Bodies in transition

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Effects of testosterone and estradiol treatment on visceral fat and cardiometabolic risk factors: results from a multicenter prospective cohort study in transgender people

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ABSTRACT

Objective

Visceral fat is strongly related to cardiometabolic risk factors such as hypertension, dyslipidemia, and type 2 diabetes. Sex hormones play a major role in the regulation of body fat, but the effect on visceral fat is unclear. Our aim was to investigate the effect of hormonal treatment on changes in visceral fat and the relation with changes in blood pressure, lipids, and insulin resistance (HOMA-IR) in adult transgender people.

Methods

In this multicenter prospective study, 179 transwomen and 162 transmen underwent whole body dual-energy X-ray absorptiometry to estimate visceral fat and laboratory measurements were performed, both before and after one year of hormonal treatment.

Results

The mean change in visceral fat in transwomen was -2 grams (95% CI -15;11) with a large range from -289 grams to +251 grams. In transmen, the mean change in visceral fat was +3 grams (95% CI -10;16), ranging from -331 grams to +179 grams. Changes in visceral fat were different between BMI categories in transwomen, with an increase in visceral fat in transwomen with a BMI<25 kg/m^2 (+12%, 95% CI 8;16) and a decrease in visceral fat in transwomen with a BMI between 25.0 and 30.0 kg/m^2 (-9%, 95% CI -13;-4, interaction: p<0.001) or with a BMI>30 kg/m^2 (-13%, 95% CI -18;-8, interaction: p<0.001). These different changes across BMI categories were not present in transmen. After adjustment for changes in total body fat and total lean body mass, changes in visceral fat were not related to changes in blood pressure, lipids, and HOMA-IR in both transwomen and transmen.

Conclusions

Estradiol and progestagen treatment increased visceral fat in transwomen with a BMI<25 kg/m^2 and decreased visceral fat in transwomen with a BMI ≥25 kg/m^2. Testosterone treatment did not affect visceral fat in transmen. Changes in visceral fat were not related to changes in cardiometabolic risk factors. Future prospective studies in transgender people should investigate risks of type 2 diabetes and cardiovascular disease.

INTRODUCTION

Abdominal obesity, and in particular an excess of visceral fat, is strongly related to cardiovascular risk factors such as hypertension, dyslipidemia, type 2 diabetes[1-3] and the development of cardiovascular disease[4,5]. The hypothesized mechanisms for a specific harmful role of excess visceral fat are its high secretion rate of pro-inflammatory cytokines, predominantly interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), and the high rate of lipolysis, resulting in an excess of free fatty acids that may be drained by the portal vein to the liver eventually leading to increased gluconeogenesis and hyperinsulinemia[1].

Sex hormones play a major role in the regulation of adipose tissue distribution and function[6]. Whereas women have more total body fat and subcutaneous fat than men, men have more visceral fat[7]. Estradiol is thought to be responsible for the typical peripheral fat distribution in women. Decreased estradiol levels in postmenopausal women are often paralleled with increases in visceral fat[8]. However, also testosterone levels might play a role in female body fat distribution, since in women with polycystic ovary syndrome, high testosterone levels are related to increased visceral fat[9]. In contrast, in hypogonadal men, low testosterone levels are associated with visceral obesity[10]. Likewise, in a recent cross-sectional study in the general population, serum testosterone levels were associated with visceral fat, in opposite directions in men and women. Altogether, these results suggest a role for sex steroid hormones in determining the deposition of visceral fat[7], although the direction of the association remains unclear.

Transwomen (individuals assigned male at birth self-identifying as female) receive anti-androgen and estradiol treatment to induce feminization and obtain the desired female sex characteristics. Transmen (individuals assigned female at birth self-identifying as male) are treated with testosterone to induce masculinization. Studies in this population may mimic a trial, in which women receive treatment with testosterone, and men with estradiol and anti-androgens. In a previous study, we showed major changes in total body fat in both groups during the first year of hormonal treatment[11], but the effects of the hormonal treatment on visceral fat are largely unknown.

In the 1990's, Elbers et al.[12] pioneered by measuring visceral fat with magnetic resonance imaging (MRI) in small groups of lean transwomen (n=20) and transmen (n=17) and showed an increase in visceral fat of 18% in transwomen and 13% in transmen after one year of hormonal treatment[12-15]. Such studies have not been performed in larger populations with

a wider BMI range, and the relation of these induced changes in visceral fat with changes in cardiometabolic risk factors is unknown.

Therefore, the aims of our study were 1) to investigate the effects of anti-androgen and estradiol treatment in transwomen and of testosterone treatment in transmen on changes in visceral fat, also stratified by BMI categories and 2) to examine to what extent changes in visceral fat were related to changes in insulin resistance and lipids in a large population of transwomen and transmen.

METHODS

Study design and study population

This study is embedded in the European Network for the Investigation of Gender Incongruence (ENIGI) project, a multicenter prospective observational study to gain more knowledge and transparency about the diagnostics and treatment of gender dysphoria[16,17]. As from 2010, people were eligible to participate in the study when they were 18 years or older, and diagnosed with gender dysphoria according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) version 4 or version 5[18,19]. People were not eligible when they started in a different treatment protocol than described below (e.g. when using spironolactone) or in case of previous gender affirming hormone use, insufficient knowledge of the spoken language, or psychological vulnerability. Participants visited the outpatient clinic every three months during the first year of hormonal treatment for clinical data collection. At the start of hormonal treatment and after one year of treatment whole body dual-energy X-ray absorptiometry (DXA) was performed[16].

Different type of DXA scanners were used in participating gender clinics (Amsterdam and Ghent: Hologic Discovery A, Oslo: Lunar, Florence: Hologic Delphi). Because the use of different types of DXA scanners results in non-comparable body composition data, only participants from Amsterdam and Ghent were selected for this present study. Further, transpeople were included if they started hormonal treatment between February 2010 and March 2015 and if they completed the first year of hormonal treatment. Also, the baseline DXA had to be performed within 90 days before the start of hormonal treatment or 31 days after the start of hormonal treatment and the follow-up DXA had to be performed between 10 months and 14 months after the start of hormonal treatment. The participant inclusion flow chart has been published before[11].

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.

