Effects of Sex Hormone Treatment on the Metabolic Syndrome in Transgender Individuals: Focus on Metabolic Cytokines

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Context: Hormonal treatment in transgender persons affects many components of the metabolic syndrome (MS).

Objective: To determine the role of direct hormonal effects, changes in metabolic cytokines, and body composition on metabolic outcomes.

Design, Setting, and Participants: 24 transwomen and 45 transmen from the European Network for the Investigation of Gender Incongruence were investigated at baseline and after 12 months of hormonal therapy.

Outcome Measures: Best predictors for changes in components of MS, applying least absolute shrinkage and selection operator regression.

Results: In transwomen, a decrease in triglyceride levels was best explained by a decrease in fat mass and an increase in fibroblast growth factor 21 (FGF-21); the decrease in total and low-density lipoprotein cholesterol levels was principally due to a decrease in resistin. A decrease in high-density lipoprotein cholesterol depended on an inverse association with fat mass. In contrast, in transmen, an increase in low-density lipoprotein cholesterol was predicted by a decrease in FGF-21 and an increase in the waist/hip ratio; a decrease in the high-density lipoprotein/total cholesterol ratio depended on a decline in adiponectin levels. In transwomen, worsened insulin resistance and increased early insulin response seemed to be due to a direct treatment effect; however, improvements in hepatic insulin sensitivity in transmen were best predicted by a positive association with chemerin, resistin, and FGF-21 and were inversely related to changes in the waist/hip ratio and leptin and adipocyte fatty acid-binding protein levels.

Conclusions: The effects of hormonal therapy on different components of the MS are sex-specific and involve a complex interplay of direct hormonal effects, changes in body composition, and metabolic cytokine secretion. (***J Clin Endocrinol Metab*** **103: 790–802, 2018)**

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Abbreviations: AFABP, adipocyte fatty acid-binding protein; AUC, area under the curve; CPA, cyproterone acetate; E2, 17-β-estradiol; FGF-21, fibroblast growth factor 21; FSH, follicle-stimulating hormone; GAHT, gender-affirming hormone treatment; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-B, homeostasis model assessment of β-cell function; LASSO, least absolute shrinkage and selection operator; LDL, low-density lipoprotein; LH, luteinizing hormone; LPL, lipoprotein lipase; MCR, metabolic clearance rate; MS, metabolic syndrome; OGTT, oral glucose tolerance test; TC, total cholesterol; TG, triglyceride; WHR, waist/hip ratio.

Transgender individuals are characterized by an incongruence between gender identity and external sexual anatomy at birth. An etiological reason for this phenomenon has yet to be identified, although psychological and biological factors have been implicated (1, 2). To mitigate the feeling of gender dysphoria, interventions such as gender-affirming hormonal therapy (GAHT) and gender-affirming surgery are often applied during the medical care of transgender persons.

GAHT in transgender persons has enabled, to some extent, investigation of the physiological role of sex steroids, although these are only partially uncoupled from other sex-specific factors that could have an influence. Regarding the metabolism, it is already known that GAHT results in impressive changes in physical appearance toward the target sex (3, 4). For example, long-term testosterone treatment in transmen increases the visceral fat mass and decreases the subcutaneous fat mass (3). In addition, it has been repeatedly shown that the classic cardiovascular risk factors belonging to the metabolic syndrome (MS), such as lipid levels, approach those of the target sex. High-density lipoprotein (HDL) levels and, in particular, the HDL/low-density lipoprotein (LDL) ratio usually increase in transwomen and decrease in transmen (5–9). In addition, distinct changes occur in the glucose metabolism after GAHT in transgender individuals (6).

Previously, it was suggested that cytokines derived from adipose tissue (*i.e.,* adipokines) and/or from the liver (*i.e.,* hepatokines) influence facets of the MS. Thus, dysregulation of these cytokines, including adiponectin, leptin, fibroblast growth factor 21 (FGF-21), and adipocyte fatty acid-binding protein (AFABP), is associated with insulin resistance and dyslipidemia [reviewed by Fasshauer and Blüher (10)]. Many of these cytokines exhibit sexual dimorphism, and it has been demonstrated previously in small cohorts that GAHT can affect circulating levels of cytokines, such as adiponectin and leptin (11, 12).

However, the underlying mechanisms and consequences are poorly understood. Thus, the extent to which changes in metabolic cytokine concentrations contribute to the observed effects on components of the MS during GAHT, in addition to subsequent changes in body composition (*e.g.,* fat mass) and the direct effects of altered sex steroids, remains unknown. Furthermore, most studies have only investigated classical adipokines, including leptin and adiponectin, and have yet to explore novel adipose tissue-secreted proteins influencing aspects of the MS.

Therefore, the present research investigated a distinct set of novel and well-established metabolic cytokines that exhibit sexual dimorphism (13) and are associated with aspects of the MS within a prospective cohort of 69 transgender individuals before and 12 months after GAHT. The aim of the present study was to determine the effect of these cytokines on the metabolic phenotype of transgender individuals undergoing GAHT, in addition to any changes in body composition and the direct effects of treatment.

Patients and Methods

Patients

The present research forms part of the European Network for the Investigation of Gender Incongruence, a collaboration of four major West European gender identity clinics (Amsterdam, Ghent, Florence, and Oslo), and a study group created to achieve greater transparency in the diagnosis and treatment of gender dysphoria. Aspects of the study design have been previously reported (4, 14). All participants recruited for the present study received a diagnosis and were treated at the Department of Endocrinology, Ghent University Hospital, Belgium, from February 2010 to March 2014. One year of follow-up data from 57 transmen and 72 transwomen were available during the study period. Patients were only selected for the present analysis if they did not have dyslipidemia, diabetes mellitus, or glucose intolerance and had not been receiving any hormonal treatment at baseline. Twenty-one transmen were already receiving 5 mg lynestrenol daily (Orgametril) or taking hormonal contraceptives to stop their menstrual cycle and were therefore not selected for the present analysis. Patients with incomplete data on body composition at any of the relevant time points were also excluded.

Finally, a total cohort of 69 transgender individuals was available for the present analysis, including 45 transwomen (male to female) and 24 transmen (female to male), who were investigated at baseline and after 12 months of GAHT. Data on the oral glucose tolerance test (OGTT) and the calculated indexes for both time points in this sample were available for 38 transmen and 17 transwomen.

