

Effects of androgens on haemostasis¹

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Abstract

Androgen deficiency is associated with an increased incidence of cardiovascular disease. There is evidence that thromboembolic disease as well as myocardial infarction in hypogonadic males are mediated by low baseline fibrinolytic activity. Hypogonadism in males is associated with an enhancement of fibrinolytic inhibition via increased synthesis of the plasminogen activator inhibitor PAI 1. On the other hand, stanozolol and danazol reduce PAI 1 and are associated with increased fibrinolytic activity. However, in male abusers of anabolic steroids the net effect on the haemostatic system may change from anti- to prothrombotic; there appears to be an individual threshold dose above which thrombogenic effects on platelets and vasomotion may overcome the profibrinolytic effects on PAI 1. There are numerous reports on weight-lifters dying of atherothrombotic ischemic heart disease while abusing anabolic steroids. Androgens are known to have profound effects on carbohydrate and lipid metabolism. In fact, much of the individual inconsistency of the effects of androgens on fibrinolytic and haemostatic activity appears to be based on the close interrelationship of these metabolic systems. Androgens may have unfavourable effects on the HDL/LDL cholesterol ratio, on triglyceride levels and on the insulin/insulin-like growth factor 1 (IGF 1) system. Hypertriglyceridemia as well as insulin resistance are both associated with low fibrinolytic activity and increased PAI 1 levels. On the other hand, lipoprotein(a), a recently acknowledged independent risk factor of CVD was shown to respond favourably to androgen treatment, in men as well as in women. In women, agonistic as well as antagonistic effects of estrogens and progestins need to be taken into account. In fact, estradiol may modulate testosterone effects on haemostasis. Androgen medication in premenopausal women, such as danazol, was found to reduce PAI 1 suggesting an improvement of the fibrinolytic activity. Also, in hormone replacement therapy (HRT) androgenic progestins or complex compounds with androgenic effects are associated with a marked reduction of PAI 1 and an improvement of fibrinolytic activity. Further improvement of fibrinolytic activity may be associated with the marked decrease of lipoprotein (a) (Lp(a)) in women on androgenic HRT. However, little is known on the interrelationship of estrogens, 19-nortestosterone or progesterone derivatives and testosterone, an interrelationship that may have substantial impact on the metabolic and particularly haemostatic net effects of a preparation. In summary, information on the effects of androgens on haemostasis is limited and may be particularly incomplete due to the fact that interaction with other

¹ Dedicated to Prof Dr med A.E. Schindler on the occasion of his 60th anniversary.

sex steroids appears to be an important confounder. In any case, there are numerous effects of synthetic androgens on the synthesis and release of haemostatic factors, namely an increase of the inhibitors of coagulation and a decrease of the inhibitor of the fibrinolytic system. However, the use of androgens in patients with congenital deficiencies of these coagulation factors or previous events of cardiovascular disease has yielded disappointing results. On the other hand, particularly the reduction of fibrinolytic inhibition (PAI 1) and Lp(a) were considered favourable effects of androgens with regard to the risk of cardiovascular disease. Differences between preparations with pronounced androgenic versus antiandrogenic effects and the effect of combined preparations need to be studied in much more detail. The profibrinolytic effects of androgens may be of particular interest with regard to favourable effects of HRT on cardiovascular disease.

Keywords: Androgen; Hormone replacement therapy; Haemostasis; Fibrinolysis; Cardiovascular disease

1. Haemostatic effects of androgens in men

The gender ratio of mortality from cardiovascular disease (CVD) before the age of 50 years was shown to be > 2 . Beside the well acknowledged beneficial effects of estrogens, hazardous effects of the androgens may also play a role [1]. In animal models and experimental studies androgens were shown to have significant effects on the extracellular matrix, namely the collagen/elastin ratio [2], extracellular NO-production [3] and the arachidonic metabolism in platelets and endothelial cells [4]. These mechanisms may have marked indirect effects on haemostatic function: an increase of blood pressure [2], vascular tone [3], and platelet aggregability [5] would translate into rather unfavourable prothrombotic effects on the cardiovascular risk profile. In fact, all these mechanisms could potentially antagonize the effects of estrogens that have recently been suggested to be responsible for the reduction of CVD in postmenopausal women on estrogen replacement therapy (ERT).

