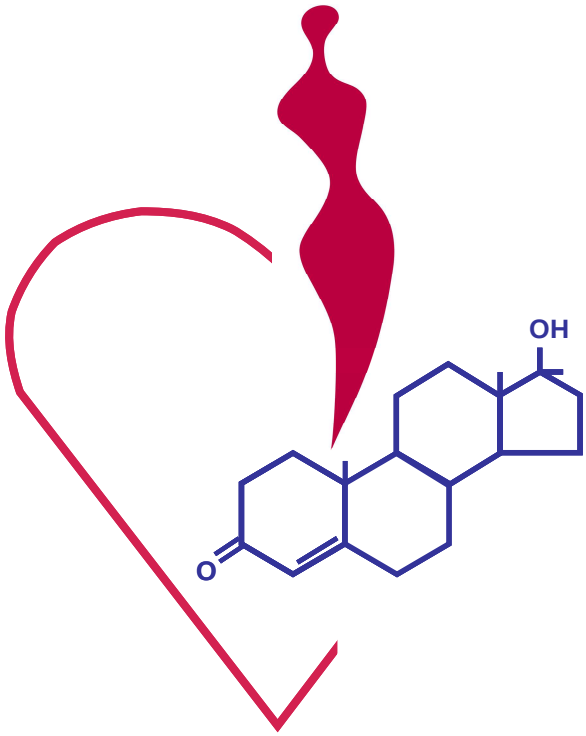
**Master's thesis**

# Testosterone, SHBG and cardiovascular health in postmenopausal women





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## 1. Introduction

Cardiovascular disease (CVD) is the leading cause of death among women worldwide. While the incidence of CVD is much lower in premenopausal women compared with men of similar age, the CVD rate in women rises steadily after the age of 50 [1,2]. Initially, this increase in CVD incidence later in life was ascribed to the menopausal decline of estrogen levels [3]. However, throughout the years, several observations have brought this theory into question. Studies examining associations between endogenous estrogen levels and CVD risk factors have yielded conflicting results [4-6] and available data on CVD events indicate a lack of association [7,8]. Furthermore, in contrast to other estrogen related diseases, the CVD rate does not show a sharp rise at time of menopause [1,2,9]. In addition, several trials have failed to demonstrate a beneficial effect of estrogen replacement therapy in postmenopausal women [10-12], further weakening the estrogen protection hypothesis.

Due to the controversial role of estrogens in women's cardiovascular health, recent research has gradually turned more focus to the potential effects of androgens. Indirect evidence for a role of androgens comes from findings of clinical studies showing an unfavourable cardiovascular risk profile in hyperandrogenic conditions such as hirsutism [13] and the polycystic ovary syndrome (PCOS) [14]. Several studies in postmenopausal women have also demonstrated a positive correlation between testosterone and various CVD risk factors [5,15-17]. These findings have raised concerns about the safety of testosterone administration to postmenopausal women [18]. However, reverse associations between testosterone and markers of atherosclerosis have been reported as well [19,20]. This review aims to summarize the current evidence on the role of endogenous testosterone in cardiovascular health in postmenopausal women and to highlight potential adverse effects of testosterone therapy.

## 2. Search strategy and selection criteria

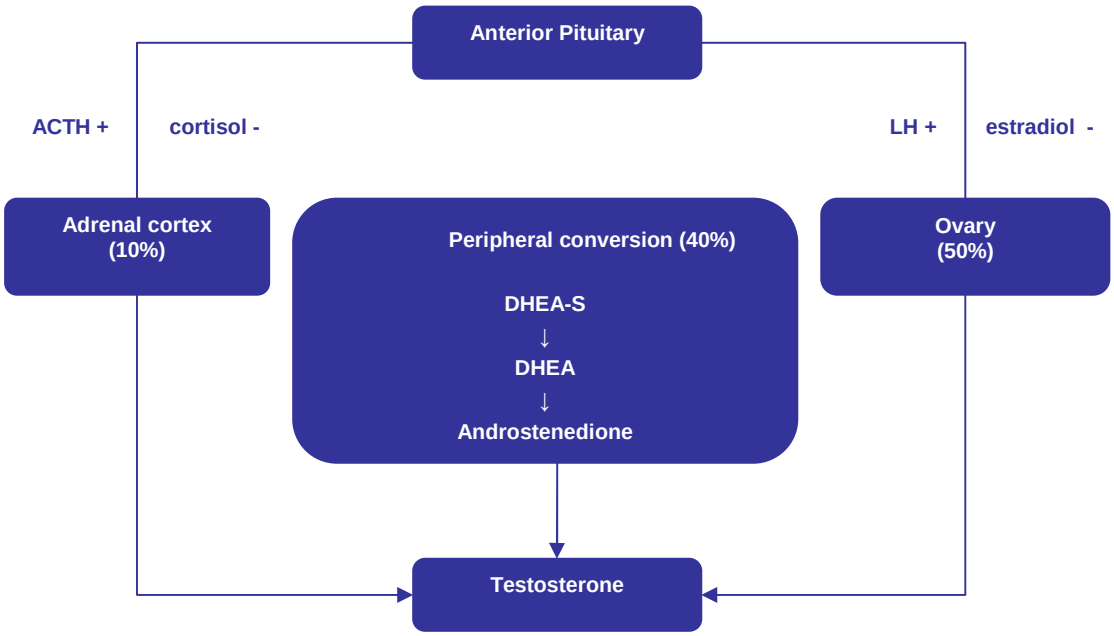
For this review we searched for English language articles in PubMed and EMBASE using the key words "androgens", "sex hormones", "sex steroids", "testosterone", "sex hormone-binding globulin" in combination with "cardiovascular" and "women". In addition, we checked reference lists of retrieved articles. To exclude confounding by postmenopausal hormone therapy (HT), we excluded observational studies that included postmenopausal women on HT. For reviewing the association between hormones and cardiovascular risk factors, we further focused on studies that excluded

