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# Sex Steroids, Insulin, and Arterial Stiffness in Women and Men

Erik J. Giltay, Jan Lambert, Louis J.G. Gooren, Jolanda M.H. Elbers,  
Mieke Steyn, Coen D.A. Stehouwer

**Abstract**—Arterial stiffness may be influenced by sex steroids and insulin; the association with fasting insulin level may be stronger in women than in men. Therefore, we analyzed the effects of sex steroid administration on (1) arterial stiffness and (2) the relationship between fasting insulin level and arterial stiffness. Twelve male-to-female transsexuals were treated with ethinyl estradiol and cyproterone acetate, and 18 female-to-male transsexuals were treated with testosterone esters, with assessments made at baseline and after 4 and 12 months. Changes in distensibility and compliance coefficients (DC and CC, respectively) of the common carotid artery, femoral artery (FA), and brachial artery (BA) were analyzed in relation to changes in fasting plasma levels of glucose, insulin, HDL-cholesterol, and triglycerides. After 4 months of estrogens and antiandrogens in men, significant reductions in the CC and DC of the FA ( $P=0.006$  and  $P=0.04$ , respectively) and BA ( $P=0.04$  and  $P=0.04$ , respectively) were observed. In women, testosterone, on average, did not affect DC or CC, but the changes in fasting insulin level were strongly negatively associated with changes in the CC and DC, especially in the FA and BA. These associations were significantly less strong in genetic men and were independent of age, mean arterial pressure, and glucose and lipid levels. This experimental study shows (1) that short-term administration of estrogens and antiandrogens increases FA and BA stiffness in men and (2) that the fasting insulin level is a stronger determinant of arterial stiffness in women than in men. (*Hypertension*. 1999;34:590-597.)

**Key Words:** arteries ■ insulin ■ gender ■ estrogen ■ testosterone ■ coefficient, distensibility  
■ coefficient, compliance

Men are at a higher risk of developing cardiovascular disease (CVD) than women, but in non-insulin-dependent diabetes mellitus, this gender difference is much less pronounced.<sup>1-4</sup> A core element of non-insulin-dependent diabetes mellitus is insulin resistance, and the question arises of whether insulin resistance or other elements clustered in the insulin resistance syndrome,<sup>5</sup> such as hyperinsulinemia, glucose intolerance, dyslipidemia, abdominal obesity, and hypertension, partially negate the normal gender difference in cardiovascular risk.

Large-artery stiffening, a major determinant of cardiac workload and systolic blood pressure,<sup>6</sup> may contribute to the development of CVD. Data on a possible gender difference in arterial stiffness are contradictory. Measurement of pulse-wave velocity, an estimate of regional arterial stiffness, indicates that arteries in men are stiffer than those in premenopausal women.<sup>7-9</sup> In contrast, local arterial stiffness of the common carotid artery (CCA), measured by ultrasound,<sup>10-12</sup> and global vascular stiffness, derived from pulse-pressure waveform analysis,<sup>13</sup> occur less in men than in women. Conflicting data on the modulating effects of endog-

enous<sup>14</sup> and exogenous<sup>15-20</sup> estrogens on regional<sup>17-20</sup> and local arterial stiffness<sup>14-16</sup> in women have also been published. Currently, there is no prospective information on the effects of estrogen or testosterone administration on arterial stiffness in men or women.

Gender differences have been found in the interrelations between fasting insulin level and arterial stiffness in cross-sectional studies. A study of 4701 men and women showed that an 80% increase in fasting insulin level was associated with an increase of 5.1% in men and of 7.5% in women of Young's elastic modulus of the CCA, a measure of local arterial stiffness controlled for wall thickness.<sup>21</sup> In a previous cross-sectional study, we found that fasting insulin level was positively and glucose utilization was negatively associated with arterial stiffness of the femoral artery (FA) in women but not in men.<sup>12</sup> In this study, we investigated prospectively (1) the effects of cross-gender sex steroid administration on arterial stiffness indices of the CCA, FA, and brachial artery (BA) and (2) the influence of gender on the interrelationships between arterial stiffness and elements of insulin resistance syndrome.

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**TABLE 1. Endocrine Variables and Vessel Wall Properties Before and After 4 and 12 Months of Estrogen and Antiandrogen Administration in M→F Transsexual Subjects**

