Bodies in transition

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General introduction and outline of this thesis

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

The overall aim of this thesis is to investigate the effects of hormonal therapy on body shape, body composition, body fat distribution, and on cardiovascular risk in transgender adults and adolescents. This general introduction describes the definition of gender dysphoria, the current knowledge on hormonal treatment and its effects on body composition and cardiovascular risk, and how the chapters in this thesis may contribute to our knowledge on these topics.

Terminology in the area of transgender health care is rapidly evolving and multiple terms are used in this thesis that need explanation (Box 1)[1].

Box 1. Terminology in transgender health care	
Sex (assigned at birth):	The biological aspect of being a man or a women. The sex of a person is assigned at birth (male or female), usually based on the appearance of the external genitalia.
Gender identity:	A person's intrinsic sense of being male, female, or an alternative gender.
Transwomen:	Persons who were assigned male at birth, but who have a female gender identity (before referred to as male-to-female transgender persons).
Transmen:	Persons who were assigned female at birth, but who have a male gender identity (before referred to as female-to-male transgender persons).
Ciswomen:	At birth assigned females with a female gender identity.
Cismen:	At birth assigned men with a male gender identity.
Gender affirming hormones (GAH):	Sex hormones (estradiol or testosterone) used to change secondary sex characteristics to affirm a person's gender identity. Sometimes we also use "cross-sex hormones".
Gender affirming surgery:	Surgery to change primary and/or secondary sex characteristics to affirm a person's gender identity.

Gender dysphoria

Definition and history

Transgender people (or trans people) may experience gender dysphoria, which refers to the discomfort or distress that is caused by a discrepancy between a person's gender identity and that person's sex assigned at birth[1].

Expressions of gender are described throughout history[2]. Hippocrates (460-370 AC) wrote about the Scythen, Indo-European nomads who lived in Central Asia. Rich Scythen men who did not get an erection anymore due to the horseback riding were "turned" into women. In the middle ages, Gregorius van Tours (538-594) wrote a story about a man who lived in the monastery as a nun and dressed and acted like a woman. François Timoléon de Choisy (1644-1724) was a French priest and writer and lived for long periods in his life as a woman. Much is known about his life since he wrote about his adventures as a young woman in "Histoire de la Comptesse de Barres". The first description of transsexuality in medical literature is from Jean-Etienne Esquirol (1772-1840) who was the founder of a psychiatric hospital in France and wrote about a transgender person in his book "Des maladies metals considérées sous le rapport medical, hygiénique, et medico-légal.". In the beginning of the 20th century, Magnus Hirschfeld (1868-1935) described that people can have both male and female characteristics and thereby started the development of tolerance towards homosexuality and transgenderism. For years it has been discussed whether the incongruity between body and mind had to be treated with psychotherapy or medical procedures on the body. Under the direction of Harry Benjamin (1885-1986), the latter approach got the upper hand, which resulted in the first medical guidelines of treatment (Standards of Care) around 1980. In the Netherlands, the first medical clinic for transgender health care was started in the 1970s in the VU Hospital by Louis Gooren[2].

Prevalence

The number of trans people increased markedly in recent years. In 1996, a prevalence of 1:11.900 in men and in 1:30.400 in women was reported[3]. A recent study on gender dysphoria in the Netherlands reported a prevalence of 1:3.800 in men and 1:5.200 in women[4]. These data however, represent a group of trans people that actively sought treatment in our clinic, and not all trans people request medical treatment. Therefore, these numbers may still be an underestimation of the true prevalence.

Etiology

The etiology of gender dysphoria is not clear. Probably, a complex interaction between biological, psychological and social factors contributes to the development of gender dysphoria[2,5,6]. Studies in the general population showed that prenatal testosterone levels in the mother predicted the degree of later male play behavior in both boys and girls[6]. Possibly, these levels also affect the development of gender identity and gender dysphoria[2]. Brain studies showed that in transwomen certain structures were similar to those in women, such as the size and volume of the bed nucleus stria terminalis and the interstitial nuclei 3 and 4 of the hypothalamus[7-9]. Also, functional magnetic resonance imaging (fMRI) showed that the exposure to certain odors activate the hypothalamus in transwomen in a female manner, possible through a different development of the brain[10]. A systematic literature review on case studies in gender dysphoric persons found that 39% of the monozygotic twins were concordant on gender dysphoria, while none of the dizygotic twins were concordant, suggesting a role for genetics in the development of gender dysphoria[11]. Besides these biological factors, recent developmental psychology models assume that psychological factors play a role too. Studies showed that anxiety and depression are more prevalent in gender dysphoric children and that psychological problems are more prevalent in their parents. Presumably, there is a complex interplay of biological and psychological factors underpinning the etiology of gender dysphoria[2].

Diagnosis

From 2000 to 2013, the Diagnostic and Statistical Manual of Mental Disorder 4th edition (DSM-IV) was in use to diagnose “gender identity disorder”[12]. In 2013, an updated version called the DSM-V became available in which the naming changed to “gender dysphoria”[13] (Box 2).

Box 2. Current diagnostic criteria for gender dysphoria (DSM-V)

Current diagnostic criteria for both adolescents and adults are (DSM-V):

A) A marked incongruence between one's experienced gender and assigned gender of at least 6 months' duration, as manifested by at least two of the following:

   1) A marked incongruence between one's experienced/expressed gender and primary and/or secondary sex characteristics (or in young adolescents, the anticipated secondary sex characteristics).
   2) A strong desire to be rid of one's primary and/or secondary sex characteristics because of a marked incongruence with one's experienced gender (or in young adolescents, a desire to prevent the development of the anticipated secondary sex characteristics).
   3) A strong desire for the primary and/or secondary sex characteristics of the other gender.
   4) A strong desire to be of the other gender (or some alternative gender different from one's assigned gender).
   5) A strong desire to be treated as the other gender (or some alternative gender different from one's assigned gender).
   6) A strong conviction that one has the typical feelings and reactions of the other gender (or some alternative gender different from one's assigned gender).

B) The condition is associated with clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Treatment

People with gender dysphoria can be treated with hormonal therapy or/and gender affirming surgery.