GAHT with 1000 mg of testosterone undecanoate (Nebido®; Jenapharm, Jena, Germany), administered every 3 months, was given to all transmen. In accordance with the Endocrine Society Guidelines (15), the chosen hormonal treatment of the transwomen was dependent on their age and included 50 mg of cyproterone acetate (CPA) administered once daily (Androcur®; Bayer, Leverkusen, Germany), in addition to 2 mg of estradiol valerate (Progynova®; Bayer) administered twice daily. Transwomen aged >45 years received 50 mg of CPA daily and a transdermal 17-β-estradiol (E2) patch releasing 100 μg every 24 hours (Dermestril®; Besins, Brussels, Belgium) (n = 17). All investigations were conducted by trained staff and included standardized questionnaires, anthropometric parameters [body mass index, waist/hip ratio (WHR), and a 75-g OGTT]. Body composition analysis was performed using dual-energy X-ray absorptiometry using a Hologic Discovery Machine (Hologic Inc., Bedford, MA). As described previously (16), the indexes of insulin sensitivity and β-cell function were calculated from the fasting and OGTT measurements of glucose and insulin.

The ethical review board of the Ghent University Hospital, Belgium approved the present research, which was conducted in

accordance with the Declaration of Helsinki. All participants gave written informed consent before inclusion. The present study is registered at www.ClinicalTrials.gov (ClinicalTrials.gov identifier, NCT01072825).

Assays

For all participants, serum samples at both time points (*i.e.*, baseline and 12-month follow-up point) were taken in the morning between 8:00 and 9:00 AM after an overnight fast. After a clotting period of 30 to 60 minutes, the serum was centrifuged and stored at −80°C until further analysis. E2 and testosterone were determined using liquid chromatography tandem mass spectrometry (AB Sciex 5500 triple quadrupole mass spectrometer; AB Sciex, Toronto, ON, Canada). Serum adipokine concentrations were determined using commercially available enzyme-linked immunosorbent assays in line with the manufacturers' instructions (adiponectin; Mediagnost, Reutlingen, Germany; leptin, progranulin, chemerin, resistin, FGF-21, and AFABP, BioVendor Inc., Brno, Czech Republic). Further immunoassays were used to determine the levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and sex hormone-binding globulin.

Routine parameters, including fasting glucose, glucose and insulin levels during the OGTT, total cholesterol (TC), HDL cholesterol, LDL cholesterol, and triglycerides (TGs), were measured using standard methods in a certified laboratory (Ghent University Hospital, Ghent, Belgium).

Statistical analysis

For statistical analysis of the anthropometric and serum data, SPSS software, version 24.0 (IBM, Armonk, NY), and R software, version 3.1.2 (17), were used. Data were examined for normality using quantile-quantile plots and, if skewed, were normalized by log transformation before further analysis. To evaluate the effects of 12 months of GAHT on the different outcome variables, mixed-effects models with repeated measures over time and incorporating a within participants design were used to compare the mean changes over time between the two groups. Most outcome parameters displayed a substantial time × sex interaction; therefore, longitudinal analyses using mixed models were performed separately for each sex. To ascertain whether changes in metabolic cytokine data occurred independently of the changes in body composition and differences in age between the transwomen and transmen, fat mass and age were used as covariates in a second mixed effects model, again performed separately for transmen and transwomen. In all the mixed models, participants were treated as a random effect. In the repeated statement, an unstructured covariance structure was used. The Spearman correlation was used to investigate univariate correlations between changes in metabolic outcomes, metabolic cytokines, and body composition.

Finally, to determine which factors best explained any shifts in metabolic parameters during the treatment, a variable selection approach was used on a change–change model. This initially required reducing the data to absolute changes from the baseline values. The derived variables were then subjected to a linear model with the change in metabolic parameters (*e.g.*, cholesterol) as an outcome variable, with changes in adipokines, lean body mass, fat mass, WHR, and age as predictor variables. Because the mode of estradiol administration in the transwomen was dependent on age, it was not adjusted for separately. However, several of the predictor variables showed strong correlations, violating the assumptions of simple linear regression. To accommodate this, least absolute shrinkage and selection operator (LASSO) regression (18) was chosen and applied. The optimal lambda was also selected, using leave-one-out cross-validation. Additionally, the LASSO penalizes unimportant predictors by applying zero coefficients and hence provides a powerful method for variable selection. Based on the selected variables, final simplified regression models were run to obtain P values and effect estimates. All statistical tests were performed with $\alpha \leq 0.05$ (two-tailed).

Results

General characteristics at baseline

At baseline, the transwomen were older ($P = 0.009$) than the transmen but did not differ in terms of any other anthropometric measure. The transmen seemed to be more active regarding sports activity than were the transwomen ($P = 0.010$), with no difference in other indicators of physical activity found. The baseline characteristics are listed in Table 1.

Adiponectin ($P = 0.047$), leptin ($P = 0.016$), resistin ($P = 0.013$), and AFABP ($P = 0.047$) levels were lower in the transwomen than in the transmen. No statistically significant differences were found for either sex regarding lipid parameters, although fasting glucose ($P = 0.030$) was higher and fasting insulin levels ($P = 0.001$) were lower in the transwomen. This also translated into a higher homeostasis model assessment (HOMA) of β-cell function (HOMA-B; $P = 0.001$) and lower HOMA of insulin resistance (HOMA-IR; $P = 0.001$).

Longitudinal analysis

Details of the longitudinal analysis are listed in Table 2.

Markers of the MS

Anthropometry. An increase in fat mass was found in the transwomen ($P < 0.001$) and a decrease in the transmen ($P = 0.048$). The lean mass increased in the transmen ($P = 0.049$) but remained unchanged in the transwomen. The hip circumference increased ($P = 0.049$) and WHR decreased ($P = 0.007$) in the transwomen, with no change found in the transmen. Although no substantial changes were found regarding physical activity measures in the transwomen, a substantial decrease regarding work and overall physical activity occurred in the transmen.

Blood pressure. A trend was seen toward an increase in systolic blood pressure in the transmen ($P = 0.065$). However, the diastolic blood pressure remained unchanged during the observation period for both sexes.

