgen balance (Stocco 2012). While E_2 to E_1 ratios were similar between users and non-users of HT, the ratios of estrogens to androgens were higher in adipose tissue of women using HT compared to those without HT (Study III). In visceral adipose tissue, these ratios

were higher with higher doses of E_2 in the HT regimen. Also in visceral adipose tissue, the local concentration of T was lower in users compared to non-users of HT. These findings suggest that differences in aromatase activity could contribute to the higher estrogen to androgen ratios in users of HT, but this would need to be confirmed by directly measuring aromatase activity in adipose tissue.

To the best of our knowledge, we were the first to describe the local adipose tissue sex steroid milieu in users of HT (Study III). Taken together, the changes in adipose tissue estrogen and androgen concentrations described above might be one mechanism through which menopausal HT attenuates the relative increase in visceral adipose tissue, which is associated with menopause (Ambikaijarah *et al*, 2019). In contrast to ovarian estrogens, ovarian production of androgens continues after menopause (Arlt, 2006; Labrie *et al*, 2011) and the increased ratio of androgens to estrogens in the circulation may contribute to the relative increase of visceral adipose tissue after menopause (Moccia *et al*, 2022). Other possible mechanisms influencing adipose tissue distribution between different areas of the body involve estrogenic effects on local lipolysis, lipogenesis, and adipogenesis, and sensitivity to insulin (Morselli *et al*, 2017; Bracht *et al*, 2020; Pedersen *et al*, 2004). Lifestyle factors and a loss of lean and muscle mass with menopause (Juppi *et al*, 2020) also play a role in increasing adiposity.

The risk of breast cancer is increased with HT consisting of estrogen and a progestin (Fournier *et al*, 2008). Whether HT induces local adipose tissue production of estrogens, possibly through increased aromatase activity, is thus relevant, as breast adipose tissue is recognized as an important regulatory factor in breast cancer development (Brown and Scherer, 2023). The extent to which adipose tissue production of estrogens elsewhere in the body contributes to the possible risk for estrogen-dependent malignancies is not established, but it is known that obesity increases the risk of estrogen-dependent postmenopausal breast (Bulun *et al*, 2012) and endometrial cancer (He *et al*, 2021). Adipose-tissue derived estrogens may contribute to this risk.

The importance of STS in producing estrone from E_1S has long been known in breast cancers (Pasqualini *et al*, 1996), but the relevance of the activity of this enzyme in benign adipose tissue of the breast or elsewhere has not been studied. Women using peroral HT showed many times higher serum concentrations of E_1S compared to women without HT (Study III) as well as to premenopausal women (Study I). The high E_1S concentrations are likely mainly a reflection of the peroral exogenous E_2 administration (Davis and Baber, 2022). Nevertheless, in HT users the high adipose tissue to serum ratios of estrogens and especially E_1 (Study III), poses the question whether adipose tissue-produced estrogens could also further contribute to serum E_1S concentrations.

Strengths and limitations

This study has limitations. One of the aims was to study differences in local sex steroid concentrations and biosynthesis between subcutaneous and visceral adipose tissue. Subcutaneous adipose tissue is the largest adipose tissue depot and spans over several regions of the body, including the abdomen, thighs, and the gluteal region (Karastergiou and Fried, 2017; Ibrahim, 2010). The samples were collected at benign gynecological surgery in the abdominal area. There may be differences in the sex steroid milieu between different types of subcutaneous adipose tissue, including the thighs and the gluteal area, as has been shown with for example aromatase activity (Killinger *et al*, 1990). To the best of our knowledge, no comprehensive comparative studies have been carried out to characterize sex steroid concentrations and biosynthesis in different subcutaneous adipose tissue depots.

Adipose tissue sampling during elective surgery enables obtaining larger samples than for example through aspiration biopsies. Benign gynecological surgery is usually laparoscopic, however, and this limits the size of especially subcutaneous adipose tissue samples. Therefore, analysis and comparison of paired adipose tissue samples (visceral and subcutaneous) from each patient was not always possible for all analyses. Minor sample to sample differences in tissue composition cannot be excluded, but it is unlikely that this has an effect on differences between distinct adipose tissue depots. Controlling for the stage of menstrual cycle of premenopausal patients (Study II) or the regimen of HT in postmenopausal patients (Study III) was not possible due to the nature of the study, but we documented the menstrual cycle stage and HT regimen from patient records, and took the information into account at data analysis.

Due to limited sample size, it was not possible to study protein levels of sex steroid-metabolizing enzymes, nor to directly study the activity of aromatase in adipose tissue. Despite this, the activities of two consecutively acting key enzymes in the metabolism of estrogens, STS and 17β-HSD enzymes, were studied (Studies I-II), and we also confirmed the action of STS in the conversion of E_1S to E_1 through the use of a specific enzyme inhibitor (Study I). In Study III, the adipose tissue samples were insufficient for measurement of 17β-HSD enzyme activity. On the other hand, the sex steroid quantitation in serum and adipose tissue was extended to include androgens and progesterone, and ratios of estrogens to androgens were used to indirectly describe aromatase activity.

Anthropometric measures were used to evaluate adiposity, as using body imaging techniques for this purpose was not available. Clinically, waist circumference is considered a reasonable indicator of abdominal obesity (Rankinen *et al*, 1999), and body adiposity index (Li *et al*, 2021; Fedewa *et al*, 2019) was included as another estimator of visceral adiposity (Study III).

Our study also has several strengths. The total numbers of subjects were mostly greater compared to other studies that have examined sex steroids in human adipose tissue (Dalla Valle *et al*, 2006; Kinoshita *et al*, 2014; Yamatani *et al*, 2013; Paatela *et al*, 2016; Pedersen *et al*, 2004; Ostinelli *et al*, 2022). The study design allowed for measurement of sex steroids in paired adipose tissue samples from subcutaneous and visceral adipose tissue, and in serum.

Overall, a major strength throughout the study is the use and development of LC-MS/MS methodology for the accurate and precise measurement of adipose tissue and serum sex steroid levels (Handelsman and Wartofsky, 2013). This included applying, advancing, and enhancing existing methodology for both the extraction and purification of adipose tissue, and for the quantification of a wide range of both estrogens and androgens with LC-MS/MS (Studies I-III). Furthermore, adding $^{13}C_3$-steroids as internal standards in place of tritiated labels eliminated the need to use of radioactive substances in the laboratory and greatly improved the reliability, specificity, and reproducibility of the sex steroid quantification (Wudy *et al*, 2018). As the sex steroid quantification methodology was adjusted several times (Studies I-III), statistical calculations between different groups were carried out for those groups of subjects whose samples had been quantified using the same methodology.

Conclusions and future perspective

The main findings of this Thesis work are described in Figure 13 and can be summarized as follows:

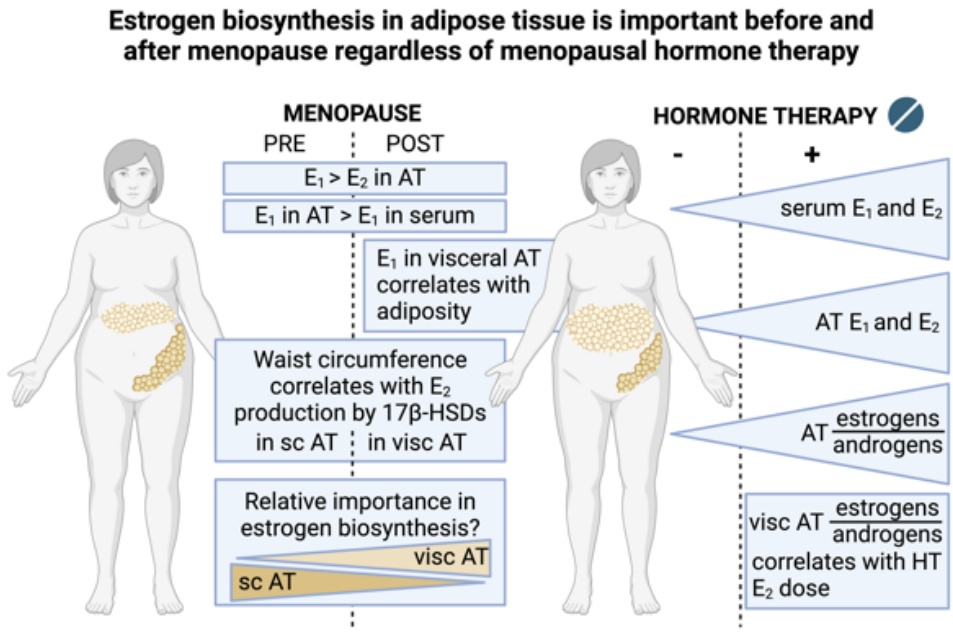


Figure 13 Main findings of Studies I-III. Created using BioRender.com. Abbreviations: PRE, premenopause; POST, postmenopause; E_1, estrone; E_2, estradiol; AT, adipose tissue; 17β-HSDs, 17β-HSD enzymes; sc AT, subcutaneous adipose tissue; visc AT, visceral adipose tissue.

Premenopausal women had considerable local concentrations of estrogens in subcutaneous and visceral adipose tissue. Higher waist circumference was associated with augmented E_2 production by reductive 17β-HSD enzymes in subcutaneous adipose tissue.

In postmenopausal women without HT, BMI was positively associated with the concentration of E_1 in visceral adipose tissue. Higher waist circumference was

associated with higher production of E_2 by reductive 17β-HSD enzymes in visceral adipose tissue.

While E_2 is the major circulating estrogen in premenopausal women, E_1 was the predominant estrogen in adipose tissue in all pre- and postmenopausal groups of women. E_1 concentrations in adipose tissue were 5 to 42 times higher than in serum in all women, including in women using peroral HT.

Women using peroral HT had higher E_1 and E_2 concentrations and higher estrogen-to-androgen ratios in adipose tissue compared to women without HT. In visceral adipose tissue, estrogen to androgen ratios increased with higher doses of E_2 in the HT regimen.

