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PHYSIOPATHOLOGY OF PLASMA ANDROSTANEDIOL-GLUCURONIDE

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Summary—Plasma androstanediol-glucuronide (ADG) is considered by many authors to be a highly reliable parameter of peripheral androgenicity. Recently, several authors have questioned the reliability of the ADG levels as a parameter of androgenicity. Our data obtained by continuous infusion experiments showed that in women the adrenal steroids, dehydroepiandrosterone sulfate, androstenedione and dehydroepiandrosterone are the major precursors of plasma ADG, accounting for almost the totality of circulating ADG. As expected, in view of its precursors, ADG levels decrease significantly with age. Dexamethasone causes a significant decrease of these levels, whereas in women with Addison's disease the levels are only 20% of normal levels; ovariectomy hardly influences ADG levels. Our data show that in women with moderate hirsutism, plasma ADG levels are no more often increased than the other androgens.

In virilizing syndromes ADG levels are higher than expected from precursor levels, suggesting an increased 5α-reductase activity. In hyperthyroidism as well as in euthyroid women with isolated suppressed thyroid stimulating hormone, ADG levels are increased without any sign of virilism.

In men, ADG levels have testosterone as a major precursor, but the adrenals contribute to ±30% of ADG levels. After transdermal dihydrotestosterone gel, free androstanediol levels increased by a factor of 40, but ADG levels were only increased by a factor of 4, suggesting that the skin is not very effective in conjugating androstanediol. It is concluded that ADG levels in women reflect essentially adrenal precursor levels as well as 5α-reductase activity in peripheral tissues inclusive of the liver.

In order to understand the physiopathology of virilizing syndromes, especially the minor syndromes such as acne and mild hirsutism, a reliable parameter of tissue androgen formation is badly needed. Indeed, whereas, when considering all virilizing syndromes as a group, testosterone (T) and even more so, free testosterone are fairly reliable parameters of androgenicity, this is no longer the case when considering mild hirsutism and acne as a subgroup

It has been suggested, mainly by Horton *et al.* [1], that androstanediol-glucuronide (ADG) is a highly reliable parameter of androgenicity and these authors claim that ADG is formed exclusively at the level of peripheral tissues and not in the liver [2] Supporting this view is the fact that it has been shown that peripheral tissues are capable of forming ADG [3], whereas Paulson *et al.* [4] observed a highly significant correlation between serum ADG levels and genital 5α-reductase activity. Several authors confirmed the value of plasma ADG as a parameter of androgenicity [5–8], but recently, a series of discordant results were published [9–12], raising some doubt concerning the reliability of the ADG level as a parameter of androgenicity in women.

Moreover, close inspection of the data of Horton's group [1], reveals that ADG levels in hirsute women observed by these authors are up to 25 times the maximal level in normal women, reaching levels higher than the mean in normal adult men. As even a severely hirsute woman is less virilized than a normal man, the fact that ADG levels in these women are higher than in men, casts some doubt on the value of ADG as a parameter of androgenicity. Moreover it suggests that the major precursor of ADG in women should be a steroid with weak intrinsic androgenicity, the levels of which in hirsute women should be greatly increased.

Therefore it appeared of interest to us, to determine, by continuous infusion experiments,

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the relative importance of different plasma androgens as precursors of ADG in women [13]. Rather surprisingly, non-conjugated plasma androstanediol (AD) appeared to be only a minor precursor of ADG. Similarly, neither plasma dihydrotestosterone (DHT) nor T were major precursors of ADG. Plasma androstenedione on the other hand, appeared to be a relatively important precursor of plasma ADG, although it accounted for only 10–15% of plasma ADG levels in women.

At this point, it is interesting to mention that Moghissi *et al.* [14] reported that in men plasma DHT and DHTG are the major precursors of ADG. From our data this does not appear to be the case in women; moreover it should be noted that neither DHT nor DHTG are secreted as such by the endocrine glands, but that in males T is the major precursor of DHT and DHTG, whereas in females androstenedione (A) and to a minor extent testosterone are the major precursors of DHT. Hence, under equilibrium conditions only A and T but neither, DHT, AD nor DHTG should be considered as precursors. Moreover, as in women plasma T originates for ±50% from plasma A, only 50% of plasma T levels should be taken into account.