Treatment protocol

Hormonal treatment in transwomen consists of cyproterone acetate (CPA) 50 mg/day in combination with oral estradiol valerate 2-4 mg/day or a transdermal estradiol patch 50-100 mcg/24h twice a week. The estradiol patch was recommended if people were above 40 years old or if they had a history of cardiovascular disease, hormone sensitive malignancies, or trombo-embolic events. Transmen were treated with testosterone gel 25-50 mg/day, testosterone undecanoate 1000 mg intramuscular (im) once per 12 weeks, or testosterone esters 250 mg im once per 2 weeks.

Clinical data collection

Body height was measured to the nearest centimeter using a Harpenden stadiometer. Body weight was measured without shoes to the nearest 0.1 kg. With these data, body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Systolic and diastolic blood pressure were determined using an electronic blood pressure monitor. At the start of hormonal treatment and after one year of hormonal treatment, whole body dual-energy X-ray absorptiometry (DXA) and laboratory measurements were performed, which are described in more detail below.

Total body fat, total lean body mass, and visceral fat estimation by DXA

Total body fat, total lean body mass, and visceral fat were estimated using DXA[20,21]. All DXA scans, before and after 12 months of hormonal treatment, were analyzed with Hologic software version 13.5.3 according to the sex assigned at birth using the user's instruction manual.

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[22]. In every pixel, the attenuation is measured and every high and low energy attenuation pair is related to a unique combination of fat mass and fat-free mass. After measuring body fat and lean body mass in every pixel, the amount of total body fat or total lean body mass is calculated. Android fat is the sum of the subcutaneous fat outside the abdominal cavity and visceral fat inside the abdominal cavity in the abdominal region. We have published a DXA figure of this region before[11]. The lower boundary of this region coincides with the

pelvic horizontal line. The height equals 20% of the distance from the pelvic horizontal line to the neck line and the lateral boundaries coincide with the arm lines[23].

To estimate 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 to estimate solely visceral fat[20,21]. 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 estimate solely the amount of visceral fat, first the amount of anterior and posterior subcutaneous fat was estimated. This was done by measuring the subcutaneous fat between the abdominal wall and the skin on both sides of the body, after which a total amount of subcutaneous fat was estimated. Then, the amount of subcutaneous fat was subtracted from the total amount of abdominal fat in order to estimate the amount of visceral fat[20,21]. Results from the DXA scans for total body fat were given in grams and percentages, results for visceral fat and total lean body mass were given in grams.

Laboratory measurements

Fasting venous blood samples were obtained at the start of hormonal treatment and after 12 months of treatment. In Ghent, fasting glucose, insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured using Roche Cobas chemistry analyzers (c701 module or c501 module, Modular, Roche Diagnostics, Mannheim, Germany). The inter-assay coefficients of variation (CV) were: glucose 0.95%, insulin 2.3%, total cholesterol 1.3%, HDL cholesterol 1.8%, and triglycerides 2.5%. The limit of quantification (LOQ) was as follows: glucose 0.1 mmol/L, insulin 0.2 mU/L, cholesterol 0.1 mmol/L, HDL cholesterol 0.08 mmol/L, and triglycerides 0.1 mmol/L. In Amsterdam, fasting glucose, total cholesterol, HDL cholesterol, and triglycerides were measured using Roche Cobas chemistry analyzers (c701 module or c501 module, Modular, Roche Diagnostics, Mannheim, Germany). The inter-assay CVs were as follows: glucose 1.1%, total cholesterol 1.4%, HDL cholesterol 0.9%, and triglycerides 1.8%. The LOQ was as follows: glucose 0.1 mmol/L, cholesterol 0.1 mmol/L, HDL cholesterol 0.08 mmol/l, and triglycerides 0.1 mmol/L. To measure insulin, an immunometric assay was used (Luminescence Advia Centaur, Siemens Medical Solutions Diagnostics, USA) with an inter-assay CV of 7% and a LOQ of

10 pmol/l. The homeostatic model assessment provides a measure for insulin resistance (HOMA-IR) which correlates very well to insulin resistance estimates from clamps which are considered as the golden standard[24]. HOMA-IR is calculated as HOMA-IR= (fasting glucose in mmol / fasting insulin in mU/L) / 22.5[25]. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula[26].

Statistical analyses

Baseline characteristics were expressed as number, percentage, or median with interquartile range (IQR) for non-normally distributed variables. To present the most representative (median) estradiol and testosterone level during the one-year treatment, the means of the estradiol and testosterone levels measured at two time points (3 months and 12 months) were calculated per participant.

First, we performed linear mixed model regression analyses with observations clustered within participants to examine changes in visceral fat, total body fat, and total lean body mass during the first year of hormonal treatment. To calculate the percentage change in visceral fat, the mean change in visceral fat was divided by the amount of visceral fat at start and multiplied by 100. These same calculations were performed for the other body composition measures.

Second, we examined the effect of BMI at baseline on the change in visceral fat. During treatment, people with overweight (BMI 25-30 kg/m^2) and obesity (BMI>30 kg/m^2) were advised to achieve and maintain a healthy lifestyle in order to lose body weight. In the latter group, this was even more emphasized because of requirements regarding body weight when applying for gender affirming surgery (BMI between 18 and 30 kg/m^2 for vaginaplasty or phalloplasty). Since these lifestyle advices might have affected changes in visceral fat, BMI at baseline, time, and the interaction between BMI at baseline and time were added to the linear mixed model to examine the influence of BMI at baseline on the change in visceral fat. BMI at baseline was categorized the following categories: BMI <25 kg/m^2, BMI 25-30 kg/m^2, and BMI >30 kg/m^2.

Third, we also performed linear mixed models to examine the changes in cardiometabolic risk factors, such as blood pressure, glucose, insulin, HOMA-IR, and lipids during the first year of hormonal treatment. The residuals of change over time in insulin and HOMA-IR in transmen were not normally distributed and therefore the natural logarithm was obtained for these analyses in transmen. For all variables, the (back transformed geometric) mean

change plus 95% confidence interval (CI) were reported. For all analyses on glucose, insulin, and HOMA-IR, people with diabetes mellitus (n=7) were excluded.

Next, we performed linear regression analyses to examine the association of changes in visceral fat, total body fat, and total lean body mass with changes in cardiometabolic risk factors. We standardized the one-unit change of these changes in body composition to a mean of zero and a standard deviation of one. In additional analyses, we tested for interaction with BMI at baseline by including interaction terms between the BMI categories and change in visceral fat, total body fat, or total lean body mass in the model. All analyses were adjusted for change in the other two body composition measures. So for example, the analyses between change in visceral fat and cardiometabolic risk factors were adjusted for change in total body fat and change in total lean body mass.