The results support the notion that adipose tissue is a key tissue for storing and producing sex steroids. Furthermore, the sex steroid profiles and metabolism in adipose tissue depend on the depot and exhibit differences during a woman's reproductive life. Based on our results, there is considerable local intracrine production of estrogens in adipose tissue in pre- and postmenopausal women including those with HT. The role of visceral adipose tissue in estrogen metabolism appears highlighted, particularly with E_1 in postmenopausal women.

Further studies should focus on local estrogen metabolism in adipose tissue from the thighs or the gluteal area in comparison with abdominal adipose tissue. Characterizing the interplay between circulating estrogens in premenopausal women and estrogen production within adipose tissue, including in relation to the menstrual cycle, also remains a topic for further research.

It would be of interest to compare peroral and transdermal estrogen administration in HT on local sex steroid metabolism in tissues. To what extent estrogens produced locally in adipose tissue are transported into the circulation and what their relative contribution to systemic estrogen balance, remains to be determined in detail.

In local sex steroid balance the enzymatic machinery is intertwined, and further studying the local concentrations of estrogens and androgens simultaneously could give rise to better understanding of their respective roles at different stages of women's reproductive life.

As adipose tissue is a heterogeneous organ comprised of multiple populations of different cells, the recent advances in methodology allowing for investigation of adipose tissue at single-cell depth provide tools for more detailed analysis of local adipose tissue sex steroid milieu. This includes the roles and interplay between adipocytes and the stromal vascular fraction. This is also of interest in both benign and cancerous breast tissue and in the interaction between breast tissue and adipose tissue of the breast.

Acknowledgements

Research towards this thesis was conducted between the years of 2015 and 2024 at the Doctoral Programme in Clinical Research, Faculty of Medicine of the University of Helsinki and the Department of Obstetrics and Gynecology, Helsinki University Hospital. I am grateful for the research facilities and framework available for doctoral candidates at these institutions and would like to thank both the former and current academic and administrative heads of the Department of Obstetrics and Gynecology for providing valuable support for academic research. Between the years of 2015 and 2021 these research projects were also conducted at Folkhälsan Research Center, and I am grateful for the infrastructure and support provided.

I express my gratitude to all the women who participated in our research over the years and without whom this project would not have been possible. Thank you to the doctors and nurses at HUS Women's Hospital for collecting the samples. I hope the results of this thesis will help us better understand the complex physiology of menopause.

I'd like to thank my official reviewers, Professor Matti Poutanen and Associate Professor Eija Laakkonen, for your time and effort in pre-evaluating my thesis, as well as your valuable feedback which greatly improved the work. I am grateful to Professor Terhi Piltonen for kindly agreeing to act as my official opponent during the public examination of my thesis, and to Professor Tomi Mikkola for acting as custos.

To my thesis supervisors, Adjunct Professor Hanna Savolainen-Peltonen and Professors Tomi Mikkola and Matti J. Tikkanen – thank you for your longstanding support and encouragement, as well as your patience during these years. I value your expertise and experience and feel fortunate to have been able to carry out my thesis under your supervision. Hanna and Tomi, I continue to be amazed by the seemingly endless time and energy that you possess in your demanding roles as clinician-researchers. Hanna, thank you for always being available even for the smaller details. Tomi, thank you for the constructive criticism and feedback, and for shortening my sentences for more concise expression. Matti, thank you for the insightful ideas and comments which many times helped crystallize the message of our results.

I want to thank my thesis committee members, Professor Oskari Heikinheimo ja Adjunct Professor Katariina Öörni, for your valuable comments and ideas which helped shape the structure of this thesis.

None of these research projects would have been possible without my co-authors and our valuable collaboration with HUSLAB. To Professor Esa Hämäläinen, Adjunct Professor Ursula Turpeinen, and Mikko Haanpää, thank you for lending your expertise and dedication to developing the LC-MS/MS methods, and for your feedback and support. To Alex, thank you for your initiative and co-operation with especially the final project of this thesis. Feng and Hanna – thank you for your support in helping me get started with

research at the laboratory and for passing on your knowledge. Veera – thank you for your support and mentorship within and outside of the research laboratory. Thank you also for your friendship and those overseas conference trips which made this research journey all the more memorable.

A warm thank you to our laboratory technicians Anne, Kirsti, and Päivi, without whose countless hours of work these projects would not have been possible. Thank you for the skills you taught me in the laboratory and for your company.

I want to express my gratitude for the valuable financial support from the following foundations, which made research towards this thesis possible: Samfundet Folkhälsan, the Finnish Medical Foundation, the Finnish Society of Obstetrics and Gynaecology (Synnytys- ja naistentautiopin tutkimussäätiö), the Jane and Aatos Erkko Foundation, the Finnish Menopause Society (Suomen Menopaussitutkimusseura), the Päivikki and Sakari Sohlberg Foundation, Juhani Ahon Lääketieteen Tutkimussäätiö, Suomalais-Norjalainen Lääketieteen Säätiö, and Kunnanlääkäri Uulo Arhion Rahasto.

While I'd like to think you can do anything, I've learned to accept you cannot do everything, at least not at once. Thank you to my superiors at Espoo hospital, especially Chief Physician Hannele Ekstam, for enabling my juggle between clinical work and research during the last few years of this project. I also appreciate the support of many of my clinical colleagues and coworkers along the way, including at, but not limited to, Espoo hospital, Kanta-Häme Central Hospital, Vanajavesi hospital, Helsinki University Hospital, and the Geriatric outpatient clinic of the City of Helsinki.

The years of working on this thesis have been numerous and have included an array of changes in personal and global circumstances, including having children, a pandemic, and changing clinical specialties. I'd like to believe that I have grown in more ways than one during and thanks to this process. Thank you to my family and friends for the support and for sharing this journey with me.

November, 2024 in Washington, DC

Natalia Hetemäki

References

"The 2022 Hormone Therapy Position Statement of The North American Menopause Society" Advisory Panel. The 2022 hormone therapy position statement of The North American Menopause Society. Menopause. 2022;29(7):767-794.

Abildgaard J, Pedersen AT, Green CJ, Harder-Lauridsen NM, Solomon TP, Thomsen C, Juul A, Pedersen M, Pedersen JT, Mortensen OH, Pilegaard H, Pedersen BK, Lindegaard B. Menopause is associated with decreased whole body fat oxidation during exercise. Am J Physiol Endocrinol Metab. 2013;304(11):E1227-E1236.

Ackerman GE, Smith ME, Mendelson CR, MacDonald PC, Simpson ER. Aromatization of androstenedione by human adipose tissue stromal cells in monolayer culture. J Clin Endocrinol Metab. 1981;53(2):412-417.

Ali AT, Hochfeld WE, Myburgh R, Pepper MS. Adipocyte and adipogenesis. Eur J Cell Biol. 2013;92(6-7):229-236.

Ambikairajah A, Walsh E, Tabatabaei-Jafari H, Cherbuin N. Fat mass changes during menopause: a metaanalysis. Am J Obstet Gynecol. 2019;221(5):393-409.e50.

Anderson DC, Thorner MO, Fisher RA, Woodham JP, Goble HL, Besser GM. Effects of hormonal treatment on plasma unbound androgen levels in hirsute women. Acta Endocronol. 1975;199(Suppl):224.

Anderson LA, McTernan PG, Barnett AH, Kumar S. The effects of androgens and estrogens on preadipocyte proliferation in human adipose tissue: influence of gender and site. J Clin Endocrinol Metab. 2001;86(10):5045-5051.

Arlt W. Androgen therapy in women. Eur J Endocrinol. 2006;154(1):1-11.

Baglietto L, English DR, Hopper JL, MacInnis RJ, Morris HA, Tilley WD, Krishnan K, Giles GG. Circulating steroid hormone concentrations in postmenopausal women in relation to body size and composition. Breast Cancer Res Treat. 2009;115(1):171-179.

Barton M, Meyer MR. Postmenopausal hypertension: mechanisms and therapy. Hypertension. 2009;54(1):11-18.

Battisti S, Guida FM, Coppa F, Vaccaro DM, Santini D, Tonini G, Zobel BB, Semelka RC. Modification of abdominal fat distribution after aromatase inhibitor therapy in breast cancer patients visualized using 3-D computed tomography volumetry. Clin Breast Cancer. 2014;14(5):365-370.

Bélanger C, Luu-The V, Dupont P, Tchernof A. Adipose tissue intracrinology: potential importance of local androgen/estrogen metabolism in the regulation of adiposity. Horm Metab Res. 2002;34(11-12):737-745.

Bellemare V, Laberge P, Noël S, Tchernof A, Luu-The V. Differential estrogenic 17beta-hydroxysteroid dehydrogenase activity and type 12 17beta-

hydroxysteroid dehydrogenase expression levels in preadipocytes and differentiated adipocytes. J Steroid Biochem Mol Biol. 2009;114(3-5):129-134.

Blouin K, Nadeau M, Mailloux J, Daris M, Lebel S, Luu-The V, Tchernof A. Pathways of adipose tissue androgen metabolism in women: depot differences and modulation by adipogenesis. Am J Physiol Endocrinol Metab. 2009;296(2):E244-E255.

Blouin K, Nadeau M, Perreault M, Veilleux A, Drolet R, Marceau P, Mailloux J, Luu-The V, Tchernof A. Effects of androgens on adipocyte differentiation and adipose tissue explant metabolism in men and women. Clin Endocrinol (Oxf). 2010;72(2):176-188.

Blouin K, Veilleux A, Luu-The V, Tchernof A. Androgen metabolism in adipose tissue: recent advances. Mol Cell Endocrinol. 2009;301(1-2):97-103.

Boardman HM, Hartley L, Eisinga A, Main C, Roqué i Figuls M, Bonfill Cosp X, Sanchez RG, Knight B. Hormone therapy for preventing cardiovascular disease in post-menopausal women. Cochrane Database Syst Rev. 2015;2015(3):CD002229.

Bowman K, Atkins JL, Delgado J, Kos K, Kuchel GA, Ble A, Ferrucci L, Melzer D. Central adiposity and the overweight risk paradox in aging: follow-up of 130,473 UK Biobank participants. Am J Clin Nutr. 2017;106(1):130-135.