	Baseline	4 mo	12 mo	<i>P</i>
17β-Estradiol, pmol/L	97±11	*	*	
Testosterone, nmol/L	21±6	1.0±0.0	1.1±0.3	<0.001
LH, IU/L	4.3±4.9	0.3±0.1	0.4±0.2	0.003
FSH, IU/L	6.3±10.6	0.6±0.2	1.0±1.3	0.06
Heart rate, bpm	65±6	73±10	69±5	0.001
Systolic blood pressure, mm Hg	120±9	122±12	120±9	0.31
Diastolic blood pressure, mm Hg	65±8	65±9	66±7	0.97
MAP, mm Hg	86±8	87±8	85±8	0.34
Pulse pressure, mm Hg	54±6	57±8	54±6	0.22
CCA				
D, mm	6.1±0.5	6.1±0.5	6.0±0.5	0.28
CC, mm <sup>2</sup> /kPa	0.84±0.17	0.80±0.17	0.79±0.25	0.49
DC, 10 <sup>-3</sup> /kPa	29.3±6.7	28.1±8.1	27.5±6.4	0.59
FA (n=9)				
D, mm	7.3±1.2	7.3±1.2	7.1±1.2	0.24
CC, mm <sup>2</sup> /kPa	1.00±0.24	0.77±0.22	0.80±0.30	0.045
DC, 10 <sup>-3</sup> /kPa	24.8±6.0	19.3±8.0	21.1±8.8	0.09
BA				
D, mm	3.0±0.5	3.1±0.5	3.1±0.4	0.13
CC, mm <sup>2</sup> /kPa	0.28±0.07	0.24±0.06	0.26±0.06	0.16
DC, 10 <sup>-3</sup> /kPa	42.8±23.6	32.3±15.7	36.3±11.3	0.10
Body mass index, m/kg <sup>2</sup>	20.4±2.9	21.2±2.7	21.4±3.0	<0.001
Glucose, mmol/L	5.3±0.6	4.9±0.4	5.1±0.5	0.10
Insulin, pmol/L	43±13	53±13	56±19	0.02
HDL-C, mmol/L	1.1±0.4	1.1±0.3	1.2±0.4	0.27
Triglyceride, mmol/L	1.0±0.8	1.6±0.9	1.7±1.1	<0.001

N=12 unless otherwise indicated. Data are mean±SD. Only data on complete sets during the 3 time points are presented. *P* values determined by ANOVA for repeated measurements.

\*Male→female transsexuals were treated with ethinyl estradiol, which is not detected by the 17β-estradiol assay used.

## Methods

We included 14 white male-to-female (M→F) transsexuals (median age, 26 years; range, 18 to 45 years) and 18 white female-to-male (F→M) transsexuals (median age, 23 years; range, 17 to 40 years). Baseline data of 24 of these subjects were reported previously.<sup>12</sup> M→F transsexuals were treated with ethinyl estradiol (100 μg/d; Lynoral, Organon) in combination with the antiandrogen cyproterone acetate (100 mg/d; Androcur, Schering). F→M transsexuals were treated with testosterone esters (250 mg every 2 weeks IM; Sustanon, Organon). One F→M transsexual reported earlier intake of oral contraceptives; all other F→M transsexuals had had regular menstrual cycles (28 to 31 days) before cross-gender sex hormone administration. There was no evidence of hypertension, CVD, or use of other sex hormones. Eight M→F transsexuals and 12 F→M transsexuals were smokers. Standardized radioimmunoassays were

**TABLE 2. Endocrine Variables and Vessel Wall Properties Before and After 4 and 12 Months of Androgen Administration in F→M Transsexual Subjects**