On the other hand, there is evidence of a positive correlation of androgen levels and fibrinolytic activity. Fibrinolytic activity is physiologically determined by the activity of free tissue-plasminogen activator (t-PA), a serine protease that converts plasminogen to the active fibrinolytic protease plasmin. Regulation of fibrinolytic activity mainly depends on the release of t-PA from the endothelial cell into the circulation and the amount of the t-PA inhibitor (PAI 1) present in the circulation [6]. The latter may be released from endothelial or synthesized by hepatic cells. High levels of PAI 1

have been shown to significantly reduce fibrinolytic reactivity (as measured by means of stimulation tests), to be associated with an increased risk of post-surgical thrombosis [7] and a poor prognosis after myocardial infarction [8,9]. The androgen-associated reduction of PAI 1 is therefore likely to be favourable with respect to CVD: fibrinolytic activity and reactivity is increased and a potential risk factor of venous or arterial thrombotic disease is reduced. However, it should be noted that this mechanism only operates as long as t-PA release is not altered. If t-PA would be reduced as well, a reduction of PAI 1 levels would have no net effect on the fibrinolytic activity.

Obviously, the effect of androgens on the haemostatic system is rather complex and factors such as dose of the androgen, agonistic or antagonistic effects of additional steroids and state of the haemostatic system prior to treatment are of crucial importance for the net clinical effect [10]. In males, clinical and experimental work in hypogonadic men, patients with inhibitor deficiencies or predisposition to CVD, as well as weight-lifters abusing anabolic steroids, have largely improved our understanding of androgenic effects due to the negligible role of other sex steroids.

1.1. Hypogonadism

Atherosclerosis is associated with low rather than high testosterone levels [11] in males. Moreover, male survivors of myocardial infarction have elevated rather than low levels of estrogens [12]. These data suggest that male hypogonadism may be associated with metabolic changes predis-

posing to atherogenesis and ischaemic heart disease. There is evidence that at least one factor is the reduced fibrinolytic activity in hypogonadic males [13]. It was demonstrated recently that the underlying mechanism is the inverse relationship of testosterone and PAI 1 in this range of testosterone concentration [14]. Thus, with low testosterone levels high PAI 1 concentrations will constitute a rather high threshold of fibrinolytic activation. The release of very high quantities of t-PA is required to overcome immediate inactivation by PAI 1 and eventually result in actual plasmin generation. PAI 1 overexpression has long been recognized as a risk marker associated with a high incidence of thromboembolic disease after hip surgery [7] and a poor prognosis in young survivors of myocardial infarction [8,9] and intravascular, mainly prothrombotic as well as extravascular atherogenetic pathomechanisms have been discussed [15]. In fact, hypogonadic men appear to suffer from a high incidence of thromboembolic disease [13].

1.2. Testosterone

Supraphysiological doses of testosterone have been investigated as a hormonal male contraceptive treatment. In a recent paper Anderson and coworkers report on changes from pretreatment baseline of several hemostatic variables during and after up to 52 weeks of treatment with 200 mg testosterone oenanthate weekly i.m. [16]. Fibrinogen levels decreased by about 15% within 16 weeks of treatment, suggesting a favourable effect on blood viscosity. However, there was a slight increase of haemoglobin concentration and hematocrit as well as white blood cell count. Also, a slight increase of antithrombin III, and a decrease of protein C and S activity was noted. The prothrombin fragment F 1 + 2 was slightly increased. The authors summarized that these changes indicate an increase of coagulatory activity which may theoretically increase the risk of thrombosis in predisposed patients. On the other hand, a decrease of PAI 1 activity was observed predominantly during the first months of treatment. While this finding was discussed as evidence of improved fibrinolytic activity and reduced arte-

rial risks, it was concluded that the overall effect of supraphysiological doses of testosterone have no marked prothrombotic effect.

1.3. Stanozolol

Anabolic steroids have long been known to enhance synthesis of some plasmatic proteins such as fibrinogen and plasminogen [17]. Much interest focused on the effects on protein C and antithrombin III, inhibitors of the coagulatory enzyme thrombin. Congenital deficiencies of these inhibitors are known predispositions to thromboembolic disease and the capacity of stanozolol and other anabolic steroids to increase plasma levels even in deficient subjects raised some expectations on potential therapeutic effects of androgens on the risk of thromboembolic complications in antithrombin III deficiency [18], protein C deficiency [19], postoperative thrombosis [20] and even in Raynaud's syndrome [21]. Unfortunately, even though stanozolol proved to increase plasma levels of antithrombin III and protein C in male and female patients suffering from a congenital deficiency syndrome, the clinical effects were rather disappointing [18]. A likely explanation is that stanozolol failed to improve the insufficient anticoagulant function. Indirect evidence for this hypothesis has been provided by studies on the effect of synthetic androgens on reaction products of coagulatory activity. Two recent reports confirmed an increase of plasma concentration and activity of inhibitors under therapy but failed to find a reduction of coagulatory activity as depicted by fibrinopeptide A (FPA) plasma levels [21,22].