First, a psychologist will examine in detail what a person expects from treatment by care providers. If a person wants to start gender affirming treatment, the psychologist will check to which extent someone meets the criteria of the DSM-V to be able to diagnose gender dysphoria. Questionnaires such as the Utrecht Gender Dysphoria Scale or the Gender Dysphoria Scale[14] can be used for this purpose. Further, psychological functioning, emotional and social stability, and the social support system are evaluated. During this psychological diagnostic phase, the person and psychologist discuss whether the wish to belong to the desired gender is consistent and whether a person is able to live in the desired gender role.

After gender dysphoria is diagnosed by the psychologist, endocrinological treatment can be started. This treatment for both adolescents and adults is described in detail in the next paragraphs. In the Netherlands, the minimum age for gender affirming surgery is 18 years. Options for surgery include mamma-augmentation, vaginoplasty, facial feminization surgery, and adam's apple reduction in transwomen. In transmen, mastectomy, uterus extirpation, ovariectomy, colpectomy, metaoidioplasty, and phalloplasty can be performed. Further, speech therapy and hair removal therapy are available. Not all persons with gender dysphoria want to undergo endocrinological or surgical treatment, the treatment needs may vary per person.

Endocrine treatment in trans people

In order to be able to understand the treatment with puberty suppressants and gender affirming hormones in trans people, we will first describe the physiological system of production of sex steroids in men and women.

The two main classes of sex steroid hormones are androgens and estrogens, of which the most important derivatives are testosterone and estradiol, respectively. Testosterone and estradiol are produced in both men and women, but in different proportions. In men, the predominant hormone is testosterone which is produced mainly by the testes and to a lesser extent by the adrenals. The aromatase enzyme, also called estrogen synthase, is responsible for the conversion of testosterone into estradiol and is present in many tissues, mainly in adipose tissue, but also in the gonads, brain, blood vessels, skin and bone in both men and women[15]. In women, the predominant hormone estradiol is mainly produced in the ovaries and a small amount of testosterone is produced in the ovaries and adrenals.

Estradiol and testosterone are the final products of the hypothalamic-pituitary-gonadal axis. In the hypothalamic-pituitary-gonadal axis (Figure 1), gonadotropin-releasing hormone (GnRH) is secreted pulsatile from the hypothalamus to activate the anterior pituitary. This gland produces luteinizing hormone (LH) and follicle-stimulating hormone (FSH) which are released to act on the gonads. In the gonads, both gametogenesis (spermatogenesis and oogenesis) as well as steroidogenesis are induced. Major sex hormones estradiol and testosterone are produced in the gonads and secreted into the blood and give feedback at the level of the hypothalamus and pituitary to control normal reproductive function[16].

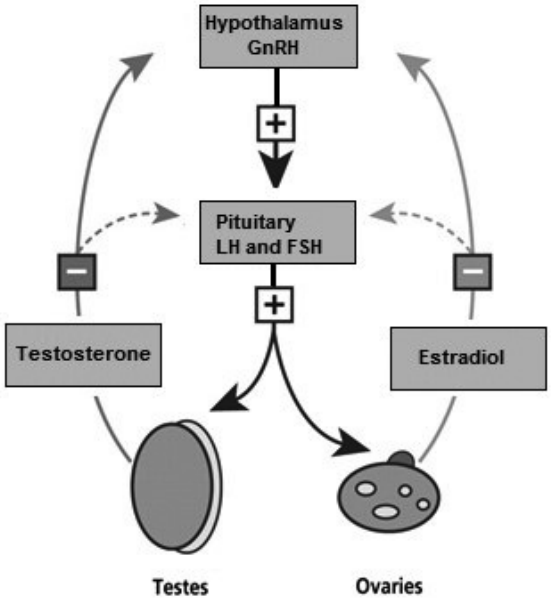

Figure 1. Hypothalamic-pituitary-gonadal axis.

Sex steroids exert their effects on target tissues through interaction with androgen receptors and estrogen receptors[16]. Target tissues for sex steroids hormones are the breast, the female reproductive tract (uterus and ovary), the male reproductive tract (testes and epididymis), bone, vascular system, central nervous system, gastrointestinal tract, immune system, skin, kidney, and lung.

Hormonal treatment in adolescents

With the onset of puberty and the start of development of the sex characteristics of the at birth assigned sex, feelings of gender dysphoria can increase in transgender adolescents. Because of this, the psychologist can decide to start treatment with puberty suppressants. This treatment prevents the development of the secondary sex characteristics of the at birth assigned sex and provides a possibility to extent the diagnostic phase until a final decision is made about the start of gender affirming hormones. Thus for adolescents, two stages of endocrine intervention can be distinguished. The first phase is puberty suppression, which is a fully reversible intervention with gonadotropin-releasing hormone agonists (GnRHa). The second phase is a partially reversible intervention with gender affirming hormonal treatment[17].

Treatment with GnRHa initially stimulates the secretion of LH and FSH, but after approximately 10 days a downregulation of the GnRH receptor is achieved with a subsequent drop in gonadotropin secretion[18]. Adolescents are eligible for this treatment with GnRHa when they are older than 12 years and when they reached Tanner stage 2 or 3, in combination with pubertal levels of sex hormones. Further, they have to be suffering from life-long gender dysphoria that had increased around puberty, they have to be psychologically stable, and they have to be supported by their social network.

After the age of 16, adolescents are eligible for gender affirming hormone treatment to induce the pubertal development of the desired sex. Adolescent transwomen are initially treated with 17β-estradiol in a dosage of 5 mcg/kg per day, increasing the dose every six months with 5 mcg/kg per day, until a dose of 2mg per day is reached. Adolescent transmen are treated with testosterone esters, starting with 25 mg/m^2 per 2 weeks intramuscular (im) which is increased with 25 mg/m^2 every six months, until a dose of 250 mg per 3-4 weeks is reached.

Hormonal treatment in adults

Transwomen are treated with anti-androgens and estradiol to induce feminization. The most commonly used anti-androgen is cyproterone acetate, a progestagen which has both an anti-gonadotropic effect through activation of the progestogen receptor on the pituitary gland and an androgen-receptor blocking effect. Other medicines with anti-androgenic action in adults are gonadotrophin-releasing hormone agonists (GnRHa), and spironolactone, a diuretic which is additionally a potent and direct antagonist of the AR and has a small antigonadotropic effect[19]. 17β-estradiol is the most potent estrogen and is given orally or transdermally. Transdermal estradiol treatment is advised in transwomen with a history of thromboembolic events, or risk factors for thromboembolic events such as a higher age[1,20].