Table 1. Baseline Characteristics

Characteristic	Transwomen (Male to Female)		Transmen (Female to Male)		*P* Value[a]
	Mean ± SE	95% CI	Mean ± SE	95% CI	
Age, y	34.8 ± 1.4	NA	27.5 ± 1.3	NA	0.009[b]
Body composition					
Fat mass, kg	14.6 ± 1.0	12.6–16.7	19.0 ± 14.2	162–21.8	0.425
Lean mass, kg	59.5 ± 1.3	56.9–62.0	45.3 ± 17.4	41.8–48.7	0.385
Weight, kg	74.6 ± 2.1	70.3–78.9	65.0 ± 2.9	59.1–70.8	0.047[b]
BMI, kg/m^2	23.8 ± 0.7	22.5–25.2	24.0 ± 0.9	22.2–25.9	0.443
Waist, cm[c]	82.8 ± 1.7	79.5–86.2	76.0 ± 2.3	71.4–80.5	0.189
Hip, cm[c]	95.3 ± 1.3	92.7–98.0	97.8 ± 1.8	94.3–101.4	0.615
WHR	0.868 ± 0.012	0.845–0.892	0.776 ± 0.016	0.744–0.808	0.221
SBP, mm Hg	127.3 ± 2.2	123.0–131.6	109.8 ± 2.9	104.1–115.6	0.507
DBP, mm Hg	77.4 ± 1.6	74.1–80.6	70.6 ± 2.2	66.2–74.9	0.584
Adipokines					
Adiponectin, μg/L[c]	7517.2 ± 657.1	6204.4–8830.0	10,799.4 ± 940.3	8920.9–12,677.9	0.047[b]
Chemerin, μg/L	267.0 ± 7.1	252.8–281.3	276.7 ± 10.2	256.3–297.2	0.540
Resistin, μg/L	6.5 ± 0.3	5.8–7.1	7.7 ± 0.5	6.7–8.6	0.013[b]
Progranulin, μg/L	36.3 ± 1.0	34.3–38.4	36.8 ± 1.5	33.8–39.7	0.465
Leptin, μg/L[c]	3.4 ± 0.4	2.5–4.2	15.1 ± 2.9	9.2–21.1	0.016[b]
FGF-21, ng/L[c]	186.1 ± 23.8	138.6–233.6	146.2 ± 34.0	78.2–214.2	0.279
AFABP, μg/L[c]	16.0 ± 2.1	11.7–20.2	18.1 ± 3.1	12.0–24.2	0.047[b]
Sex hormones					
LH, U/L[c]	10.8 ± 1.2	2.9–7.7	10.8 ± 12.6	8.5–15.1	0.001[b]
FSH, U/L[c]	5.3 ± 1.1	3.2–7.4	6.7 ± 7.2	4.9–10.7	0.000[b]
E2, ng/L[c]	29.7 ± 6.6	16.5–42.8	123.1 ± 77.8	105.2–141.1	0.003[b]
Testosterone, ng/dL[c]	504.3 ± 23.6	457.3–551.3	44.2 ± 42.4	20.2–108.5	< 0.001[b]
SHBG, nmol/L[c]	39.6 ± 5.1	29.3–49.6	75.5 ± 45.5	61.9–89.1	0.310
Lipids					
TG, mg/dL[c]	117.7 ± 15.9	86.1–149.3	94.1 ± 22.8	48.8–139.4	0.486
TC, mg/dL	195.2 ± 5.8	183.7–206.7	175.4 ± 8.2	158.9–191.8	0.163
HDL, mg/dL	56.5 ± 2.1	52.4–60.6	59.2 ± 2.9	53.3–65.0	0.579
HDL, %	30.0 ± 1.4	27.3–32.7	35.3 ± 2.0	31.4–39.2	0.582
LDL, mg/dL[c]	115.0 ± 4.6	105.8–124.1	98.7 ± 6.5	85.6–111.7	0.120
Physical activity					
Sport	3.0 ± 0.2	2.6–3.3	2.4 ± 0.1	2.2–2.6	0.010[b]
Leisure time	2.9 ± 0.2	2.6–3.2	3.1 ± 0.1	2.9–3.2	0.478
Work	2.7 ± 0.1	2.4–3.0	2.7 ± 0.1	2.4–2.9	0.997
Total	8.6 ± 0.4	7.8–9.2	8.1 ± 0.2	7.7–8.6	0.303
Glucose metabolism, OGTT					
Glucose 0 min, mmol/L	4.9 ± 0.1	4.7–5.0	4.6 ± 0.6	4.4–4.8	0.030[b]
Glucose 30 min, mmol/L	8.7 ± 0.3	8.2–9.2	7.8 ± 1.6	7.1–8.5	0.668
Glucose 60 min, mmol/L	8.6 ± 0.3	7.9–9.3	7.5 ± 2.5	6.6–8.5	0.816
Glucose 120 min, mmol/L	6.0 ± 0.3	5.4–6.6	5.3 ± 1.9	4.5–6.1	0.228
Insulin 0 min, SI[c]	56.7 ± 5.5	45.7–67.8	79.1 ± 53.2	64.3–94.0	0.001[b]
Insulin 30 min, SI[c]	452.7 ± 39.6	373.6–531.8	475.0 ± 276.4	365.6–584.4	0.952
Insulin 60 min, SI[c]	628.4 ± 54.8	519.0–737.8	548.9 ± 248.6	400.8–697.1	0.176
Insulin 120 min, SI[c]	369.1 ± 40.5	288.3–449.9	512.2 ± 347.8	402.8–621.6	0.126
Indexes					
AUC glucose[c]	904.2 ± 27.1	850.0–958.4	799.4 ± 38.4	722.8–876.1	0.796
AUC insulin[c]	52,940.1 ± 4019.9	44,904.0–60,976.2	50,750.9 ± 5773.5	39,207.3–62,294.6	0.731
HOMA-IR[c]	1.7 ± 0.2	1.3–2.1	2.4 ± 0.3	1.8–3.0	0.001[b]
HOMA-B[c]	212.3 ± 25.0	162.2–262.4	170.9 ± 37.7	95.4–246.4	0.001[b]
HOMA-SEC[c]	152.8 ± 15.7	121.3–184.2	236.7 ± 22.0	192.7–280.7	0.675
Insulinogenic index[c]	145.6 ± 26.5	92.6–198.7	108.0 ± 40.1	27.5–188.4	0.297
Stumvoll MCR[c]	7.8 ± 0.3	7.1–8.5	7.3 ± 0.5	6.4–8.3	0.128

Abbreviations: CI, confidence interval; DBP, diastolic blood pressure; HOMA-SEC, HOMA of first-phase insulin secretion; NA, not applicable; SBP, systolic blood pressure; SHBG, sex hormone-binding globulin; SI, International System of Units.

[a]Student's *t* test.

[b]Statistically significant.

[c]Variables normalized by log-transformation before analysis.