On the other hand, the enhancement of fibrinolytic activity [24] by synthetic androgens was confirmed by studies in patients with defective fibrinolysis [25,26]. Stanozolol was shown to decrease the plasma levels and activity of PAI 1 thus increasing the fibrinolytic activity by reduction of the inhibitory threshold [27]. However, the clinical use of androgens for prevention of thromboembolic disease was limited by early reports on thrombotic complications of high dose stanozolol [28]. These clinical data suggested a dose dependency of the net effects of androgens: low doses of

stanozolol may improve the fibrinolytic activity but high doses may have predominantly procoagulatory effects [10]. Further evidence for this concept was derived from studies in male abusers of anabolic steroids.

1.4. Anabolic steroids

In general, young male athletes, namely weight-lifters, carry very few risk factors for CVD. It is conceivable that athletes themselves tend to believe that in spite of adverse effects on lipids [29] and carbohydrates [30] the abuse of anabolic steroids would not be harmful. However, after a first report on a case of myocardial infarction in a 22-year-old weight-lifter using anabolic steroids [31], several other cases of ischaemic heart disease, stroke and arterial thromboembolism were published [10]. The absence of classical risk factors, the youth of the patients and the lack of atherosclerotic lesions suggested a atherthrombotic rather than atherogenic pathomechanism raising questions on the prothrombotic effects of high dose androgens [32].

It has recently been suggested that an increased platelet aggregability *in vivo* might mediate this prothrombotic effect of high dose androgens: platelet aggregability with adenosin diphosphate and collagen was significantly increased in abusers of anabolic steroids when compared to their fellow male weight-lifters who did not use androgens [33]. Platelet aggregability is largely dependent on the arachnidonic acid metabolism and an effect of high dose androgens on both platelet [5], as well as vascular cyclooxygenase activity [34] has been demonstrated. Thus, high dose anabolic steroids are capable of increasing vascular tone and reactivity, i.e. blood pressure, and platelet aggregability (i.e. blood coagulability). Obviously, these effects are clearly prothrombotic and in opposition to the antithrombotic effects via improved fibrinolytic action.

In summary, the numerous cases of arterial thrombotic disease in abusers of androgens clearly indicate that at least in some individuals the net effect of high dose anabolic steroids on lipids, carbohydrates, platelets and vascular function may be prothrombotic, i.e. may overcome the

antithrombotic effects on the fibrinolytic capacity. So, the dose dependency of the male response to androgens provides further evidence for the clinical significance of the individual predisposition.

2. Haemostatic effects of androgens in women

Among the most important factors that may modulate the hemostatic as well as all metabolic effects of steroid hormones is gender and the hormonal milieu. In principle, the hormonal milieu may modulate the effects of a certain steroid by lowering the impact of the competing steroid on a particular target cell (i.e. the effects on bioavailability and receptor expression) or by competing on the metabolic level (i.e. at the protein-level either directly or via second messengers). Examples for the latter are the modulation of lipid profiles or vascular tone resp flow in estrogen versus estrogen/progestin-androgen combinations [35]. So, the effects of androgens on the haemostatic system in women may be quite different from those in men.

2.1. Hyperandrogenism

The syndrome of polycystic ovaries (PCO), a clinical diagnosis frequently associated with hyperandrogenism in premenopausal women, is frequently associated with a complex dysfunction of the carbohydrate metabolism [36]. In fact, hyperinsulinemia and high levels of the insulin-like growth factor 1 (IGF 1) have been shown to sustain the dysregulation of ovarian function by interfering with the pulsatility of gonadotrophin release. In premenopausal women hyperandrogenemia is frequently associated with an impaired glucose tolerance, a metabolic dysregulation that is known to have distinct effects on fibrinolytic function [37,38]. Evidence from *in vitro* and *in vivo* studies has suggested that hyperinsulinemia as well as high levels of IGF 1 increase synthesis and release of PAI 1. It has been concluded that much of the cardiovascular risk associated with insulin resistance [39] is mediated by this reduction of fibrinolytic activity [40]. Unfortunately, studies of the hemostatic system in hyperandro-