Bracht JR, Vieira-Potter VJ, De Souza Santos R, Öz OK, Palmer BF, Clegg DJ. The role of estrogens in the adipose tissue milieu. Ann N Y Acad Sci. 2020;1461(1):127-143.

Bray GA. Beyond BMI. Nutrients. 2023;15(10):2254.

Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. Endocr Rev. 2009;30(5):465-493.

Brown KA, Scherer PE. Update on Adipose Tissue and Cancer. Endocr Rev. 2023;44(6):961-974.

Bulun SE, Chen D, Moy I, Brooks DC, Zhao H. Aromatase, breast cancer and obesity: a complex interaction. Trends Endocrinol Metab. 2012;23(2):83-89.

Bulun SE, Simpson ER. Competitive reverse transcription-polymerase chain reaction analysis indicates that levels of aromatase cytochrome P450 transcripts in adipose tissue of buttocks, thighs, and abdomen of women increase with advancing age. J Clin Endocrinol Metab. 1994;78(2):428-432.

Bäckdahl J, Franzén L, Massier L, Li Q, Jalkanen J, Gao H, Andersson A, Bhalla N, Thorell A, Rydén M, Ståhl PL, Mejhert N. Spatial mapping reveals human adipocyte subpopulations with distinct sensitivities to insulin. Cell Metab. 2021;33(11):2301.

Camhi SM, Bray GA, Bouchard C, Greenway FL, Johnson WD, Newton RL, Ravussin E, Ryan DH, Smith SR, Katzmarzyk PT. The relationship of waist circumference and BMI to visceral, subcutaneous, and total body fat: sex and race differences. Obesity (Silver Spring). 2011;19(2):402-408.

Campos DB, Palin MF, Bordignon V, Murphy BD. The 'beneficial' adipokines in reproduction and fertility. Int J Obes (Lond). 2008;32(2):223-231.

Carr MC. The emergence of the metabolic syndrome with menopause. J Clin Endocrinol Metab. 2003;88(6):2404-2411.

Casiano Evans EA, Hobson DTG, Aschkenazi SO, Alas AN, Balgobin S, Balk EM, Dieter AA, Kanter G, Orejuela FJ, Sanses TVD, Rahn DD. Nonestrogen Therapies for Treatment of Genitourinary Syndrome of Menopause: A Systematic Review. Obstet Gynecol. 2023;142(3):555-570.

Charatcharoenwitthaya N, Khosla S, Atkinson EJ, McCready LK, Riggs BL. Effect of blockade of TNF-alpha and interleukin-1 action on bone resorption in early postmenopausal women. J Bone Miner Res. 2007;22(5):724-729.

Chazenbalk G, Singh P, Irge D, Shah A, Abbott DH, Dumesic DA. Androgens inhibit adipogenesis during human adipose stem cell commitment to preadipocyte formation. Steroids. 2013;78(9):920-926.

Cherubini A, Ostadreza M, Jamialahmadi O, Pelusi S, Rrapaj E, Casirati E, Passignani G, Norouziesfahani M, Sinopoli E, Baselli G, Meda C, Dongiovanni P, Dondossola D, Youngson N, Tourna A, Chokshi S, Bugianesi E; EPIDEMIC Study Investigatrs; Della Torre S, Prati D, Romeo S, Valenti L. Interaction between estrogen receptor-α and PNPLA3 p.I148M variant drives fatty liver disease susceptibility in women. Nat Med. 2023;29(10):2643-2655.

Chew NWS, Ng CH, Tan DJH, Kong G, Lin C, Chin YH, Lim WH, Huang DQ, Quek J, Fu CE, Xiao J, Syn N, Foo R, Khoo CM, Wang J-W, Dimitriadis GK, Young DY, Siddiqui MS, Lam CSP, Wang Y, Figtree GA, Chan MY, Cummings DE, Noureddin M, Wong VW-S, Ma RCW, Mantzoros CS, Sanyal A, Muthiah MD. The global burden of metabolic disease: Data from 2000 to 2019. Cell Metab. 2023;35(3):414-428.e3.

Cleland WH, Mendelson CR, Simpson ER. Effects of aging and obesity on aromatase activity of human adipose cells. J Clin Endocrinol Metab. 1985;60(1):174-177.

Cox-York KA, Erickson CB, Pereira RI, Bessesen DH, Van Pelt RE. Region-specific effects of oestradiol on adipose-derived stem cell differentiation in post-menopausal women. J Cell Mol Med. 2017;21(4):677-684.

Cristancho AG, Lazar MA. Forming functional fat: a growing understanding of adipocyte differentiation. Nat Rev Mol Cell Biol. 2011;12(11):722-734.

Cypess AM. Reassessing Human Adipose Tissue. N Engl J Med. 2022;386(8):768-779.

Dai W, Li Y, Zheng H. Estradiol/Testosterone imbalance: impact on coronary heart disease risk factors in postmenopausal women. Cardiology. 2012;121(4):249-254.

Dalla Valle L, Toffolo V, Nardi A, Fiore C, Bernante P, Di Liddo R, Parnigotto PP, Colombo L. Tissue-specific transcriptional initiation and activity of steroid sulfatase complementing dehydroepiandrosterone sulfate uptake and intracrine steroid activations in human adipose tissue. J Endocrinol. 2006;190(1):129-139.

Davis SR, Baber RJ. Treating menopause - MHT and beyond. Nat Rev Endocrinol. 2022;18(8):490-502.

Davis SR, Baber R, Panay N, Bitzer J, Cerdas Perez S, Islam RM, Kaunitz AM, Kingsberg SA, Lambrinoudaki I, Liu J, Parish SJ, Pinkerton J, Rymer J, Simon JA, Vignozzi L, Wierman ME. Global Consensus Position Statement on the Use of Testosterone Therapy for Women. J Clin Endocrinol Metab. 2019;104(10):4660-4666.

Davis SR, Bell RJ, Robinson PJ, Handelsman DJ, Gilbert T, Phung J, Desai R, Lockery JE, Woods RL, Wolfe RS, Reid CM, Nelson MR, Murray AM,

McNeil JJ; ASPREE Investigator Group. Testosterone and Estrone Increase From the Age of 70 Years: Findings From the Sex Hormones in Older Women Study. J Clin Endocrinol Metab. 2019;104(12):6291-6300

Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. J Clin Endocrinol Metab. 2005;90(7):3847-3853.

de Villiers TJ, Stevenson JC. The WHI: the effect of hormone replacement therapy on fracture prevention. Climacteric. 2012;15(3):263-266.

Depypere H, Inki P. The levonorgestrel-releasing intrauterine system for endometrial protection during estrogen replacement therapy: a clinical review. Climacteric. 2015;18(4):470-482.

Després JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006;444(7121):881-887.

DiStefano JK. NAFLD and NASH in Postmenopausal Women: Implications for Diagnosis and Treatment. Endocrinology. 2020;161(10):bqaa134.

Drolet R, Richard C, Sniderman AD, Mailloux J, Fortier M, Huot C, Rhéaume C, Tchernof A. Hypertrophy and hyperplasia of abdominal adipose tissues in women. Int J Obes (Lond). 2008;32(2):283-291.

Duerre DJ, Galmozzi A. Deconstructing Adipose Tissue Heterogeneity One Cell at a Time. Front Endocrinol (Lausanne). 2022;13:847291.

Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, Sul HS. Regulation of lipolysis in adipocytes. Annu Rev Nutr. 2007;27:79-101.

Edlefsen KL, Jackson RD, Prentice RL, Janssen I, Rajkovic A, O'Sullivan MJ, Anderson G. The effects of postmenopausal hormone therapy on serum estrogen, progesterone, and sex hormone-binding globulin levels in healthy postmenopausal women. Menopause. 2010;17(3):622-629.

Edman CD, MacDonald PC. Am J Obstet Gynecol. 1978;130(4):456-461.

Edwards DP. Regulation of signal transduction pathways by estrogen and progesterone. Annu Rev Physiol. 2005;67:335-376.

Eisenhofer G, Peitzsch M, Kaden D, Langton K, Pamporaki C, Masjkur J, Tsatsaronis G, Mangelis A, Williams TA, Reincke M, Lenders JWM, Bornstein SR. Reference intervals for plasma concentrations of adrenal steroids measured by LC-MS/MS: Impact of gender, age, oral contraceptives, body mass index and blood pressure status. Clin Chim Acta. 2017;470:115-124

El Khoudary SR, Aggarwal B, Beckie TM, Hodis HN, Johnson AE, Langer RD, Limacher MC, Manson JE, Stefanick ML, Allison MA; American Heart Association Prevention Science Committee of the Council on Epidemiology and Prevention; and Council on Cardiovascular and Stroke Nursing. Menopause Transition and Cardiovascular Disease Risk: Implications for Timing of Early Prevention: A Scientific Statement From the American Heart Association. Circulation. 2020;142(25):e506-e532.

El Khoudary SR, Greendale G, Crawford SL, Avis NE, Brooks MM, Thurston RB, Karvonen-Gutierrez C, Waetien E, Matthews K. The menopause transition and women's health at midlife: a progress report from the Study of Women's Health Across the Nation (SWAN). Menopause. 2019;26(10):1213-1227.

Emont MP, Jacobs C, Essene AL, Pant D, Tenen D, Colleluori G, Di Vincenzo A, Jorgensen AM, Dashti H, Stefek A, McGonagle E, Strobel S, Laber S,

Agrawal S, Westcott GP, Kar A, Veregge ML, Gulko A, Srinivasan H, Kramer Z, De Filippis E, Merkel E, Ducie J, Boyd CG, Gourash W, Courcoulas A, Lin SJ, Lee BT, Morris D, Tbias A, Khera AV, Claussnitzer M, Pers TH, Giordano A, Ashenberg O, Regev A, Tsai LT, Rosen ED. A single-cell atlas of human and mouse white adipose tissue. Nature. 2022;603(7903):926-933.

Ensrud KE, Larson JC, Guthrie KA, Crandall CJ, LaCroix AZ, Reed SD, Bhasin S, Mitchel CM, Joffe H. Changes in serum endogenous estrogen concentrations are mediators of the effect of low-dose oral estradiol on vasomotor symptoms. Menopause. 2022;29(9):1014-1020.