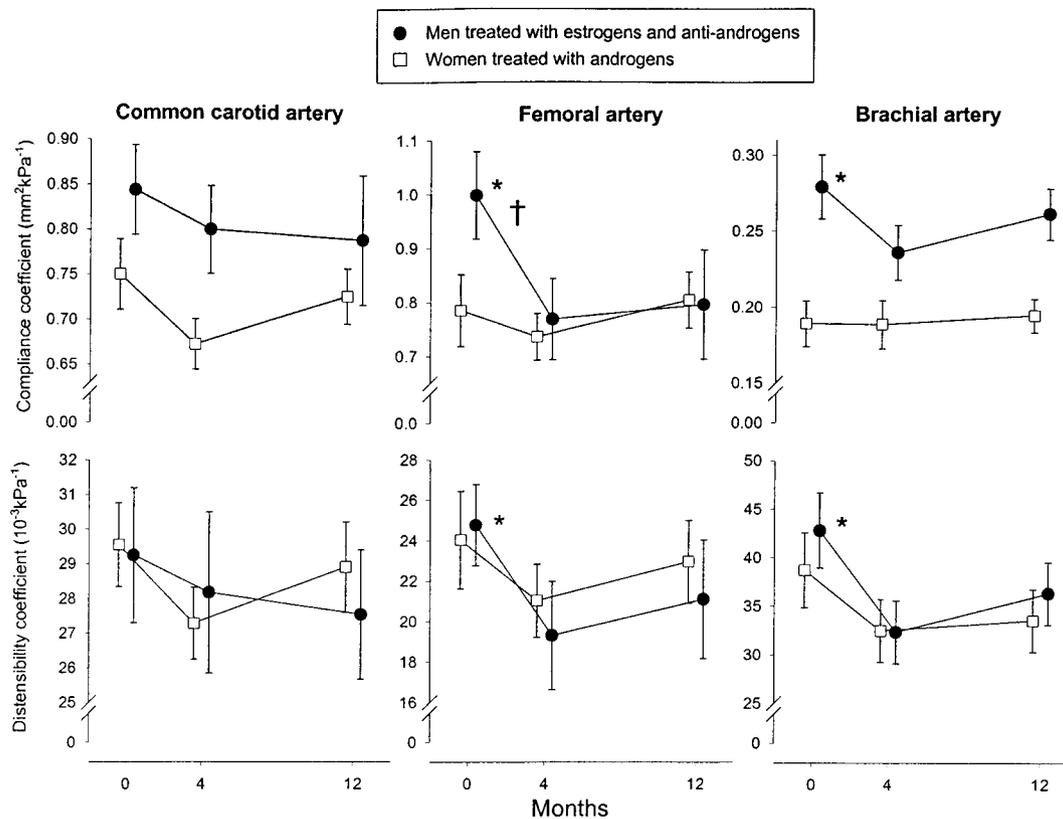
	Baseline	4 mo	12 mo	<i>P</i>
17β-Estradiol, pmol/L	212±150	127±33	131±36	0.01
Testosterone, nmol/L	1.6±0.5	26±16	30±14	<0.001
LH, IU/L	4.7±2.1	2.4±2.1	2.1±1.7	<0.001
FSH, IU/L	4.6±1.1	4.0±1.6	3.3±1.9	0.04
Heart rate, bpm	69±10	67±12	68±7	0.83
Systolic blood pressure, mm Hg	114±9	114±9	116±12	0.63
Diastolic blood pressure, mm Hg	62±6	61±6	61±7	0.58
MAP, mm Hg	82±7	80±8	82±8	0.56
Pulse pressure, mm Hg	52±6	54±5	55±6	0.054
CCA (n=17)				
D, mm	5.7±0.4	5.6±0.4	5.7±0.4	0.25
CC, mm <sup>2</sup> /kPa	0.75±0.16	0.67±0.11	0.73±0.13	0.14
DC, 10 <sup>-3</sup> /kPa	29.5±5.0	27.3±4.2	28.9±5.4	0.32
FA (n=17)				
D, mm	6.6±0.7	6.8±0.7	6.8±0.7	0.04
CC, mm <sup>2</sup> /kPa	0.79±0.27	0.74±0.18	0.80±0.21	0.44
DC, 10 <sup>-3</sup> /kPa	24.0±10.0	21.0±7.4	23.0±8.4	0.22
BA (n=17)				
D, mm	2.6±0.4	2.8±0.4	2.8±0.4	0.01
CC, mm <sup>2</sup> /kPa	0.19±0.06	0.19±0.07	0.19±0.05	0.89
DC, 10 <sup>-3</sup> /kPa	38.7±15.9	32.5±13.4	33.5±13.3	0.09
Body mass index, m/kg <sup>2</sup>	23.7±5.2	24.7±4.9	24.5±4.5	0.001
Glucose, mmol/L	4.9±0.6	4.3±0.7	4.8±0.6	0.001
Insulin, pmol/L	55±30	49±23	52±25	0.55
HDL-C, mmol/L	1.3±0.3	1.0±0.2	1.0±0.2	<0.001
Triglyceride, mmol/L	0.8±0.5	0.9±0.4	0.9±0.4	0.52

N=18 unless otherwise indicated. Data are mean±SD. Only data on complete sets during the 3 time points are presented. *P* values determined by ANOVA for repeated measurements.

used to measure serum levels of 17β-estradiol and testosterone. Serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by immunometric luminescence assays. Informed consent was obtained from all subjects, and the study was approved by the Ethical Review Committee of the University Hospital Vrije Universiteit.

## Hemodynamic Measurements

The distensibility coefficient (DC), reflecting intrinsic vascular wall elasticity, and the compliance coefficient (CC), reflecting buffering capacity of the vessel wall, were calculated from the arterial diameter (*D*) and changes in arterial diameter during the heart cycle ( $\delta D$ ; ie, distension) and pulse pressure ( $\delta P$ ) as follows:  $DC = (2 \times \delta D) / (D \times \delta P)$  and  $CC = (\pi \times \delta D \times D) / (2 \times \delta P)$ .



**Figure 1.** Plots showing the DC and CC (with bars representing SEM) of the CCA, FA, and BA at baseline and after 4 and 12 months in 12 M→F transsexuals receiving estrogens and antiandrogens and 18 F→M transsexuals receiving androgens. After 4 months of estrogen and antiandrogen administration, significant reductions, as compared with baseline, of CC and DC of the FA and BA were found. Interaction analysis showed that estrogenic and androgenic effects were not significantly different. \* $P < 0.05$  (paired sample  $t$  test comparing baseline and 4-month values). † $P < 0.05$  (ANOVA for repeated measurements comparing baseline and 4- and 12-month values).

All hemodynamic measurements were performed with use of a noninvasive ultrasound system as previously described<sup>11,12,22,23</sup> after at least 15 minutes of supine rest in a temperature-controlled, quiet room. All subjects refrained from smoking or consuming caffeine for at least 4 hours before examination. The within-subject coefficients of variation for DC and CC are 7.7% and 8.3%, respectively, for the CCA; 13.4% and 12.5%, respectively, for the FA; and 16.1% and 15.6%, respectively, for the BA.<sup>22,23</sup> The systolic, diastolic, pulse, and mean arterial pressure (MAP) were assessed 4 times at 5-minute intervals with an automatic oscillometric device (BP-8800, Colin) and then averaged. Twelve-month measurements were not obtained in 2 M→F transsexuals, and some other measurements could not be obtained successfully for practical reasons (detailed in Tables 1 to 3).

