Cardiovascular disease in transsexual persons treated with cross-sex hormones: reversal of the traditional sex difference in cardiovascular disease pattern

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Abstract

Objective: The incidence of heart disease increases with age, but is lower in women than in men up to 75 years. A protective effect of female sex hormones or, alternatively, acceleration in male heart disease by testosterone at younger ages, could explain this sex difference. In contrast with the above, male-to-female transsexual subjects (MtoF) treated with estrogens (+ anti-androgens) show more cardiovascular pathology than female-to-male transsexual subjects (FtoM) receiving testosterone. Why MtoF suffer more frequently from cardiovascular disease than females is as yet unclear. The mode of cross-sex hormone treatment may be a factor, and, if so, it may need adaptations.

Subjects and methods: Studies in transsexual people on the effects of cross-sex hormone treatment on surrogate cardiovascular risks and on clinical endpoints were reviewed. With regard to MtoF, a parallel was sought with men with prostate cancer, undergoing androgen deprivation and estrogen administration.

Results: Exposure of FtoM to testosterone was not associated with a strong increase in cardiovascular events. Aging and pre-existing cardiovascular pathology contributed to the risk of cardiovascular disease in MtoF. Use of the synthetic biopotent compound ethinyl estradiol in a dose two to four times of oral contraceptives increased cardiovascular risk substantially. The route of administration of estrogens (oral vs transdermal) may have impacted on the risks.

Conclusion: MtoF should not be treated with oral ethinyl estradiol. Transdermal estrogens are probably safer than oral estrogens. Pre-existing cardiovascular risks should be taken into consideration when prescribing and choosing the type of estrogens in cross-sex hormone administration (oral vs transdermal). In addition, risk factors, as they emerge with aging, should be addressed.

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Introduction

This contribution reviews the, at first sight somewhat unexpected, finding that in transsexual subjects cardiovascular pathology is more prevalent in male-to-female transsexual subjects (MtoF) receiving treatment with estrogen (+ anti-androgens, sometimes with progestational properties) than in female-to-male transsexual subjects (FtoM) receiving testosterone. This contrasts

with the general population wherein, at all ages, men, at least up to about 65–75 years of age, have a survival disadvantage relative to women, usually attributed to more cardiovascular pathology in men, while the latter is also occurring at a younger age in men.

The present analysis started with the underlying assumption that cardiovascular disease patterns of MtoF

treated with estrogens show similarities with those of women and, conversely, patterns of FtoM treated with testosterone similarities to men's patterns. However, this is not the case in transsexual subjects. In this contribution, we will examine a number of factors related to cross-sex hormone treatment MtoF and FtoM receive. It will be analyzed whether these factors could account for the higher prevalence of cardiovascular disease in MtoF. If so, modifications of cross-sex hormone treatment may be necessary. The sex difference in cardiovascular mortality favoring women has been observed across a diversity of human populations and also in other species (1). However, how the sex gap evolves with individual aging is still poorly understood (2, 3). Studies of cause-specific mortality indicate that sex differences in cardiovascular diseases are for a large part responsible for the sex gap in adult mortality and also why this gap narrows at old ages (4, 5, 6) when women are aging and suffer increasingly frequently from heart disease. Women have a lower heart disease mortality at any age, but when they reach their mid-seventies, they have survived large numbers of men. Also in women heart disease mortality increases with aging and because they outnumber men at that age, the number of women dying of heart disease becomes similar to the mortality in men once they reach the age of 75 (7). In men, there is an about 10-year head start and cardiovascular disease is also more severe. The classic Kalin & Zumoff (8) study showed a rather consistent MtoF ratio of 2:1 for fatal coronary heart disease in countries with very different rates of heart disease. Interestingly, over the last 2 decades it has become clear that men with low testosterone concentrations are high-risk individuals with regard to cardiovascular disease and mortality from all causes (9).

If it is to be believed that the sex difference in cardiovascular pathology may be related to the differences in sex steroid milieu between men and women; this protective effect with regard to cardiovascular disease in women would be expected to be lost at menopause. Postmenopausally, there is no increase in cardiovascular disease above the age-related increase. This is in contrast with the rate of breast malignancies which decline upon the postmenopausal decline of estrogen production (for review: (10, 11, 12)). Alternatively, acceleration in male heart disease mortality at younger ages could explain sex differences rather than any postmenopausal changes in women. But nonhormone-mediated effects of genes located on the sex chromosomes may be involved as well.