subjects with prevalent metabolic and cardiovascular conditions (e.g. diabetes, heart disease and stroke) or studies that adjusted for these conditions in their analyses.

### 3. Testosterone production in women

In women, testosterone circulates in levels that are about 5% of those observed in men. There are three main sources of testosterone production in women: 1. the ovary, 2. the adrenal cortex, 3. peripheral conversion of androgen precursor hormones (androstenedione, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEA-S)). The secretion of testosterone from the ovary is stimulated by luteinizing hormone (LH), with estradiol exerting negative feedback. In the adrenal cortex, the secretion of testosterone is stimulated by adrenocorticotropic hormone (ACTH) with negative feedback by cortisol. In premenopausal women, 25% of testosterone is derived from the ovary, 25% from the adrenals and 50% is produced by peripheral conversion. These 3 sources of circulating testosterone are slightly redistributed in postmenopausal women, due to the menopause and age-related atrophy of the adrenal cortex [21].

**Figure 1.** Testosterone production in postmenopausal women.




Abbreviations: ACTH, adrenocorticotropic hormone; LH, luteinizing hormone; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulphate. +, stimulatory; -, inhibitory.

With the follicular depletion during menopause, estradiol production decreases rapidly, leading to a loss of negative feedback at the pituitary. The resulting increase in LH levels drives the ovarian production of testosterone [22,23]. Despite the persistent ovarian activity in naturally postmenopausal women, testosterone levels decrease gradually [24,25] due to the age-related atrophy of the adrenal cortex which causes a decline in adrenocortical secretion of testosterone and androgen precursor hormones. This explains the lower proportion of testosterone that is derived from the adrenal cortex (10%) and peripheral conversion (40%) in postmenopausal women (Figure 1).

**4. Measuring testosterone levels**

Given the low circulating levels of testosterone, highly sensitive and accurate assays are required to obtain reliable measurements. Mass spectrometry is the gold standard for measurement of total testosterone (TT). Nowadays, direct immunoassays are widely used across laboratories. While being more rapid and economical, their reliability in female samples has been questioned because of their high imprecision in the lower end of the testosterone range [26,27]. Direct immunoassays assays often suffer from calibration and specificity problems, which generally result in an overestimation of TT levels in women [26]. Specificity problems can be partly solved by adding an extraction or chromatography step prior to the immunoassay, which removes some of the interferences by cross-reacting substances [28,29].

For economic and logistic reasons, most researchers use single blood samples for testosterone analyses. Although this may cause a larger degree of measurement error (due to diurnal variation), a single measure obtained by extracted immunoassays is thought to be reliable for ranking postmenopausal women in epidemiological studies [30,31].

In the circulation, testosterone is specifically bound to SHBG (~66%) and non-specifically to albumin (~33%), leaving only a small fraction unbound (~1-2%). Unbound free testosterone is considered to be the bioactive fraction able to diffuse across cellular membranes. However, bioactive roles for albumin and even SHBG bound testosterone have also been suggested by studies showing cellular uptake of bound testosterone fractions [32,33]. Since SHBG is present in such a large excess in postmenopausal women, free testosterone levels are primarily driven by SHBG. The hepatic production of SHBG is positively regulated by estradiol and negatively regulated by testosterone. Therefore, increased TT

levels in hyperandrogenic conditions not only raise free testosterone levels directly, but also indirectly by lowering SHBG levels.

Accurate measurements of free testosterone and bioavailable testosterone (free and albumin bound testosterone) rely on the accuracy of TT assays. Equilibrium dialysis and the ammonium sulphate precipitation technique are the gold standards for measurement of free testosterone (FT) and bioavailable testosterone (BT). Both methods are not routinely used as they are time-consuming and expensive. As an alternative, several algorithms based on the law of mass action (Sodergard [34] and Vermeulen [35]) have been proposed to calculate FT and BT using concentrations of total testosterone, SHBG and albumin. These algorithms have been shown to correlate well with FT and BT measured by the gold standards [35,36]. Free testosterone can also be measured directly using radioimmunoassay. The reliability of this assay, however, is uncertain due to its lack of accuracy and precision [35,36]. Another way to estimate bioavailable testosterone (BT) is the free-androgen index (FAI) which represents the ratio of total testosterone (nmol/L) to SHBG (nmol/L). In contrast to men, this index is a reliable indicator of the amount of bioavailable testosterone in women [29,35,37].