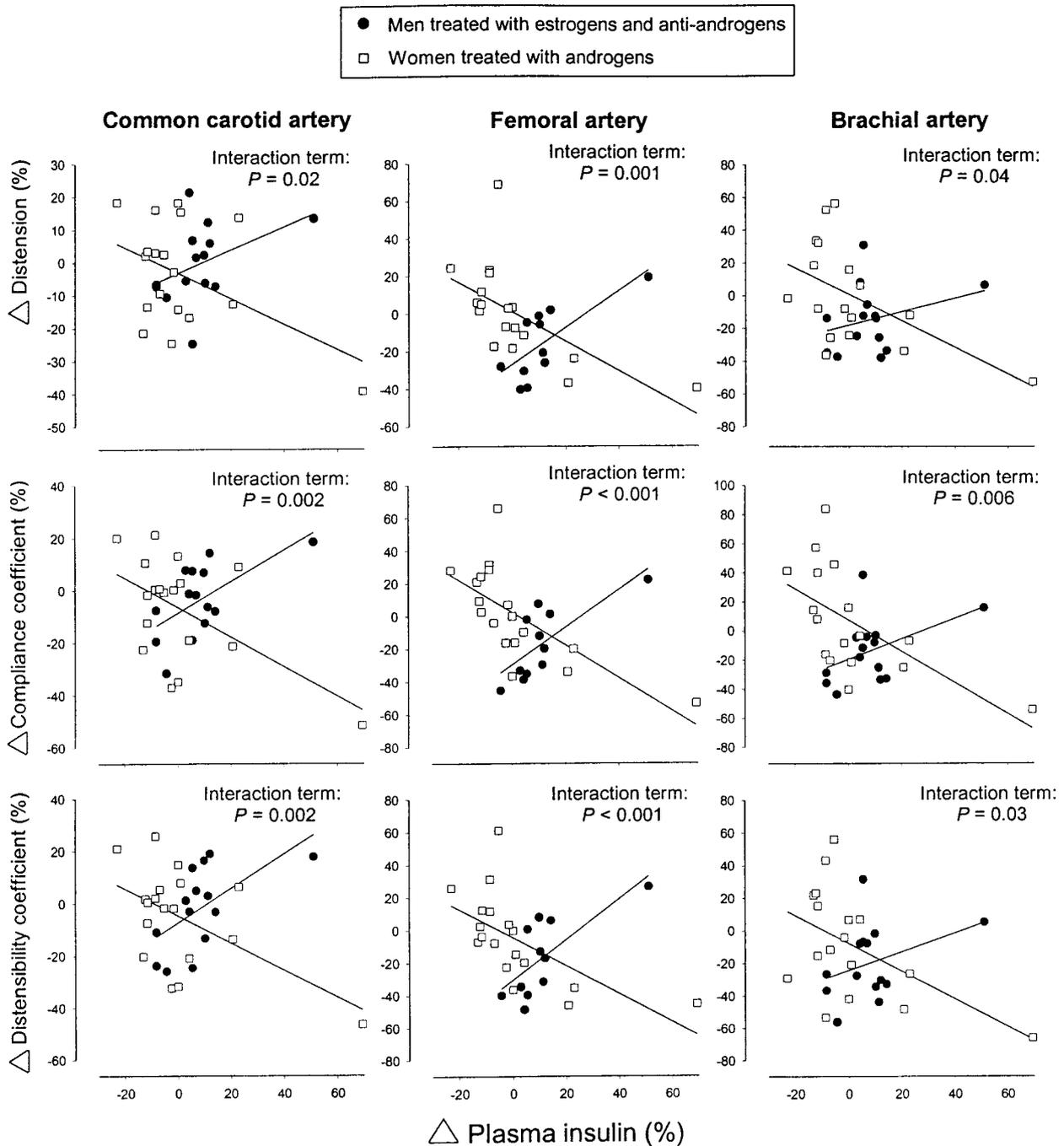
### Elements Clustered in the Insulin Resistance Syndrome

Fasting blood samples were obtained in all subjects to measure plasma levels of glucose, insulin (using an immunoradiometric assay, Biosource Diagnostics), HDL cholesterol (HDL-C), and triglycerides (using enzymatic colorimetric methods, Boehringer Mannheim). Body mass index was assessed ( $\text{weight}/\text{height}^2$ ), and lean body mass and total body fat were estimated using bioelectrical impedance analysis (BIA 101/S, RJL Systems). Body circumferences were measured in duplicate to calculate the waist-to-hip ratio. Areas of abdominal subcutaneous and visceral fat (using a magnetic resonance imaging technique) and glucose utilization rate ( $M$  [expressed as  $\text{mg glucose}/\text{kg lean body mass} \cdot \text{min}$ ]; with a 2-hour hyperinsulinemic euglycemic clamp) were assessed in 9 M→F

and 12 F→M transsexuals at baseline and after 12 months as previously described.<sup>12</sup>