Fedewa MV, Nickerson BS, Esco MR. Associations of body adiposity index, waist circumference, and body mass index in young adults. Clin Nutr. 2019;38(2):715-720.

Ferrara CM, Lynch NA, Nicklas BJ, Ryan AS, Berman DM. Differences in adipose tissue metabolism between postmenopausal and perimenopausal women. J Clin Endocrinol Metab. 2002;87(9):4166-4170.

Forney JP, Milewich L, Chen GT, Garlock JL, Schwartz BE, Edman CD, MacDonald PC. Aromatization of androstenedione to estrone by human adipose tissue in vitro. Correlation with adipose tissue mass, age, and endometrial neoplasia. J Clin Endocrinol Metab. 1981;53(1):192-199.

Foryst-Ludwig A, Kintscher U. Metabolic impact of estrogen signalling through ERalpha and ERbeta. J Steroid Biochem Mol Biol. 2010;122(1-3):74-81.

Foster PA. Steroid Sulphatase and Its Inhibitors: Past, Present, and Future. Molecules. 2021;26(10):2852.

Fouad Mansour M, Blanchette S, Pelletier M, Poirier D, Tchernof A. 17β-hydroxysteroid dehydrogenase type 2 activity, expression and cellular localization in abdominal adipose tissues from women. Clin Endocrinol (Oxf). 2023;98(2):229-237.

Fournier A, Berrino F, Clavel-Chapelon F. Unequal risks for breast cancer associated with different hormone replacement therapies: results from the E3N cohort study. Breast Cancer Res Treat. 2008;107(1):103-111.

Frayn KN. Adipose tissue as a buffer for daily lipid flux. Diabetologia. 2002;45(9):1201-1210.

Gartoulla P, Worsley R, Bell RJ, Davis SR. Moderate to severe vasomotor and sexual symptoms remain problematic for women aged 60 to 65 years. Menopause. 2015;22(7):694-701.

GBD 2015 Obesity Collaborators, Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, Marczak L, Mokdad AH, Moradi-Lakeh M, Naghavi M, Salama JS, Vos T, Abate KH, Abbafati C, Ahmed MB, Al-Aly Z, Alkerwi Al, Al-Raddadi R, Amare AT, Amberbir A, Amegah AK, Amini E, Amrock SM, Anja RM, Ärnlöv J, Asayesh H, Banerjee A, Barac A, Baye E, Bennet DA, Beyene AS, Biadgilign S, Birvukov S, Bjertness E, Boneva DJ, Campos-Nonato I, Carrero JJ, Cecilio P, Cercy K, Ciobanu LG, Cornaby L, Damtew SA, Dandona L, Dandona R, Dharmaratne SD, Duncan BB, Eshrati B, Esteghamati A, Feigin VL, Fernandes JC, Fürst T, Gebrehiwot TT, Gold A, Gona PN, Goto A, Habtewold TD, Hadush KT, Hafezi-Nejad N, Hay SI, Horino M, Islami F, Kamal R, Kasaeian A, Katikireddi SV, Kengne AP, Kesavachandran CN, Khader YS, Khang Y-H, Khubchandani J, Kim D, Kim YJ, Kinfu Y, Kosen S, Ku T, Defo BK, Kumar GA, Larson HJ, Leinsalu M, Liang X, Lim SS, Liu P, Lopez AD, Lozano R, Majeed A, Malekzadeh R, Malta DC, Mazidi M, McAlinden C, McGarvey ST, Mengistu DT, Mensah

GA, Mensink GBM, Mezgebe HB, Mirrakhimov EM, Mueller UO, Noubiap JJ, Obermeyer CM, Ogbo FA, Owolabi MO, Patton GC, Pourmalek F, Oorbani M, Rafav A, Rai RK, Ranabhat CL, Reinig N, Safiri S, Salomon JA, Sabaria JR, Santos IS, Sartorius B, Sawhney M, Schmidhuber J, Schutte AE, Schmidt MI, Sepanlou SG, Shamsizadeh M, Sheikhbahaei S, Shin M-J, Shiri R, Shiue I, Roba HS, Silva DAS, Silverberg JI, Singh JA, Stranges S, Swaminathan S, Tabarés-Seisdedos R, Tades F, Tedla BA, Tegegne BS, Terkawi AS, Thakur JS, Tonelli M, Topor-Madry R, Tyrovolas S, Ukwaia KN, Uthman OA, Vaeghasemi M, Vasankari T, Vlassov VV, Vollset SE, Weiderpass R, Werdecker A, Wesana J, Westerman R, Yano Y, Yonemoto N, Yonga G, Zaidi Z, Zenebe ZM, Zipkin B, Murray CJL. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. N Engl J Med. 2017;377(1):13-27.

Ghaben AL, Scherer PE. Adipogenesis and metabolic health. Nat Rev Mol Cell Biol. 2019;20(4):242-258.

Gliniak CM, Pedersen L, Scherer PE. Adipose tissue fibrosis: the unwanted houseguest invited by obesity. J Endocrinol. 2023;259(3):e230180.

Goldman AL, Bhasin S, Wu FCW, Krishna M, Matsumoto AM, Jasuja R. A Reappraisal of Testosterone's Binding in Circulation: Physiological and Clinical Implications. Endocr Rev. 2017;38(4):302-324.

Goossens GH, Jocken JWE, Blaak EE. Sexual dimorphism in cardiometabolic health: the role of adipose tissue, muscle and liver. Nat Rev Endocrinol. 2021;17(1):47-66.

Grabner GF, Xie H, Schweiger M, Zechner R. Lipolysis: cellular mechanisms for lipid mobilization from fat stores. Nat Metab. 2021;3(11):1445-1465.

Gruber CJ, Tschugguel W, Schneeberger C, Huber JC. Production and actions of estrogens. N Engl J Med. 2002;346(5):340-352.

Gu P, Xu A. Interplay between adipose tissue and blood vessels in obesity and vascular dysfunction. Rev Endocr Metab Disord. 2013;14(1):49-58.

Gustafson B, Nerstedt A, Smith U. Reduced subcutaneous adipogenesis in human hypertrophic obesity is linked to senescent precursor cells. Nat Commun. 2019;10(1):2757.

Haarbo J, Marslew U, Gotfredsen A, Christiansen C. Postmenopausal hormone replacement therapy prevents central distribution of body fat after menopause. Metabolism. 1991;40(12):1323-1326.

Hall JE. Endocrinology of the Menopause. Endocrinol Metab Clin North Am. 2015;44(3):485-496.

Hammond GL. Plasma steroid-binding proteins: primary gatekeepers of steroid hormone action. J Endocrinol. 2016;230(1):R13-R25.

Handelsman DJ, Wartofsky L. Requirement for mass spectrometry sex steroid assays in the Journal of Clinical Endocrinology and Metabolism. J Clin Endocrinol Metab. 2013;98(10):3971-3973.

Hanson SR, Best MD, Wong CH. Sulfatases: structure, mechanism, biological activity, inhibition, and synthetic utility. Angew Chem Int Ed Engl. 2004;43(43):5736-5763.

Harlow SD, Gass M, Hall JE, Lobo R, Maki P, Rebar RW, Sherman S, Sluss PM, de Villiers TJ; STRAW + 10 Collaborative Group. Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished

agenda of staging reproductive aging. J Clin Endocrinol Metab. 2012;97(4):1159-1168.

He Q, Xia B, Liu A, L M, Zhou Z, Cheung EC, Kuo ZC, Wang B, Li F, Tang Y, Zheng Z, Sun R, Hu YJ, Meng W, He Y, Yuan J, Zhang C. Association of body composition with risk of overall and site-specific cancers: A population-based prospective cohort study. Int J Cancer. 2021;149(7):1435-1447.

Heilbronn L, Smith SR, Ravussin E. Failure of fat cell proliferation, mitochondrial function and fat oxidation results in ectopic fat storage, insulin resistance and type II diabetes mellitus. Int J Obes Relat Metab Disord. 2004;28 Suppl 4:S12-S21.

Heinosalo T, Saarinen N, Poutanen M. Role of hydroxysteroid (17beta) dehydrogenase type 1 in reproductive tissues and hormone-dependent diseases. Mol Cell Endocrinol. 2019;489:9-31.

Herold J, Kalucka J. Angiogenesis in Adipose Tissue: The Interplay Between Adipose and Endothelial Cells. Front Physiol. 2021;11:624903.

Hirschberg AL. Approach to Investigation of Hyperandrogenism in a Postmenopausal Woman. J Clin Endocrinol Metab. 2023;108(5):1243-1253.

Hirschberg AL. Hyperandrogenism and Cardiometabolic Risk in Pre- and Postmenopausal Women-What Is the Evidence?. J Clin Endocrinol Metab. 2024;109(5):1202-1213.

Hodis HN, Mack WJ, Henderson VW, Shoupe D, Budoff MJ, Hwang-Levine J, Li Y, Feng M, Dustin L, Kono N, Stanczyk FZ, Selzer RH, Azen SP; ELITE Research Group. Vascular Effects of Early versus Late Postmenopausal Treatment with Estradiol. N Engl J Med. 2016;374(13):1221-1231.

Honour JW. Development and validation of a quantitative assay based on tandem mass spectrometry. Ann Clin Biochem. 2011;48(Pt 2):97-111.

Hwang I, Kim JB. Two Faces of White Adipose Tissue with Heterogeneous Adipogenic Progenitors. Diabetes Metab J. 2019;43(6):752-762.

Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. Obes Rev. 2010;11(1):11-18.

Ihunnah CA, Wada T, Philips BJ, Ravuri SK, Gibbs RB, Kirisci L, Rubin JP, Marra KG, Xie W. Estrogen sulfotransferase/SULT1E1 promotes human adipogenesis. Mol Cell Biol. 2014;34(9):1682-1694.

Janssen I, Powell LH, Jasielec MS, Kazlauskaite R. Covariation of change in bioavailable testosterone and adiposity in midlife women. Obesity (Silver Spring). 2015;23(2):488-494.