It is further of note that the pathology leading to ischemic heart disease has a long latency period before it leads to manifest clinical disease and death, and it is,

therefore, difficult to interpret momentary cross-sectional associations between sex, age, hormones, and mortality.

The reversal of this sex difference in the transsexual population receiving cross-sex hormones, and the elevated cardiovascular pathology in MtoF, prompted us to review the role of sex steroids (their molecular structures, dose and mode of administration, and age of commencement of cross-sex hormone administration) with regard to their potential role in the elevated cardiovascular pathology in MtoF. Table 1 presents the characteristics of the commonly used estrogens in MtoF. And, *vice versa*, the lack of apparent unfavorable cardiovascular effects of testosterone administration to FtoM, at least for the term we have been able to follow these effects.

Cross-sex hormone treatment of transsexual persons

MtoF subjects receive treatment with a compound that suppresses the production/action of testosterone (LHRH agonists or antagonists, cyproterone acetate, progestins) accompanied by estrogenic substances. The pharmacological nature of these estrogens and the route of administration may vary considerably. FtoM subjects are treated with testosterone preparations and, if menstrual bleeding does not stop, a progestational compound may be added, which is no longer needed when surgical sex reassignment including hysterectomy and ovariectomy has taken place. Testosterone molecules are unmodified and circulating testosterone in FtoM is, in part, aromatized to estradiol and circulating estradiol levels do not decline substantially compared with pretreatment levels (for review of cross-sex hormone treatment (13)). If long-term exposure

Table 1 Characteristics of commonly used estrogen preparations in male-to-female transsexual people.

	Transdermal 17 β -estradiol	Oral 17 β -estra- diol valerate/ hemi-hydrate	17 α -ethinyl estradiol (oral or vaginal)
Dose	50–100 μ g	2–4 mg	50–100 μ g
Biopotency	+	+	+++
Venous throm- bosis risk	–	–	↑↑
Effect on lipids	Neutral	Neutral to negative	Very negative
Coagulation	–	↑	↑↑
Fibrinolysis	–	↑	↑
Inflammatory markers	–	↑	↑↑
Sex hormone- binding globulin	–	↑	↑↑

to sex steroids is a significant factor in the sex difference in cardiovascular disease, it is of note that transsexual persons start their cross-sex hormone treatment usually between 20 and 40 years of age. The clinic in Amsterdam has started providing treatment to transsexual persons in 1975 and, consequently, we have a significant number of subjects on file having had exposure to cross-sex hormones for a substantial part of their adult lives, but populations that can be studied remain relatively small (14).

When initiating sex reassignment treatment, all subjects attending our clinics had agreed that active participation in a research project was subject to their informed consent, but that their anonymized data could potentially be used in future scientific analysis with the provision that data could not be related to an individual person. This procedure was reviewed and approved by the hospital's ethical review board.

Effects of cross-sex hormone treatment on cardiovascular health in transsexual persons

Cross-sex hormone treatment both improves and impairs several surrogate cardiovascular risk markers in both MtoF and FtoM (see Tables 2 and 3), but it is not yet clear how these changes in surrogate cardiovascular risk markers

translate into the clinical endpoints of cardiovascular disease. Over the past years, several studies have also addressed morbidity and mortality of cross-sex hormone therapy in transsexual persons (Tables 4 and 5).

The current evidence largely suggests that administration of cross-sex hormone therapy to MtoF (15, 16) is associated with increased cardiovascular risk. Two studies, assessing mortality in transsexual persons, indeed observed an increased cardiovascular mortality in MtoF compared with the general population (14, 17). Interestingly, the Kaplan–Meier curve diverged after about 10 years of follow-up, which could explain previous research that observed no higher cardiovascular mortality after on average 9 years of follow-up (15). The observation of increased cardiovascular morbidity and mortality during cross-sex hormone therapy in MtoF subjects is not entirely unexpected in as far as a parallel can be drawn with hormone use in women. The majority of observational studies and randomized controlled trials show an increased risk of venous thrombosis (18, 19) and arterial disease (stroke and myocardial infarction) (20, 21) among current contraceptive users and older postmenopausal women using hormone replacement therapy. But age might be a significant variable, because use of hormone therapy in younger menopausal women is associated with a decreased cardiovascular morbidity and mortality (22).

Table 2 Short-term changes in metabolic and cardiovascular risk factors in MtoF transsexual persons.