**5. Cardiovascular risk factors and disease**

Over the past years, several observational studies have examined the relationship between endogenous testosterone and cardiovascular disease and its risk factors. Tables 1 - 5 summarize the reported associations of TT, SHBG, BT and FT with various indicators of cardiovascular disease risk. It is important to emphasize the limitations of the observational studies being summarized here. Study populations were heterogeneous and selection criteria diverse. For example, the type of menopause (surgical, natural or mixed) varied between study populations. Furthermore, adjustment for potential confounding factors was not always adequate. The majority of studies did not adjust for estradiol (E2), which could be a potential confounder because postmenopausal estradiol is mainly derived from peripheral aromatization of testosterone. Another limitation is that most studies used direct immunoassays (without extraction) which are suboptimal for the measurement of TT levels in postmenopausal women. Furthermore, the selection of poorly matched controls may have introduced bias in case-control studies because cardiovascular disease patients are more likely to be on medication and to have modified their lifestyle. Finally, atherogenic changes may cause a decline in

serum testosterone levels in patients with cardiovascular disease, increasing the likelihood of spurious associations in case-control studies.

5.1 Body composition and blood pressure

Obesity and hypertension are important predictors of cardiovascular morbidity and mortality in postmenopausal women [38,39]. Cross-sectional findings on the relationship between TT and markers of obesity are somewhat conflicting with either an increase or no change in body mass index (BMI), waist circumference and waist to hip ratio (WHR) with increasing TT levels (Table 1). None of the studies, however, suggested that high TT levels were associated with decreased obesity. Associations with SHBG, BT and FT seem to be more consistent: increased BMI, waist circumference and WHR are related to an increase in BT and FT levels, and a decrease in SHBG levels. It has been suggested that testosterone and SHBG are more strongly related to abdominal obesity than general obesity [40,41]. In the study of Kaye et al. [40], the association between SHBG and WHR remained significant after adjusting for BMI.

Since all studies used a cross-sectional design, the causal direction of the reported associations cannot be ascertained. The reversal direction, for instance, is supported by studies showing a decrease in testosterone levels following weight and body fat loss in overweight women [42,43]. Furthermore, adipose tissue, with its 17β-hydroxysteroid dehydrogenase activity, has been suggested to be an important site of peripheral testosterone production [44,45]. On the other hand, androgen treatment has been reported to increase visceral fat in healthy obese postmenopausal women [46]. Likewise, high doses of exogenous testosterone increase BMI and visceral fat mass in female cynomolgus monkeys [47]. Recent findings from the study of Zang et al. [48] also support a causal role for testosterone in postmenopausal obesity. In this study testosterone was found to down-regulate hormone sensitive lipase expression in subcutaneous adipose tissue. Additionally, testosterone increased the expression of phosphodiesterase-3B, an enzyme involved in the anti-lipolytic action of insulin in adipocytes.

The relationship between androgenicity and blood pressure has been studied less frequently and with less consistent results (Table 1). Two large cross-sectional studies failed to demonstrate an association between testosterone and blood pressure. In contrast, Haffner et al. [17] found a positive association of TT with systolic and diastolic blood pressure. Furthermore, high FT and low SHBG

levels have been reported in postmenopausal women with hypertension [49,50]. Testosterone may influence blood pressure through induction of obesity. However, several studies [17,49] have demonstrated that the association between testosterone and blood pressure is independent of BMI, suggesting a direct effect on the renin-angiotensin-aldosterone system (RAAS). Experimental data also support a direct effect of testosterone. In female rats, testosterone treatment increases renin activity and angiotensinogen expression [51,52]. Moreover, high testosterone levels have been associated with increased vasoconstriction in sucrose fed female rats [53] and increased levels of endothelin, a potent vasoconstrictor [15].

**Table 1.** Associations of endogenous testosterone and SHBG levels with body composition and blood pressure: results from observational studies.

| | *BMI* | *Waist circumference* | *WHR* | *DBP* | *SBP* |
|---|---|---|---|---|---|
| **TT** | ↑ [15,41,54-57]<br>= [40,58-61] | ↑ [15,41,54]<br>= [55,59,60] | ↑ [54]<br>= [40,58-60] | ↑ [15,17]<br>= [5,59] | ↑ [15,17,62]<br>= [5,59] |
| **SHBG** | ↓ [41,50,54-58,60,61,63-68] | ↓ [40,41,54,55,60,63,64] | ↓ [40,54,58,60,63,64,67,69]<br>= [65] | ↓ [5,17]<br>= [70] | ↓ [5]<br>= [17,70] |
| **BT** | ↑ [54,58,59,65,68] | ↑ [54,59] | ↑ [54,58,59,65] | ↑ [59]<br>= [5] | = [5,59] |
| **FT** | ↑ [55,56,60]<br>= [61] | ↑ [55]<br>= [60] | = [60] | = [17] | ↑ [49,62]<br>= [17] |

Abbreviations: TT, total testosterone; SHBG, sex hormone-binding globulin; BT, bioavailable testosterone; FT, free testosterone; BMI, body mass index; WHR, waist to hip ratio; DBP, diastolic blood pressure; SBP, systolic blood pressure. ↑, positive association; ↓, negative association; =, no significant association.