Finally, to assess the influence of outliers, we repeated all analyses excluding data points more than five standard deviations from the mean.

RESULTS

In this study, 179 transwomen and 162 transmen were included. Baseline characteristics at the start of hormonal treatment are shown in Table 1. Of transwomen, 63% had a BMI<25 kg/m^2 (n=113), 23% had a BMI 25-30 kg/m^2 (n=42), and 14% had a BMI>30 kg/m^2 (n=24). Of transmen, 56% had a BMI<25 kg/m^2 (n=91), 21% had a BMI 25-30 kg/m^2 (n=34), and 23% had a BMI>30 kg/m^2 (n=37). Mean follow-up time was 377 days (SD ± 22) in transwomen and 380 days (SD ± 22) in transmen. Table 2 shows the changes in cardiometabolic risk factors during the first year of hormonal treatment.

Table 1. General characteristics

	TRANSWOMEN	TRANSMEN
At baseline		
Age (years)	29 (IQR 23-43)	24 (IQR 21-33)
BMI (kg/m^2)	23.0 (IQR 20.5-26.6)	24.6 (IQR 21.7-29.1)
Smokers (%)	25	28
Alcohol (U/week)	0 (IQR 0-2)	0 (IQR 0-2)
Caucasian (%)	98%	93%
Median hormone levels at start Amsterdam		
Estradiol level (pmol/l)	97 (IQR 80-116)	144 (IQR 76-311)
Testosterone level (nmol/l)	18.9 (IQR 14.0-22.0)	1.3 (IQR 1.0-1.6)
Median hormone levels at start Ghent		
Estradiol level (pmol/l)	30 (IQR 24-36)	45 (IQR 28-112)
Testosterone level (nmol/l)	18.9 (IQR 13.9-21.3)	1.1 (IQR 0.7-1.3)
Median hormone levels during treatment Amsterdam		
Estradiol level (pmol/l)	225 (IQR 150-317)	182 (IQR 141-257)
Testosterone level (nmol/l)	0.7 (IQR 0.6-0.9)	27.0 (IQR 18.1-38.3)
Median hormone levels during treatment Ghent		
Estradiol level (pmol/l)	223 (IQR 168-317)	137 (IQR 122-187)
Testosterone level (nmol/l)	(IQR 0.5-2.6)	17.5 (IQR 13.7-21.9)

BMI: body mass index, IQR: inter quartile range, U: units, CPA: cyproterone acetate.

Table 2. Change in body composition and cardiometabolic risk factors in transwomen and transmen.

TRANSWOMEN	Start of treatment	Mean change (95% CI)
Body composition		
Visceral fat (grams)	353 (322;384)	-2 (-15;11)
Android fat (kg)	1.6 (1.4;1.7)	+0.1 (0.1;0.2)
Total body fat (kg)	19.1 (17.9;20.2)	+4.0 (3.4;4.7)
Total lean body mass (kg)	57.2 (56.0;58.4)	-1.7 (-2.1;-1.3)
Cardiometabolic risk factors		
Systolic blood pressure (mmHg)	129 (127;131)	-4 (-6;-2)
Diastolic blood pressure (mmHg)	79 (78;81)	-2 (-3;0)
Glucose (mmol/l)	5.3 (5.2;5.5)	-0.1 (-0.2;0.0)
Insulin (mU/L)	8.9 (7.8;10.0)	+3.9 (2.7;5.1)
HOMA-IR	2.3 (1.9;2.7)	+0.9 (0.4;1.2)
Total cholesterol (mmol/l)	4.6 (4.5;4.8)	-0.6 (-0.7;-0.5)
HDL cholesterol (mmol/l)	1.4 (1.3;1.4)	-0.2 (-0.2;-0.1)
LDL cholesterol (mmol/l)	2.7 (2.6;2.9)	-0.3 (-0.4;-0.3)
Triglycerides (mmol/l)	1.1 (1.0;1.2)	-0.2 (-0.3;-0.1)

TRANSMEN	Start of treatment	Mean change (95% CI)
Body composition		
Visceral fat (grams)	277 (249;306)	+3 (-10;16)
Android fat (kg)	2.0 (1.8;2.1)	-0.1 (-0.1;0.0)
Total body fat (kg)	26.0 (24.4;27.6)	-2.8 (-3.5;-2.2)
Total lean body mass (kg)	46.9 (45.7;48.2)	+4.7 (4.2;5.1)
Cardiometabolic risk factors		
Systolic blood pressure (mmHg)	124 (122;127)	0 (-2;2)
Diastolic blood pressure (mmHg)	76 (75;78)	+1 (-1;2)
Glucose (mmol/l)	5.1 (5.0;5.2)	-0.2 (-0.3;-0.1)
Insulin (mU/L)	9.5 (8.7;10.5)	-2.2 (-3.0;-1.3)
HOMA-IR	2.1 (1.9;2.4)	-0.5 (-0.7;-0.3)
Total cholesterol (mmol/l)	4.6 (4.4;4.7)	0.0 (-0.1;0.1)
HDL cholesterol (mmol/l)	1.5 (1.5;1.6)	-0.2 (-0.3;-0.2)
LDL cholesterol (mmol/l)	2.6 (2.5;2.7)	+0.2 (0.1;0.3)
Triglycerides (mmol/l)	9(0.8;1.0)	+0.2 (0.1;0.3)

Data are shown as (backtransformed) mean (95% confidence interval).
HOMA-IR: homeostatic model assessment, HDL: High density lipoprotein, LDL: Low density lipoprotein.

Change in visceral fat

The mean change in visceral fat in transwomen was -2 grams (95% CI -15;11) with a large range from -289 grams to +251 grams. In transmen, the mean change in visceral fat was +3 grams (95% CI -10;16), ranging from -331 grams to +179 grams. Percentage changes in visceral fat, android fat, total body fat, and total lean body mass are shown in Figure 1. Changes in visceral fat were different between BMI categories in transwomen, with an increase in visceral fat in transwomen with a BMI<25 kg/m^2 (+12%, 95% CI 8;16) and a decrease in visceral fat in transwomen with a BMI between 25 and 30 kg/m^2 (-9%, 95% CI -13;-4, interaction: p<0.001) or with a BMI>30 kg/m^2 (-13%, 95% CI -18;-8, interaction: p<0.001) (Figure 2). These different changes across BMI categories were not present in transmen, with no change in transmen with a BMI<25 kg/m^2 (+6%, 95% CI -4;15), or a BMI 25.0-30.0 kg/m^2 (0%, 95% CI -8;6, interaction: p=0.36), or with a BMI>30 kg/m^2 (-2%, 95% CI -8;4, interaction: p=0.62) (Figure 2).