Transmen are treated with androgens to induce masculinization. To achieve testosterone values in the normal male range, either intramuscular or transdermal preparations are used. A frequently used testosterone preparation is a mix of testosterone esters with testosterone propionate 30 mg, testosterone fenylpropionate 60 mg, testosterone isocoproaat 60mg, and testosterone decanoate 100 mg. Also, testosterone undecanoate alone or testosterone gel are prescribed frequently.

Physical effects of hormonal treatment

GnRHa therapy in adolescents suppresses further puberty development and can even reduce already developed sex characteristics such as breast tissue in girls and testicular tissue in boys.

Feminizing effects in transwomen include breast growth, redistribution of body fat, a decrease in muscle mass and strength, a decrease in libido and erections, a decrease in testicular volume and sperm production, a decrease in body hair growth, and softening of the skin[20]. Side effects that can occur are fatigue, mood swings, or depressive feelings. Masculinizing effects in transmen include voice deepening, facial and body hair growth, hair loss on the scalp, an increase in muscle mass and strength, a decrease in body fat and redistribution of body fat, clitoral enlargement, and cessation of menses[20]. Side effects of testosterone treatment contain the occurrence of acne, fatty skin, increase of sweating, irritability, or aggression.

The effect of sex hormones on body composition and body shape

Women have, on average, more body fat than men[21]. This difference is already seen at birth, when girls, proportionally, have more body fat than boys. This difference persists in childhood and becomes even larger in puberty when girls start to develop more body fat and boys mainly increase in lean body mass[21,22]. In young adulthood, Dutch women and men have a body fat percentage of 35% and 11%, respectively[23]. Further in life, aging is associated with an increase in body fat in both men and women. In men, this increase is about 1% increase in body fat per year. In women, an increase in body fat of 9% after menopause is seen[24-26]. Besides sex differences in the amount of body fat, sexual dimorphism is also seen in body fat distribution and this is already present before puberty. Prepubertally, girls have already less waist fat (android fat) and more hip fat (gynoid fat) than boys[27]. During puberty, the magnitude of this difference is amplified[27]. Girls mainly store body fat in the hips and thighs resulting in a peripheral body fat distribution, while men depose body fat in the abdominal region resulting in a central body fat distribution[21], including more intra-abdominal fat, also referred to as visceral fat[28]. After menopause in women, a shift of body fat from the hip region to the abdominal region is seen[24-26].

It is well-established that sex steroids play a major role in the regulation of total body fat and body fat distribution, illustrated by these large changes in body fat distribution during

puberty, menopause[25,29], and in trans people treated with sex hormones, as shown in previous studies.

In adolescent trans people, only two studies are performed on the effects of GnRHa and sex hormone treatment on body composition and body shape. One pilot study in transwomen (n=10)[17] showed in the first year of GnRHa treatment an increase in total body fat and a decrease in lean body mass. The second study examined transwomen treated for one to three years (n=28) and showed a decrease in standardized body mass index (BMI), no changes in body fat percentage or lean body mass, and a decrease in waist-hip-ratio[30].

In adult trans people treated with gender affirming hormones, several studies have been performed on body composition, specifically total body fat and total lean body mass[31-36], and on body shape, determined with waist-hip-ratio[31-33,37]. In general, in transwomen an increase in total body fat was seen with a decrease in total lean body mass. In transman generally, a decrease in total body fat was observed with an increase in total lean body mass. Waist-hip ratio was examined in three previous studies in transwomen which found divergent results from a small increase in waist-hip ratio[31] to a decrease in waist-hip ratio[33]. In transmen, no difference in waist-hip ratio[32,34] or an increase in waist-hip ratio were found[33,37].

However, most of these studies on both body composition and body shape included only a small number of participants ranging from 15 to 29[31,32,34,37,38]. Further, several of these studies used an outdated treatment protocol, prescribing ethinyl estradiol to transwomen[31,37]. Therefore, larger studies are needed to examine changes in total body fat, total lean body mass, and waist-hip ratio under current treatment protocols.

In addition, it is unknown whether hormonal treatment in adults affects body composition equally in different body regions. Since men and women have a different body fat distribution, the preferable site of fat deposition might change during hormonal treatment, resulting in a different body fat distribution and body shape than before treatment. However, the percentage change in body fat and lean body mass in different body regions has never been studied before.

Also, little research has been performed on the effects and timing of hormonal treatment in adolescents[39]. An important question is whether a better result is achieved when treatment is started at a younger age. In the current protocol, before hormonal treatment is initiated,

puberty must have started[1]. However, trans people can experience a progressive puberty as a time bomb which can cause complications in the diagnostic trajectory with the psychologist. For clarity for both care provider and patient, it is important to know whether starting treatment at an earlier pubertal stage affects physical appearance in adulthood.

Further, because different treatment modalities are available, in clinical care, many trans people ask their care providers whether these treatment modalities exert different effects on physical changes, for example on body shape. One study in transmen studied effects of three testosterone modalities (testoviron depot, testosterone gel, and testosterone undecanoate)[34] after one year of treatment and showed an increase in waist-hip ratio in the testoviron group versus a decrease in waist-hip ratio in the testosterone undecanoate group. Nevertheless, only 15 transmen were in each treatment group. In transwomen, Wierckx et al.[33] described changes in waist and hip circumferences stratified by oral or transdermal estradiol use. Both groups showed a decrease in waist-hip ratio, although no comparison was made between them. Different effects of types of estradiol treatment (oral or transdermal) and types of testosterone treatment (transdermal or parenteral) are suggested in postmenopausal women and older men[40-42] and therefore one might expect different changes in the trans population.

Another question frequently asked in clinical care is whether the level of testosterone or the level of estradiol influences physical changes, for example in body composition. In ciswomen, endogenous testosterone is positively associated with lean body mass[43], so this might be similar in transmen receiving testosterone treatment. In cismen, estradiol levels are associated with body fat[44], so possibly this is the same in transwomen receiving estradiol treatment. However, this has never been studied before in trans people.