Jiang M, Klein M, Zanger UM, Mohammad MK, Cave MC, Gaikwad NW, Dias NJ, Selcer KW, Guo Y, He J, Zhang X, Shen Q, Qin W, Li J, Li S, Xie W. Inflammatory regulation of steroid sulfatase: A novel mechanism to control estrogen homeostasis and inflammation in chronic liver disease. J Hepatol. 2016;64(1):44-52.

Jones ME, Thorburn AW, Britt KL, Hewitt KN, Misso ML, Wreford NG, Proietto J, Oz OK, Leury BJ, Robertson KM, Yao S, Simpson ER. Aromatase-deficient (ArKO) mice accumulate excess adipose tissue. J Steroid Biochem Mol Biol. 2001;79(1-5):3-9.

Joyner J, Hutley L, Cameron D. Intrinsic regional differences in androgen receptors and dihydrotestosterone metabolism in human preadipocytes. Horm Metab Res. 2002;34(5):223-228.

Juppi HK, Sipilä S, Cronin NJ, Karvinen S, Karppinen JE, Tammelin TH, Aukee P, Kovanen V, Kujala UM, Laakkonen EK. Role of Menopausal Transition and Physical Activity in Loss of Lean and Muscle Mass: A Follow-Up Study in Middle-Aged Finnish Women. J Clin Med. 2020;9(5):1588.

Kanneganti TD, Dixit VD. Immunological complications of obesity. Nat Immunol. 2012;13(8):707-712.

Karastergiou K, Fried SK. Cellular Mechanisms Driving Sex Differences in Adipose Tissue Biology and Body Shape in Humans and Mouse Models. Adv Exp Med Biol. 2017;1043:29-51.

Karpe F, Pinnick KE. Biology of upper-body and lower-body adipose tissue--link to whole-body phenotypes. Nat Rev Endocrinol. 2015;11(2):90-100.

Karvonen-Gutierrez C, Kim C. Association of Mid-Life Changes in Body Size, Body Composition and Obesity Status with the Menopausal Transition. Healthcare (Basel). 2016;4(3):42.

Ke Y, Bélanger A, Simard JN, Gonthier R, Martel C, Vaillancourt M, Labrie F. Concentration range of serum sex steroids in normal postmenopausal women and those with diagnosis of vulvovaginal atrophy. Menopause. 2018;25:293-300.

Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89(6):2548-2556.

Kersten S. Mechanisms of nutritional and hormonal regulation of lipogenesis. EMBO Rep. 2001;2(4):282-286.

Killinger DW, Perel E, Danilescu D, Kharlip L, Lindsay WR. Influence of adipose tissue distribution on the biological activity of androgens. Ann N Y Acad Sci. 1990;595:199-211.

Kim SM, Kim SE, Lee DY, Choi D. Serum estradiol level according to dose and formulation of oral estrogens in postmenopausal women. Sci Rep. 2021;11(1):3585.

Kinoshita T, Honma S, Shibata Y, Yamashita K, Watanabe Y, Maekubo H, Okuyama M, Takashima A, Takeshita N. An innovative LC-MS/MS-based method for determining CYP 17 and CYP 19 activity in the adipose tissue of pre- and postmenopausal and ovariectomized women using 13C-labeled steroid substrates. J Clin Endocrinol Metab. 2014;99(4):1339-1347.

Kleider C, Calderón Giraldo J, Pemp D, Esch HL, Lehmann L. Validation of a GC- and LC-MS/MS based method for the quantification of 22 estrogens and its application to human plasma. Steroids. 2022;186:109077.

Kolatorova L, Vitku J, Suchopar J, Hill M, Parizek A. Progesterone: A Steroid with Wide Range of Effects in Physiology as Well as Human Medicine. Int J Mol Sci. 2022;23(14):7989.

Kuhl H. Pharmacology of estrogens and progestogens: influence of different routes of administration. Climacteric. 2005;8 Suppl 1:3-63.

Kushnir MM, Rockwood AL, Roberts WL, Yue B, Bergquist J, Meikle AW. Liquid chromatography tandem mass spectrometry for analysis of steroids in clinical laboratories. Clin Biochem. 2011;44(1):77-88.

Kwok KH, Lam KS, Xu A. Heterogeneity of white adipose tissue: molecular basis and clinical implications. Exp Mol Med. 2016;48(3):e215.

Labrie F. Intracrinology. Mol Cell Endocrinol. 1991;78(3):C113-C118.

Labrie F. All sex steroids are made intracellularly in peripheral tissues by the mechanisms of intracrinology after menopause. J Steroid Biochem Mol Biol. 2015;145:133-138.

Labrie F, Bélanger A, Bélanger P, Bérubé R, Martel C, Cusan L, Gomez J, Candas B, Castiel I, Chaussade V, Deloche C, Leclaire J. Androgen glucuronides, instead of testosterone, as the new markers of androgenic activity in women. J Steroid Biochem Mol Biol. 2006;99(4-5):182-188

Labrie F, Bélanger A, Luu-The V, Labrie C, Simard J, Cusan L, Gomez JL, Candas B. DHEA and the intracrine formation of androgens and estrogens in peripheral target tissues: its role during aging. Steroids. 1998;63(5-6):322-328.

Labrie F, Bélanger A, Pelletier G, Martel C, Archer DF, Utian WH. Science of intracrinology in postmenopausal women. Menopause. 2017;24(6):702-712.

Labrie F, Cusan L, Gomez JL, Côté I, Bérubé R, Bélanger P, Martel C, Labrie C. Effect of intravaginal DHEA on serum DHEA and eleven of its metabolites in postmenopausal women. J Steroid Biochem Mol Biol. 2008;111(3-5):178-194

Labrie F, Luu-The V, Lin SX, Simard J, Labrie C. Role of 17 beta-hydroxysteroid dehydrogenases in sex steroid formation in peripheral intracrine tissues. Trends Endocrinol Metab. 2000;11(10):421-427.

Labrie F, Martel C, Balser J. Wide distribution of the serum dehydroepiandrosterone and sex steroid levels in postmenopausal women: role of the ovary? Menopause. 2011;18(1):30-43.

Laforest S, Pelletier M, Denver N, Poitier B, Nguyen S, Walker SR, Durocher F, Homer NZM, Diorio C, Tchernof A, Andrew R. Simultaneous quantification of estrogens and glucocorticoids in human adipose tissue by liquid-chromatography-tandem mass spectrometry. J Steroid Biochem Mol Biol. 2019;195:105476.

Lee CG, Carr MC, Murdoch SJ, Mitchell E, Woods NF, Wener MH, Chandler WL, Boyko EJ, Brunzell JD. Adipokines, inflammation, and visceral adiposity across the menopausal transition: a prospective study. J Clin Endocrinol Metab. 2009;94(4):1104-1110.

Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. Mol Aspects Med. 2013;34(1):1-11.

Leeners B, Geary N, Tobler PN, Asarian L. Ovarian hormones and obesity. Hum Reprod Update. 2017;23(3):300-321.

Lewis JR, McNab TJ, Liew LJ, Tan J, Hudson P, Wang JZ, Prince RL. DNA methylation within the I.4 promoter region correlates with CYPl19A1 gene expression in human ex vivo mature omental and subcutaneous adipocytes. BMC Med Genet. 2013;14:87.

Li J, Papadopoulos V, Vihma V. Steroid biosynthesis in adipose tissue. Steroids. 2015;103:89-104.

Li X, Liu J, Zhou B, Li Y, Wu Z, Meng H, Wang G. Sex Differences in the Effect of Testosterone on Adipose Tissue Insulin Resistance From Overweight to Obese Adults. J Clin Endocrinol Metab. 2021;106(8):2252-2263.

Liu JH. The Role of Progestogens in Menopausal Hormone Therapy. Clin Obstet Gynecol. 2021;64(4):772-783.

Lovejoy JC, Champagne CM, de Jonge L, Xie H, Smith SR. Increased visceral fat and decreased energy expenditure during the menopausal transition. Int J Obes (Lond). 2008;32(6):949-958.

Luu-The V, Tremblay P, Labrie F. Characterization of type 12 17beta-hydroxysteroid dehydrogenase, an isoform of type 3 17beta-hydroxysteroid dehydrogenase responsible for estradiol formation in women. Mol Endocrinol. 2006;20(2):437-443.

MacDonald PC, Edman CD, Hemsell DL, Porter JC, Siiteri PK. Effect of obesity on conversion of plasma androstenedione to estrone in postmenopausal women with and without endometrial cancer. Am J Obstet Gynecol. 1978;130(4):448-455.

Mahendroo MS, Mendelson CR, Simpson ER. Tissue-specific and hormonally controlled alternative promoters regulate aromatase cytochrome P450 gene expression in human adipose tissue. J Biol Chem. 1993;268(26):19463-19470.

Maniyadath B, Zhang Q, Gupta RK, Mandrup S. Adipose tissue at single-cell resolution. Cell Metab. 2023;35(3):386-413.

Manrique-Acevedo C, Chinnakotla B, Padilla J, Martinez-Lemus LA, Gozal D. Obesity and cardiovascular disease in women. Int J Obes (Lond). 2020;44(6):1210-1226.

Marchais-Oberwinkler S, Henn C, Möller G, Klein T, Negri M, Oster A, Spadaro A, Werth R, Wetzel M, Xu K, Frotscher M, Hartmann RW, Adamski J. 17β-Hydroxysteroid dehydrogenases (17β-HSDs) as therapeutic targets: protein structures, functions, and recent progress in inhibitor development. J Steroid Biochem Mol Biol. 2011;125(1-2):66-82.

Marchand GB, Carreau AM, Laforest S, Côté J-A, Daris M, Cianflone K, Prehn C, Adamski J, Tchernof A. Circulating steroid levels as correlates of adipose tissue phenotype in premenopausal women. Horm Mol Biol Clin Investig. 2018;34(1):10.1515/hmbci-2017-0082.

Marina L, Sojat AS, Maseroli E, Spaggiari G, Pandurevic S, Santi D. Hormonal profile of menopausal women receiving androgen replacement therapy: a meta-analysis. J Endocrinol Invest. 2020;43(6):717-735.