genomic women are scarce. In a preliminary study on the hemostatic effects of oral contraceptives we investigated the fibrinolytic system in 29 premenopausal women prior to OC therapy. PAI 1 was positively correlated with testosterone levels suggesting a different regulation of PAI 1 than in men. However, we did not study the carbohydrate metabolism in these women. So, increased PAI 1 in our hyperandrogenic patients may be induced by impaired glucose tolerance rather than testosterone levels. In any case, the interrelationship of these both mechanisms may result in a decrease of fibrinolytic activity in hyperandrogenic women.

2.2. Danazol

Danazol is a weak androgen. Beside its clinical use in endometriosis and mastalgia danazol has well acknowledged effects on the synthesis of certain haemostatic factors. There are favourable effects in deficiencies of the first component of the complement system C1, in protein C deficiency, antithrombin III deficiency and even in factor VIII deficiency [41]. However, with the exception of the C1 deficiency, apparently only laboratory findings were found to improve in long term danazol therapy of these deficiencies, while hemostatic function and clinical symptoms did not.

In a study of 20 women on danazol we have focused on the effect on haemostatic function as well as risk markers of CVD. We failed to find any prothrombotic effect of this weak androgen. In fact, we demonstrated a significant reduction of PAI 1 concentration and activity and a decrease of all reaction products of thrombin activity and fibrin turnover [42]. So, these findings in premenopausal women without haemostatic disease were very much in line with what has been found in low dose stanozolol therapy in males: no increase in reaction products of thrombin generation and activity associated with a decrease of the fibrinolytic threshold suggesting an improvement of the fibrinolytic capacity may be considered beneficial in the context of venous thromboembolism. Similar conclusions were drawn by a recent study of Ford and coworkers in 18 women on 600 mg danazol for treatment of endometriosis [23]. However, in this study, an increase of the red

cell count, haemoglobin levels, the hematocrit and the platelet count (not platelet function) was observed. The authors concluded that a potential adverse rheological effect should be considered in the treatment of individuals at risk from arterial cardiovascular disease.

2.3. Hormone replacement, testosterone implants and complex androgenic steroids

Menopause is known to be a risk factor for cardiovascular disease. There is evidence of prothrombotic changes associated with the cessation of ovarian function, mainly an increase of factor VII [43], fibrinogen [44] and of PAI 1 [45]. The beneficial effect of hormone replacement therapy (HRT) was suggested to be in part mediated by a reduction of these prothrombotic changes. Moreover, differential effects of the various steroids, namely the differential androgenic potency of each combination may yield different metabolic effects justifying a new evaluation of the various preparations with regard to the reduction of cardiovascular risks.

Such differential effects must be considered mainly when progestins are added. Sporrang and coworkers compared the effects of estradiol in combination with norethisterone acetate versus megestrol acetate. The 19-nortestosterone derivative was associated with a reduction of F VII and AT III activity, while no such changes were seen in women on megestrol acetate [46]. The authors considered these changes to be minor and in accord with the concept of reduced CVD in HRT users. However, among their 60 patients, two cases of thromboembolic disease were observed during the observation period of 1 year. Both occurred in the group treated with the progesterone derivative megestrol acetate. So even though there may be unfavourable effects of androgenic progestins on the lipid profile, the risk of thrombotic complications may be reduced in users of 19-nortestosterone derivatives containing HRT preparations.

Fibrinolytic activity as well as t-PA and PAI 1 have been studied in postmenopausal women treated with a complex steroid with androgenic as well as estrogenic and progestational properties

(tibolone). These effects were studied longitudinal [47] as well as in a controlled comparative study. The latter compared haemostatic effects of this compound with those of an estradiol-cyproterone acetate (CPA) combination [48]. While CPA in combination with estrogen had no effects on the fibrinolytic activity, the androgenic compound was associated with a decrease of PAI 1 suggesting an increased fibrinolytic activity. In this study an increased rate of plasmin generation was confirmed by measurements of the plasmin-antiplasmin complexes, which were increased in women on tibolone. It may be argued that the reduction of a well known risk marker of CVD and the enhancement of the fibrinolytic activity contributes to an improvement of the overall effect on CVD risk factors.