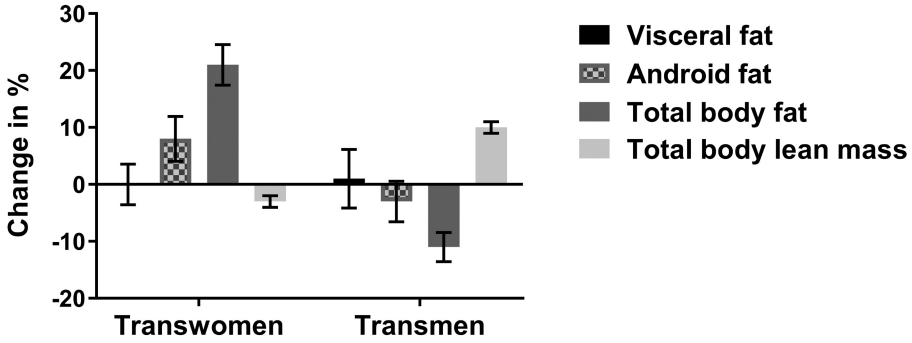

Figure 1. Percentage change in visceral fat, android fat, and total body fat during one year of hormonal treatment.
Transwomen: Visceral fat: +0% (95% CI -4;3), android fat +8% (95% CI 4;12), total body fat +21% (95% CI 18;25), total lean body mass: -3% (95% CI -4;-2). Transmen: Visceral fat: +1% (95% CI -4;6), android fat: -3% (95% CI -7;0), total body fat: -11% (95% CI -13;-8), total lean body mass +10% (95% CI 9;11).

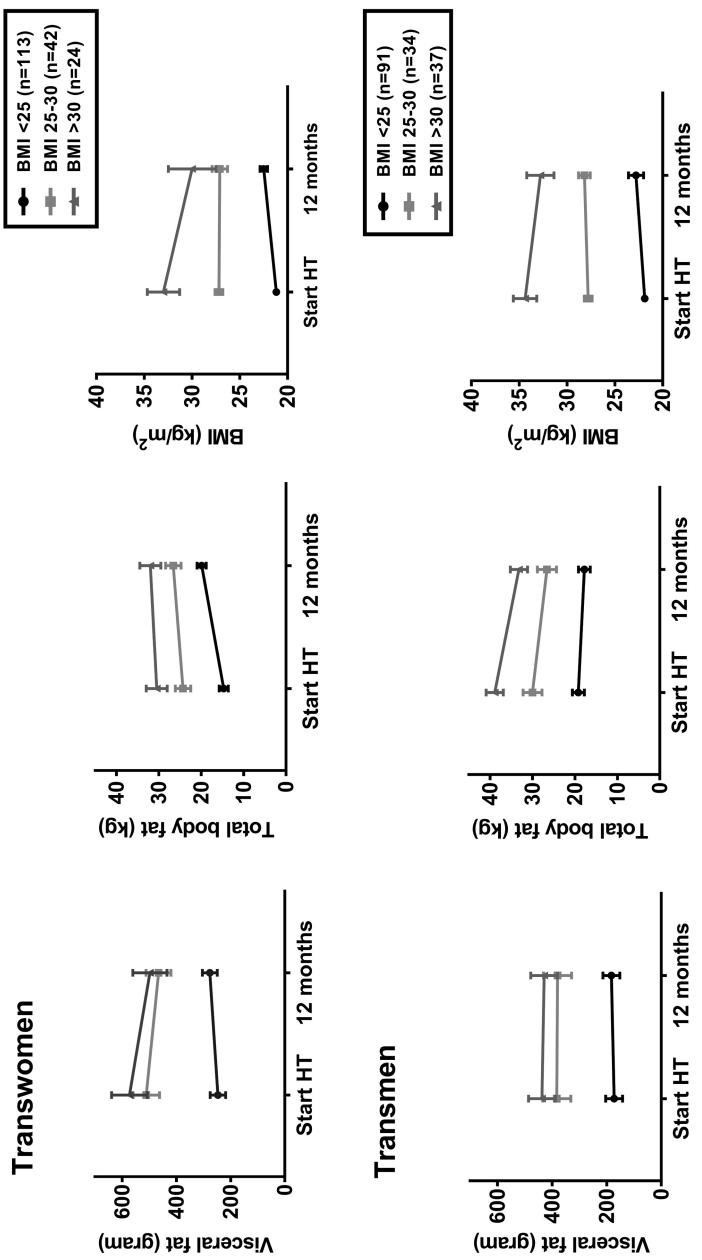

Figure 2. Change in visceral fat, total body fat, and body mass index (BMI) per BMI category in transwomen and transmen during one year of hormonal treatment.

Transwomen: Total body fat: BMI<25 kg/m^2: +35% (95% CI 30;41), BMI 25-30 kg/m^2: +10% (95% CI 4;15), BMI>30 kg/m^2: +5% (95% CI 0;11). BMI: BMI<25 kg/m^2: +6% (95% CI 5;7), BMI 25-30 kg/m^2: -1% (95% CI -3;2), BMI>30 kg/m^2: -1% (95% CI -5;4). **Transmen**: Total body fat: BMI<25 kg/m^2: -7% (95% CI -12;-3), BMI 25-30 kg/m^2: -12% (95% CI -16;-7), BMI>30 kg/m^2: -15% (95% CI -18;-11). BMI: BMI<25 kg/m^2: +4% (95% CI 1;7), BMI 25-30 kg/m^2: 1% (95% CI -1;3), BMI>30 kg/m^2: -4% (95% CI -8;-1). *HT: hormonal treatment, BMI: body mass index, CI: confidence interval.*

Relation between changes in measures of body composition with changes in cardiometabolic risk factors

The number of included participants for each analysis on the relation between change in body composition and change in cardiometabolic risk factors is shown in the subscriptions of Supplemental table 1 and Supplemental table 2. Figure 3 shows scatterplots of the change in visceral fat and the change in risk factors in the total group of transwomen and the total group of transmen. After adjustment for change in the other body composition measures, there were no associations between changes in visceral fat, total body fat, and total lean body mass and changes in cardiometabolic risk factors (Supplemental table 1). The interaction between BMI at start and change in visceral fat was significant for 3 out of 36 associations with cardiometabolic risk factors, and therefore we performed stratified analyses. Also, within strata of BMI at start there were no associations between changes in visceral fat and changes in cardiometabolic risk factors after adjustment for change in total body fat (Supplemental table 2).