Table 2. Longitudinal Analysis

	Transwomen (Male to Female) at 12 mo						
			Crude Analysis[a]		Adjusted[a,b]		
Variable	Mean ±SE	95% CI	F	*P* Value	F	*P* Value	Direction[c]
Body composition							
Fat mass, kg	18 ± 1.1	15.8 to 20.2	19.675	< 0.001[d]			↑
Lean mass, kg	57.5 ± 1.8	53.9 to 61.2	23.739	< 0.001[d]			↓
BMI, kg/m^2	24.2 ± 0.7	22.8 to 25.6	0.217	0.643			
Waist, cm	82.2 ± 1.9	78.7 to 86.2	0.163	0.688			
Hip, cm	98.7 ± 1.4	95.8 to 101.6	4.090	0.049[d]			↑
WHR	0.827 ± 0.01	0.8 to 0.853	7.931	0.007[d]			↓
SBP, mm Hg	120.6 ± 3	114.6 to 126.6	2.768	0.103			
DBP, mm Hg	75.7 ± 1.6	72.6 to 79.1	0.591	0.446			
Adipokines							
Adiponectin, μg/L	8604.9 ± 547	7513 to 9697	4.307	0.044[d]	4.294	0.044[d]	↑
Chemerin, μg/L	247.9 ± 11	225.9 to 269.9	4.642	0.037[d]	4.642	0.037[d]	↓
Resistin, μg/L	6.5 ± 0.3	5.9 to 7.2	0.083	0.774	0.083	0.774	
Progranulin, μg/L	33.4 ± 1.4	30.6 to 36.3	6.089	0.017[d]	4.297	0.053	↓
Leptin, μg/L	9.3 ± 1.2	6.9 to 11.6	6.089	< 0.001[d]	113.1	< 0.001[d]	↑
FGF-21, ng/L	137.3 ± 19.4	98.7 to 176	113.103	0.010[d]	7.142	0.010[d]	↓
AFABP, μg/L	14.8 ± 1.7	11.3 to 18.2	7.142	0.631	0.234	0.631	
Sex hormones							
LH, U/L	1.7 ± 2.1	−2.4 to 5.9	0.234	< 0.001[d]			↓
FSH, U/L	1.6 ± 2	−2.4 to 5.7	114.979	< 0.001[d]			↓
E2, ng/L	108.9 ± 16.2	76.6 to 141.1	130.199	< 0.001[d]			↑
Testosterone, ng/dL	53.8 ± 23.5	7.3 to 100.9	59.570	< 0.001[d]			↓
SHBG, nmol/L	40.7 ± 2.4	36 to 45.4	0.801	0.376			
Lipids							
TG, mg/dL	82.5 ± 17.5	48.2 to 116.7	9.143	0.004[d]			↓
TC, mg/dL	164.6 ± 5.2	154.3 to 174.9	42.200	< 0.001[d]			↓
HDL, mg/dL	48.2 ± 1.6	45 to 51.3	21.809	< 0.001[d]			↓
HDL, %	30.4 ± 1.2	28 to 32.7	0.336	0.565			
LDL, mg/dL	99.3 ± 4.5	90.4 to 108.2	27.532	< 0.001[d]			↓
Physical activity							
Sport	2.9 ± 0.2	2.5 to 3.2	2.9	0.618			
Leisure	3.0 ± 0.2	2.7 to 3.3	3.0	0.480			
Work	2.8 ± 0.1	2.5 to 3.0	2.8	0.538			
Total	8.7 ± 0.4	7.9 to 9.4	8.7	0.755			
Glucose metabolism[e]							
Glucose, mmol/L							
0 min	4.8 ± 0.1	4.6 to 5	1.350	0.252			
30 min	8 ± 0.3	7.3 to 8.7	3.509	0.068			
60 min	8 ± 0.3	7.3 to 8.6	3.006	0.091			
120 min	6.2 ± 0.3	5.7 to 6.8	0.438	0.511			
Insulin, SI							
0 min	78.9 ± 5.2	68.4 to 89.3	19.588	< 0.001[d]			↑
30 min	469 ± 39.6	397.4 to 556	0.528	0.472			
60 min	642.6 ± 53.8	529.8 to 744.9	0.421	0.520			
120 min	553.6 ± 52.2	448.7 to 657.7	17.342	< 0.001[d]			↑
Indexes[e]							
AUC glucose	851.4 ± 26.7	797.9 to 904.8	2.568	0.117			
AUC insulin	59,956 ± 4753	50,438 to 69,474	3.016	0.090			
HOMA-IR	2.5 ± 0.2	2 to 2.9	12.739	0.001[d]			↑
HOMA-B	132.1 ± 13.6	104.9 to 159.3	22.675	< 0.001[d]			↓
HOMA-SEC	245.5 ± 28.8	187.9 to 303.1	22.675	< 0.001[d]			↑
Insulinogenic index	119.8 ± 14.2	91.4 to 148.2	1.902	0.175			
Stumvoll MCR	6.5 ± 0.3	5.8 to 7.2	5.172	0.028[d]			↓

(*Continued*)

Lipids. In the transwomen, the TG levels ($P = 0.004$) and TC levels ($P < 0.001$) decreased, although they remained stable in the transmen. A statistically significant decrease was found in HDL in both sexes ($P < 0.001$ for both). However, although the HDL/TC ratio (HDL%) in the transwomen remained unchanged, it decreased in the

Table 2. Continued

	Transmen (Female to Male) at 12 mo						
			Crude Analysis[a]		Adjusted[a,b]		
Variable	**Mean ± SE**	**95% CI**	**F**	***P* Value**	**F**	***P* Value**	**Direction[c]**
Body composition							
Fat mass, kg	16.4 ± 15.4	14.3 to 20.4	4.380	0.048[d]			↓
Lean mass, kg	60.3 ± 25.5	56.5 to 66.6	26.641	< 0.001[d]			↑
BMI, kg/m^2	25.1 ± 1	23.1 to 27	4.352	0.048[d]			
Waist, cm	77.8 ± 2.6	72.5 to 82.9	1.990	0.171			
Hip, cm	97.5 ± 2	93.9 to 101.7	0.009	0.925			
WHR	0.795 ± 0.018	0.76 to 0.831	3.025	0.095			
SBP, mm Hg	117 ± 4.4	108.3 to 125.7	3.769	0.065			
DBP, mm Hg	74 ± 2.4	69.3 to 78.7	1.995	0.172			
Adipokines							
Adiponectin, μg/L	6142.5 ± 782.1	4580 to 7705	40.332	< 0.001[d]	35.735	< 0.001[d]	↓
Chemerin, μg/L	238.2 ± 15.7	206.8 to 269.7	6.181	0.020[d]	6.627	0.014[d]	↓
Resistin, μg/L	7.8 ± 0.5	6.9 to 8.8	0.168	0.686	0.012	0.915	
Progranulin, μg/L	34.9 ± 1	32.9 to 36.9	13.910	0.001[d]	12.017	0.002[d]	↓
Leptin, μg/L	6.5 ± 1.3	3.8 to 9.2	63.498	< 0.001[d]	76.419	< 0.001[d]	↓
FGF-21, ng/L	176.8 ± 27.7	121.4 to 232.1	0.164	0.689	0.302	0.588	
AFABP, μg/L	19.6 ± 2.5	14.6 to 24.5	0.291	0.595	0.277	0.604	
Sex hormones							
LH, U/L	11 ± 2.9	5.3 to 16.7	10.112	0.004[d]			↓
FSH, U/L	11.7 ± 2.8	6.1 to 17.2	1.492	0.235			
E2, ng/L	108.9 ± 22.7	6.3 to 96.5	19.864	< 0.001[d]			↓
Testosterone, ng/dL	656.6 ± 32.4	592 to 721.5	380.128	< 0.001[d]			↑
SHBG, nmol/L	40.7 ± 5.1	29.3 to 49.6	58.930	< 0.001[d]			↓
Lipids							
TG, mg/dL	90.3 ± 12	66.9 to 113.8	0.422	0.522			
TC, mg/dL	177.3 ± 7.5	162.4 to 192.3	0.129	0.723			
HDL, mg/dL	51 ± 2.3	46.4 to 55.6	20.579	< 0.001[d]			↓
HDL, %	29.8 ± 1.7	26.4 to 33.2	30.172	< 0.001[d]			↓
LDL, mg/dL	109.5 ± 6.4	96.8 to 122.3	7.871	0.01[d]			↑
Physical activity							
Sport	2.4 ± 0.1	2.1 to 2.6	0.358	0.553			
Leisure	3.0 ± 0.1	2.8 to 3.1	1.236	0.273			
Work	2.5 ± 0.1	2.2 to 2.7	5.653	0.022[d]			↓
Total	7.8 ± 0.2	7.3 to 8.2	5.521	0.022[d]			↓
Glucose metabolism[e]							
Glucose, mmol/L							
0 min	4.4 ± 0.1	4.2 to 4.7	1.258	0.273			
30 min	8.3 ± 8.3	7.4 to 9.2	0.689	0.415			
60 min	8.1 ± 0.5	7.2 to 9.1	0.683	0.417			
120 min	5.7 ± 0.4	4.9 to 6.4	0.285	0.599			
Insulin, SI							
0 min	51.9 ± 7.5	39.1 to 69.1	9.994	0.005[d]			↓
30 min	469.6 ± 55.8	369.9 to 593	0.045	0.834			
60 min	735.1 ± 76.9	596.8 to 903.8	9.896	0.005[d]			↓
120 min	463.3 ± 72.6	357 to 647.5	0.151	0.702			
Indexes[e]							
AUC glucose	885.7 ± 37.3	811 to 960.4	0.628	0.433			
AUC insulin	59,275.5 ± 7054.2	45,155.5 to 73,395.6	1.887	0.187			
HOMA-IR	1.5 ± 0.3	0.8 to 2.2	10.382	0.004[d]			↓
HOMA-B	132.1 ± 13.6	104.9 to 159.3	0.744	0.399			
HOMA-SEC	197.7 ± 43.4	187.9 to 303.1	0.744	0.399			
Insulinogenic index	133.6 ± 20.4	92.9 to 174.3	3.063	0.096			
Stumvoll MCR	7.3 ± 0.5	6.2 to 8.3	0.006	0.944			