Another possible candidate as an important precursor of plasma ADG is dehydroepiandrosterone (DHEA). However from the conversion rates obtained, DHEA also appears to account for only a small fraction of plasma ADG in women.

Finally we evaluated the role of DHEA sulfate (DHEAS) as a precursor of ADG, and much to our surprise DHEAS appeared to be the major precursor of ADG, although only a mean of 0.068% of plasma DS was converted to plasma ADG.

In order to confirm that the plasma DHEA was not an important intermediate in the conversion of DHEAS to ADG, we measured the conversion of plasma DHEAS to plasma DHEA. Our data show that this conversion was largely insufficient to account for the plasma ADG levels observed, in other words that free DHEA is not a major intermediate. As plasma DHEA is not a major precursor of ADG in our studies, this suggests that hydrolysis of DHEAS should occur at the cellular level, comparable to the role of estrone sulfate as a precursor of intratissular estrone.

Table 1. Contribution of plasma precursors to plasma ADG levels in women

Precursor	Mean Co in women	% $CR_{BB}^{\rightarrow ADG}$	Mean contribution
DHEAS	4.65 μmol/l	0.068	3.16 nmol/l
DHEA	19 nmol/l	1.77	0.33 nmol/l
A	5 nmol/l	8.8	0.44 nmol/l
T (50%)	1 nmol/l	16.4	0.08 nmol/l
			4.01 nmol/l

% Of ADG accounted for by the CRs = 4.01/4.10* = 98.0%;
*4.10 nmol/l = mean ADG level, in young women.

This leads to the origin of ADG in normal women shown in Table 1.

Having identified the major plasma precursors of plasma ADG in women, we studied ADG levels in women under different conditions. First we determined ADG levels in healthy ($n = 32$; age 16–46 years, mean: 32 years) young women during reproductive life. We observed a mean ADG concentration of 3.76 ± 1.69 (SD) nmol/l with an upper limit of 7.15 nmol/l. This value decreases rapidly in aging women and in postmenopausal women ($n = 25$, age 50–78 years, mean = 64 years) the mean value was only 1.10 ± 0.24 nmol/l (SE). In fact there is a continuous age dependent decrease in ADG levels from age 40 onwards, parallelling plasma DHEAS levels, as we observed a statistically highly significant correlation ($r = 0.74$, $n = 50$) between DHEAS and ADG levels in young as well as in elderly women. As the adrenal is the major source of ADG precursors, it is not surprising that in women with Addison's disease ($n = 12$, age 39–62 years, mean = 48 years), ADG levels (0.12–0.39 nmol/l) are only about 20% of levels in normal women of similar age (mean: 1.88 nmol/l). As expected, dexamethasone suppression, even at a low dose (0.5 mg/day for 5 days) causes a significant decrease in ADG levels. Ovariectomized women, on the other hand, have plasma ADG

Table 2. Mean contribution of precursors to basal plasma ADG levels (nmol/l) using conversion rates obtained in non-hirsute women

Precursor	DS	D	NSB-T[a]	A	Total precursor	ADG measured	% Of ADG measured[b]
Normal women ($n = 12$) mean	2.98	0.39	0.09	0.49	3.89	3.76	104 ± 12
Dysfunctional hirsutism ($n = 14$)							
Successful treatment ($n = 19$)	4.62	0.47	0.16	0.69	5.94	8.39	74 ± 11
Unsuccessful treatment ($n = 5$)	4.43	0.42	0.13	0.87	5.85	6.64	86 ± 10
PCO ($n = 10$)	3.23	0.38	0.17	0.97	4.75	7.94	64 ± 14

[a]Non-SHBG bound T.
[b]Mean ± SD.