Martel C, Labrie F, Archer DF, Ke Y, Gonthier R, Simard JN, Lavoie L, Vaillancourt M, Montesino M, Balser J, Moyneur E, other participating members of the Prasterone Clinical Research Group. Serum steroid concentrations remain within normal postmenopausal values in women receiving daily 6.5mg intravaginal prasterone for 12 weeks. J Steroid Biochem Mol Biol. 2016;159:142-153.

Martel C, Rhéaume E, Takahashi M, Trudel C, Couët J, Luu-The V, Simard J, Labrie F. Distribution of 17 beta-hydroxysteroid dehydrogenase gene expression and activity in rat and human tissues. J Steroid Biochem Mol Biol. 1992;41(3-8):597-603.

Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. Endocr Rev. 2013;34(3):309-338.

McTiernan A, Wu L, Chen C, Chlebowski R, Mossavar-Rahmani Y, Modugno F, Perri MG, Stanczyk FZ, Van Horn L, Wang CY; Women's Health Initiative Investigators. Relation of BMI and physical activity to sex hormones in postmenopausal women. Obesity (Silver Spring). 2006;14(9):1662-1677.

Menazza S, Murphy E. The Expanding Complexity of Estrogen Receptor Signaling in the Cardiovascular System. Circ Res. 2016;118(6):994-1007.

Mezzullo M, Pelusi C, Fazzini A, Repaci A, Di Dalmazi G, Gambineri A, Pagotto U, Fanelli F. Female and male serum reference intervals for challenging sex and precursor steroids by liquid chromatography - tandem mass spectrometry. J Steroid Biochem Mol Biol. 2020;197:105538.

Mikkola TS, Tuomikoski P, Lyytinen H, Korhonen P, Hoti F, Vattulainen P, Gissler M, Ylikorkala O. Estradiol-based postmenopausal hormone therapy and risk of cardiovascular and all-cause mortality. Menopause. 2015;22(9):976-983.

Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr Rev. 2011;32(1):81-151.

Misso ML, Jang C, Adams J, Tran J, Murata Y, Bell R, Boon WC, Simpson ER, Davis SR. Adipose aromatase gene expression is greater in older women and is unaffected by postmenopausal estrogen therapy. Menopause. 2005;12(2):210-215.

Moeller G, Adamski J. Integrated view on 17beta-hydroxysteroid dehydrogenases. Mol Cell Endocrinol. 2009;301(1-2):7-19.

Moeller G, Adamski J. Multifunctionality of human 17beta-hydroxysteroid dehydrogenases. Mol Cell Endocrinol. 2006;248(1-2):47-55.

Morigny P, Houssier M, Mouisel E, Langin D. Adipocyte lipolysis and insulin resistance. Biochimie. 2016;125:259-266.

Morselli E, Santos RS, Criollo A, Nelson MD, Palmer BF, Clegg DJ. The effects of oestrogens and their receptors on cardiometabolic health. Nat Rev Endocrinol. 2017;13(6):352-364.

Mueller JW, Gilligan LC, Idkowiak J, Arlt W, Foster PA. The Regulation of Steroid Action by Sulfation and Desulfation. Endocr Rev. 2015;36(5):526-563.

Nagy B, Szekeres-Barthó J, Kovács GL, Sulyok E, Farkas B, Várnagy Á, Vértes V, Kovács K, Bódis J. Key to Life: Physiological Role and Clinical Implications of Progesterone. Int J Mol Sci. 2021;22(20):11039.

Nanba AT, Rege J, Ren J, Auchus RJ, Rainey WE, Turcu AF. 11-Oxygenated C19 Steroids Do Not Decline With Age in Women. J Clin Endocrinol Metab. 2019;104(7):2615-2622.

National Institutes of Health. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health. Obes Res. 1998;6 Suppl 2:51S-209S.

Neeland IJ, Ross R, Després JP, Matsutsawa Y, Yamashita S, Shai I, Seidell J, Magni P, Santos RD, Arsenault B, Cuevas A, Hu FB, Griffin B, Zambon A, Barter P, Fruchart JC, Eckel RH; International Atherosclerosis Society; International Chair on Cardiometabolic Risk Working Group on Visceral Obesity. Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement. Lancet Diabetes Endocrinol. 2019;7(9):715-725.

Neeland IJ, Yokoo T, Leinhard OD, Lavie CJ. 21st Century Advances in Multimodality Imaging of Obesity for Care of the Cardiovascular Patient. JACC Cardiovasc Imaging. 2021;14(2):482-494.

Nelson HD, Humphrey LL, Nygren P, Teutsch SM, Allan JD. Postmenopausal hormone replacement therapy: scientific review. JAMA. 2002;288(7):872-881.

Nelson LR, Bulun SE. Estrogen production and action. J Am Acad Dermatol. 2001;45(3 Suppl):S116-S124.

Newell-Fugate AE. The role of sex steroids in white adipose tissue adipocyte function. Reproduction. 2017;153(4):R133-R149.

Nilsson M, Dahlman I, Rydén M, Nordström EA, Gustafsson J-A, Arner P, Dahlman-Wright K. Oestrogen receptor alpha gene expression levels are reduced in obese compared to normal weight females. Int J Obes (Lond). 2007;31(6):900-907.

O'Brien SN, Welter BH, Mantzke KA, Price TM. Identification of progesterone receptor in human subcutaneous adipose tissue. J Clin Endocrinol Metab. 1998;83(2):509-513.

O'Lone R, Frith MC, Karlsson EK, Hansen U. Genomic targets of nuclear estrogen receptors. Mol Endocrinol. 2004;18(8):1859-1875.

O'Malley BW. Mechanisms of action of steroid hormones. N Engl J Med. 1971;284(7):370-377.

Olde B, Leeb-Lundberg LM. GPR30/GPER1: searching for a role in estrogen physiology. Trends Endocrinol Metab. 2009;20(8):409-416.

Ostinelli G, Laforest S, Denham SG, Gauthier M-F, Drolet-Labelle V, Scott E, Hould F-S, Marceau S, Homer NZM, Bégin C, Andrew R, Tchernof A. Increased Adipose Tissue Indices of Androgen Catabolism and Aromatization in Women With Metabolic Dysfunction. J Clin Endocrinol Metab. 2022;107(8):e3330-e3342.

Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011;11(2):85-97.

Paatela H, Wang F, Vihma V, Savolainen-Peltonen H, Mikkola TS, Turpeinen U, Hämäläinen E, Jauhiainen M, Tikkanen MJ. Steroid sulfatase activity in subcutaneous and visceral adipose tissue: a comparison between pre- and postmenopausal women. Eur J Endocrinol. 2016;174(2):167-175.

Palmer BF, Clegg DJ. The sexual dimorphism of obesity. Mol Cell Endocrinol. 2015;402:113-119.

Payne AH, Hales DB. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr Rev. 2004;25(6):947-970.

Pedersen SB, Kristensen K, Hermann PA, Katzenellenbogen JA, Richelsen B. Estrogen controls lipolysis by up-regulating alpha2A-adrenergic receptors directly in human adipose tissue through the estrogen receptor alpha. Implications for the female fat distribution. J Clin Endocrinol Metab. 2004;89(4):1869-1878.

Penell JC, Kushnir MM, Lind L, Bergquist J, Bergquist J, Lind PM, Naessen T. Concentrations of nine endogenous steroid hormones in 70-year-old men and women. Endocr Connect. 2021;10(5):511-520.

Pi-Sunyer FX. The epidemiology of central fat distribution in relation to disease. Nutr Rev. 2004;62(7 Pt 2):S120-S126.

Piltonen T, Koivunen R, Morin-Papunen L, Ruokonen A, Huhtaniemi IT, Tapanainen JS. Ovarian and adrenal steroid production: regulatory role of LH/HCG. Hum Reprod. 2002;17(3):620-624.

Poutanen M. Understanding the diversity of sex steroid action. J Endocrinol. 2012;212(1):1-2.

Poutanen M, Hagberg Thulin M, Härkönen P. Targeting sex steroid biosynthesis for breast and prostate cancer therapy. Nat Rev Cancer. 2023;10.1038/s41568-023-00609-y.

Poutanen M, Penning TM. Biology and clinical relevance of Hydroxysteroid (17beta) dehydrogenase enzymes. Mol Cell Endocrinol. 2019;489:1-2

Price T, Aitken J, Head J, Mahendroo M, Means G, Simpson E. Determination of aromatase cytochrome P450 messenger ribonucleic acid in human breast tissue by competitive polymerase chain reaction amplification. J Clin Endocrinol Metab. 1992;74(6):1247-1252.

Purohit A, Wang DY, Ghilchik MW, Reed MJ. Regulation of aromatase and sulphatase in breast tumour cells. J Endocrinol. 1996;150 Suppl:S65-S71.

Purohit A, Woo LW, Potter BV. Steroid sulfatase: a pivotal player in estrogen synthesis and metabolism. Mol Cell Endocrinol. 2011;340(2):154-160.

Raison J, Basdevant A, Sitt Y, Guy-Grand B. Regional differences in adipose tissue lipoprotein lipase activity in relation to body fat distribution and menopausal status in obese women. Int J Obes. 1988;12(5):465-472.

Rankinen T, Kim SY, Pérusse L, Després JP, Bouchard C. The prediction of abdominal visceral fat level from body composition and anthropometry: ROC analysis. Int J Obes Relat Metab Disord. 1999;23(8):801-809.

Reed MJ, Purohit A, Woo LW, Newman SP, Potter BV. Steroid sulfatase: molecular biology, regulation, and inhibition. Endocr Rev. 2005;26(2):171-202.

Reinecke I, Hofmann B, Mesic E, Drenth HJ, Garmann D. An Integrated Population Pharmacokinetic Analysis to Characterize Levonorgestrel Pharmacokinetics After Different Administration Routes. J Clin Pharmacol. 2018;58(12):1639-1654.

Rizk J, Sahu R, Duteil D. An overview on androgen-mediated actions in skeletal muscle and adipose tissue. Steroids. 2023;199:109306.