Sex steroids, body composition, and cardiovascular disease

Cardiovascular disease is a main cause of premature death and disability in the general population, resulting in 17.3 million deaths per year worldwide[45,46]. The traditional risk factors such as overweight and obesity are still the most important risk factors for cardiovascular disease in both men and women[45]. Obesity is associated with cardiovascular mortality and morbidity such as coronary heart disease and the development of type 2 diabetes[28,47]. Further, studies have shown that obesity and total body fat are positively associated with cardiovascular risk factors, such as hypertension and dyslipidemia. Even within normal

BMI range, weight gain during adult life or even during childhood and adolescence seems to have a great effect on cardiovascular risk[48]. However, it is believed that it is not obesity per se, but rather specific locations and dysfunction of body fat, through the associations with metabolic alterations, that promote future cardiovascular disease[49]. Even more than general obesity or total body fat, abdominal obesity is associated with cardiovascular risk factors, morbidity, and mortality[50]. Abdominal fat contains subcutaneous fat and visceral fat. Both are associated with cardiovascular risk factors[51], but visceral fat is supposed to be most harmful.

Visceral fat is shown to be a strong, independent predictor of all-cause mortality[52]. An excess of visceral fat is associated with several cardiovascular complications, such as impaired glucose and insulin metabolism, insulin resistance, atherogenic dyslipidemia, hypertension, and metabolic syndrome[28,53]. An hypothesized mechanism for the harmful role of visceral fat is that the hyperlipolytic visceral fat is drained by the portal vein to the liver[54]. This results in an overexposure of nonesterified fatty acids to the liver and to impairment of liver function, such as an overproduction of triglycerides, increased hepatic glucose production, and reduced hepatic degradation of insulin contributing to systemic hyperinsulinemia. Also, visceral obesity is associated with a low-grade inflammatory state. Both inflammatory cells (macrophages) and adipocytes in visceral fat secrete hormones and pro-inflammatory cytokines, such as interleukin-6 and TNF-α, that can impact tissues locally and systematically[28,48]. This chronic state of inflammation alters glucose and lipid metabolism and is thought to contribute to the higher cardiovascular risk of individuals with visceral obesity[28].

In general, men have more visceral fat than women[28,51], possibly due to a different hormonal status. Studies in men and women using hormone replacement therapy show that visceral fat is affected by sex steroids[55,56]. Older men using testosterone experienced a small significant decrease in visceral fat, while older men in the placebo group increased in visceral fat after one year[55]. Postmenopausal women using estradiol treatment (plus an exercise intervention) decreased more in visceral fat than postmenopausal women only receiving the exercise intervention[56]. Further, an observational study showed associations between androgens levels and visceral fat, although they differed by sex[57]. Higher testosterone levels were associated with more visceral fat in women, and with less visceral fat in men[57]. Thus, androgens may have different effects on visceral fat in men and women.

Besides visceral fat, sex steroids also affect other cardiovascular risk factors. Estrogens have a regulating effect on metabolic factors such as lipids, inflammatory markers, coagulation, and vasodilation[58]. The menopause, when a decline in estradiol levels occurs, is associated with a deterioration of cardiovascular risk factors, for example an increase in systolic blood pressure and increases in total cholesterol and LDL cholesterol levels[58]. These changes are thought to contribute to the higher prevalence of cardiovascular disease in postmenopausal women in comparison with premenopausal women. Similar as in visceral fat, androgens appear to have an opposite effect on other cardiovascular risk factors in men and women[59]. A female androgen excess and male androgen deficiency are both associated with an adverse metabolic phenotype, including insulin resistance, type 2 diabetes mellitus, and non-alcoholic fatty liver disease[59]. A female androgen excess is seen in women with for example polycystic ovary syndrome (PCOS)[60-62] or non-classic congenital adrenal hyperplasia[63]. PCOS women with an androgen excess have a higher risk for insulin resistance[60], and higher levels of LDL cholesterol[64] and triglycerides[64] compared with PCOS women without an androgen excess. This suggests an adverse effect of high androgen levels in women on their cardiovascular profile. On the other hand, in men, low testosterone levels are associated with high total cholesterol, LDL cholesterol, and triglyceride levels, and low HDL cholesterol levels[59]. Further, increased arterial stiffness in older hypogonadal men has been reported compared to age- and weight-matched controls, which was partly reversible by testosterone supplementation[59]. Thus, although the effects of sex steroids might differ per sex, it is evident that sex steroids affect visceral fat and other cardiovascular risk factors.

In adolescent trans people using GnRHa treatment with subsequent gender affirming hormones, only two studies examined changes in cardiovascular risk factors. Delemarre et al.[17] described no changes in lipid metabolism in both transwomen (n=10) and transmen (n=11). The other study examining 16 transwomen after three years of GnRHa and estradiol treatment, found an increase in BMI of 0.7 kg/m^2, and no changes in systolic blood pressure[30]. In adolescent transmen treated with only testosterone, two studies found a trend towards an increase in total cholesterol, LDL cholesterol, triglycerides[65,66], and HOMA-IR[65], with a decrease in HDL cholesterol[66]. In two studies in adolescent transwomen using other types of anti-androgens and estradiol, no changes in total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were found[66,67]. Regarding standardized BMI scores (BMI-SDS), some studies showed a trend towards an increase in BMI-SDS[68], while others found no change[69], or a decrease in BMI-SDS[30]. In transmen, both BMI-SDS as

absolute BMI scores increased during GnRHa treatment in combination with testosterone or solely testosterone treatment.

In adult trans people, one study group[70] investigated the change in visceral fat in 18 transwomen and 17 transmen using magnetic resonance imaging (MRI). After one year of treatment, visceral fat increased in both transwomen and transmen[70]. Ten transmen were re-examined after 3 years and then showed an even larger increase in visceral fat[71].

In the previous studies in cardiovascular risk factors in adolescents, only small groups of transwomen (n=10 to n=49) and transmen (n=11 to n=72) were examined, mostly with only GnRHa or only sex hormone treatment, and over a short period of time (max 1-3 years)[17,30,65-67,69]. Long term studies in a large number of adolescent trans people are needed to examine whether the current treatment protocol affects cardiovascular risk profile. Further, because previously a higher BMI in young adult trans people was found compared to their age-matched peers[68], the prevalence of obesity or dyslipidemia in young adulthood should be assessed. This will provide clinicians with more knowledge on the cardiovascular risk in transgender adolescents.

The previous studies on visceral fat in adult trans people were performed in a small group of trans people, with an outdated treatment protocol in transwomen (ethinyl estradiol). Also, these studies were performed in a group of lean transwomen and transmen, while obesity seems more prevalent in trans people compared with their peers[72]. It is unknown whether the same changes in visceral fat would be observed in overweight or obese trans people. Moreover, the relations between change in visceral fat and change in blood pressure, lipid levels, or insulin resistance have never been studied. Such as study will provide us with a better understanding of the possible mechanisms of how hormonal treatment affects cardiovascular risk factors.