Abbreviations: CI, confidence interval; DBP, diastolic blood pressure; HOMA-SEC, HOMA of first-phase insulin secretion; SBP, systolic blood pressure; SHBG, sex hormone-binding globulin; SI, International System of Units.

[a]Mixed model analysis.

[b]Adjusted for age and fat mass.

[c]Arrows indicate direction of statistically significant changes between baseline and 12 mo.

[d]Statistically significant.

[e]Data on OGTT and calculated indexes for both time points were available for 17 transmen and 38 transwomen.

transmen ($P < 0.001$). Furthermore, LDL cholesterol decreased in the transwomen ($P < 0.001$) and increased in the transmen ($P = 0.010$).

Glucose metabolism. An increase occurred in the fasting insulin levels ($P < 0.001$) in the transwomen, with a decrease in the transmen ($P = 0.005$). In contrast, neither fasting glucose nor the glucose and insulin levels during OGTT [area under the curve (AUC)] were affected by 12 months of GAHT for either sex. In transwomen, HOMA-IR ($P = 0.001$) and HOMA of first-phase insulin secretion ($P < 0.001$) increased. In contrast, a decrease occurred in the Stumvoll metabolic clearance rate (MCR; $P = 0.028$) and the HOMA-B ($P < 0.001$). In transmen, the HOMA-IR decreased ($P = 0.004$).

Sex hormones

A decrease occurred in LH levels in both sexes ($P < 0.001$ for transwomen; $P = 0.004$ for transmen), and FSH decreased in transwomen ($P < 0.001$) but not in transmen. As expected, an increase in E2 occurred in the transwomen and a decrease in the transmen ($P < 0.001$). In contrast, a decrease in testosterone occurred in the transwomen, with an increase in the transmen ($P < 0.001$). Also, a decrease occurred in sex hormone-binding globulin in the transmen ($P < 0.001$), with no statistically significant change in the transwomen ($P = 0.376$).

Metabolic cytokines

The adiponectin levels increased in the transwomen ($P = 0.044$) but strongly decreased in the transmen ($P < 0.001$). The chemerin levels decreased in both sexes ($P = 0.037$ for transwomen, $P = 0.020$ for transmen), but no change was found in resistin levels in either sex, with resistin levels greater in the transmen at both time points. Circulating progranulin decreased in transwomen ($P = 0.017$) and in transmen ($P = 0.001$). The FGF-21 serum concentration decreased in the transwomen ($P = 0.010$) but remained unchanged in the transmen. All statistically significant changes, except for the decrease in progranulin levels in transwomen, remained statistically significant after adjusting for age and fat mass. No relevant change was observable for AFABP (Fig. 1). Univariate correlations between the changes in metabolic outcome parameters and changes in metabolic cytokines and body composition are shown in Fig. 2.