### Statistical Analysis

Variables with skewed distributions (abdominal subcutaneous fat area and plasma levels of insulin and triglycerides) were logarithmically transformed before analysis to normalize their distributions. Student's  $t$  test for independent and paired samples was used to compare differences between men and women and between different artery sites. In the M→F and the F→M groups (analyzed separately), an ANOVA for repeated measurements was used to analyze the effects of cross-gender sex hormones. Interaction terms were included in an ANOVA to test whether the effects of cross-gender sex hormones on the CC and DC differed between genetic men and women. An ANCOVA for repeated measurements was used to analyze the influence of elements clustered in the insulin resistance syndrome on hemodynamic measurements at the 3 time points (with  $\epsilon^2$  as the measure of effect size). Univariate and bivariate linear regression analyses were used to explore interrelationships between proportional changes, at 4 months, of hemodynamic measurements and elements clustered in the insulin resistance syndrome. Interaction terms of genetic gender and insulin changes were included in linear regression analyses to test whether the associations of the proportional changes of hemodynamic measurements and fasting insulin levels differed between genetic men and women. If a value was below the lower limit of detection, that value was used for statistical calculations (for LH, 0.3 IU/L; FSH, 0.5 IU/L; 17 $\beta$ -estradiol, 90 pmol/L; and testosterone, 1.0 nmol/L).  $P < 0.05$  (2-way) was considered statistically significant. The software used was SPSS for Windows, version 8.0.



**Figure 2.** Scatter plots showing the association between the proportional changes, at 4 months vs baseline, of plasma insulin levels and those of  $\delta D$ , CC, and DC. Univariate regression lines in 14 M→F and in 18 F→M transsexuals are given. Interaction terms in linear regression analyses showed that the effects of cross-sex hormones in genetic men and women were all significantly different. When the 1 M→F transsexual and the 1 F→M transsexual with a proportional change in fasting insulin level >50% were excluded from the analyses, interaction terms were still statistically significant determinants for DC of the CCA and for  $\delta D$ , CC, and DC of the FA (for all,  $P < 0.05$ ), and there was a statistical trend for CC of the BA ( $P = 0.07$ ).

**Results**

**Pretreatment Values**

All subjects were eugonadal at baseline according to clinical and laboratory criteria. *D* and CC of the BA were statistically significantly larger in men than in women ( $P = 0.009$  and  $P = 0.001$ , respectively). No significant gender differences were found in other hemodynamic measurements, nor in smoking status, body mass index,

and plasma levels of glucose, insulin, triglyceride, and HDL-C (Tables 1 and 2).

**Effects of Cross-Gender Sex Hormone Administration**

After estrogen and antiandrogen administration to M→F transsexuals, serum levels of testosterone, LH, and FSH decreased (Table 1). In M→F transsexuals, 4 months of

**TABLE 3. Associations Between Proportional Changes, of 4 Months versus baseline, of D, CC, and DC and Proportional Changes of Elements Clustered in the Insulin Resistance Syndrome**

	CA				FA			
	n	D, %	CC, %	DC, %	n	D, %	CC, %	DC, %
<b>M→F transsexuals</b>								
MAP, %	14	0.10 (0.72)	0.16 (0.59)	0.13 (0.67)	11	0.71 (0.01)	-0.39 (0.24)	-0.48 (0.13)
Glucose, %	13	-0.32 (0.28)	0.39 (0.19)	0.49 (0.09)	10	0.37 (0.29)	0.06 (0.86)	-0.02 (0.95)
Insulin, %	14	-0.08 (0.78)	0.61 (0.02)	0.59 (0.03)	11	-0.19 (0.58)	0.75 (0.008)	0.73 (0.01)
HDL-C, %	14	0.08 (0.79)	-0.11 (0.70)	-0.12 (0.68)	11	-0.01 (0.98)	0.37 (0.27)	0.33 (0.32)
Triglyceride, %	14	-0.28 (0.34)	0.79 (0.001)	0.80 (0.001)	11	0.07 (0.83)	0.10 (0.76)	0.07 (0.83)
Body mass index, %	14	-0.21 (0.48)	0.46 (0.10)	0.47 (0.09)	11	-0.21 (0.53)	-0.12 (0.73)	-0.07 (0.85)
Waist-to-hip ratio, %	11	-0.28 (0.40)	0.32 (0.33)	0.38 (0.25)	9	0.36 (0.34)	-0.19 (0.63)	-0.25 (0.52)
Lean body mass, %	11	-0.02 (0.95)	0.36 (0.28)	0.31 (0.35)	9	0.35 (0.36)	-0.57 (0.11)	-0.58 (0.10)
Total body fat, %	11	-0.31 (0.35)	0.39 (0.24)	0.46 (0.16)	9	-0.49 (0.19)	0.60 (0.09)	0.64 (0.07)
<b>F→M transsexuals</b>								
MAP, %	18	-0.33 (0.18)	-0.49 (0.04)	-0.48 (0.045)	18	-0.05 (0.84)	-0.40 (0.10)	-0.41 (0.09)
Glucose, %	18	0.01 (0.99)	-0.17 (0.49)	-0.20 (0.44)	18	-0.30 (0.23)	-0.05 (0.85)	0.04 (0.89)
Insulin, %	18	-0.40 (0.10)	-0.57 (0.02)	-0.55 (0.02)	18	-0.24 (0.33)	-0.70 (0.001)	-0.64 (0.005)
HDL-C, %	18	0.10 (0.70)	0.06 (0.83)	0.02 (0.94)	18	-0.03 (0.89)	-0.17 (0.50)	-0.17 (0.51)
Triglyceride, %	18	-0.04 (0.89)	-0.21 (0.42)	-0.20 (0.43)	18	-0.07 (0.78)	-0.20 (0.44)	-0.17 (0.50)
Body mass index, %	18	0.07 (0.77)	0.18 (0.47)	0.19 (0.45)	18	-0.03 (0.90)	0.22 (0.38)	0.25 (0.31)
Waist-to-hip ratio, %	13	-0.02 (0.95)	-0.18 (0.56)	-0.18 (0.55)	13	0.29 (0.34)	-0.04 (0.89)	-0.18 (0.55)
Lean body mass, %	13	-0.05 (0.86)	0.28 (0.35)	0.31 (0.31)	13	-0.18 (0.55)	0.56 (0.048)	0.62 (0.03)
Total body fat, %	13	-0.02 (0.96)	-0.28 (0.36)	-0.27 (0.37)	13	-0.04 (0.89)	-0.13 (0.66)	-0.09 (0.78)