Sensitivity analyses

There was one outlier in the insulin concentrations and thus HOMA-IR values of transmen. After excluding this outlier, associations were similar between one SD increase in visceral fat and insulin concentrations (+0.1 mU/l, 95% CI -1.3;1.6) or HOMA-IR values (+0.1, 95% CI -0.3;0.5). In addition, similar associations were seen between one SD increase in total body fat and insulin concentrations (+0.6 mU/l, 95% CI -1.1;2.6) or HOMA-IR values (+0.1, 95% CI -0.4;0.5). Whereas before the exclusion of this outlier, a strong significant association was found between one SD change in total lean body mass and insulin concentrations, this was not seen any more after exclusion (insulin: +0.9 mU/l, 95% CI -0.6;2.4 and HOMA-IR: +0.3, 95% CI -0.1;0.7).

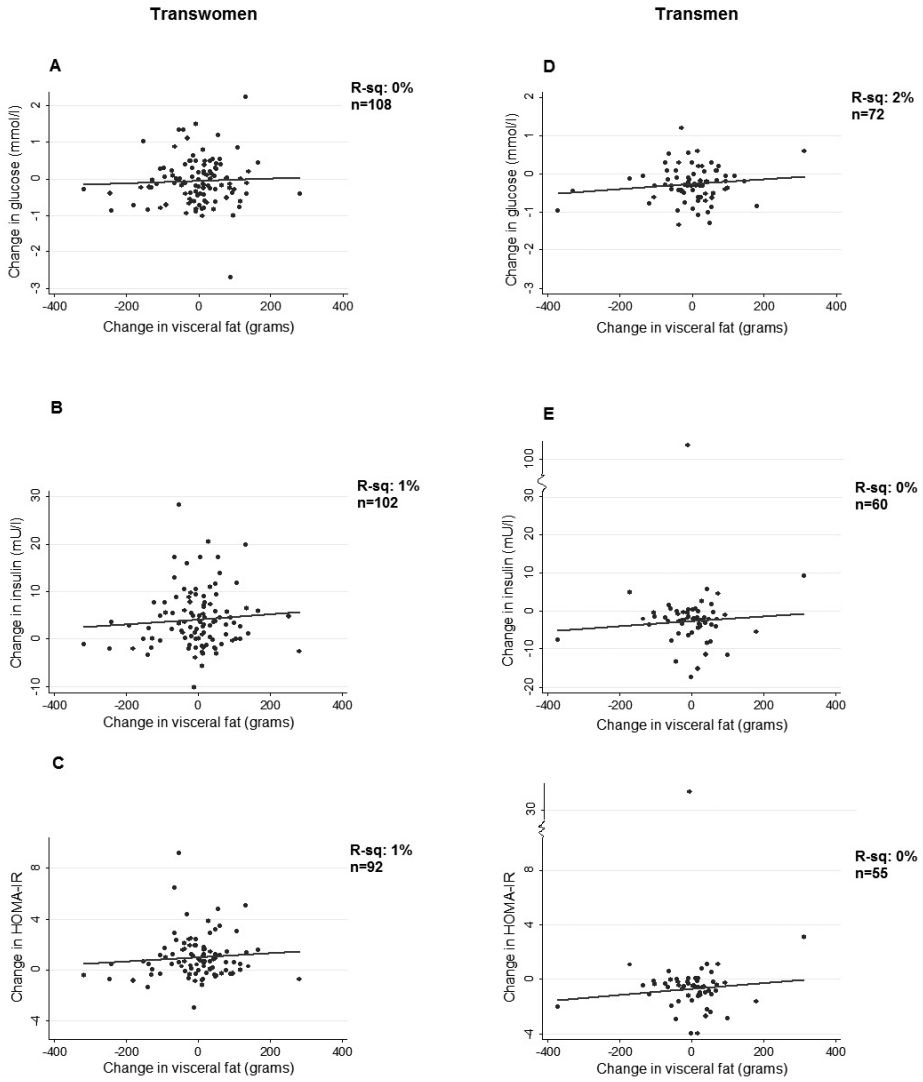

Figure 3a. Scatterplots of the change in visceral fat against the change in glucose, ln-insulin, and ln-HOMA-IR during one year of hormonal treatment in transwomen and transmen.

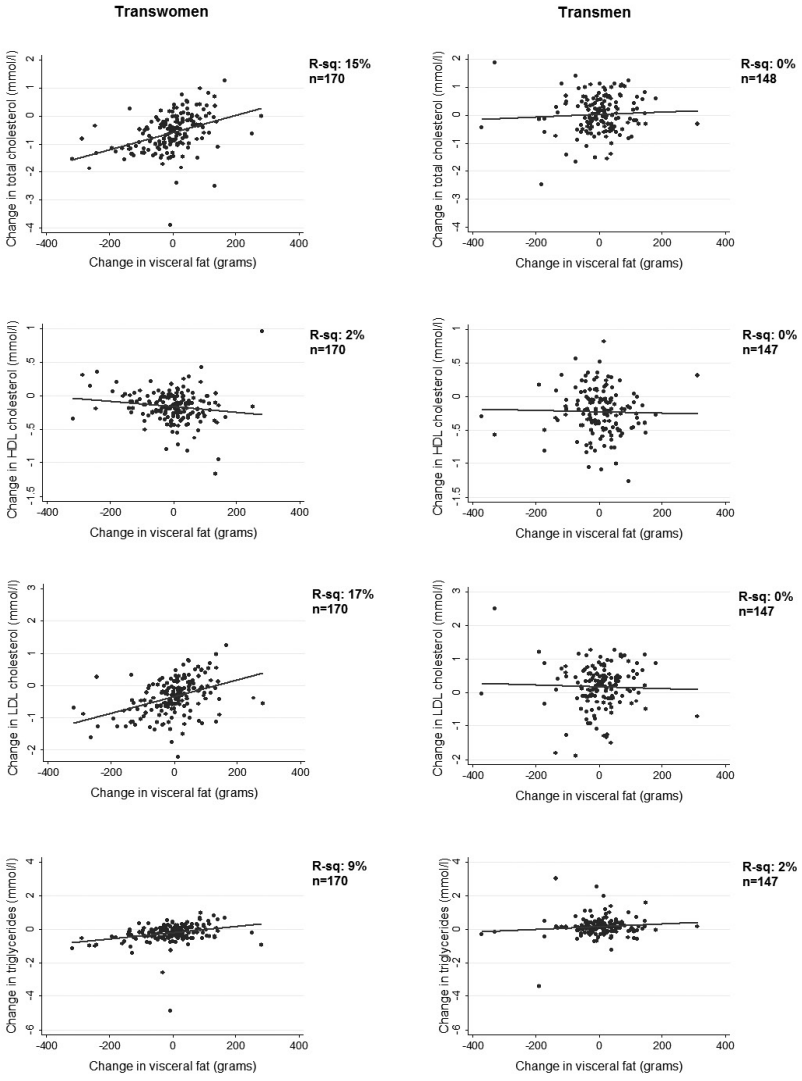

Figure 3b. Scatterplots of the change in visceral fat against the change in total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides during one year of hormonal treatment in transwomen and transmen.