## 5.2 Glucose and insulin metabolism

Table 2 summarizes the cross-sectional and longitudinal studies that examined the association between testosterone, SHBG and markers of glucose and insulin metabolism. In most studies no significant association with TT was found. Some of these studies, however, may have been limited by a small sample size and lack of control for confounders. Lambrinoudaki et al. [5] observed an independent association between TT and insulin resistance in a large study of 598 postmenopausal women. Similarly, another study including a large number of incident diabetes cases showed a significant association between TT and type 2 diabetes after multivariable adjustment [71]. Unlike TT, SHBG and bioactive fractions of testosterone show stronger associations with markers of glucose and insulin metabolism. BT, FT and SHBG have repeatedly been associated with type 2 diabetes,

although one study failed to demonstrate an association with SHBG [62]. This study may have been underpowered (n = 49) to detect an association.

The underlying nature of the observed associations appears to be complex. The associations may in part be mediated by obesity. Although the associations of SHBG with insulin sensitivity and type 2 diabetes seem to be independent [16,50,61,63], data on the independence of associations with BT and FT are less consistent. In several studies associations of BT and FT with type 2 diabetes [59,71,75] and insulin resistance [5,59,61] remained significant after controlling for measures of obesity (e.g. BMI, waist circumference), whereas others could not demonstrate an independent association [16,73].

**Table 2.** Associations of endogenous testosterone and SHBG levels with measures of glucose and insulin metabolism: results from observational studies.

| | *Fasting glucose* | *Fasting insulin* | *Insulin resistance* | *HbA1c* | *Type 2 diabetes* |
|---|---|---|---|---|---|
| **TT** | = [16,58,59,72,73] | = [58,59,61,73] | ↑ [5]<br>= [16,58,59,61,73] | = [17,74] | ↑ [71*]<br>= [16,59,62,65,75] |
| **SHBG** | ↓ [16,58,66,70,72]<br>= [63] | ↓ [58,61,66,67,70]<br>= [63] | ↓ [5,16,58,61,68] | ↓ [17,74]<br>= [50] | ↓ [16,50,63,65,75]<br>= [62] |
| **BT** | ↑ [58,59,65,72]<br>= [16,59,73] | ↑ [58,59,73]<br>= [59,61] | ↑ [5,16,58,59,61,73,76] | ↑ [74] | ↑ [16,59,65,75] |
| **FT** | = [73] | ↑ [73]<br>= [61] | ↑ [73]<br>= [61] | = [17] | ↑ [62,71*] |

Abbreviations: TT, total testosterone; SHBG, sex hormone-binding globulin; BT, bioavailable testosterone; FT, free testosterone.; HbA1c, glycated hemoglobin. ↑, positive association; ↓, negative association; =, no significant association; *, longitudinal study.

The temporal nature of the associations is also not completely resolved. Longitudinal studies support a causal role for testosterone and SHBG in glucose and insulin metabolism. Androgen administration to healthy women reduces insulin sensitivity [77] and peripheral glucose uptake in female to male transsexuals [78]. Likewise, anti-androgen therapy partially improves insulin sensitivity in hyperandrogenic women [79,80]. In addition, rat experimental data show that testosterone impairs insulin-mediated glucose uptake at the skeletal muscle by reducing the expression of glycogen synthase [81,82]. There is, however, also some evidence that hyperinsulinemia could give rise to increased androgen levels. Insulin inhibits hepatic SHBG production *in vitro* [83]. Simultaneously, insulin stimulates ovarian testosterone production [84] and LH release from pituitary cells [85]. Furthermore, suppression of insulin levels by metformin therapy reduces androgen levels in PCOS women [86]. These observations suggest that the association of androgenicity with insulin resistance and type 2 diabetes may be an

epiphenomenon, with insulin determining circulating levels of SHBG and testosterone. Remarkably, none of the studies relating testosterone or SHBG to type 2 diabetes adjusted for this potential confounding effect of insulin. However, in middle aged women lower SHBG levels have been associated with type 2 diabetes risk independent of fasting insulin levels [87]. Recent findings from a mendelian randomization study show an association between SHBG polymorphisms and type 2 diabetes, further supporting a causal role of SHBG in glucose and insulin metabolism [50].

5.3 Lipid profile

Results from observational studies do not support a major role for TT in lipid metabolism. A few studies reported an inverse association of TT with high-density lipoprotein cholesterol (HDL-C) and a positive association with total cholesterol, low-density lipoprotein cholesterol (LDL-C) and trigylcerides, but in most studies no significant correlation between TT and lipid parameters was found (Table 3). In contrast, low levels of SHBG have consistently been associated with a pro-atherogenic lipid profile, namely increased triglyceride and decreased HDL-C levels. The mechanisms behind these associations are not completely understood, but there are some indications for a direct regulatory effect of SHBG on hepatic and lipoprotein lipases.