Values are standardized regression coefficients ( $\beta$ ), with  $P$  value between brackets, determined with univariate regression analysis.

administration of estrogens and antiandrogens, as compared with baseline, was associated with significant reductions of  $\delta D$ , CC, and DC of the FA ( $P=0.02$ ,  $P=0.006$ , and  $P=0.04$ , respectively) and BA ( $P=0.01$ ,  $P=0.04$ , and  $P=0.04$ , respectively; Figure 1) and a significant increase in heart rate ( $P=0.005$ ; Table 1). The proportional changes, at 4 months, in  $\delta D$  of the FA and BA were significantly different than the change in  $\delta D$  of the CCA ( $P=0.047$  and  $P=0.01$ , respectively), whereas changes in  $\delta D$  were similar for the FA and BA ( $P=0.85$ ). The proportional changes, at 4 months, in heart rate were not significantly associated with any proportional change of the DC or CC of the CCA, FA, and BA, except for the DC of the FA ( $\beta = -0.64$ ,  $P=0.04$ ). After 12 months, the CC of the FA remained significantly decreased ( $P=0.03$ ; Figure 1) and heart rate remained significantly increased ( $P=0.02$ ) as compared with baseline values. No significant changes were seen in  $D$  values (Table 1).

After testosterone administration to F→M transsexuals, the serum testosterone level increased markedly, whereas serum levels of  $17\beta$ -estradiol, LH, and FSH decreased only slightly (Table 2). The CC and DC of the CCA, FA, and BA did not change significantly over time (Figure 1). The  $D$  values of the FA and BA increased significantly after 4 and 12 months of androgen administration in an ANOVA for repeated measurements (Table 2). Interaction terms in an ANOVA for repeated measurements showed that the effects on CC and DC did not differ significantly between administration of estrogens+antiandrogens or androgens (for all,  $P\geq 0.23$ ; Figure 1), except for the CC of the FA, which tended

to decrease in genetic men as compared with genetic women ( $P=0.07$ ).

### Associations With Elements Clustered in the Insulin Resistance Syndrome

Table 3 shows the associations between proportional changes, at 4 months, of  $D$ , CC, and DC with proportional changes of elements clustered in the insulin resistance syndrome. The proportional change of fasting insulin level was the most robust determinant of the proportional changes of  $\delta D$ , CC, and DC, with positive associations in M→F transsexuals and negative associations in F→M transsexuals (Table 3 and Figure 2). Interaction analysis showed that these associations differed significantly between the genetic sexes (Figure 2). Associations of proportional changes of insulin level with those of the CC and DC of the FA in M→F transsexuals and with those of the CC and DC of the FA and the CC of the BA in F→M transsexuals were independent of smoking status, age, and proportional changes of MAP, body mass index, and glucose, HDL-C, and triglyceride levels. To establish this, several bivariate regression analyses were performed with proportional changes of the CC or DC as the dependent variable and with proportional changes of fasting insulin level combined with each of the possible confounding variables as the 2 independent variables.