DISCUSSION

In this large prospective cohort study we investigated the effects of one year hormonal treatment with estradiol and progestogen and treatment with testosterone on the amount of visceral fat and the relation with cardiometabolic risk factors in 179 transwomen and 162 transmen with a large range of BMI. On average, visceral fat did not change in both transwomen and transmen. Nevertheless, there was a large individual range in change in visceral fat in both transwomen and transmen. BMI at baseline affected the change in visceral fat in transwomen, but not in transmen. The amount of visceral fat increased in transwomen with a BMI<25 kg/m^2, and decreased in transwomen with a BMI≥25 kg/m^2. The change in visceral fat, however, was not related to changes in cardiometabolic risk factors in both transwomen and transmen, also not within strata of BMI.

The few previous studies reporting on the effects of hormonal treatment on visceral fat showed an increase in visceral fat in small groups of transwomen (+17% to +18%) and transmen (+6% to +18%) after one year of hormonal treatment[13-15]. Participants in these studies were lean with a mean BMI of 21 kg/m^2. In our study, there was no mean change in visceral fat, but with a large inter-individual range of -289 grams to +251 grams in transwomen and a range of -331 grams to +179 in transmen. One of the possible explanations for this large variation is the lifestyle advice that persons with a BMI≥25 kg/m^2 have received. This may have resulted in a decrease in visceral fat despite the hormonal treatment[27]. Within the BMI category of 20 to 25 kg/m^2, hormonal treatment resulted in increases in visceral fat in both transwomen (+12%) and transmen (+4%), although these increases were smaller than reported in previous studies. Possibly, the small sample size in previous studies may have resulted in these larger changes in visceral fat by change. Further, these previous studies used transverse MRI images to measure visceral fat, while DXA estimates the whole volume of visceral fat. Possibly, the increase in visceral fat on transverse MRI images may not result in similar increases in the total amount of visceral fat.

As indicated, in the present study, changes in visceral fat depended on BMI at baseline in transwomen. Whereas obese transwomen (BMI>30 kg/m^2) increased in total body fat (+6%) and decreased in visceral fat (-12%), transwomen with a BMI between 20 and 25 kg/m^2 increased in both total body fat (+36%) and visceral fat (+12%). Although the exact mechanism is unknown and potential lifestyle advices may have played a role, this may suggest a shift from visceral fat to subcutaneous fat in obese transwomen, while in normal-weight transwomen body fat increased in both compartments.

Similar as in previous studies[13,28], estradiol and progestagen treatment resulted in an increase in insulin resistance and decreases in serum total cholesterol, LDL cholesterol, and triglycerides in transwomen. Opposite effects were seen in transmen, thus a decrease in insulin resistance and a deterioration in lipid profile after testosterone treatment. Based on previous literature[1,7,29-33], we hypothesized that changes in visceral fat or total body fat during the first year of hormonal treatment would result in changes in cardiometabolic risk factors. However, changes in visceral fat were not related to changes in cardiometabolic risk factors during the first year of treatment, neither in transwomen nor transmen. Even within transwomen who did increase in visceral fat after one year of hormonal treatment, this increase was not related with changes in cardiometabolic risk factors.

We realized that testosterone treatment in transmen also resulted in a large increase in lean body mass, mostly muscle mass, and we hypothesized that this may explain the decrease in insulin resistance in transmen. However, changes in lean body mass were also not associated with changes in insulin resistance. An alternative explanation could be that hormonal treatment directly affects glucose and lipid metabolism in the liver[34,35] or insulin resistance in the muscle[36], rather than via changes in body fat depots. In that case, our results may imply that previous observed cross-sectional associations between visceral fat and cardiometabolic risk factors in the general population are explained by confounding.

In observational studies in PCOS women, testosterone levels were associated with increased visceral fat, insulin resistance, and lipid levels. In contrast, we observed no change in visceral fat in transmen treated with testosterone. Also contrary to what we expected, insulin resistance decreased and there were only small unfavorable changes in lipids in transmen. This may suggest that high doses of androgens might not have such a harmful influence on cardiometabolic risk in women as suggested before in observational studies. An explanation for the discrepancy between our findings and observations in PCOS women might be provided by a theory that high testosterone levels, as seen in transmen, have less harmful effects than lower testosterone levels, as seen in PCOS women. This range of testosterone levels is associated with metabolically disadvantageous effects leading to the concept of the “metabolic valley of death”[37]. It is suggested that both men and women within this testosterone range have an increased metabolic risk compared to persons with higher testosterone levels. If this theory applies, then that might explain why in transmen, with equal testosterone levels as eugonadal men, no harmful effects of hormonal treatment are seen.

On the other hand, two small intervention studies in postmenopausal women treated with low dosages of testosterone showed no disadvantageous effects[38,39], specifically no changes in visceral fat and insulin resistance with small changes in lipids. Although these studies were performed in small groups (n=8 and n=21), these results contradict the theory that low testosterone levels have harmful effects. Altogether, it is still unclear what the effects is of (the height of) testosterone levels on visceral fat in women.