Aims and outline of this thesis

Aims

The overall aim of this thesis was to investigate the changes in body shape, body composition, body fat distribution, and cardiovascular risk profile in trans people. Changes in total and regional body composition affect both physical appearance as metabolism and cardiovascular risk. Insight in these aspects is important in transgender health care in order to provide information on the extent to which physical changes will occur and on

what to expect of the results after treatment. In addition, it is important for care providers to know which metabolic changes take place, so appropriate cardiovascular risk management can be provided.

Outline

In **chapter 2**, we performed a meta-analysis to summarize the effects of hormonal treatment on total body weight, total body fat, and total lean body mass from previously performed small studies that reported on changes in total body weight and body composition in trans people.

Chapter 3 and chapter 4 focus on physical changes during hormone treatment in the context of a feminine or masculine appearance such as body shape and body fat distribution. In **chapter 3**, we investigated the changes in body shape, total body fat, and total lean body mass in transgender adolescents treated with GnRHa and gender affirming hormones. Further, we examined in this chapter whether they obtained the body shape and body composition of the affirmed gender in young adulthood and whether this was influenced by the pubertal stage at start of treatment. Further, in a large prospective multi-center study, the ENIGI study, we evaluated one-year changes in body shape and regional body composition in adult trans people. In **chapter 4*,*** we investigated the changes in waist and hip circumferences, and waist-hip ratio as measures of body shape and changes in regional body fat and lean body mass in the arm, leg, trunk, android region, and gynoid region in transgender adults after one year of hormonal treatment. Also, we evaluated whether factors such as medication type, serum sex hormone levels, BMI at start, and age affect changes in aforementioned measures.

Chapter 5 and chapter 6 focus on changes during hormone treatment in the context of cardiovascular risk. In **chapter 5**, we examined changes in cardiovascular risk factors such as BMI, lipids, insulin resistance, and blood pressure in transgender adolescents treated with GnRHa and gender affirming hormones from adolescence until young adulthood. Changes in visceral fat in adult trans people after one year of hormonal treatment and the relations with changes in cardiovascular risk factors such as glucose, insulin resistance, lipids, and blood pressure were investigated in **chapter 6**.

Chapter 7 provides a summary of the main findings of this thesis, discusses its strengths and limitations, and provides implications and recommendations for future research.

Study populations and data used in this thesis

Cohort of transgender adolescents

Chapter 3 and chapter 5 use data from the cohort of transgender adolescents that started treatment with GnRHa and subsequently gender affirming hormones in the VU Medical Center. In this retrospective study, the medical files of all transgender adolescents who attended the VU Medical Center gender clinic from 1975 to 2015 were checked. Hospital registries provided clinical data such as medical diagnoses, medication prescriptions, surgical interventions, laboratory test results, and visit dates. All medical files were checked manually to record aforementioned data when missing in the hospital registries. Also other clinical data were noted such as medical history, medication use, prior gender affirming hormone use, and type of gender affirming hormone treatment. Of these people additional anthropometric data were retrieved from the medical records such as body weight, body height, waist circumference, and hip circumferences if measured. The local ethics committee waived the necessity for informed consent.

European Network of the Investigation of Gender Incongruence (ENIGI)

Chapter 4 and chapter 6 use data from the European Network for the Investigation of Gender Incongruence (ENIGI) project. The ENIGI project is an unique scientific collaboration between mental health professionals and endocrine specialists working in multiple European gender clinics[73]. It is an ongoing multicenter prospective cohort study, which started in 2010 and inclusion is still ongoing. The ENIGI project is performed at the Ghent University Hospital in Ghent (Belgium), the Rikshospitalet in Oslo (Norway), the University Hospital in Florence (Italy), and the VU University Medical Center in Amsterdam (The Netherlands). The same mental health and endocrine treatment protocol was used in all centers. Data collection included clinical measurements (every visit), blood samples (at start, and after 3 and 12 months), questionnaires (at start, and after 3, 6, and 12 months), and dual-energy X-ray absorptiometry (at start and after 12 months). The endocrine protocol has been published before[74]. The Ethics Committee of Ghent University Hospital, Belgium approved the overall study protocol. The other participating centers also obtained approval of their local ethical committees. Informed consent was obtained according to the institutional guidelines.

Assessment of body composition by dual-energy X-ray absorptiometry (DXA)

For examining body composition, simple measurements such as skinfold thickness, waist circumference, or calculating BMI are useful as easy baseline and follow-up measurements. Though, they have poor accuracy and don't allow distinction between (a change in) body fat and lean body mass. Magnetic resonance imaging (MRI) and computed tomography (CT) have shown to provide very accurate assessments of body fat and lean body mass[75,76]. However, these methods are limited available, are expensive, time-consuming and/or require radiation[77]. Dual-energy X-ray absorptiometry (DXA), on the other hand, is easily available, relatively inexpensive, requires minimal radiation exposure, and body composition parameters obtained by DXA are strongly related to those obtained by CT or MRI in adults and adolescents of normal weight[78-80]. Also, in a validation study in 272 South African women with a mean age of 29 years, DXA was shown a valid method to estimate visceral fat compared to CT (r=0.93)[81].

Whole body DXA is a two-dimensional method to examine body composition. The DXA scanner produces two beams of high and low energies that are attenuated in the body[82]. Body fat has a different x-ray attenuation than lean body mass, since body fat has a higher hydrogen content. In every pixel, the attenuation is measured, and every high and low energy attenuation pair is related to a unique combination of body fat and lean body mass. After measuring body fat and lean body in every pixel, the amount of total body fat or total lean body mass is calculated.

For a quantification of visceral fat, we aimed to measure only the adipose tissue within the abdominal cavity. When body fat is measured as described above, the body fat in this two-dimensional image is a sum of the visceral fat inside the abdominal cavity and the subcutaneous fat on the anterior and posterior side of the body. Therefore, additional software was used[81,83]. A 5 centimeter wide region was placed across the abdomen just above the iliac crest at a level that approximately coincides with the fourth lumbar vertebrae. In this region, the abdominal cavity is indicated by a lighter grey color because the musculature in the abdominal wall appears lighter than the (darker) subcutaneous fat tissue outside the abdominal cavity. To calculate solely the amount of visceral fat, the amount of anterior and posterior subcutaneous fat is estimated by measuring the subcutaneous fat on the sides of the body. Then, a total amount of subcutaneous fat is estimated and this is subtracted from the total amount of abdominal fat which gives the amount of visceral fat[81,83].