When both the M→F and F→M transsexuals with a proportional change in fasting insulin level  $>50\%$  (Figure 2) were excluded from the analyses, the results were similar: Proportional changes in fasting insulin level were still posi-

TABLE 3. Continued

n	BA		
	D, %	CC, %	DC, %
14	-0.38 (0.18)	0.09 (0.77)	0.29 (0.32)
13	-0.16 (0.60)	0.60 (0.03)*	0.62 (0.03)*
14	0.05 (0.87)	0.45 (0.11)	0.36 (0.21)
14	0.26 (0.37)	0.27 (0.34)	0.09 (0.76)
14	0.19 (0.52)	0.32 (0.27)	0.16 (0.58)
14	0.03 (0.91)	0.28 (0.33)	0.23 (0.43)
11	0.06 (0.86)	0.32 (0.34)	0.30 (0.38)
11	0.24 (0.47)	0.35 (0.29)	0.18 (0.60)
11	0.05 (0.89)	0.30 (0.37)	0.25 (0.45)
17	0.06 (0.82)	-0.34 (0.18)	-0.33 (0.20)
17	-0.22 (0.41)	0.07 (0.80)	0.13 (0.62)
17	-0.00 (1.00)	-0.61 (0.009)*	-0.53 (0.03)*
17	0.37 (0.15)	-0.07 (0.79)	-0.24 (0.36)
17	-0.52 (0.03)*	-0.20 (0.44)	0.11 (0.67)
17	0.25 (0.34)	0.35 (0.17)	0.15 (0.56)
13	-0.13 (0.68)	-0.22 (0.46)	-0.14 (0.65)
13	0.60 (0.03)*	0.58 (0.04)*	0.15 (0.63)
13	-0.32 (0.28)	-0.02 (0.95)	0.25 (0.41)

tively associated with those of the CC of the FA ( $\beta=0.64$ ,  $P=0.048$ ) in M→F transsexuals and negatively associated with those of the CC and DC of the FA ( $\beta=-0.72$ ,  $P=0.001$  and  $\beta=-0.64$ ,  $P=0.006$ , respectively) and of the CC of the BA ( $\beta=-0.56$ ,  $P=0.02$ ) in F→M transsexuals.

ANCOVA of values at baseline and after 4 and 12 months of cross-gender sex hormone administration was used to analyze the variability in DC and CC explained by the fasting insulin level. In M→F transsexuals, fasting insulin level significantly determined the variability of the CC and DC of the CCA ( $\epsilon^2=0.25$ ,  $P=0.04$  and  $\epsilon^2=0.35$ ,  $P=0.003$ , respectively). In F→M transsexuals, fasting insulin level significantly determined the variability of the CC of the CCA ( $\epsilon^2=0.15$ ,  $P=0.02$ ), CC and DC of the FA ( $\epsilon^2=0.27$ ,  $P=0.002$  and  $\epsilon^2=0.15$ ,  $P=0.03$ , respectively), and CC and DC of the BA ( $\epsilon^2=0.35$ ,  $P<0.001$  and  $\epsilon^2=0.29$ ,  $P=0.001$ , respectively). Visual interpretation of relationships between fasting insulin level and the CC and DC indicated that these were largely positively related in genetic men and inversely related in genetic women, both before and during hormone administration (data not shown).

With the use of ANCOVA, we also analyzed whether the variability of  $D$ , DC, or CC could be explained by glucose utilization, the abdominal subcutaneous fat area, or the visceral fat area. None of these covariables could significantly explain the variability of the hemodynamic measurements of the CCA, FA, or BA (data not shown).

### Discussion

We prospectively studied the effects of cross-gender sex steroid administration on arterial stiffness indices in healthy

women and men. Because of the heterogeneity of the arterial tree, we studied 3 sites. On the basis of the elastin, collagen, and smooth muscle content in the vessel wall, the CCA is regarded as an elastic vessel, whereas the FA and BA are regarded as muscular vessels.<sup>6</sup> Our findings suggest that administration of high-dose estrogens and antiandrogens to young men may have short-term deteriorating effects on FA and BA compliance and distensibility. Estrogenic effects on arterial stiffness may partly account for the somewhat increased short-term risk of CVD associated with relatively high-dose estrogen administration in men with prostate cancer<sup>24</sup> or myocardial infarction.<sup>25</sup> In addition, administration of estrogens and progestogens to postmenopausal women with<sup>26</sup> or without<sup>27</sup> coronary disease does not decrease and may even increase the short-term risk of CVD, which contradicts epidemiological data.<sup>28</sup> In genetic women, we found that androgen administration did not affect arterial stiffness, whereas the  $D$  of the FA and BA increased, which may be an adaptation to increased water retention<sup>29</sup> and muscle mass. An increased muscle mass might affect the  $D$  of the FA and BA more than that of the CCA, because the FA and BA supply blood to muscular territories.