Outcome variable	Observed changes	References	Effect on cardiovascular morbidity
Body composition			
Weight	Increase	(28, 40, 45, 66)	↑
Visceral fat	Increase	(45)	↑
Total body fat	Increase	(28, 66)	↑
Insulin metabolism			
Fasting glucose	No effect	(28, 40)	–
Fasting insulin	Increase	(28, 40, 66)	↑
Insulin sensitivity	Decrease	(28, 66)	↑
Lipid spectrum			
Total cholesterol	No effect	(28, 45, 66)	–
LDL cholesterol	No effect/increase	(28)/(66)	–/↓
HDL cholesterol	Increase	(28, 66)	↓
VLDL cholesterol	No effect	(28)	–
Triglycerides	Increase?	(40, 45)	↑
Fish fatty acid (DHA)	Increase	(66)	↓
Other CVD risk factors			
Heart rate	No effect	(40)	–
Diastolic blood pressure	No effect/increase	(28)/(40)	–/↑
Systolic blood pressure	No effect/increase	(28)/(40)	–/↑
Arterial stiffness	No effect	(40)	–
Hemostasis/fibrinolysis	Increase	(22, 45)	↑
Total homocysteine	Decrease	(48)	↓
Inflammation markers	No effect/increase	(48)/(66)	–/↑

MtoF, male-to-female; DHA, docosahexaenoic acid; CVD, cardiovascular disease.

Table 3 Short-term changes in metabolic and cardiovascular risk factors in FtoM transsexual persons.

Outcome variable	Observed changes	References	Effect on cardiovascular morbidity
Body composition			
Weight/BMI	No effect/increase	(28)/(40, 45, 66)	↑
Visceral fat	Slight increase	(66)	↑
Total body fat	No effect/increase	(28)/(66)	↑
Insulin metabolism			
Fasting glucose	Decrease	(28, 40)	↓
Fasting insulin	No effect	(28, 40, 66)	–
Insulin sensitivity	No effect/slight decrease	(28)/(66)	–/↑
Lipid spectrum			
Total cholesterol	No effect	(28, 48, 66)	–
LDL cholesterol	No effect	(28, 40, 48, 66)	–
HDL cholesterol	Decrease	(28, 40, 66)	↑
VLDL cholesterol	No effect	(28)	–
Triglycerides	Increase	(40, 66)	↑
Fish fatty acid (DHA)	Decrease	(66)	↑
Other CVD risk factors			
Heart rate	–	(40)	–
Diastolic blood pressure	No effect	(28, 40, 66)	–
Systolic blood pressure	No effect/increase	(28, 40)/(66)	–/↑
Arterial stiffness	No effect	(40)	–
Hemostasis/fibrinolysis	No effect	(22, 45)	–
Total homocysteine	Increase	(48)	↑
Inflammation markers	Increase	(66)	↑

FtoM, female-to-male; DHA, docosahexaenoic acid; CVD, cardiovascular disease.

A detailed study from the gender clinic in Gent (Belgium) reported on cardiovascular disease in 100 transsexual persons (50 MtoF and 50 FtoM), with an average of 10 years of cross-sex hormone therapy (23). Only three of the MtoF used ethinyl estradiol, and others used transdermal estradiol or oral estrogens (estradiol valerate, estriol). Therefore, it is unlikely that use of ethinyl estradiol has played a significant role in the patterns of elevated cardiovascular pathology in this transsexual population. Three MtoF subjects had experienced thromboembolism (two cerebral and one deep venous thrombosis) during hormone treatment. In addition, in another four MtoF transsexual subjects with other cardiovascular diseases: transient ischemic attack ($n=1$), venous ulcer ($n=1$), and myocardial infarction ($n=2$) were observed. One MtoF subject had a myocardial infarction before hormone therapy (23). Another MtoF underwent surgery for peripheral arterial disease during the course of hormone treatment, but the presence of diabetes mellitus was a likely contributing factor in this person. All participants except one, who experienced thromboembolic or other cardiovascular events, were smokers at the time of event (on average 24 smoking years). In conclusion, cross-sex hormone administration to MtoF, using a variety of estrogens, was associated with a higher degree of cardiovascular morbidity.