Roepke TA, Malyala A, Bosch MA, Kelly MJ, Rønnekleiv OK. Estrogen regulation of genes important for K+ channel signaling in the arcuate nucleus. Endocrinology. 2007;148(10):4937-4951.

Ronconi V, Turchi F, Bujalska IJ, Giacchetti G, Boscaro M. Adipose cell-adrenal interactions: current knowledge and future perspectives. Trends Endocrinol Metab. 2008;19(3):100-103.

Rosen ED, Spiegelman BM. What we talk about when we talk about fat. Cell. 2014;156(1-2):20-44.

Rosner W, Hankinson SE, Sluss PM, Vesper HW, Wierman ME. Challenges to the measurement of estradiol: an endocrine society position statement. J Clin Endocrinol Metab. 2013;98(4):1376-1387.

Rothman MS, Carlson NE, Xu M, Wang C, Swerdloff R, Lee P, Goh VHH, Ridgway EC, Wierman ME. Reexamination of testosterone, dihydrotestosterone, estradiol and estrone levels across the menstrual cycle and in postmenopausal women measured by liquid chromatography-tandem mass spectrometry. Steroids. 2011;76(1-2):177-182

Rubinow KB. An intracrine view of sex steroids, immunity, and metabolic regulation. Mol Metab. 2018;15:92-103.

Saely CH, Geiger K, Drexel H. Brown versus white adipose tissue: a mini-review. Gerontology. 2012;58(1):15-23.

Sakers A, De Siqueira MK, Seale P, Villanueva CJ. Adipose-tissue plasticity in health and disease. Cell. 2022;185(3):419-446.

Saloniemi T, Jokela H, Strauss L, Pakarinen P, Poutanen M. The diversity of sex steroid action: novel functions of hydroxysteroid (17β) dehydrogenases as revealed by genetically modified mouse models. J Endocrinol. 2012;212(1):27-40.

Salpeter SR, Walsh JM, Ormiston TM, Greyber E, Buckley NS, Salpeter EE. Meta-analysis: effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women. Diabetes Obes Metab. 2006;8(5):538-554.

Santen RJ. Vaginal administration of estradiol: effects of dose, preparation and timing on plasma estradiol levels. Climacteric. 2015;18(2):121-134.

Santner SJ, Feil PD, Santen RJ. In situ estrogen production via the estrone sulfatase pathway in breast tumors: relative importance versus the aromatase pathway. J Clin Endocrinol Metab. 1984;59(1):29-33.

Santoro N, Roeca C, Peters BA, Neal-Perry G. The Menopause Transition: Signs, Symptoms, and Management Options. J Clin Endocrinol Metab. 2021;106(1):1-15.

Savolainen-Peltonen H, Rahkola-Soisalo P, Hoti F, Vattulainen P, Gissler M, Ylikorkala O, Mikkola TS. Use of postmenopausal hormone therapy and risk of Alzheimer's disease in Finland: nationwide case-control study. BMJ. 2019;364:l665.

Savolainen-Peltonen H, Tuomikoski P, Korhonen P, Hoti F, Vattulainen P, Gissler M, Ylikorkala O, Mikkola TS. Cardiac Death Risk in Relation to the Age at Initiation or the Progestin Component of Hormone Therapies. J Clin Endocrinol Metab. 2016;101(7):2794-2801.

Savolainen-Peltonen H, Vihma V, Leidenius M, Wang F, Turpeinen U, Hämäläinen E, Tikkanen MJ, Mikkola TS. Breast adipose tissue estrogen metabolism in postmenopausal women with or without breast cancer. J Clin Endocrinol Metab. 2014;99(12):E2661-E2667.

Savolainen-Peltonen H, Vihma V, Wang F, Turpeinen U, Hämäläinen E, Haanpää M, Leidenius M, Tikkanen MJ, Mikkola TS. Estrogen biosynthesis in breast adipose tissue during menstrual cycle in women with and without breast cancer. Gynecol Endocrinol. 2018;34(12):1039-1043.

Scarabin PY. Progestogens and venous thromboembolism in menopausal women: an updated oral versus transdermal estrogen meta-analysis. Climacteric. 2018;21(4):341-345.

Scheja L, Heeren J. The endocrine function of adipose tissues in health and cardiometabolic disease. Nat Rev Endocrinol. 2019;15(9):507-524.

Schierbeck LL, Rejnmark L, Tofteng CL, Stilgren L, Eiken P, Mosekilde L, Kober L, Beck Jensen J-E.. Effect of hormone replacement therapy on cardiovascular events in recently postmenopausal women: randomised trial. BMJ. 2012;345:e6409.

Schiffer L, Arlt W, O'Reilly MW. Understanding the Role of Androgen Action in Female Adipose Tissue. Front Horm Res. 2019;53:33-49.

Schiffer L, Arlt W, Storbeck KH. Intracrine androgen biosynthesis, metabolism and action revisited. Mol Cell Endocrinol. 2018;465:4-26.

Schiffer L, Barnard L, Baranowski ES, Gilligan LC, Taylor AE, Arlt W, Shackleton CHL, Storbeck K-H. Human steroid biosynthesis, metabolism and excretion are differentially reflected by serum and urine steroid

metabolomes: A comprehensive review. J Steroid Biochem Mol Biol. 2019;194:105439.

Schiffer L, Kempegowda P, Sitch AJ, Adaway JE, Shaheen F, Ebbehoj A, Singh S, McTaggart MP, O'Reilly MW, Prete A, Hawley JM, Keevil BG, Bancos I, Taylor AE, Arlt W. Classic and 11-oxygenated androgens in serum and saliva across adulthood: a cross-sectional study analyzing the impact of age, body mass index, and diurnal and menstrual cycle variation. Eur J Endocrinol. 2023;188(1):lvac017.

Schleinitz D, Krause K, Wohland T, Gebhardt C, Linder N, Stumvoll M, Blüher M, Bechmann I, Kovacs P, Gericke M, Tönjes A. Identification of distinct transcriptome signatures of human adipose tissue from fifteen depots. Eur J Hum Genet. 2020;28(12):1714-1725.

Seeber B, Ziehr SC, Gschließer A, Moser C, Mattle V, Seger C, Griesmacher A, Concin N, Concin H, Wildt L. Quantitative levonorgestrel plasma level measurements in patients with regular and prolonged use of the levonorgestrel-releasing intrauterine system. Contraception. 2012;86(4):345-349.

Sherman BM, West JH, Korenman SG. The menopausal transition: analysis of LH, FSH, estradiol, and progesterone concentrations during menstrual cycles of older women. J Clin Endocrinol Metab. 1976;42(4):629-636.

Shifren JL, Desindes S, McIlwain M, Doros G, Mazer NA. A randomized, open-label, crossover study comparing the effects of oral versus transdermal estrogen therapy on serum androgens, thyroid hormones, and adrenal hormones in naturally menopausal women. Menopause. 2007;14(6):985-994.

Shifren JL, Rifai N, Desindes S, McIlwain M, Doros G, Mazer NA. A comparison of the short-term effects of oral conjugated equine estrogens versus transdermal estradiol on C-reactive protein, other serum markers of inflammation, and other hepatic proteins in naturally menopausal women. J Clin Endocrinol Metab. 2008;93(5):1702-1710.

Siiteri PK. Adipose tissue as a source of hormones. Am J Clin Nutr. 1987;45(1 Suppl):277-282.

Simon JA. Estrogen replacement therapy: effects on the endogenous androgen milieu. Fertil Steril. 2002;77 Suppl 4:S77-S82.

Simpson E, Rubin G, Clyne C, Robertson K, O'Donnel L, Jones M, Davis SR. The role of local estrogen biosynthesis in males and females. Trends Endocrinol Metab. 2000;11(5):184-188.

Simpson ER, Davis SR. Minireview: aromatase and the regulation of estrogen biosynthesis--some new perspectives. Endocrinology. 2001;142(11):4589-4594.

Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito Y, Michael MD. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocr Rev. 1994;15(3):342-355.

Simpson ER, Zhao Y, Agarwal VR, Michael MD, Bulun SE, Hinshelwood MM, Graham-Lorence S, Sun T, Fisher CR, Qin K, Mendelson CR. Aromatase expression in health and disease. Recent Prog Horm Res. 1997;52:185-214.

Simpson ER. Sources of estrogen and their importance. J Steroid Biochem Mol Biol. 2003;86(3-5):225-230.

Sites CK, Brochu M, Tchernof A, Poehlman ET. Relationship between hormone replacement therapy use with body fat distribution and insulin sensitivity in obese postmenopausal women. Metabolism. 2001;50(7):835-840.

Skiba MA, Bell RJ, Islam RM, Handelsman DJ, Desai R, Davis SR. Androgens During the Reproductive Years: What Is Normal for Women?. J Clin Endocrinol Metab. 2019;104(11):5382-5392.

Slater CC, Hodis HN, Mack WJ, Shoupe D, Paulson RJ, Stanczyk FZ. Markedly elevated levels of estrone sulfate after long-term oral, but not transdermal, administration of estradiol in postmenopausal women. Menopause. 2001;8(3):200-203.

Soldano S, Montagna P, Brizzolara R, Sulli A, Parodi A, Seriolo B, Paolino S, Villagio B, Cutolo M. Effects of estrogens on extracellular matrix synthesis in cultures of human normal and scleroderma skin fibroblasts. Ann N Y Acad Sci. 2010;1193:25-29.

Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Näslund E, Britton T, Concha H, Hassan M, Rydén M, Frisén J, Arner P. Dynamics of fat cell turnover in humans. Nature. 2008;453(7196):783-787.

Spoletini I, Vitale C, Pelliccia F, Fossati C, Rosano GM. Androgens and cardiovascular disease in postmenopausal women: a systematic review. Climacteric. 2014;17(6):625-634.

Stanczyk FZ, Jurow J, Hsing AW. Limitations of direct immunoassays for measuring circulating estradiol levels in postmenopausal women and men in epidemiologic studies. Cancer Epidemiol Biomarkers Prev. 2010;19(4):903-906.