There is evidence that PAI 1 is mainly regulated by the hepatocyte and may only to a very limited extent be affected by transdermal ERT. Oral conjugated equine estrogens (CEE) were shown to be associated with reduced PAI 1 in a short term crossover protocol, a transdermal estradiol delivering system had no effect [49]. This finding was recently confirmed by a comparative study with a new transdermal estradiol delivering system [50]. The authors speculate that the lack of the hepatic first pass on the hepatic insulin-like growth factor 1 (IGF 1) synthesis [51] might have prevented a comparable effect of the transdermal estradiol. Stephenson and coworkers have recently confirmed that transdermal estradiol application in combination with progestins is associated with less impact on hepatic regulation of lipoproteins and carbohydrates than oral CEE [52]. However, androgens may modulate the hepatic response. It may be argued that a reduction of the estrogen induced triglyceride enhancement and an improvement of the fibrinolytic activity with androgenic steroids is a valuable contribution to the general antithrombotic effects of HRT [45] and may be particularly interesting in women predisposed to thrombotic disease.

Thom and coworkers compared the effects of testosterone implants in combination with HRT with various regimen of HRT without testosterone implants. Although the number of variables studied was rather limited, the lack of

differential effects on factor X activity suggested that the androgen did not induce surplus coagulatory activity [53]. Unfortunately, nothing is known about the fibrinolytic response to testosterone implants. Further research on these therapeutic options is badly needed: a potential beneficial effect of testosterone implants on fibrinolytic capacity may have been overlooked and may turn out to be particularly favourable in women with certain risk profiles.

Recently, the interrelationship of the fibrinolytic system with lipoprotein(a) (Lp(a)) has gained considerable interest. Lp(a) is an independent risk factor of CVD in male high risk populations and possibly also in postmenopausal women [54]. There is a remarkable homology of Lp(a) with the proenzyme of the fibrinolytic system, plasminogen. Atherothrombotic effects of Lp(a) may be mediated by competitive binding at plasminogen binding sites both on the endothelium and within the fibrin matrix of fresh clots [55]. Additionally, an Lp(a) induced up-regulation of the endothelial PAI 1 synthesis may increase the threshold of fibrinolytic inhibition [56]. The relative contribution of each of these mechanisms is still not clear. In an animal model, increased Lp(a) serum levels were shown to be associated with inhibition of the fibrinolytic response and atherogenesis [57].

The finding of decreased Lp(a) concentration in HRT is therefore considered beneficial. Androgens such as stanozolol and danazol have been shown to reduce the serum concentration markedly [58]. While estrogen replacement alone, as well as in combination with progesterone derivatives, appears to reduce Lp(a) only by up to 20% [59], tibolone was reported to reduce Lp(a) in longitudinal studies by 50% [60,61]. However, comparative trials on the differential effects of these preparations are still scarce. Moreover, several reports on hormonal effects on Lp(a) have noticed that these effects appear to depend on the pretreatment Lp(a) level, i.e. the effects were most pronounced in women with markedly elevated pretreatment values [58,60,61]. Thus, differential hormonal effects on Lp(a) may be biased and should be studied in a subgroup of women with elevated (>0.3 g/l) Lp(a) baseline values.

3. Summary

Data on the haemostatic effects of androgens are scarce. There is evidence of a dose response with regard to the effects on blood pressure, platelet aggregability, haemoglobin, platelet and red cell count, and coagulatory activation which was found only in men on superphysiological doses of testosterone and abusers of anabolic steroids. High dose danazol therapy is still considered in patients with C1 deficiency and autoimmune hemolytic anemia, but not in AT III or protein C/S deficiency. A thrombotic risk, particularly an arterial risk cannot be ruled out in these dose ranges, mainly in predisposed patients. However, with lower doses a more antithrombotic profile emerged with an increase of coagulation inhibitors and a decrease of fibrinolysis inhibitors. Also, even at low doses a decrease of fibrinogen levels was found. It has been noted that these changes are in contrast with the effects of ethinylestradiol [16], suggesting that combination therapy may alleviate unfavourable effects of estrogens. Unfortunately, there is only insufficient data on the effects of combination therapies. Studies addressing these issues need to take into account potential confounders such as the pretreatment risk profile with regard to the hemostatic system as well as the lipid and carbohydrate metabolism. Pretreatment Lp(a) as well as insulin-like growth factor 1 levels may have substantial impact on the net effect of such combination therapies and may be of particular interest in postmenopausal women.

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