The ground-breaking scientific work of Barrett-Connor has high-lighted the role of diabetes mellitus as a risk factor in cardiovascular disease, more so for women than for men (24). Women with diabetes mellitus without known heart disease have a similar risk of developing congestive heart failure as nondiabetic women with known heart disease (25). Diabetes confers about a twofold excess risk for a wide range of vascular diseases, independently from other conventional risk factors (26). In both MtoF and FtoM, diabetes mellitus was prevalent in about one-third of the population studied (27). Indeed, one study found that the combination of ethinyl estradiol and cyproterone acetate may induce a degree of insulin resistance in MtoF and be a factor in the development of diabetes mellitus (28) (see also Tables 2 and 3).

In two reports on health status (15, 16), analyzing also cardiovascular disease in the transsexual population in the gender clinic in Amsterdam, we were unable to establish an increase in cardiovascular pathology. However, in a report on mortality in the same transsexual subjects published in 2011 (14), we found that ischemic heart disease was the cause of death in 18 MtoF subjects, among the 966 MtoF followed during a mean of 19.4 years of cross-sex hormone treatment. So, it would seem, unsurprisingly, that aging of the same population analyzed in these three studies is a factor in the emergence of cardiovascular disease. Aging

Clinical Study	L J Gooren and others	Sex steroids and cardiovascular disease	170:6	813
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Table 4 Studies on cardiovascular endpoints in MtoF transsexuals compared with general population or control group.

Reference	n	Follow-up	Treatment regimen	Outcome
(16)	303	Median duration HRT of 4.4 years	Ethinyl estradiol 100 µg/day and cyproterone acetate 100 mg/day	45-fold increase in VT and/or PE No increased cardiovascular morbidity and mortality
(15)	816	Mean duration HRT of 9.5 years	Ethinyl estradiol 100 µg/day or transdermal 17β-estradiol 100 µg/twice a week and cyproterone acetate 100 mg/day	20-fold increase in venous thrombosis and/or pulmonary embolism No increased cardiovascular morbidity or mortality rate
(14)	966	Median duration HRT of 18.5 years*	Ethinyl estradiol 100 µg/day or transdermal 17β-estradiol 100 µg/twice a week and cyproterone acetate 100 mg/day	Higher mortality due to ischemic heart disease; SMR 1.64 (1.43–1.87) Higher mortality due to CVD; SMR 2.11 (1.32–3.21) in age group 40–64 years
(17)	191	Median time since SRS of 9.1 years*	Not specified	Higher mortality due to cardiovascular disease compared with controls
(56)	58	Mean duration HRT of 6.5 ± 7.9 years	Different estrogen regimens and cyproterone acetate 50 mg/day	Lower cardiovascular morbidity compared with control male and female population
(27)	214	Median duration HRT of 6 years	Different estrogen regimens and cyproterone acetate 50 mg/day	Higher number of AMI compared with control women Higher number of CVD compared with control men and women

HRT, hormone replacement therapy; VT, venous thrombosis; PE, pulmonary embolism; AMI, acute myocardial infarction; CVD, cerebrovascular disease
median duration of HRT not separately reported for MtoF and MtoF.

appeared also to be a factor in the study of Wierckx *et al.* (27) in the gender clinic in Belgium.

In our previous study (14), the mean age of occurrence of the lethal ischemic cardiac event was 59.7 years (range: 42–79 years). The mean duration of estrogen use was 13.2 years (range: 2–42 years). Eleven of these subjects (61%) had been using ethinyl estradiol during a mean period of 9.7 years (range: 2–16 years), whereas the other seven had used transdermal estrogen ($n=2$), stilbestrol ($n=1$), tibolone ($n=1$), or conjugated estrogens ($n=3$) for a mean period of 16.9 years (range: 5–42 years). Five MtoF subjects died from stroke. All of them had been using ethinyl estradiol. In the Cox proportional hazard analysis of the type of estrogen treatment in MtoF, current use of ethinyl estradiol was significantly associated with cardiovascular mortality, but not with an increased risk of all-cause mortality or mortality due to other causes. The threefold increased hazard ratio of cardiovascular mortality in current users, compared with never and former users of ethinyl estradiol, remained significant after adjustment for covariates. The fact that only the report of 2011 could establish a significant increased cardiovascular mortality in MtoF indicates that, not surprisingly, there is a time span between use of cross-sex hormones and the manifestation of cardiovascular pathology.

The calculated incidence of cardiovascular mortality was higher in MtoF than in FtoM. The incidence rates were 123 per 100 000 person-years in MtoF (95% CI: 73–173) and 15 per 100 000 person-years in FtoM (95% CI: 1–68).