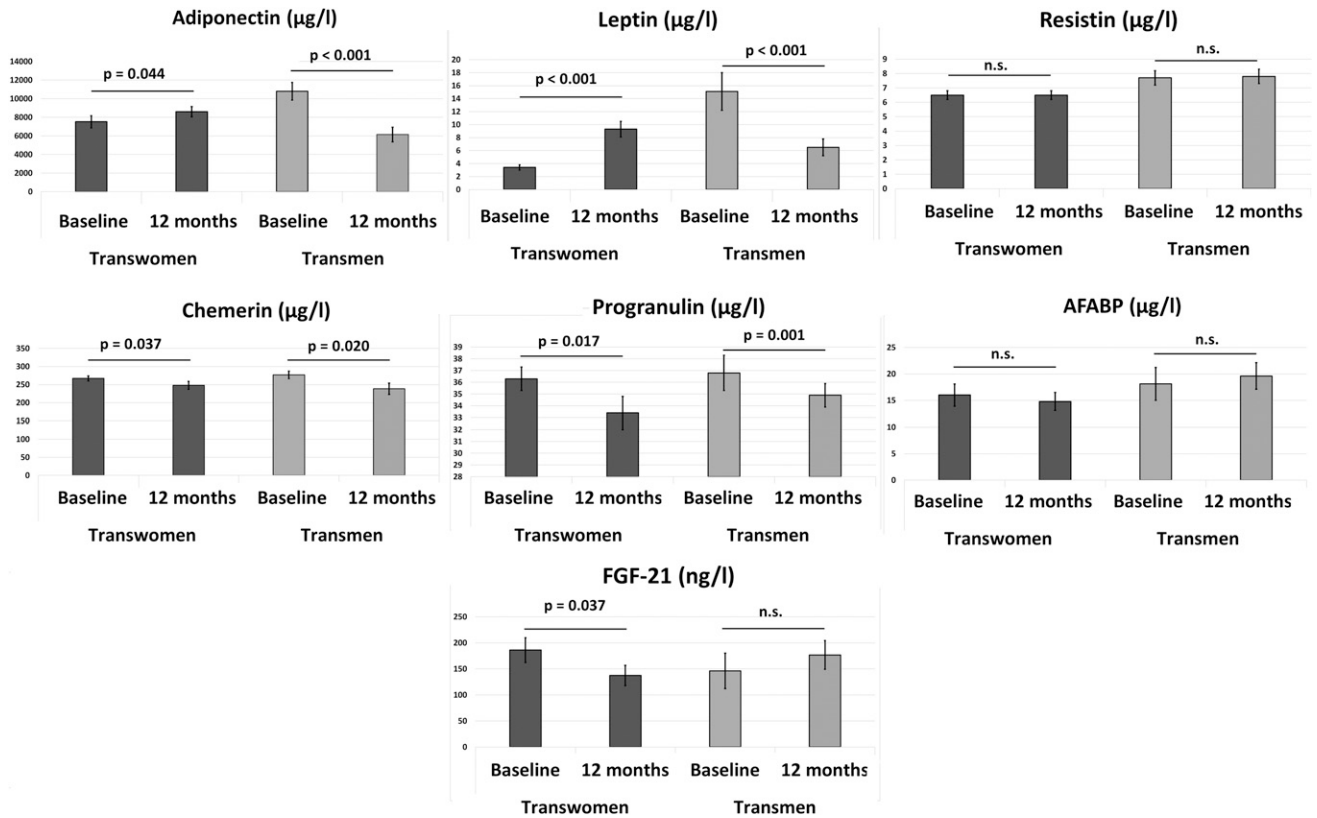

Figure 1. Changes in metabolic cytokines. Adiponectin levels increased in transwomen ($P = 0.044$) but strongly decreased in transmen ($P < 0.001$). A decrease was found in chemerin levels in both sexes ($P = 0.037$ for transwomen, $P = 0.020$ for transmen), with no change in resistin levels in either sex, with resistin levels higher in transmen at both time points. Circulating progranulin decreased in transwomen ($P = 0.017$) and in transmen ($P = 0.001$). FGF-21 serum concentrations decreased in transwomen ($P = 0.010$) but remained unchanged in transmen. All statistically significant changes, except for the decrease in progranulin levels in transwomen, remained statistically significant after adjusting for age and fat mass. No substantial change was observable for AFABP. Data presented as mean ± standard error of the mean.

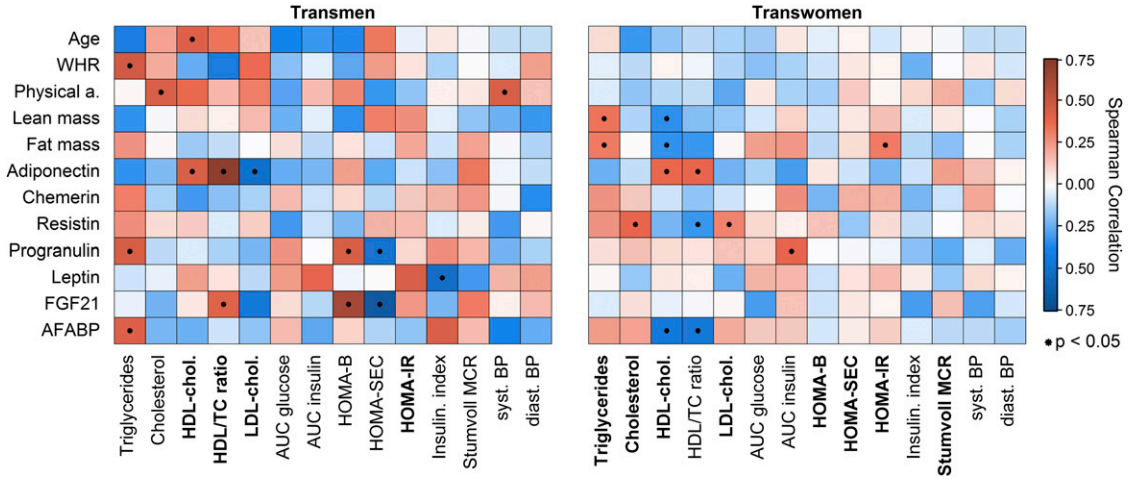

Figure 2. Univariate Spearman correlations of changes in potential predictors and changes in metabolic parameters. Heatmaps of univariate Spearman correlations of changes in potential predictors (*y*-axis) and changes in metabolic parameters (*x*-axis). Colors indicate either positive (red) or negative (blue) associations. Statistically significant correlations are indicated by black dots. Bold characters indicate statistically significant differences ($P < 0.05$) in metabolic parameters from baseline values.

Multivariate analysis of outcome variables by LASSO

As depicted in Fig. 3, most outcome variables were, as expected, strongly affected by their baseline values. The β and P values are listed in Supplement Tables 1 to 4.

Lipid parameters

In the adjusted models, in the transwomen, a decrease in TGs was associated with a decrease in fat mass (P = 0.01) and an increase in FGF-21 levels ($P < 0.001$), such that a decrease of 1 kg in fat body mass was associated with a decrease in TG levels of 4 mg/dL and an increase in 1 U of FGF-21 with a decrease of 0.1 mg/dL (Supplement Table 1).

In contrast, in the transmen, no substantial changes were seen in TGs during the observation period; however, a positive association was seen with changes in FGF-21 levels. Our model showed that a change in 1 U of FGF-21 would be paralleled by a change of 0.09 mg/dL in TG levels (P = 0.02). In addition, a positive association was found with AFABP levels, with a change of 1 U paralleled by a change of 2.5 mg/dL in TG levels (P = 0.04).