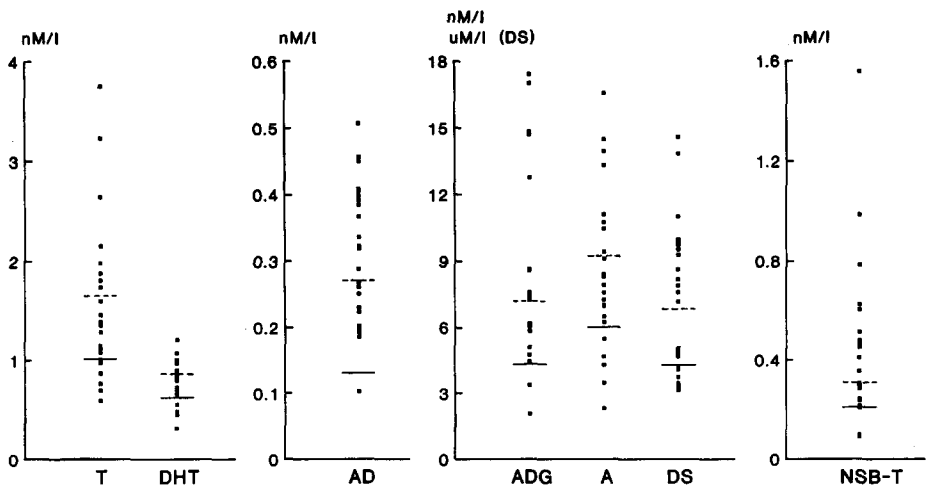

Fig. 1. Plasma androgen levels in women with mild dysfunctional hirsutism (F and G 7–15). NSB-T: non-SHBG bound T; ——, mean; and – – –, mean + 2 SD in non-hirsute women.

levels which are ±0.85 nmol/l lower than normal women of similar age, suggesting a minor contribution of ovarian androgens to plasma ADG levels. During pregnancy, in accordance with the progressive decrease in DHEAS levels, plasma ADG levels decrease, from a mean of 2.75 ± 1.27 (SD) nmol between pregnancy week 5–10 to 0.80 ± 0.32 (SD) nmol/l between week 30 and delivery [15]. This is in complete disagreement with data of Braunstein and Horton [16], the discrepancy for which we have no explanation. In all these circumstances, and in different age groups, applying the conversion rates (CRs) as obtained as described, we can account for between 75 and 104% of measured plasma ADG levels (Table 2). As to women with mild dysfunctional hirsutism, i.e. with a Ferriman and Gallwey score 7–15, although mean ADG levels were significantly higher than the upper limit of normal levels in a group of healthy women of comparable age, ADG levels were only above the upper limit of normal in 50% of the cases, and appeared not to be a more reliable parameter of androgenicity than any other androgen (Fig. 1). Free AD levels on the other hand were increased in 75% of the cases and appeared to be a much more reliable, albeit imperfect, parameter of androgenicity. The frequency of increased androgen levels in our patients with mild hirsutism is comparable to the frequency reported by Meikle *et al.* [17]. Also in women with proven polycystic ovary syndrome (PCO), defined as obese, hirsute women with irregular menses or amenorrhoe and increased androstenedione levels, ADG levels were only increased in 2/3 of the cases.

On the other hand our data does suggest that in hirsute women conversion of DHEAS and androstenedione to ADG is increased, as using the CRs observed in normal women we account for only 80% of plasma ADG in women with idiopathic hirsutism and ±60% in hirsute women with PCO (Table 3).

Treatment of mild dysfunctional hirsutism with a combination pill, containing cyproterone acetate (CyAc) 2 mg and ethinyloestradiol (EE) 35 μg, from day 5 to 25 of the cycle for 6 months, results in a significant decrease of ADG levels in these women. Besides precursor levels, also the 5α-reductase activity appears to be decreased, as applying the CRs observed in normal women, to precursor levels, results in an important overestimation of measured ADG levels (Table 3). It is well known that thyroid

Table 3. Contribution of precursors to plasma ADG levels in hirsute women using conversion rates obtained in non-hirsute women