REFERENCES

1. Coleman E, Bockting W, Botzer M, Cogen-Kettenis P, DeCuypere G, Feldman J, et al. Standards of Care for the Health of Transsexual, Transgender, and Gender-Noncomforming People, Version 7. International Journal of Transgenderism. 2012;13:165-232.
2. T'Sjoen G, Van Trotsenburg MAA, Gijs L. Transgenderzorg: Uitgeverij Acco; 2013.
3. van Kesteren PJ, Gooren LJG, Megens JAJ. An Epidemiological and Demographic Study of Transsexuals in the Netherlands. Archives of Sexual Behavior. 1996;25(6):589-600.
4. Wiepjes CM, Nota NM, de Blok CJM, Klaver M, De Vries ALC, Wensing-Kruger SA, et al. The Amsterdam Cohort of Gender Dysphoria Study (1972-2015): Trends in Prevalence, Treatment, and Regrets. The Journal of Sexual Medicine. 2018;15(4):582-90.
5. Meyer-Bahlburg HFL. From mental disorder to iatrogenic hypogonadism: Dilemmas in conceptualizing gender identity variants as psychiatric conditions. Archives of Sexual Behavior. 2010;39:461-76.
6. Hines M. Gender development and the human brain. Annual Review of Neuroscience. 2011;34:69-88.
7. Garcia-Falgueras A, Swaab DF. A sex difference om the hypothalamic uncinate nucleus: reltionship to gender identity. Brain. 2008;131:3132-46.
8. Kruijver FPM, Zhou J, Pool CW, Hofman MA, Gooren LJG, Swaab DF. Male-to-female transsexuals have female neuron numbers in a limbic nucleus The Journal of Clinical Endocrinology & Metabolism. 2000;85(5):2034-41.
9. Zhou J, Hofman MA, Gooren LJG, Swaab DF. A sex difference in the human brain and its relation to transsexuality. Nature. 1995;378:68-70.
10. Burke SM, Cohen-Kettenis PT, Veltman DJ, Klink DT, Bakker J. Hypothalamic response to the chemo-signal androstadienone in gender dysphoric children and adolescents. Frontiers in endocrinology. 2014;5:1-10.
11. Heylens G, De Cuypere G, Zucker KJ, Schelfaut C, Elaut E, Vanden Bossche H, et al. Gender Identity Disorder in Twins: A Review of the Cae Report Literature. International Society for Sexual Medicine. 2011;9:751-7.
12. American Psychiatric Association Diagnostic and Statistical manual of Mental Disorders. 4th edition, text revision (DSM-IV-TR). Association. WDAP, editor2000.
13. Diagnostic and Statistical Manual of Mental Disorders. 5th Edition. Association AAP, editor2015.
14. Schneider C, Cerwenka SC, Nieder TO, Broken P, Cohen-Kettenis PT, DeCuypere G, et al. Measuring Gender Dysphoria: A Multicenter Examination and Comparison of the Utrecht Gender Dysphoria Scale and the Gender Identity/Gender Dysphoria Questionnaire for Adolescents and Adults. Archives of Sexual Behavior. 2016;45:551-8.
15. Czajka-Oraniec I, Simpson E. Aromatase research and its clinical significance. Polish Journal of Endocrinology. 2010;61(1):126-34.
16. Wierman ME. Sex steroid effects at target tissues: mechanisms of action. Advances in Physiology Education. 2007;31:26-33.

17. Delemarre-van de Waal HA, Cohen-Kettenis PT. Clinical management of gender identity disorder in adolescents: a protocol on psychological and paediatric endocrinology aspects. European Journal of Endocrinology. 2006;155:131-7.

18. Magon N. Gonadotropin releasing hormone agonists: Expanding vistas. Indian Journal of Endocrinology and Metabolism. 2011;15(4):261-7.

19. Loriaux DL, Menard R, Taylor AE, Pita JC, Santen R. Spironolactone and endocrine dysfuncrion. Annals of Internal Medicine. 1976;85:630-6.

20. Hembree WC, Cohen-Kettenis P, Delemarre-van de Waal HA, Gooren LJG, Meyer III WJ, Spack NP, et al. Endocrine Treatment of Transsexual Persons: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2009;94(9):3132-54.

21. Wells JCK. Sexual dimorphism of body composition. Best Practice & Research Clinical Endocrinology & Metabolism. 2007;21(3):415-30.

22. Taylor RW, Gold E, Manning P, Goulding A. Gender differences in body fat content are present well before puberty. International Journal of Obesity. 1997;21:1082-4.

23. van der Sluis IM, de Ridder MAJ, Boot AM, Krenning EP, de Muinck Keizer-Schrama SMPF. Reference data for bone density and body composition measured with dual energy x ray absorptiometry in white children and young adults. Arch Dis Child. 2002;87:341-7.

24. Svendsen OL, Hassager C, Christiansen C. Age- and Menopause- associated variations in body composition and fat distribution in healthy women as measured by Dual-Energy X-Ray Absorptiometry. Metabolism. 1995;44(3):369-73.

25. Tchernof A, Poehlmand ET. Effects of the menopause transition on body fatness and body fat distribution. Obesity research. 1998;6(3):246-54.

26. Zamboni M, Armellini F, Harris T, Turcato E, Micciolo R, Bergamo-Andreis IA, et al. Effects of age on body fat distribution and cardiovascular risk factors in women. The American Journal of Clinical Nutrition. 1997;66:111-5.

27. Taylor RW, Grant AM, Williams SM, Goulding A. Sex differences in regional body fat distribution from pre- to postpuberty. Obesity (Silver Spring). 2010;18(7):1410-6.

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

29. Sipilä S. Body composition and muscle performance during menopause and hormone replacement therapy. Journal of Endocrinological Investigation. 2003;26:893-901.

30. Hannema SE, Schagen SEE, Cohen-Kettenis PT, Delemarre-van de Waal HA. Efficacy and safety of pubertal induction using 17b-estradiol in transgirls. Journal of Clinical Endocrinology and Metabolism. 2017;102(7):2356-63.

31. Giltay EJ, Elbers JMH, Gooren LJG, Emeis JJ, Kooistra T, Asscheman H, et al. Visceral Fat Accumulation is an inportant determinant of PAI-1 levels in young, nonobese men and women. Arterioscler Thromb Vasc Biol. 1998;18:1716-22.