Parallels of MtoF receiving cross-sex hormones with prostate cancer patients receiving androgen deprivation and/or estrogen treatment

Androgen deprivation treatment (ADT) is a standard therapy for palliation of metastatic prostate cancer. It is of concern that several retrospective studies have associated ADT with increased risk of diabetes and, possibly, cardiovascular events. Insulin resistance, diabetes, and metabolic syndrome have emerged as the complications of ADT. Some data also suggests that ADT might be responsible for incident cardiovascular disease as found in a population-based cohort of over 70 000 men with prostate cancer with a follow-up of 4.6 years. Treatment with LHRHa was associated with increased risk of incident diabetes by 1.44 (95% CI: 1.34–1.55), incident coronary heart disease by 1.16 (95% CI: 1.10–1.21), incident myocardial infarction by 1.11 (95% CI: 1.01–1.21), and incident sudden cardiac

Clinical Study	L J Gooren and others	Sex steroids and cardiovascular disease	170:6	814
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Table 5 Studies on cardiovascular endpoints in FtoM transsexuals compared with general population or control population.

Reference	n	Follow-up	Treatment regimen	Outcome
(16)	122	Median duration HRT of 4.4 years ^a	Testosterone esters 250 mg i.m. every 2 weeks or testosterone undecanoate 120–160 mg/day	No increased cardiovascular morbidity
(15)	293	Mean duration HRT of 8.2 years	Testosterone esters 250 mg i.m. every 2 weeks or testosterone undecanoate 160 mg/day	No increased cardiovascular morbidity or mortality rate
(14)	365	Median duration HRT of 18.5 years ^a	Testosterone esters 250 mg i.m. every 2 weeks or testosterone undecanoate 160 mg/day	No increased cardiovascular mortality rate
(17)	133	Median time since SRS was 9.1 years ^a	Not specified	Higher mortality due to cardiovascular disease compared with controls
(56)	37	Mean duration HRT of 4.9 ± 4.6 years	Different testosterone preparations	No difference in cardiovascular morbidity compared with control men and women
(27)	138	Median duration HRT of 6 years	Different testosterone preparations	No difference in cardiovascular morbidity compared with control men and women

HRT, hormone replacement therapy.

^aMedian duration of HRT not separately reported for MtoF and MtoF.

death by 1.16 (95% CI: 1.05–1.27) (29). Similar results have been reported (30), including a review (31).

MtoF people undergo ADT and also receive estrogen treatment. However, this is also the case in some patients with prostate cancer. In a recent study (32), patients were randomly assigned to receive LHRHa or estrogen patches. At 3 months, 93% receiving LHRHa and 92% receiving estrogen had achieved castrate testosterone concentrations. After a median follow-up of 19 months, 24 cardiovascular events were reported, six events in six (7.1%) men in the LHRHa group (95% CI: 2.7–14.9) and 18 events in 17 (10.1%) men in the estrogen-patch group (95% CI: 6.0–15.6). Nine (50%) of 18 events in the estrogen group occurred after crossover to LHRHa. Mean 12-month changes in fasting glucose concentrations were 0.33 mmol/l (5.5%) in the LHRHa group and –0.16 mmol/l (–2.4%) in the estrogen-patch group ($P=0.004$), and for fasting cholesterol were 0.20 mmol/l (4.1%) and –0.23 mmol/l (–3.3%) respectively ($P<0.0001$). The results show that parenteral estrogen administered via patches can lead to castrate testosterone concentrations similar to those achieved with LHRHa in men with prostate cancer. In this study, a significant point was that patients with high baseline risks of cardiovascular events were excluded. The rate of cardiovascular complications in men receiving estrogen patches was similar to that in men receiving LHRHa. In addition, it was lower than rates observed with oral estrogen by the Veterans Administration Cooperative Urological Research Group using (oral) diethylstilbestrol (33).

A meta-analysis provided reassuring results. Twenty trials were included in the review. The trials differed with regard to the included patients, formulation and dose of parenteral estrogen, comparator used, outcome measures reported, and the duration of follow-up. The results provide no evidence to suggest that parenteral estrogen is consistently associated with an increase in cardiovascular mortality compare with orchidectomy or LHRHa (34).