Hepatic lipase (HL) and lipoprotein lipase (LPL) are involved in the regulation of plasma triglycerides and HDL-C with opposing effects: LPL activity is associated with a decrease in triglycerides and an increase in HDL-C, whereas HL activity increases triglyceride and decreases HDL-C levels. In the HERITAGE family study [91] a strong inverse association between SHBG and HL activity was found. In the same study, high SHBG levels were found to be associated with increased LPL activity.

The relationship of SHBG with triglycerides and HDL-C may also be mediated indirectly through their associations with obesity. Yasui et al. [73] found that associations of SHBG with HDL-C and triglycerides were no longer significant after controlling for BMI. In another study, a similar lack of independence was observed for the association with HDL-C [89]. Conversely, in several studies associations between SHBG and triglycerides [5,6,69,89] and HDL-C cholesterol [5,17] persisted after adjustment for BMI or WHR.

Another possible mediator is insulin. Insulin is known to exert a direct regulatory effect on hepatic lipase (HL) and lipoprotein lipase (LPL) [92,93]. Interestingly, only a few studies [6,67,70] adjusted for the effect of insulin. Mudali et al. [6] demonstrated that adjustments for insulin, BMI and other covariates did not significantly influence associations of SHBG with HDL-C and triglycerides. On the other hand,

Soler et al. [67] found that associations between SHBG and triglycerides were lost after controlling for WHR, insulin and estradiol. In the study of Haffner et al [70] the association of SHBG with HDL-C was independent of fasting insulin levels, but the relationship with triglycerides lost significance after adjusting for insulin.

**Table 3.** Associations of endogenous testosterone and SHBG levels with plasma lipids: results from observational studies.

| | *Total cholesterol* | *LDL cholesterol* | *HDL cholesterol* | *Trigylcerides* |
|---|---|---|---|---|
| **TT** | ↑ [5,17,62]<br>= [6,54,67,69,73,88] | ↑ [5,62]<br>= [6,54,69,73,88,89] | ↓ [5,59]<br>= [6,17,54,67,69,73,88,89] | ↑ [5]<br>= [6,59,67,69,73,88,89] |
| **SHBG** | ↓ [6,17]<br>= [5,54,69,70,73,90] | ↓ [6,50]<br>= [5,54,69,70,73,89,90] | ↑ [5,6,17,50,54,67,69,70,73,89,90] | ↓ [5,6,50,67,69,70,73,89]<br>= [90] |
| **BT** | ↑ [5,54]<br>= [6,73] | ↑ [5,6,54]<br>= [73] | ↓ [5,54]<br>= [6,59,73] | ↑ [5]<br>= [6,59,73] |
| **FT** | ↑ [62]<br>= [17,67,73] | = [73,89] | ↓ [17]<br>= [67,73,89] | = [67,73,89] |

Abbreviations: TT, total testosterone; SHBG, sex hormone-binding globulin; BT, bioavailable testosterone; FT, free testosterone; LDL cholesterol, low density lipoprotein cholesterol; HDL cholesterol, high density lipoprotein cholesterol. ↑, positive association; ↓, negative association; =, no significant association.

## 5.4 Other cardiovascular risk factors

Besides traditional risk factors, a growing number of studies have started to examine associations with other markers of cardiovascular risk such as C-reactive protein (CRP), fibrinogen and white blood cell count (WBC) (Table 4). CRP is an inflammatory marker and independent predictor of cardiovascular events in postmenopausal women [94]. In several studies, higher BT levels and lower SHBG levels [50,54,95,96] have been associated with an increase in CRP levels. In addition, a positive association between TT and CRP has been reported in healthy postmenopausal women [15,54]. Conversely, Joffe et al. [97] found an opposite association in women referred to coronary angiography, with low testosterone levels being associated with an increase in CRP levels. Interestingly, this inverse association with testosterone was not present in women who remained CVD-free. These findings suggest that the association between testosterone and CRP depends on CVD status, with a potential confounding effect of subclinical cardiovascular disease. Early atherogenic changes may affect testosterone production by the ovaries and adrenals through restriction of the blood supply. This may explain the presence of the reverse association among women with subclinical CVD.

Data on the association between testosterone and the clotting factor fibrinogen are inconclusive. In two small studies [15,98], TT levels were not related to plasma fibrinogen levels. However, in a larger study including 317 postmenopausal women [96], fibrinogen levels were approximately 10% higher in highest TT quartile compared with the lowest quartile, but no association between SHBG and fibrinogen was found. Interestingly, high TT levels in this study were also associated with an increase in WBC, another promising marker for cardiovascular disease.