$CR\%_{BB}^{\rightarrow ADG}$ in non-hirsute women	DS (0.068)	D (1.77)	A (8.8)	NSB-T[a] (40.2)	Total ADG calculated from CR (nmol/l)	ADG measured (nmol/l)	% Plasma ADG accounted for CR of non-hirsute women
Basal	4.90	0.47	0.74	0.09	6.20	7.60	82
1 m	3.80	0.38	0.58	0.02	4.78	3.08	155
3 m	3.43	0.40	0.61	0.02	4.46	2.81	159
6 m	3.37	0.36	0.65	0.03	4.41	3.05	145
	Contribution to plasma ADG in nmol/l						

[a]Non-SHBG bound T.

hormones increase hepatic 5α-reductase activity and James *et al.* [10] has demonstrated that hyperthyroidism is accompanied by an increase in plasma ADG levels. As ADG, in contrast to AD, is not bound to sex hormone-binding globulin (SHBG), this increase is not the consequence of an increased binding, but reflects an increased ADG formation, probably at the hepatic level. We confirmed these data and moreover could show that also in so called "euthyroid women with suppressed thyroid stimulating hormone levels", ADG levels, as well as SHBG levels are significantly increased, which implies that these women are in fact hyperthyroid as far as the influence of thyroid hormone on hepatic enzymes is concerned.

In young severely obese (non-hirsute) women, notwithstanding the fact that free T levels were significantly increased, as a consequence of the decreased SHBG levels, the mean ADG level was similar to the mean level in non-obese women of comparable age.

In summary, our data does not confirm the claimed almost absolute reliability of plasma ADG levels as a parameter of peripheral androgenicity. ADG levels are influenced by the levels of the androgen precursors and by the 5α-reductase activity, both in peripheral tissues and in the liver, as suggested by the increased ADG levels in hyperthyroidism. Unfortunately, we have to conclude that in 1990 we are still in search of a reliable parameter of tissue androgenicity in women.

It is evident that in men the potential interest of plasma ADG levels as a parameter of androgenicity is limited, in the view of the reliability of (free) T levels as a parameter(s) of androgenicity. Therefore, we did not study conversion rates of plasma precursors to plasma ADG in men. We recall that Moghissi *et al.* [14] reported that T (and DHT) was an important precursor of plasma ADG in men. We found the mean ADG level in young healthy men to be 16.25 ± 8.70 (SD) nmol/l, slightly lower than T levels. They decrease with age, in parallel with free T levels, and in the group of men over 65 years old, the mean plasma level was 9.30 nmol/l. In young orchidectomized men, ($n = 6$, mean age: 34 years) plasma ADG levels were around 4.0 nmol/l, rather similar to the mean value in women, suggesting that about 25% of ADG levels in men have an adrenal origin.

It is interesting to mention that Belanger *et al.* [18] observed in castrated males with prostatic cancer a correlation of ADG levels with plasma D(S) levels. The levels (mean = 6.30 nmol/l; range 5.21–9.66 nmol/l) in men with Addison's disease ($n = 12$; age 40–60 years; mean: 46 years) were lower than expected from the levels in orchidectomized men, but as T levels were also lower than in normal subjects (mean = 10.20 nmol/l) this may be related to an immunological impairment of Leydig cell function, in analogy to the autoimmune ovarian failure in women with Addison's disease. Finally and surprisingly, whereas transdermal application of a DHT gel (125 mg/day for 8 days), resulted in a 10-fold increase in plasma DHT and a 40-fold increase in AD levels, ADG levels are hardly double the levels observed in normal men, whereas DHTG levels are about 4 times basal levels. This strongly suggests that peripheral tissues, more specifically the skin, are not very effective in conjugating DHT and AD, respectively.

In conclusion ADG levels have not fulfilled the expectation to be a highly reliable parameter of androgenicity in women. In women they have as a major precursor plasma DHEAS. ADG levels reflect essentially adrenal precursor levels as well as tissue 5α-reductase activity, but not only peripheral tissues, also the liver is probably an important site of ADG formation.

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