There is indeed a difference between metabolic effects of oral and parenteral estrogens; although oral estrogen therapy is known to be associated with thromboembolic complications, studies of parenteral estrogen in men with prostate cancer suggest that the use of parenteral estrogen achieves target androgen suppression, does not adversely affect prothrombotic protein levels, and is not associated with adverse metabolic, skeletal, and body compositional changes when compared with conventional ADT (35).

Oral estrogens were the treatment of choice for carcinoma of the prostate for over 4 decades, but were abandoned because of an excess of cardiovascular and thromboembolic toxicity (33). It is now recognized that most of this toxicity is related to the first-pass portal circulation, which upregulates the hepatic metabolism of hormones, lipids, and coagulation proteins. Most of this toxicity can be avoided by parenteral (i.m. or transdermal) administration of estrogen, which avoids hepatic enzyme induction. It also seems that a short-term but modest increase in cardiovascular morbidity (but not mortality) is compensated for by a long-term cardioprotective

benefit, which accrues progressively as vascular remodeling develops over time (36). But this view has to be nuanced if pretreatment cardiovascular morbidity is included in the analysis. Patients with previous cardiovascular disease are at considerable risk of cardiovascular events during treatment with high-dose estrogen polyestradiol phosphate (PEP) and even during complete androgen deprivation therapy. Patients without pretreatment cardiovascular morbidity have a moderate cardiovascular risk during estrogen PEP treatment and could be considered for this treatment (37). Also in the study of Wierckx *et al.* (27) the presence of one or more cardiovascular risk factors predicted cardiovascular disease.

In patients with locally advanced prostatic cancer, PEP estrogen therapy is associated with a statistically significant higher risk of cardiovascular complications compared with orchidectomy (38). The relative risk of diabetes mellitus is increased in men treated with LHRHa by 44% and the relative risk of cardiovascular morbidity by 10–20%. However, on balance it would seem that estrogen treatment of men with prostate cancer is safe in terms of absolute risk as long as they do not have cardiovascular pathology, but men with cardiovascular pathology may run a clinically important risk.

Relevance of chemical structure and route of estrogen administration to MtoF subjects

The special role of ethinyl estradiol

As ethinyl estradiol stood out as a risk factor in MtoF in the study on mortality (14), we have reviewed our earlier studies of the effects of cross-sex hormone administration on surrogate cardiovascular risks (28, 39, 40, 41, 42, 43, 44, 45) included in Tables 2 and 3. The estrogen used in our own studies was ethinyl estradiol. The impression is given that the combination of cyproterone acetate + ethinyl estradiol in MtoF had more deleterious effects on variables of cardiovascular risk when compared with testosterone in FtoM. The deleterious effects of ethinyl estradiol are not only the result of its oral administration and its first-pass hepatic effect, but are also due to its long half-life and very high biopotency compared with 17 β -estradiol (46).

Four studies have been conducted in the Amsterdam clinic comparing the effects of oral ethinyl estradiol with transdermal 17 β -estradiol. One study showed that oral ethinyl estradiol treatment of MtoF (compared with transdermal 17 β -estradiol) was associated with adverse changes in activated protein C resistance and plasma levels of protein S and C, while testosterone had

antithrombotic effects in FtoM (44). The other study found that administration of oral ethinyl estradiol, but not transdermal 17 β -estradiol, lowered tissue-type plasminogen activator levels in humans without affecting endothelial synthesis (47). The third study found that both oral ethinyl estradiol and transdermal 17 β -estradiol lowered plasma total homocysteine in MtoF (39, 48). Finally, oral ethinyl estradiol but not transdermal 17 β -estradiol increased plasma levels of the acute-phase reactant C-reactive protein (CRP). Remarkably, testosterone administration also increased CRP levels in FtoM (49).

Alarmed by the high prevalence of venous thrombosis in MtoF receiving treatment with cyproterone acetate and various types of estrogens, we undertook a study in 2003 which showed that ethinyl estradiol has much stronger prothrombotic effects than oral estrogen preparation such as estradiol valerate or transdermal estrogens (44). This was a reason to halt further administration of ethinyl estradiol to all transsexual subjects under our care. Some transsexual persons, however, continued ethinyl estradiol at their own initiative, receiving prescriptions from other health care providers.