**Table 4**. Associations of endogenous testosterone and SHBG with C-reactive protein, fibrinogen and white blood cell count: results from observational studies.

| | *C-reactive protein* | *Fibrinogen* | *White blood cell count* |
|---|---|---|---|
| **TT** | ↑ [15,54]<br>↓ [97]<br>= [89,95,96] | ↑ [96]<br>= [15,98] | ↑ [96] |
| **SHBG** | ↓ [54,95-97] | = [96] | = [96] |
| **BT** | ↑ [95,97] | | |
| **FT** | = [89] | | |

Abbreviations: TT, total testosterone; SHBG, sex hormone-binding globulin; BT, bioavailable testosterone; FT, free testosterone. ↑, positive association; ↓, negative association; =, no significant association.

5.5 Atherosclerosis

Studies investigating the relation between testosterone and atherosclerotic indices (carotid, aortic, coronary and peripheral atherosclerosis) have yielded contradictory results (Table 5).

Phillips et al. [99] examined the correlation between testosterone and the degree of coronary atherosclerosis in a cross-sectional design among 60 patients with coronary artery disease (CAD). In this study, increasing FT levels were associated with CAD severity, independent of estradiol, BMI and other cardiovascular risk factors. In the WISE study [100] positive associations of TT and FT with CAD turned significant after adjustment for estradiol levels. Ouyang et al. [101] focused on a population of postmenopausal women without clinically evident CVD and found that high TT and BT levels and low SHBG were associated with subclinical atherosclerosis. The associations with TT and BT were independent of age, BMI and cardiovascular risk factors, but the association with SHBG lost significance after adjustment for HDL and LDL cholesterol. In the Rotterdam Study [102], higher levels of TT also tended to be associated with atherosclerosis in postmenopausal women, although this association was diluted after adjustments for cardiovascular risk factors.

On the other hand, several studies have reported opposite results. Bernini et al. [19] demonstrated an inverse association between FT and carotid intima media thickness (cIMT) in 44 postmenopausal women. Similar findings were reported by Debing et al. [20] who found that cases with carotid atherosclerosis had lower levels of FT than atherosclerotic free controls. In another case-control study [103] carotid atherosclerosis was also found to be more common in women in the lowest TT quartile than in the highest TT quartile. However, results from this latter study need to be interpreted with caution, as extreme outliers in hormone levels (which are likely to result from measurement errors) were not removed from analyses and may have caused spurious relations. Furthermore, it should be noted that reverse causality is a bigger issue in case-control studies. Atherosclerosis may affect testosterone production by impairing the blood flow to androgen producing organs. This could partly explain the discrepancy in results. Alternatively, the contradictory findings may indicate the presence of a U-shaped relationship in which very low testosterone levels (below the physiological range) and high testosterone levels compromise arterial function. This theory is supported by studies showing impaired endothelial function in postmenopausal women with low testosterone levels [104] and increased carotid atherosclerosis and endothelial dysfunction in PCOS women with supraphysiological testosterone levels [105,106].

**Table 5.** Associations of endogenous testosterone and SHBG levels with indices of atherosclerosis: results from observational studies.

| | *Carotid atherosclerosis* | *Aortic atherosclerosis* | *Coronary atherosclerosis* | *Peripheral atherosclerosis* |
|---|---|---|---|---|
| **TT** | ↑ [101]<br>↓ [19,20,103] | ↑ [102*] | ↑ [100]<br>= [99] | = [107] |
| **SHBG** | = [19,20]<br>↓ [101,103] | | = [99] | = [107] |
| **BT** | ↑ [101] | = [102*] | | = [107] |
| **FT** | ↓ [20] | | ↑ [99,100] | |

Abbreviations: TT, total testosterone; SHBG, sex hormone-binding globulin; BT, bioavailable testosterone; FT, free testosterone. ↑, positive association; ↓, negative association; =, no significant association; *, longitudinal study.

Results from experimental studies show a similar pattern. Bruck et al [108] demonstrated an increase in plaque size following testosterone treatment in female rabbits on an atherogenic diet. This increase was independent of changes in plasma lipids. Similarly, testosterone treatment doubled the extent of coronary atherosclerosis in female cynomolgus monkeys fed an atherogenic diet for 24 hours [47]. In

addition, testosterone has been reported to increase vasoconstriction and to decrease vasodilatation in surcrose fed female rats, suggesting an adverse effect of testosterone on endothelial function [53]. *In vitro* data further suggest that testosterone may increase monocyte adhesion to the vascular endothelium [109]. On the other hand, testosterone has been reported to induce relaxation in rabbit coronary artery and aorta rings [110]. In addition, administration of physiological testosterone levels to androgen deficient female rats improves the vasodilatory reserve of the vascular endothelium [111].