A strength of our study is that we were able to examine the effect of hormonal treatment on changes in visceral fat in a large cohort of transgender people. We were able to examine the effects on visceral fat in different BMI categories and showed heterogeneous effects on change in visceral fat between BMI categories. Nevertheless, changes in visceral fat were not related to changes in cardiometabolic risk factors, also not within strata of BMI. A limitation of this study is the fact that it is not a randomized trial including a control group, but can merely be considered as a natural experiment. Nevertheless, confounding of the treatment effect is unlikely to have played a role, because the participants opted for hormonal treatment for other reasons than their amount of visceral fat or cardiometabolic health status. However, without control groups we were not able to control for the natural course of change in visceral fat over time. In the general population, change in visceral fat over time has hardly been studied. One study in 436 nondiabetic middle-aged Japanese Americans with a mean BMI of 24.2 kg/m^2 found a mean yearly increase in visceral fat of 2.8%, measured by computed tomography[40], but this has never been studied in a younger population. Therefore we were unable to assess to which extent the yearly change in visceral fat in transmen and transwomen could be contributed to natural change over time or the effect of the hormonal treatment. Another limitation is that DXA estimates visceral fat by using other measures of body fat and sex-specific formulas[20]. 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)[21], but DXA has not been validated yet for measurement of change in visceral fat over time. In addition, we used the sex assigned at birth to analyze the DXA scans, both at baseline and after one year, and the influence of the hormonal treatment on the estimation of visceral fat by DXA is unclear. Therefore, studies on the effect of hormonal treatment in trans people with direct assessment of changes in visceral fat using MRI, a method that does not rely on sex, are warranted.

The mean observed changes in visceral fat and cardiometabolic risk factors were not clinically relevant. Nevertheless, we observed a small increase in insulin resistance in transwomen and small increases in LDL cholesterol and triglycerides with a decrease in

HDL cholesterol in transmen. Long-term studies should indicate whether these changes will result in increased risks of development of cardiometabolic diseases. In the meantime, we do advise routine measurement of glucose and lipids in the transgender population on hormonal treatment, although for most people this might be of limited clinical relevance.

In conclusion, on average anti-androgen plus estradiol treatment in transwomen and testosterone treatment in transmen did not change visceral fat, albeit with a large individual variation in change in visceral fat, as assessed with DXA. In transwomen, but not in transmen, BMI at baseline affected the change in visceral fat, with an increase in transwomen with a BMI<25 and a decrease in transwomen with a BMI≥25. The changes in visceral fat were not associated with changes in cardiometabolic risk factors. Future prospective follow-up studies in transwomen and transmen are needed to directly assess changes in visceral fat using MRI, and to investigate risks of type 2 diabetes and cardiovascular disease.

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Supplemental table 1. Change in risk factors per +1 standard deviation in visceral fat, total body fat, and total lean body mass.

	TRANSWOMEN			TRANSMEN		
	Visceral fat	*Total body fat*	*Total lean body mass*	*Visceral fat*	*Total body fat*	*Total lean body mass*
Systolic blood pressure (mmHg)	1 (-1;3)	2 (0;4)	5 (3;7)	-1 (-3;1)	1 (-1;3)	1 (-1;3)
Adjusted*	0 (-2;2)	-1 (-3;2)	5 (3;8)	-2 (-4;0)	2 (0;5)	1 (-1;3)
Diastolic blood pressure (mmHg)	1 (-1;2)	2 (0;3)	2 (1;4)	1 (-1;2)	1 (-1;2)	1 (-1;2)
Adjusted*	0 (-2;2)	1 (-2;3)	2 (1;4)	1 (-1;2)	0 (-2;2)	1 (-1;2)
Total cholesterol (mmol/L)	0.3 (0.2;0.4)	0.3 (0.2;0.4)	0.2 (0.1;0.3)	0.0 (-0.1;0.2)	0.1 (0.0;0.2)	0.0 (-0.1;0.1)
Adjusted*	0.2 (0.0;0.3)	0.2 (0.0;0.3)	0.0 (-0.1;0.1)	0.0 (-0.1;0.1)	0.1 (-0.1;0.2)	0.0 (-0.1;0.1)
HDL cholesterol (mmol/L)	0.0 (-0.1;0.0)	0.0 (-0.1;0.0)	0.0 (-0.1;0.0)	0.0 (-0.1;0.1)	0.0 (-0.1;0.0)	0.0 (-0.1;0.0)
Adjusted*	0.0 (-0.1;0.0)	0.0 (-0.1;0.0)	0.0 (-0.1;0.0)	0.0 (-0.1;0.1)	0.0 (-0.1;0.1)	0.0 (-0.1;0.0)
LDL cholesterol (mmol/L)	0.2 (0.1;0.3)	0.2 (0.2;0.3)	0.2 (0.1;0.2)	0.0 (-0.1;0.1)	0.1 (-0.1;0.2)	0.0 (-0.1;0.1)
Adjusted*	0.1 (0.0;0.2)	0.1 (0.0;0.2)	0.1 (0.0;0.1)	-0.1 (-0.2;0.1)	0.1 (0.0;0.3)	0.0 (-0.2;0.1)
Triglycerides (mmol/L)	0.2 (0.1;0.3)	0.2 (0.1;0.2)	0.1 (0.0;0.2)	0.1 (0.0;0.2)	0.1 (0.0;0.2)	0.1 (0.0;0.2)
Adjusted*	0.1 (0.0;0.2)	0.1 (0.0;0.2)	0.0 (-0.1;0.1)	0.0 (-0.1;0.2)	0.0 (-0.1;0.1)	0.1 (0.0;0.2)
Glucose (mmol/L)	0.0 (-0.1;0.1)	0.1 (-0.1;0.2)	0.0 (-0.1;0.2)	0.1 (0.0;0.2)	0.1 (-0.1;0.2)	0.1 (0.0;0.2)
Adjusted*	0.0 (-0.2;0.1)	0.1 (-0.1;0.3)	0.0 (-0.2;0.1)	0.0 (-0.1;0.1)	0.0 (-0.1;0.2)	0.1 (0.0;0.2)
Insulin (mU/L)	0.5 (-0.7;1.7)	0.0 (-3.9;3.8)	2.0 (0.7;3.3)	1.2 (-3.2;5.6)**	1.9 (0.8;3.1) **	6.4 (2.3;10.5) **
Adjusted*	-0.7 (-2.0;0.7)	1.6 (-0.3;3.5)	1.3 (-0.1;2.7)	-1.3 (-5.7;3.1) **	0.9 (-4.2;6.0) **	6.5 (2.2;10.7) **
HOMA-IR	0.1 (-0.2;0.5)	0.0 (-1.2;1.2)	0.7 (0.3;1.1)	0.3 (-1.1;1.7) **	0.4 (0.1;0.8) **	2.0 (0.7;3.4) **
Adjusted*	-0.2 (-0.6;0.2)	0.7 (0.1;1.3)	0.1 (-0.3;0.5)	-0.4 (-1.8;1.1) **	0.1 (-1.5;1.8) **	2.1 (0.7;3.5) **

Data were expressed as regression coefficient + 95% confidence interval. The standard deviation of the change in visceral fat was 87.4 grams in transwomen and 83.3 grams in transmen. The standard deviation of the change in total body fat was 4468 grams in transwomen and 4333 grams in transmen. The standard deviation of the change in total lean body mass was 2832 grams in transwomen and 2721 grams in transmen. Number of included transwomen: n=92 to n=171. Number of included transmen: n=55 to n=157.