The commonly prescribed dose of ethinyl estradiol to MtoF was 100 μ g/day, sometimes lowered to 50 μ g/day after sex reassignment surgery. The ethinyl estradiol content of oral contraceptives has gone down over time from 50 μ g/day to 30 to 20 μ g/day. A recent large study on thrombotic stroke and myocardial infarction with oral contraception has concluded that although the absolute risks of thrombotic stroke and myocardial infarction associated with the use of hormonal contraception were low, the risk was increased by 0.9–1.7 with oral contraceptives that contained ethinyl estradiol at a dose of 20 μ g/day and by a factor of 1.3–2.3 with those that contained ethinyl estradiol at a dose of 30–40 μ g/day, with relatively small differences in risk according to progestin type (50). Both the risks of thrombotic stroke and myocardial infarction were increased. This suggests that there is a dose–response effect, and that MtoF transsexual persons using 50–100 μ g are at higher risk of cardiovascular disease than women using modern oral contraceptives with 20–30 μ g ethinyl estradiol. With other types of estrogens available, it should therefore be strongly recommended against the use of ethinyl estradiol in MtoF subjects who may use estrogens over several decades, which increases both venous and arterial thrombotic events. Adverse effects of ethinyl estradiol on cardiovascular risk are likely due to its prothrombotic, dyslipidemia-inducing and proinflammatory actions (46). There are safer alternatives.

Route of administration of estrogens

While ethinyl estradiol has its own typical deleterious effect on cardiovascular risks (due to its chemical profile rather than oral administration), route of administration of 17 β -estradiol is a factor to be considered. In the literature on oral contraceptives, it has been well documented that the type of estrogen and its route of administration has an impact on risk factors of venous thrombosis and cardiovascular disease. This issue has also been addressed earlier in the section on observations in prostate cancer patients treated with estrogens. Oral estradiol increases the markers of fibrinolytic activity and induces potentially antiatherogenic changes in lipids and lipoproteins but also changes markers of coagulation toward hypercoagulability and increases serum CRP concentrations. Transdermal estradiol has no effects on any of these parameters (51). Ethinyl estradiol, compared with 17 β -estradiol, is much more resistant to metabolic degradation and has less favorable effects on lipids, hepatic proteins, angiotensinogen, and on the markers of hemostasis (52). Two studies in transsexual populations indicate that MtoF treated with ethinyl estradiol, compared with other types of estrogen, are more prone to venous thrombosis (44) and to cardiovascular mortality (14). The effects of various types of estrogens and their route of administration in transsexual persons have been excellently reviewed (53).

The effects of testosterone administration on cardiovascular morbidity and mortality in FtoM persons

Testosterone administration to FtoM affected some cardiovascular risk factors negatively (Table 2) but, notably, there was no induction of insulin resistance, which is the characteristic of hyperandrogenic conditions in women, such as polycystic ovarian syndrome (54). In comparison with certain estrogenic compounds, testosterone is chemically unmodified and administered through non-oral routes (injections or transdermal). There is little information on the oral testosterone undecanoate which has become obsolete because of the low serum testosterone levels achieved.

In a clinical study from Belgium, a substantial degree of cardiovascular risk factors was encountered in FtoM receiving testosterone. Hypercholesterolemia (cholesterol ≥ 190 mg/dl or >4.9 mmol/l) was observed in 64% of FtoM. Serum triglycerides were significantly higher (55) in FtoM than in MtoF. A similar number of MtoF and FtoM had an elevated blood pressure at the time of investigation and/or used antihypertensive medication (26 vs 28%). But

both systolic and diastolic blood pressure were significantly higher in FtoM than in MtoF. None of the FtoM had experienced cardiovascular events such as myocardial infarction, cerebrovascular disease, or deep venous thrombosis.

In other studies of FtoM transsexual persons, a lower or similar cardiovascular morbidity was observed compared with a control population (15, 16, 56), although one study (17) found a higher cardiovascular mortality rate in FtoM whereas another, in a larger population with a longer term follow-up, did not confirm this (14). Most of the evidence suggests that testosterone treatment in FtoM is relatively safe at short and medium follow-up, although it should be noted that outcome studies in transsexual men are carried out in smaller sample sizes and at significantly younger age of FtoM compared with MtoF. Future research will show whether a parallel can be drawn with men receiving testosterone treatment and who showed an increased risk of a cardiovascular-related event (55). Our data collection is not large enough and the design of the studies not suited to arrive at a firm conclusion, but the impression is given that exposure of FtoM to testosterone over significant periods of their lifetime is not associated with a strong increase in cardiovascular events. Hyperandrogenic women with the polycystic ovarian syndrome have an established increased risk of developing type 2 diabetes (57) and many cardiovascular risks (58) but a still debated increased risk of cardiovascular disease (59). It is still not clear why, in view of the many cardiovascular risk factors, there is no overwhelming increase in cardiovascular morbidity and mortality in women with the polycystic ovarian syndrome (60). In a study comparing testosterone-treated FtoM with hyperandrogenic women with polycystic ovarian syndrome, we found that testosterone administration induced a decline in serum HDL and increase in triglycerides, but had no effect on insulin resistance, a frequent feature of PCOS (54). Remarkably, cohort studies of premenopausal women followed through the menopause transition suggest that women with oophorectomy are at greater risk for coronary heart disease than intact women, pointing to a greater risk from deficiency of testosterone produced by the postmenopausal ovary than from reduced postmenopausal estradiol levels (61). In summary, it is difficult to establish definitively negative effects of testosterone on cardiovascular disease in females.