5.6 Cardiovascular events and mortality

Relatively few data are available on the relationship between endogenous testosterone and cardiovascular events and mortality in postmenopausal women, which may indicate publication bias. In the Rancho-Bernardo Study [8], TT and BT levels did not differ between cases with and without CVD at baseline and did not predict cardiovascular mortality over a 19-year follow-up. Although the number of cardiovascular deaths was relatively high in this study (n = 176), stratification for estrogen replacement therapy may have reduced power to detect an association. Contrary to the data on CVD risk factors, Haffner et al. [112] found that diabetic women in the lowest TT quartile had an increased risk of ischemic heart disease (IHD) mortality, although this association was no longer significant in multivariable adjusted analyses. As the authors pointed out, the lack of a positive association may result from a plateau effect in which variations in testosterone levels may not contribute to IHD mortality in diabetic women who are already androgenized. In contrast, in a large nested case-control study [7] higher BT levels were associated with an increased risk of cardiovascular events, although this association was not independent of BMI, hypertension and diabetes. Data on the relationship between SHBG and CVD are also mixed. Haffner et al. [112] failed to demonstrate an association between SHBG and IHD mortality in diabetic women. In the Gothenburg Study [113], a U-shaped association between SHBG and myocardial infarction was found, with a high incidence of myocardial infarction (MI) in the lowest decile of SHBG. However, this study did not adjust for BMI. In the Rancho Bernardo study [114], which adjusted for BMI, no significant association between SHBG and CVD mortality was found; however, women with higher SHBG levels had slightly lower CVD and IHD mortality rates. Similarly, Rexrode et al.[7] found that low SHBG levels increased the risk of CVD events, although this relationship was not independent of BMI.

## 6. Testosterone replacement therapy

There is increasing interest in the use of testosterone as part of postmenopausal hormone therapy. In several studies addition of testosterone to estrogen therapy has been reported to have beneficial effects on sexual function and bone mineral density in postmenopausal women [115,116]. In some of these studies, possible adverse effects of this combination therapy were also examined. Overall, the cardiovascular effects of testosterone supplementation appear to depend on the route of administration and duration of exposure (Table 6). No significant change in lipid parameters has been observed after co-administration with testosterone patches or implants [115,117-120], whereas oral methyl-testosterone [121-123] and testosterone undecanoate therapies [124] have been associated with a decrease in HDL-cholesterol levels. In addition, an increase in fibrinogen levels has been reported with methyl testosterone therapy [125]. These differential effects may be attributed to a first-pass liver effect, which is bypassed by implants and transdermal patches. Interestingly, oral preparations of methyltestosterone cause a favourable decrease in triglyceride levels [121-123,125], an effect which is not observed with transdermal testosterone and testosterone undecanoate. Available data on body composition are mixed. An increase in lean body mass and decrease in fat mass have been reported after co-administration with oral methyltestosterone [123] and testosterone implants [116]. In contrast, Leao et al [126] described an increase in body weight and visceral fat mass after the addition of 1.25 mg methyl-testosterone to percutanous estradiol.

In long-term studies including naturally postmenopausal women more adverse effects have been reported. In the study of Penotti et al. [124] 8-month supplementation of 40 mg testosterone undecanoate counteracted the beneficial effect of estrogen on cerebral vascular reactivity, by increasing the pulsatile index (PI) of the middle cerebral artery. In a retrospective study, Hak et al. [127] found an adverse effect of long-term, high dose intramuscular estrogen-testosterone therapy on aortic atherosclerosis. These findings indicate that high dose testosterone replacement may affect naturally postmenopausal women more adversely than surgically postmenopausal women.

Despite the large number of studies examining the side effects of testosterone co-administration, the long-term cardiovascular safety of testosterone supplementation is not well established. The follow-up period in most studies was less than 6 months. Furthermore, the co-administration with estrogens may have counteracted possible adverse effects of exogenous testosterone. Estrogen therapy causes an increase in SHBG levels and a decrease in testosterone levels by suppressing luteinizing hormone

(LH) secretion [128,129]. For this reason, the effects of combined estrogen and testosterone use may not adequately represent the individual testosterone-related risks. Only recently, the effect of single testosterone administration was investigated in the APHRODITE study [130]. In this study, no difference in lipid profiles and carbohydrate metabolism was observed between women treated with 150 and 300 µg transdermal testosterone compared with placebo.

**Table 6.** Trials examining the effect of exogenous testosterone on cardiovascular risk parameters in healthy postmenopausal women.