32. van Caenegem E, Wierckx K, Taes Y, Schreiner T, Vandewalle S, Toye K, et al. Body composition, bone turnover, and bone mass in trans men during testosterone treatment: 1-year follow-up data from a prospective case-controlled study (ENIGI). European Journal of Endocrinology. 2015;172(2):163-71.

33. Wierckx K, van Caenegem E, Schreiner T, Haraldsen IR, Fisher AD, Toye K, et al. Cross-Sex Hormone Therapy in Trans Persons is safe and Effective at Short-Time Follow-Up: Results from the European Network for the Investigation of Gender Incongruence. Journal of Sexual Medicine. 2014;11:1999-2011.

34. Pelusi C, Costantino A, Martelli V, Lambertini M, Bazzocchi A, Ponti F, et al. Effects of three different testosterone formulations in female-to-male transsexual persons. Journal of Sexual medicine. 2014;11:3002-11.

35. Mueller A, Haeberle L, Zollver H, Claassen T, Kronawitter D, Oppelt PG, et al. Effects of Intramuscular Testosterone Undecanoate on body composition and bone mineral density in female-to-male transsexuals. J Sex Med. 2010;7:3190-8.

36. Mueller A, Zollver H, Kronawitter D, Oppelt PG, Claassen T, Hoffmann I, et al. Body composition and bone mineral density in male-to-female transsexuals during cross-sex hormone therapy using gonadotrophin-releasing hormone agonist. Clin Endocrinol Diabetes. 2011;119:95-100.

37. Elbers JMH, Asscheman H, Seidell JC, Gooren LJG. Effects of sex steroids hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals. American Journal of Physiology. 1999;276:317-25.

38. Cupisti S, Giltay EJ, Gooren LJG, Kronawitter D, Oppelt PG, Beckmann MW, et al. The impact of testosterone administration to female-to-male transsexuals on insulin resistance and lipid parameters compared with women with polycystic ovary syndrome. Fertility and Sterility. 2010;94(7):2647-53.

39. Cohen-Kettenis PT, Klink DT. Adolescents with gender dysporia. Best Practice & Research Clinical Endocrinology & Metabolism. 2015;29:485-95.

40. Hänggi W, Lippuner K, Jaeger P, Brikhäuser MH, Horber FF. Differential impact of conventional oral or transdermal hormone replacement therapy or tibolone on body composition in postmenopausal women. Clinical Endocrinology. 1998;48:691-9.

41. O'Sullivan AJ, Crampton LJ, Freund J, Ho KKY. The route of estrogen replacement therapy confers divergent effects on substrate oxidation and body composition in postmenopausal women. Journal of Clinical Investigation. 1998;102(5):1035-40.

42. Saad F, Gooren LJ, Haider A, Yassin A. A Dose-Response Study of Testosterone on Sexual Dysfunction and Feautures of the Metabolic Syndrome Using Testosterone Gel and Parental Testosterone Undecanoate. Journal of Andrology. 2008;29(1):102-5.

43. Sowers MF, Beebe JL, McConnell D, Randolph J, Jannausch M. Testosterone concentrations in women aged 25-50 years: associations with lifestyle, body composition, and ovarian status. American Journal of Epidemiology. 2001;153(3):256-64.

44. Vermeulen AA. The aging male : the official journal of the International Society for the Study of the Aging Male2002.

45. Appelman YE, van Rijn BB, ten Haaf ME, Boersma E, Peters SAE. Sex differences in cardiovascular risk factors and disease prevention. Atherosclerosis. 2015;241:211-8.

46. World Health Organization G. Global Status Report on Noncommunicable Diseases. 2011.

47. Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obeisty as an independent risk factor for cardiovascular disease: A 26-year follow-up of participants in the Framingham Heart Study. Obesity and Cardiovascular disease. 1983;67(5):968-77.

48. van Gaal LF, Mertens IL, de Block CE. Mechanisms linking obesity with cardiovascular disease. Nature. 2006;444(14):875-80.

49. De Larochellière E, Cote J, Gilbert G, Bineau K, Ross M, Dion-Roy V, et al. Visceral/epicardio adiposity in nonobese and apparently healthy young adults: Association with the cardiometabolic profile. Atherosclerosis. 2014;234:23-9.

50. van Pelt RE, Evans EM, Schechtman KB, Ehsani AA, Kohrt WM. Contributions of total and regional fat mass to risk for cardiovascular disease in older women. American Journal of Physiology-Endocrinology and Metabolism. 2002;282:1023-8.

51. De Mutsert R, Gast K, Widya R, de Koning E, Jazet I, Lamb H, et al. Associations of abdominal subcutaneous and visceral fat with insulin resistance and secretion differ between men and women: The Netherlands Epidemiology of Obesity Study. Metabolic syndrome and related disorders. 2018;16(1):54-63.

52. Kuk JL, Katzmarzyk PT, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral Fat Is an Independent Predictor of All-cause Mortality in Men. Obesity. 2006;14(2):336-41.

53. Shuster A, Patlas M, Pinthus JH, Mourtzakis M. The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis. The British Journal of Radiology. 2012;85:1-10.

54. Björntorp P. "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. Atherosclerosis. 1990;10:493-6.

55. Allan CA, Strauss BJG, Burger HG, Forbes EA, McLachlan RI. Testosterone Therapy Prevents Gain in Visceral Adipose Tissue and Loss of Skeletal Muscle in Nonobese Aging Men. Journal of Clinical Endocrinology and Metabolism. 2008;93(1):139-46.

56. Green JS, Stanforth PR, Rankinen T, Lean AS, Raq DC, Skinner JS, et al. The Effects of Exercise Training on Abdominal Visceral Fat, Body Composition, and Indicators of the Metabolic Syndrome in Postmenopausal Women With and Without Estrogen Replacement Therapy: The HERITAGE Family Study. Metabolism. 2004;53(9):1192-6.

57. Mongraw-Chaffin ML, Andersson CAM, Allison MA, Ouyang P, Szklo M, Vaidya D, et al. Association between sex hormones and adiposity: Qualitative differences in women and men in the Multi-Ethnic study of atherosclerosis. Journal of Clinical Endocrinology and Metabolism. 2015;100(4):596-600.

58. Maas AHEM, Appelman YE. Gender differences in coronary heart disease. Netherlands Heart Journal. 2010;18(12):598-603.