* Analyses between change in visceral fat and risk factors were adjusted for change in total body fat and change in total lean body mass. Analyses on total body fat and risk factors were adjusted for change in visceral fat and change in total lean body mass. Analyses on total lean body mass and risk factors were adjusted for change in visceral fat and change in total body fat.

** Sensitivity analyses in transmen were performed in which one outlier was excluded. These data are shown in the result section.

Supplemental table 2. Changes in cardiovascular risk factors per one standard deviation of the mean change in visceral fat shown per BMI category (<25 kg/m², 25-30 kg/m², >30 kg/m²).

	TRANSWOMEN			**TRANSMEN**		
	BMI <25	*BMI 25-30*	*BMI>30*	*BMI<25*	*BMI 25-30*	*BMI>30*
Systolic blood pressure (mmHg)	-1 (-5;3)	2 (-2;7)	2 (-2;6)	2 (-2;6)	-1 (-5;3)	-2 (-4;1)
Adjusted*	-1 (-6;4)	1 (-4;6)	0 (-4;5)	0 (-5;6)	-2 (-7;3)	-3 (-6;1)
Diastolic blood pressure (mmHg)	0 (-3;3)	-2 (-5;1)	0 (-3;2)	3 (0;5)	-2 (-5;1)	1 (-1;3)
Adjusted*	0 (-4;4)	-3 (-6;0)	-1 (-5;2)	1 (-2;5)	-3 (-7;1)	2 (-1;5)
Total cholesterol (mmol/L)	0.3 (0.1;0.5)	0.1 (-0.1;0.3)	0.2 (0.0;0.3)	0.0 (-0.2;0.2)	0.2 (-0.1;0.4)	0.0 (-0.2;0.2)
Adjusted*	0.1 (-0.1;0.3)	0.1 (-0.2;0.3)	0.2 (0.0;0.4)	-0.2 (-0.4;0.1)	0.2 (-0.1;0.4)	0.1 (-0.2;0.3)
HDL cholesterol (mmol/L)	-0.1 (-0.1;0.0)	0.1 (0.0;0.1)	-0.1 (-0.1;0.0)	-0.1 (-0.2;0.1)	0.0 (-0.2;0.1)	0.0 (0.0;0.1)
Adjusted*	-0.1 (-0.2;0.0)	0.1 (0.0;0.2)	-0.1 (-0.2;0.0)	-0.1 (-0.2;0.1)	0.0 (-0.2;0.1)	0.0 (-0.1;0.1)
LDL cholesterol (mmol/L)	0.3 (0.1;0.4)	0.0 (-0.2;0.1)	0.2 (0.1;0.3)	0.1 (-0.1;0.2)	0.1 (-0.1;0.3)	-0.2 (-0.4;0.0)
Adjusted*	0.2 (0.0;0.3)	-0.1 (-0.2;0.1)	0.2 (0.1;0.4)	0.0 (-0.3;0.2)	0.2 (-0.1;0.4)	-0.1 (-0.4;0.2)
Triglycerides (mmol/L)	0.2 (0.1;0.3)	0.1 (-0.2;0.3)	0.1 (0.0;0.2)	0.0 (-0.2;0.1)	0.1 (-0.1;0.3)	0.1 (-0.1;0.4)
Adjusted*	0.1 (0.0;0.2)	0.1 (-0.2;0.3)	0.0 (-0.1;0.2)	-0.1 (-0.2;0.1)	0.0 (-0.2;0.3)	0.1 (-0.2;0.5)
Glucose (mmol/L)	-0.1 (-0.3;0.2)	0.1 (-0.1;0.3)	0.0 (-0.5;0.5)	0.0 (-0.3;0.2)	0.0 (-0.2;0.1)	0.1 (0.0;0.3)
Adjusted*	-0.1 (-0.4;0.2)	0.0 (-0.2;0.2)	0.1 (-0.5;0.6)	0.1 (-0.2;0.4)	-0.1 (-0.4;0.1)	0.0 (-0.2;0.2)
Insulin (mU/l)	-0.6 (-3.0;1.8)	0.6 (-1.5;2.7)	0.0 (-2.8;2.8)	-0.5 (-2.5;1.5)	-1.0 (-4.0;2.0)	0.2 (-12.6;12.9)**
Adjusted*	-1.4 (-4.6;1.8)	-0.5 (-2.6;1.7)	-0.6;-3.7;2.4)	-0.1 (-2.4;2.3)	-1.1 (-4.9;2.6)	-4.3 (-20.7;12.0)***
HOMA	-0.2 (-0.9;0.5)	0.1 (-0.5;0.7)	-0.3 (-1.9;1.3)	-0.2 (-0.7;0.4)	-0.3 (-1.0;0.5)	0.0 (-4.1;4.2)**
Adjusted*	-0.5 (-1.4;0.5)	-0.1 (-0.7;0.5)	-0.3 (-1.7;1.1)	0.0 (-0.6;0.6)	-0.3 (-1.2;0.6)	-1.5 (-7.2;4.1)***

Data were expressed as regression coefficient + 95% confidence interval. The standard deviation of the change in visceral fat was 87.4 grams in transwomen and 83.3 grams in transmen. Number of included transwomen: BMI<25: range from n=57 to n=106; BMI 25.1-30: n=26 to n=41; BMI>30: n=9 to n=24. Number of included transmen: BMI<25: range from n=27 to n=88; BMI 25.1-30: n=17 to n=34; BMI>30: n=11 to n=35.

* Analyses between change in visceral fat and risk factors were adjusted for change in total body fat and change in total lean body mass.

** After excluding one outlier; change in insulin (1.6, 95% CI -0.9;4.0), change in HOMA-IR (0.5, 95% CI -0.2;1.2)

*** After excluding one outlier; change in insulin (0.8, 95% CI -3.6;5.2), change in HOMA-IR (0.3, 95% CI -1.0;1.7)