In previous cross-sectional studies, postmenopausal estrogen replacement therapy was associated with higher CCA distensibility<sup>15,16</sup> and lower aortofemoral and leg pulse-wave velocity,<sup>18</sup> whereas no differences were found in brachial and aortodorsalis pulse-wave velocity<sup>19</sup> and CCA distensibility<sup>16</sup> with estrogen-progesterone combination therapy. Furthermore, natural fluctuations of estrogen levels during the menstrual cycle did not influence the CC and DC of the CCA and FA,<sup>14</sup> and long-term use of the synthetic steroid tibolone did not influence aortic pulse-wave velocity.<sup>17</sup> A longitudinal study showed that short-term withdrawal of estrogen-containing therapy, either alone or combined with progesterone, in postmenopausal women decreased systemic arterial compliance and increased leg pulse-wave velocity, both of which were restored to baseline values after reinstatement of therapy.<sup>20</sup>

At baseline, we found a higher CC in men than in women, which was significant for the BA and nonsignificant for the FA and CCA (48%, 27%, and 13%, respectively) and which may have been due to the relatively larger  $D$  that is part of the numerator of the equation for CC (Tables 1 and 2). Larger artery calibers and a lower heart rate<sup>13</sup> in men as compared with women may be related to the greater body height of men.<sup>12,30</sup>

### Possible Determinants of Changes in Arterial Stiffness

The mechanism of action of sex steroids on arterial stiffness is not well understood. First, estrogens could act directly on vascular smooth muscle and endothelial cells, which contain estrogen receptors.<sup>31,32</sup> Second, estrogens,<sup>33</sup> like androgens,<sup>29</sup> increase body water retention. Estrogens also increase arginine vasopressin release,<sup>33</sup> myocardial contractility, and stroke volume<sup>34</sup> and, in our subjects, increased heart rate. This possibly reduced stroke volume as well as the time interval in which the pulsatile pressure expands the arterial wall. Because of the dynamic viscoelastic properties of the vessel wall, the maximum increase in  $D$  falls as a conse-

quence of increased heart rate. This could have led to a decreased  $\delta D$  value at 4 months, as was found in the FA and BA. In anesthetized rats, it has been shown that acute increases in heart rate are accompanied by reductions in arterial compliance and distensibility.<sup>35</sup>

Third, the effects may be mediated by an action on insulin sensitivity or circulating insulin. Estrogens may induce insulin resistance<sup>36</sup> and, in our subjects, increased the fasting insulin level, which is a fair marker of insulin resistance among subjects with normal glucose tolerance.<sup>37</sup> We previously concluded that arterial stiffening in women is positively associated with fasting insulin levels or insulin resistance,<sup>12</sup> which is supported by the present experimental data. Insulin resistance may lead to a disturbance of cellular cation transport (or be a marker thereof), which may promote vasoconstriction and arterial stiffening,<sup>38</sup> affecting the muscular FA and BA more than the elastic CCA. However, insulin may also directly affect the vessel wall. Insulin induces hypertrophy of vascular smooth muscle in vitro<sup>39</sup>; insulin receptors have been found in the arterial wall<sup>40</sup>; and CCA wall thickness, which is inversely associated with CCA distensibility,<sup>16</sup> is positively related to insulin level in vivo.<sup>41</sup>

The contrasting associations in men and women between fasting insulin level and arterial stiffness suggest that being a genetic woman (ie, the presence of 2 X chromosomes) determines the relationship between fasting insulin level and arterial stiffness. Alternatively, the presence of circulating estrogens, which were not substantially reduced in genetic women (cf Table 2), may be the basis for the stronger relationship between fasting insulin level and arterial stiffness in women than in men. Our findings may be relevant to the observation that diabetic women, as compared with diabetic men, are at a relatively higher risk of developing CVD<sup>1,2</sup> and have a worse prognosis after myocardial infarction.<sup>3,4</sup>

### Limitations and Strengths of Study

Our analyses were limited by the fact that because of the nature of the treatment indication, we could include only relatively small numbers of subjects, did not include a control group, and used an open study design. However, a regression toward the mean effect seems unlikely with regard to the effects on FA and BA stiffness in men treated with estrogens and antiandrogens, because shifts in FA and BA distension differed significantly from those of the CCA. Local pulse pressure was not measured, but BA pulse pressure did not appear to modulate the association between fasting insulin level and arterial stiffness, as illustrated by the significantly stronger associations in women than in men between shifts of fasting insulin level and locally measured  $\delta D$  (not corrected for  $\delta P$  and  $D$ ). We applied statistical analyses many times by including several covariates, without correcting for their multiplicity. However, our main finding, the association between fasting insulin level and arterial stiffness, was strong, consistent in the 2 muscular arteries, robust during and after sex steroid challenges, and significantly different between the genetic sexes. Moreover, this is, to the best of our knowledge, the first report from an experimental study of the stronger association in women than in men between muscular

artery stiffness and fasting insulin level, which has been reported in cross-sectional studies.<sup>12,21</sup>

### Conclusions

Estrogen and antiandrogen administration for 4 months increases FA and BA stiffness in men, whereas the effects wear off somewhat after 12 months. In women, testosterone administration did not affect CC or DC, but the proportional changes of fasting insulin level were strongly negatively associated with the proportional changes of CC and DC. These associations were consistent in the FA and BA, were significantly less strong in genetic men, and were independent of age, MAP, and glucose and lipid levels. Fasting insulin level in the presence of estrogens may be a stronger determinant of arterial stiffness in women than in men.

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