59. Schiffer L, Kempegowda P, Arlt W, O'Reilly M. The sexually dimorphic role of androgens in human metabolic disease. European Journal of Endocrinology. 2017;177:125-43.

60. O'Reilly M, Taylor AE, Crabtree NJ, Hughes BA, Capper F, Crowley RK, et al. Hyperandrogenemia predict metabolic phenotype in polycystic ovary syndrome: The utility of serum androstenedione. Journal of Clinical Endocrinology and Metabolism. 2014;99:1027-36.

61. Legro RS. Polycystic Ovary Syndrome and Cardiovascular Disease: A Premature Association? Endocrine Reviews. 2003;24(3):302-12.

62. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The Prevalence and Features of the Polycystic Ovary Syndrome in an Unselected Population. Journal of Clinical Endocrinology and Metabolism. 2004;89(6):2745-9.

63. Falhammer H, Nordenström A. Nonclassic congenital adrenal hyperplasia due to 21-hydroxylase deficiency: clinical presentation, diagnosis, treatment, and outcome. Endocrine. 2015;50:32-50.

64. Jones H, Sprung VS, Pugh CJA, Daousi C, Irwin A, ZAziz N, et al. Polycystic Ovary Syndrome with Hyperandrogenism Is Characterized by an Increased Risk of Hepatic Steatosis Compared to Nonhyperandrogenic PCOS Phenotypes and Healthy Controls, Independent of Obesity and Insulin Resistance. Journal of Clinical Endocrinology and Metabolism. 2012;97(10):3709-16.

65. Tack LJW, Craen M, Dhondt K, Vanden Bossche H, Laridaen J, Cools M. Consecutive lynestrenol and cross-sex hormone treatment in biological female adolescents with gender dysphoria: a retrospective analysis. Biology of Sex Differences. 2016;7(14).

66. Jarin J, Pine-Twaddell E, Trotman G, Stevens J, Conard L, Tefera E, et al. Cross-sex hormones and metabolic parameters in adolescents with gender dysphoria. Pediatrics. 2017;139(5).

67. Tack LJW, Heyse R, Craen M, Dhondt K, Bossche Vd, Laridaen J, et al. Consecutive cyproterone acetate and estradiol treatment in late-pubertal transgender female adolescents. The Journal of Sexual Medicine. 2017;14:747-57.

68. Klink D, Caris M, Heijboer AC, Van Trotsenburg MAA, Rotteveel J. Bone mass in young adulthood following gonadotropin-releasing hormone analog treatment and cross-sex hormone treatment in adolescents with gender dysphoria. Journal of Clinical Endocrinology and Metabolism. 2015;100(2):270-5.

69. Schagen SEE, Cohen-Kettenis P, Delemarre-van de Waal HA, Hannema SE. Efficacy and Safety of Gonadotropin-Releasing Hormone Agonist Treatment to Suppress Puberty in Gender Dysphoric Adolescents. Journal of Sexual Medicine. 2016;13:1125-32.

70. Elbers JHH, Giltay EJ, Teerlink T, Scheffer PG, Asscheman H, Seidell JC, et al. Effects of sex steroids on components of the insulin resistance syndrome in transsexual subjects. Clinical Endocrinology. 2003;58:562-71.

71. Elbers JMH, Asscheman H, Seidell JC, Megens JAJ, Gooren LJG. Long-term testosterone administration increases visceral fat in female to male transsexuals. Journal of Endocrinology and Metabolism. 1997;82(7):2044-447.

72. VanKim NA, Erickson DJ, Eisenberg ME, Lust K, Rosser SBR, Laska MN. Weight-related disparities for transgender college students. Health Behavior Policy Review. 2014;1(2):161-71.

73. Kreukels BPC, Haraldsen IR, De Cuypere G, Richter-Appelt H, Gijs L, Cohen-Kettenis PT. A European network for the investigation of gender incongruence: The ENIGI initiative. European Psychiatry. 2012;27:445-50.

74. Dekker MJHJ, Wierckx K, van Caenegem E, Klaver M, Kreukels BPC, Elaut E, et al. A European Network for the Investigation of Gender Incongruence: Endocrine Part. The Journal of Sexual Medicine. 2016:1-6.

75. Abate N, Burns D, Peshock RM, Garg A, Grundy SM. Estimation of adipose tissue mass by magnetic resonance imaging: validation against dissection in human cadavers. Journal of Lipid Research. 1994;35:1490-6.

76. Rössner S, Bo W, Hiltbrandt E, Hinson W, Karstaedt N, Santago P, et al. Adipose tissue determinations in cadavers--a comparison between cross-sectional planimetry and computed tomography. International Journal of Obesity. 1990;14(10):893-902.

77. Bredella MA, Ghomia RH, Thomas BJ, Torriani M, Brick DJ, Gerweck AV, et al. Comparison of DXA and CT in the Assessment of Body Composition in Premenopausal Women With Obesity and Anorexia Nervosa. Obesity. 2010;18(11):2227-33.

78. Fuller N, Hardingham C, Graves M, Screaton N, Dixon A, Ward L, et al. Assessment of limb muscle and adipose tissue by dualenergy X-ray absorptiometry using magnetic resonance imaging for comparison. Intenational Journal of Obesity. 1999;23:1295-302.

79. Glickman SG, Marn CS, Supiano MA, Dengel DR. Validity and reliability of dual-energy X-ray absorptiometry for the assessment of abdominal adiposity. Journal of Applied Physiology. 2004;97:509-14.

80. Levine LA, Abboud L, Bary M, Reed JE, Sheedy PF, Jensen MD. Measuring leg muscle and fat mass in humans: comparison of CT and dual-energy X-ray absorptiometry. Journal of Applied Physiology. 2000;88:452-6.

81. Mickelsfield LK, Goedecke JH, Punyanitya M, Wilson KE, Kelly TL. Dual-Energy E-Ray Performs as Well as Clinical Computed Tomography for the Measurement of Visceral Fat. Obesity. 2012;20(5):1109-14.

82. Laskey MA, Phil D. Dual-Energy X-Ray Absorptiometry and body composition. Nutrition & Diabetes. 1996;12(1):45-51.

83. Kelly TL, Wilson KE, Ruth C. Patent Application Publication Estimating Visceral Fat by Dual-Energy X-Ray Absorptiometry US 2010/0234719 A1. 2010.