In our evaluation of effects of cross-sex hormones on cardiovascular risk, we assessed effects studied in adult life, but the potentiality of prenatal/neonatal factors may be considered as well (62, 63). Males are subjected to testosterone exposure prenatally at the time of the formation of the genitalia and again perinatally. FtoM have not had these early exposures.

There is further a timing hypothesis: differential effects of sex steroids on early and later stages of atherosclerotic disease. Atherosclerosis is characterized by the gradual loss of vascular protective mechanisms and the emergence of advanced, unstable lesions (64). Effects of sex steroids on the endothelium and its protective functions, vascular smooth muscle cells, and inflammatory cells differ depending on the stage of atherosclerosis in the underlying blood vessel.

In addition, not all cardiovascular sex differences are related to differences in sex steroid milieu. In a sex steroid-free environment, there are intrinsic sex-related differences in gene expression and cellular phenotype by microvascular endothelial cells. These intrinsic cell-sex specifics likely contribute significantly to sexual dimorphism in cardiovascular function (65).

Conclusions

The present analysis does not permit firm conclusions as to the unexpected higher rate of cardiovascular morbidity and mortality in MtoF persons compared with FtoM persons, showing a contrast with the finding of a higher prevalence of cardiovascular disease in men than in women in the general population. Nonetheless, a few speculations can be reasonably offered:

- 1) In the Amsterdam clinic, increased mortality becomes only apparent in the third-wave analysis. The analyses of 1989 (16) and 1997 (15) had not shown an increase in cardiovascular disease, but the analysis of 2011 (14) clearly produced evidence of increased cardiovascular mortality, so a longer follow-up and aging of the transsexual population – increasing statistical power to analyze cardiovascular endpoints – was in all likelihood a factor, also observed by Wierckx *et al.* (27).
- 2) Our studies on the effects of cardiovascular risk factors in the transsexual population (66) and the study with mortality as an endpoint (14) clearly singled out oral ethinyl estradiol as a culprit of the increased rate of cardiovascular pathology. So, this estrogen should no longer be prescribed to MtoF subjects.
- 3) MtoF subjects are treated with androgen ablation and receive estrogen administration. It is not unreasonable to draw a parallel with men with prostate cancer who receive similar treatment, though transsexual people are considerably younger when they start treatment. In men with prostate cancer, it has appeared that parenteral estrogens are safer with regard to the development of cardiovascular disease (32). Further, estrogen administration was much safer in patients

who had no pre-existing cardiovascular disease, at least in terms of absolute risks (37). The available data in MtoF do not allow to determine whether this applies to the MtoF population, but it is not improbable and this was also encountered in the study of Wierckx *et al.* (27).

- 4) Diabetes mellitus in women adds considerably to the risk of cardiovascular disease (24, 25). Diabetes was often encountered in the study population of MtoF and FtoM in the study of Wierckx *et al.* (27).
- 5) It is remarkable that women with hyperandrogenism have a high number of surrogate cardiovascular risk factors, but there is limited convincing evidence that high endogenous or exogenous androgens increase cardiovascular pathology (60). In FtoM, testosterone administration does not induce hyperinsulinism (54). So, if a parallel can be drawn with hyperandrogenic women, it may explain that cardiovascular pathology in FtoM is not significantly increased in most studies. However, more studies are necessary with longer follow-up periods as cohorts of FtoM were relatively small to draw firm conclusions from.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

All authors, having served as authors of a large number of papers cited here, have made significant contributions to the research of this paper. L J Gooren was leading the draft of the paper which was carefully scrutinized by the other authors.

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