Stanczyk FZ, Sriprasert I, Karim R, Hwang-Levine J, Mack WJ, Hodis HN. Concentrations of endogenous sex steroid hormones and SHBG in healthy postmenopausal women. J Steroid Biochem Mol Biol. 2022;223:106080.

Stocco C. Tissue physiology and pathology of aromatase. Steroids. 2012;77(1-2):27-35.

Storbeck KH, O'Reilly MW. The clinical and biochemical significance of 11-oxygenated androgens in human health and disease. Eur J Endocrinol. 2023;188(4):R98-R109.

Strott CA. Steroid sulfotransferases. Endocr Rev. 1996;17(6):670-697.

Stuenkel CA, Davis SR, Gompel A, Lumsden MA, Murad MH, Pinkerton JV, Santen RJ. Treatment of Symptoms of the Menopause: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2015;100(11):3975-4011.

Sturdee DW, Panay N; International Menopause Society Writing Group. Recommendations for the management of postmenopausal vaginal atrophy. Climacteric. 2010;13(6):509-522.

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249.

Szmuilowicz ED, Stuenkel CA, Seely EW. Influence of menopause on diabetes and diabetes risk. Nat Rev Endocrinol. 2009;5(10):553-558.

Talaulikar V. Menopause transition: Physiology and symptoms. Best Pract Res Clin Obstet Gynaecol. 2022;81:3-7.

Tang QQ, Lane MD. Adipogenesis: from stem cell to adipocyte. Annu Rev Biochem. 2012;81:715-736.

Taylor AE, Keevil B, Huhtaniemi IT. Mass spectrometry and immunoassay: how to measure steroid hormones today and tomorrow. Eur J Endocrinol. 2015;173(2):D1-D12.

Tchernof A, Brochu D, Maltais-Payette I, Fouad Mansour M, Marchand GB, Carreau A-M, Kapeluto J. Androgens and the Regulation of Adiposity and Body Fat Distribution in Humans. Compr Physiol. 2018;8(4):1253-1290.

Tchernof A, Després JP. Pathophysiology of human visceral obesity: an update. Physiol Rev. 2013;93(1):359-404.

Tchernof A, Mansour MF, Pelletier M, Boulet MM, Nadeau M, Luu-The V. Updated survey of the steroid-converting enzymes in human adipose tissues. J Steroid Biochem Mol Biol. 2015;147:56-69.

Tchkonia T, Thomou T, Zhu Y, Karagiannides I, Pothoulakis C, Jensen MD, Kirkland JL. Mechanisms and metabolic implications of regional differences among fat depots. Cell Metab. 2013;17(5):644-656.

Tchoukalova YD, Koutsari C, Karpyak MV, Votruba SB, Wendland E, Jensen MD. Subcutaneous adipocyte size and body fat distribution. Am J Clin Nutr. 2008;87(1):56-63.

Tchoukalova YD, Koutsari C, Votruba SB, Tchkonia Y, Giorgadze N, Thomou T, Kirkland JL, Jensen MD. Sex- and depot-dependent differences in adipogenesis in normal-weight humans. Obesity (Silver Spring). 2010;18(10):1875-1880.

Terveys, toimintakyky ja hyvinvointi Suomessa: FinTerveys 2017 -tutkimus. https://urn.fi/URN:ISBN:978-952-343-105-8 Accessed: 20.10.2023

Thomas MP, Potter BV. The structural biology of oestrogen metabolism. J Steroid Biochem Mol Biol. 2013;137:27-49.

Tsachaki M, Odermatt A. Subcellular localization and membrane topology of 17β-hydroxysteroid dehydrogenases. Mol Cell Endocrinol. 2019;489:98-106.

Valenzuela PL, Carrera-Bastos P, Castillo-García A, Lieberman DE, Santos-Lozano A, Lucia A. Obesity and the risk of cardiometabolic diseases. Nat Rev Cardiol. 2023;20(7):475-494.

Van Pelt RE, Gozansky WS, Schwartz RS, Kohrt WM. Intravenous estrogens increase insulin clearance and action in postmenopausal women. Am J Physiol Endocrinol Metab. 2003;285(2):E311-E317.

Vázquez-Martínez ER, Mendoza-Garcés L, Vergara-Castañeda E, Cerbón M. Epigenetic regulation of Progesterone Receptor isoforms: from classical models to the sexual brain. Mol Cell Endocrinol. 2014;392(1-2):115-124.

Verdonk SJE, Vesper HW, Martens F, Sluss PM, Hillebrand JJ, Heijboer AC. Estradiol reference intervals in women during the menstrual cycle, postmenopausal women and men using an LC-MS/MS method. Clin Chim Acta. 2019;495:198-204.

Vigneswaran K, Hamoda H. Hormone replacement therapy - Current recommendations. Best Pract Res Clin Obstet Gynaecol. 2022;81:8-21.

Vihma V, Wang F, Savolainen-Peltonen H, Turpeinen U, Hämäläinen E, Leidenius M, Mikkola TS, Tikkanen MJ. Quantitative determination of estrone by liquid chromatography-tandem mass spectrometry in subcutaneous

adipose tissue from the breast in postmenopausal women. J Steroid Biochem Mol Biol. 2016;155(Pt A):120-125.

Vijay J, Gauthier MF, Biswell RL, Louiselle DA, Johnston JJ, Cheung WA, Belden B, Pramatorova A, Biertho L, Gibson M, Simon M-M, Djambazian H, Staffa A, Bourque G, Laitinen A, Nystedt J, Vohl M-C, Fraser JD, Pastinen T, Tchernof A, Grundberg E. Single-cell analysis of human adipose tissue identifies depot and disease specific cell types. Nat Metab. 2020;2(1):97-109.

Vinogradova Y, Coupland C, Hippisley-Cox J. Use of hormone replacement therapy and risk of venous thromboembolism: nested case-control studies using the QResearch and CPRD databases. BMJ. 2019;364:k4810.

Vuppaladhadiam L, Lager J, Fiehn O, Weiss S, Chesney M, Hasdemir B, Bhargava A. Human Placenta Buffers the Fetus from Adverse Effects of Perceived Maternal Stress. Cells. 2021;10(2):379.

Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev. 2000;21(6):697-738.

Wang J, Feng Y, Liu B, Xie W. Estrogen sulfotransferase and sulfatase in steroid homeostasis, metabolic disease, and cancer. Steroids. 2024;201:109335.

Wang F, Koskela A, Hämäläinen E, Turpeinen U, Savolainen-Peltonen H, Mikkola TS, Vihma V, Adlercreutz H, Tikkanen MJ. Quantitative determination of dehydroepiandrosterone fatty acyl esters in human female adipose tissue and serum using mass spectrometric methods. J Steroid Biochem Mol Biol. 2011;124(3-5):93-98.

Wang F, Vihma V, Badeau M, Savolainen-Peltonen H, Leidenius M, Mikkola T, Turpeinen U, Hämäläinen E, Ikonen E, Wähälä K, Fledelius C, Jauhiainen M, Tikkanen MJ. Fatty acyl esterification and deesterification of 17β-estradiol in human breast subcutaneous adipose tissue. J Clin Endocrinol Metab. 2012;97(9):3349-3356.

Wang F, Vihma V, Soronen J, Turpeinen U, Hämäläinen E, Savolainen-Peltonen H, Mikkola TS, Naukkarinen J, Pietiläinen KH, Jauhiainen M, Yki-Järvinen H, Tikkanen MJ. 17β-Estradiol and estradiol fatty acyl esters and estrogen-converting enzyme expression in adipose tissue in obese men and women. J Clin Endocrinol Metab. 2013;98(12):4923-4931.

Wawrzkiewicz-Jałowiecka A, Lalik A, Soveral G. Recent Update on the Molecular Mechanisms of Gonadal Steroids Action in Adipose Tissue. Int J Mol Sci. 2021;22(10):5226.

Wise PM, Krajnak KM, Kashon ML. Menopause: the aging of multiple pacemakers. Science. 1996;273(5271):67-70.

World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser. 2000;894:i-253.

World Health Organization. World Health Statistics 2022: Monitoring Health for the SDGs, Sustainable Development Goals. 2022; World HealthOrganization. https://iris.who.int/bitstream/handle/10665/356584/9789240051140-eng.pdf?sequence=1 Accessed 20.10.2023.

Wudy SA, Schuler G, Sánchez-Guijo A, Hartmann MF. The art of measuring steroids: Principles and practice of current hormonal steroid analysis. J Steroid Biochem Mol Biol. 2018;179:88-103.

Xu Y, Lin J, Wang S, Xiong J, Zhu Q. Combined estrogen replacement therapy on metabolic control in postmenopausal women with diabetes mellitus. Kaohsiung J Med Sci. 2014;30(7):350-361.

Yamatani H, Takahashi K, Yoshida T, Takata K, Kurachi H. Association of estrogen with glucocorticoid levels in visceral fat in postmenopausal women. Menopause. 2013;20(4):437-442.

Yanes Cardozo LL, Romero DG, Reckelhoff JF. Cardiometabolic Features of Polycystic Ovary Syndrome: Role of Androgens. Physiology (Bethesda). 2017;32(5):357-366.

Zhang Y, Nadeau M, Faucher F, Lescelleur O, Biron S, Daris M, Rhéaume C, Luu-The V, Tchernof A. Progesterone metabolism in adipose cells. Mol Cell Endocrinol. 2009;298(1-2):76-83.

Zhao Y, Mendelson CR, Simpson ER. Characterization of the sequences of the human CYP19 (aromatase) gene that mediate regulation by glucocorticoids in adipose stromal cells and fetal hepatocytes. Mol Endocrinol. 1995;9(3):340-349.

Zhu L, Jiang X, Sun Y, Shu W. Effect of hormone therapy on the risk of bone fractures: a systematic review and meta-analysis of randomized controlled trials. Menopause. 2016;23(4):461-470.

Zwick RK, Guerrero-Juarez CF, Horsley V, Plikus MV. Anatomical, Physiological, and Functional Diversity of Adipose Tissue. Cell Metab. 2018;27(1):68-83.