The relevant decreases in TC and LDL cholesterol levels in transwomen were positively associated with changes in resistin levels. A decrease of 1 U of resistin would result in a decrease of 5.2 mg/dL TC (P = 0.004) and 4.1 mg/dL LDL cholesterol (P = 0.005; Supplement Table 1). In contrast, an increase in LDL cholesterol in the transmen was dependent on a decrease in FGF-21 levels (-0.08 mg/dL/Δunit; $P < 0.001$), an increase in physical

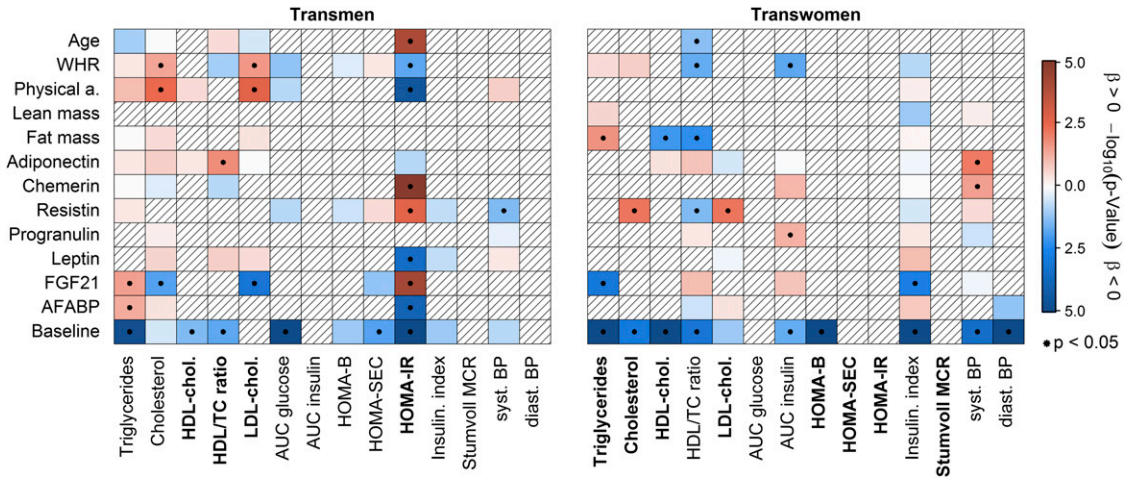

Figure 3. Variables selected as predictors from a LASSO to explain the change in metabolic parameters after treatment. Heatmaps of variables selected as predictors (*y*-axis) from a LASSO to explain the change in metabolic parameters (*x*-axis) after treatment of (Left) female to male or (Right) male to female. Colors indicate either positive (red) or negative (blue) associations. Gray shades indicate variables not selected. Statistically significant associations in the final model are indicated by black dots. Bold characters indicate statistically significant differences ($P < 0.05$) in metabolic parameters from baseline values.

activity (10.5 mg/dL/Δunit; $P = 0.001$), and an increase in the WHR (1.8 mg/dL/Δ0.01; $P = 0.02$).

The decrease in the HDL% in the transmen was best explained by the decrease in adiponectin levels (Fig. 2), which translated into a decrease of 0.6% per 1000 units ($P = 0.01$; Supplement Table 2). In the transwomen, HDL % did not change significantly, although it was influenced by a variety of independent variables in an inverse manner, namely resistin (−0.7%/Δunit; $P = 0.04$), fat mass (−0.53%/Δkg; $P = 0.005$), WHR (−0.2%/Δ0.01; $P = 0.02$), and age (−0.12/y; $P = 0.04$).

An increase in HDL in the transwomen was dependent on an inverse association with fat mass (−0.64 mg/dL/Δkg; $P = 0.007$). No good predictor for the decrease in HDL in the transmen could be identified.

Glucose metabolism

No relevant, independent predictors for the changes in HOMA-B, HOMA-IR, HOMA of first-phase insulin secretion, and Stumvoll MCR in the transwomen were identified. However, an inverse association was found of the insulinogenic index with the FGF-21 levels (−0.43/Δunit; $P = 0.002$) and a positive association was found of the insulin AUC with the progranulin levels (2378.3/Δunit; $P = 0.04$).

In the transmen, a statistically significant decrease in the HOMA-IR was predicted by a variety of changes in independent variables. A positive association was found with age (0.04/y; $P < 0.001$) and chemerin (0.006/Δunit; $P < 0.001$), resistin (0.08/Δunit; $P = 0.001$), and FGF-21 (0.003/Δunit; $P < 0.001$) levels and was inversely related to changes in WHR (−0.03/0.01; $P = 0.01$), physical activity (−0.42/Δunit; $P < 0.001$), leptin (−0.06/Δunit, $P < 0.001$), and AFABP (−0.06/Δunit; $P < 0.001$).

Blood pressure

Changes in systolic blood pressure were best explained by changes in resistin levels in the transmen (2.8 mm Hg/unit decrease in resistin levels; $P = 0.04$) and changes in adiponectin (3 mm Hg/Δ1000 units; $P = 0.005$) and chemerin levels (4 mm Hg/Δunit; $P = 0.02$) in the transwomen.

Discussion

Effect of GAHT on metabolic cytokine expression

From the findings, it is clear that GAHT resulted in a complete reversal of the observed sexual dimorphism for adiponectin and leptin, independent of any changes in anthropometry. This finding is in accordance with earlier studies in this population (12) and supported by the fact that testosterone and estradiol can directly regulate leptin and adiponectin secretion from adipose tissue samples in both women and men (19, 20).

In contrast, chemerin, progranulin, and FGF-21 levels did not differ between the transmen and transwomen at baseline. Although chemerin and progranulin had decreased after GAHT in both sexes, FGF-21 decreased only in the transwomen. Sexual dimorphism for resistin remained unaffected by 12 months of treatment, in line with findings from earlier studies (21) and indicating that sex steroids do not play a major role in its regulation. In contrast to previous studies of epidemiological samples (22), a sex difference was not observed. Furthermore, no change was seen over time in AFABP levels among our cohort, indicating that AFABP is not affected by GAHT in either sex.

All these metabolic cytokines have, in epidemiological studies, been associated with parameters of the MS (10, 23, 24) and showed several correspondingly relevant correlations on univariate analysis in our sample and also could explain the changes in the parameters of the MS.

Lipids

Substantial alterations in lipid profiles were observed in both sexes during treatment. The TC levels decreased in the transwomen, primarily owing to a reduction in LDL cholesterol. In contrast, the LDL cholesterol levels increased in the transmen, resulting in a decrease in the HDL/TC ratio among the members of this group. These findings are in accordance with those from earlier studies (9) and also with the gender dimorphism reported for lipoproteins in the general population (25).

The decrease in TGs in transwomen was best explained by a relative change in fat mass and FGF-21 levels, after accounting for other potential confounders. The positive association with fat mass is in accordance with studies showing that the secretion of TG-rich lipoproteins and their degradation is, among others, determined by lipoprotein lipase (LPL) in adipose tissue (26). A possible explanation for the negative effect of FGF-21 on TGs in our lean transwomen might be an FGF-21-dependent, accelerated lipoprotein catabolism in adipose tissues, thereby reducing TGs, such as has been demonstrated in mice (27). In contrast, our data suggest the opposite associations in our transmen cohort, in whom, although remaining stable during the observation period, FGF-21 was positively correlated with changes in TG levels. Our findings, therefore, might suggest a sex-dependent mechanism with regard to the metabolic effects of FGF-21. Larson *et al.* (28) have recently demonstrated in a rodent model that FGF-21 regulation and its metabolic effects are highly dependent on the sex steroid milieu. Furthermore, sex has been demonstrated as a major predictor of FGF-21 serum levels in cross-sectional data (29).