| *Trial* | *Menopause* | *Drug* | *Route* | *Dose* | *Follow-up* | *Cardiovascular effect* |
|---|---|---|---|---|---|---|
| ***Co-administered with estrogen/progesterone replacement therapy*** | | | | | | |
| Shifren et al, 2000 [117] | Surgical | T | Transdermal (patch) | 150/300 µg/day | 12 weeks | - |
| Buster et al, 2005 [118] | Surgical | T | Transdermal (patch) | 300 µg/day | 24 weeks | - |
| Braunstein et al, 2005 [119] | Surgical | T | Transdermal (patch) | 150/300/450 µg/day | 24 weeks | - |
| Simon et al, 2005 [120] | Surgical | T | Transdermal (patch) | 300 µg/day | 24 weeks | - |
| Davis et al, 2006 [131] | Surgical | T | Transdermal (patch) | 300 µg/day | 24 weeks | - |
| Shifren et al, 2006 [115] | Natural | T | Transdermal (patch) | 300 µg/day | 24 weeks | - |
| Davis et al, 2006 [132] | Surgical | T | Transdermal (gel) | 2 mg | 16 weeks | - |
| Nathorst-Boost et al, 2006[133] | Natural | T | Transdermal (gel) | 10 mg | 3 months | - |
| Burger et al, 1987 [134] | Natural/ Surgical | T | Implant | 50 mg | 6 weeks | - |
| Davis et al, 1995 [116] | Natural/ Surgical | T | Implant | 50 mg | 24 months | ↓ fat mass |
| Hickok et al, 1993 [135] | - | MT | Oral | 1.25 mg/day | 6 months | ↓ HDL-C |
| Watts et al, 1995 [136] | Surgical | MT | Oral | 2.5 mg/day | 24 months | ↓ HDL-C, triglycerides |
| Basaria et al, 2002 [125] | Natural/ Surgical | MT | Oral | 2.5 mg/day | 16 weeks | ↓ HDL-C, triglycerides ↑ fibrinogen |
| Dobs et al, 2002 [123] | Natural/ Surgical | MT | Oral | 2.5 mg/day | 16 weeks | ↓ HDL-C, triglycerides, fat mass |
| Lobo et al, 2003 [121] | Natural/ Surgical | MT | Oral | 1.25 mg/day | 16 weeks | ↓ HDL-C, triglycerides |
| Warnock et al, 2005 [122] | Surgical | MT | Oral | 1.25 mg/day | 8 weeks | ↓ HDL-C, triglycerides |
| Leao et al, 2006 [126] | Surgical | MT | Oral | 1.25 mg/day | 12 months | ↓ HDL-C ↑ visceral fat mass |
| Penotti et al, 2001 [124] | Natural | TU | Oral | 40 mg/day | 8 months | ↓ HDL-C, ↑ pulsatile index |
| ***Without estrogen/progesterone replacement therapy*** | | | | | | |
| Davis et al, 2008 [130] | Natural/ Surgical | T | Transdermal (patch) | 150/300 µg/day | 52 weeks | - |

Abbreviations: T, testosterone; MT, methyltestosterone, TU, testosterone undecanoate; HDL-C, high density lipoprotein-cholesterol; CRP, C-reactive protein. ↑, positive association; ↓, negative association.

## 7. Conclusions

Studies reviewed in this thesis suggest that increased androgenicity (increased testosterone and decreased SHBG levels) has a neutral to adverse effect on cardiovascular health in postmenopausal women. Most studies, however, have been limited by a cross-sectional design and therefore no firm conclusions on the temporal association can be drawn. Furthermore, studies examining potential adverse effects of exogenous testosterone are often restricted by a short follow-up period. Since testosterone replacement therapy is increasingly being used for the treatment of sexual dysfunction in postmenopausal women, its long-term effects on cardiovascular risk markers need to be studied more thoroughly.

The observed associations between increased androgenicity and cardiovascular disease raise questions about the mechanisms through which testosterone and SHBG contribute to CVD risk. Epidemiological and experimental data support both direct and indirect effects of testosterone and SHBG. Part of the observed associations may also be mediated through estrogens, as testosterone is the primary source of postmenopausal estradiol. Research into non-traditional CVD risk factors is growing and may help identify alternative pathways through which androgens affect cardiovascular health.

Of particular interest is that SHBG is often more strongly related to CVD risk than testosterone itself. Although this may imply an important contribution of SHBG to the observed associations, this could also reflect the reliability of the current testosterone assays being used. Increasing the accuracy and sensitivity of direct testosterone assays remains a challenge, and as long as the use of direct immunoassays has not been validated in women, reliable measurements rely on mass spectrometry methods. Moreover, despite the strong inverse associations between SHBG and cardiovascular risk, the precise role of SHBG remains uncertain. SHBG may influence CVD risk indirectly by modulating the biologic effects of testosterone or exert more direct effects through its own SHBG receptor.

In conclusion, more large-scale longitudinal studies are required to determine the temporal relationship between testosterone, SHBG and cardiovascular risk and to ascertain the safety of testosterone replacement in postmenopausal women. In addition, mendelian randomization studies may help to determine the likelihood of causality. Finally, further elucidation of the underlying mechanisms is needed to clarify why postmenopausal women are more prone to CVD risk than their premenopausal counterparts, and to indicate potential means of prevention and intervention.

## 8. Abstract

Cardiovascular disease (CVD) affects men and women differently with women having a lower incidence and later onset of disease. Research has recently refocused interest into the cardiovascular role of androgens. The purpose of this review is to summarize the evidence available on the association between testosterone and cardiovascular health in postmenopausal women. Published studies relating testosterone and sex-hormone binding globulin (SHBG) levels to cardiovascular disease and its risk factors were reviewed. Studies included in this review suggest that increased androgenicity, characterized by high testosterone and low SHBG levels, has a neutral to adverse effect on cardiovascular health in postmenopausal women. However, long-term data on cardiovascular effects of endogenous and exogenous testosterone are scarce and many studies are limited by the use of insensitive and inaccurate testosterone assays. Large-scale, longitudinal studies relating testosterone and SHBG levels to cardiovascular risk factors and endpoints are needed to determine the temporal relationship between androgenicity and cardiovascular risk and to ascertain the efficacy and safety of testosterone therapy in postmenopausal women.

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