In addition, a positive association was found for AFABP levels in the transmen, with a change of 1 U paralleled by a change of 2.5 mg/dL in TG levels. This is supported by earlier epidemiological studies that found an independent positive association of AFABP and TGs (22, 30), although in one study this was only true for males (22).

The substantial decrease in TC and LDL cholesterol levels and the HDL% in the transwomen was positively associated with changes in resistin levels. This is in line with previous research demonstrating that resistin might reduce LDL cholesterol clearance by downregulating the hepatic LDL receptor, in part via proprotein convertase subtilisin/kexin type 9 (31). The increase in HDL cholesterol, in contrast, was dependent on a negative association with fat mass, a well-established association, and might among other mechanisms result from a decrease in plasma cholesteryl ester transfer protein expression (32).

However, an increase in LDL cholesterol levels in the transmen was dependent on a decrease in FGF-21 levels and an increase in physical activity and the WHR. It has been shown in rodent models that FGF-21 deficiency results in an increase in hepatic cholesterol biosynthesis and a shift from HDL to LDL, potentially again mediated via the proprotein convertase subtilisin/kexin type 9 pathway (33). The decrease in the HDL/TC ratio was best explained by a decrease in adiponectin levels, potentially mediated by adiponectin's effects on hepatic LPL activity (34).

It was not possible to identify an independent predictor for the decrease in HDL cholesterol in the transmen, indicating that those changes were directly attributable to GAHT, in line with earlier reports of the effects of exogenous androgen administration in hypogonadal men (35) and transmen (6) and, again, potentially mediated via increasing LPL activity.

Blood pressure

Although changes in blood pressure were not substantial across whole groups, individual changes could be explained by an inverse association with changes in resistin levels in the transmen and a positive association with adiponectin and chemerin levels in the transwomen. Previous studies have revealed that hypoadiponectinemia is an independent risk factor for arterial hypertension (27). However, the role of adiponectin in hypertension is not yet fully understood. Thus, an association between adiponectin multimer composition and hypertension has been suggested by Baumann *et al.* (36). Chemerin has been linked to hypertension in epidemiological samples (37), and preclinical data have indicated that it might be involved in amplifying sympathetic nerve-mediated arterial contractions (38).

The findings regarding resistin were, however, unexpected, because resistin has been linked to promoting hypertension, possibly via activation of the renin–angiotensin system (39).

Glucose metabolism

GAHT in the transwomen resulted in an increase in the markers of insulin resistance, first-phase insulin secretion, and a decrease in insulin sensitivity markers. In contrast, a substantial decrease in the HOMA-IR was found as a measure of insulin resistance in the transmen.

Most changes in parameters of glucose metabolism in the transwomen seemed to be directly attributable to the reversal in the sex steroid milieu and not via indirect treatment effects such as metabolic cytokine expression or changes in body composition.

Fasting glucose metabolism indexes such as the HOMA-IR predominately measure hepatic insulin sensitivity, but dynamic OGTT-based indexes such as the Stumvoll MCR measure both hepatic and muscle insulin sensitivity (40). Thus, these findings indicate that GAHT in the transwomen decreased hepatic and muscle insulin sensitivity, and testosterone treatment in the transmen improved hepatic insulin resistance. These findings are in accordance with earlier research of transgender individuals in whom E2 and CPA treatment increased fasting insulin and decreased glucose usage during a hyperinsulinemic euglycemic clamp, but the fasting glucose levels were unaffected (41).

In the transwomen, progranulin levels were positively associated with insulin AUC during the OGTT, and the insulinogenic index, as a measure of β-cell function, was negatively affected by changes in FGF-21. Although it is quite well-established that progranulin contributes to insulin resistance (42), a bidirectional link might exist between FGF-21 and glucose metabolism. Although FGF-21 has been shown to have protective effects on islet cell functioning and insulin secretion in chronic hyperglycemia in rodents (43), FGF-21 also serves as an independent predictor of the MS and type 2 diabetes mellitus in apparently healthy white individuals (44). Hypothetically, the negative association between FGF-21 and the insulinogenic index could represent a beneficial metabolic status of insulin sensitivity or, alternatively, FGF-21 resistance (45).

In the transmen, the substantial decrease in the HOMA-IR was determined by a variety of changes in body composition, metabolic cytokine expression, and behavioral measures, as indicated by the negative association with the individual's physical activity parameters. Although the positive association of chemerin, resistin, and FGF-21 with HOMA-IR indicate a negative effect of these cytokines on insulin resistance, the opposite was true for leptin and

AFABP. Leptin is an adipokine that reverses insulin resistance in metabolic disease states such as lipodystrophy (46). In contrast to leptin, the negative association of AFABP with HOMA-IR is counterintuitive, because AFABP is regarded an insulin resistance-inducing adipokine (47) and AFABP inhibition improves insulin sensitivity (48).

One strength of our research was that the findings were obtained from a well-defined cohort of transgender individuals undergoing a standardized protocol, including dynamic measures of glucose metabolism, body composition measurements, and liquid chromatography mass spectrometry sex steroid measurements. It could be argued, perhaps, regarding the burgeoning number of newly identified metabolic cytokines in recent years, that those investigated in our study represent only an arbitrary selection. However, to the best of our knowledge, ours is the first study of this type of population to investigate such parameters comprehensively concerning the contribution of these metabolic cytokines to sex steroid-driven metabolic regulation.

Nevertheless, the present study had some limitations that should be considered. First, the general transferability of the results to the general population, in terms of the effects of sex steroids on the outcomes investigated, could be limited. This is potentially because GAHT, for most transwomen, includes antiandrogenic co-medication. Therefore, it might be that some of the observed effects are not primary attributable to the effects of estradiol and/or androgen withdrawal but instead to the intrinsic effects of CPA. We could not exclude that the different routes of application of estradiol in the transwomen might have had an effect on the outcomes we investigated. Because the type of estradiol used was dependent on the age of the transwomen, we did not separately control for estradiol type. According to the published data, the dosages used in our study are comparable regarding overall E2 exposure (49). We also did not observe any relevant differences regarding serum steroid levels or FSH and LH as surrogate markers for adequate hormone substitution between the two groups (Supplemental Table 7).

Additionally, the cycle phase in the transmen group was not controlled, which might have further compromised the detection of clear hormonal effects. Future studies using larger samples should account for such differences. Finally, because the 2 groups were of unequal size (*i.e.*, more transmen than transwomen), we could not rule out that we missed some treatment effects in the smaller group owing to missing power.

Conclusions

One of the most in-depth analyses to date has, in our study, succeeded in further disentangling the direct and indirect effects of GAHT on the components of the MS in transgender individuals. Many effects of GAHT on the components of the MS seem to be directly attributable to changes in the sex steroid milieu. However, we also found indirect sex-specific effects involving mediators such as changes in body composition and metabolic cytokine secretion, or a combination of both of these factors.

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