

# Lp(a) and lipids in adult Turner's syndrome: impact of treatment with 17 $\beta$ -estradiol and norethisterone

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## Abstract

Turner's syndrome is associated with a high incidence of cardiovascular disease and hypothyreosis; conditions which are associated with abnormal lipid metabolism. To test whether alterations of lipid metabolism is present in healthy Turner's women, we compared lipids in a group of adult women with Turner's syndrome with an age matched group of healthy women. In addition the impact of sex steroid replacement therapy was studied in the women with Turner's syndrome. Patients were studied before and during treatment with hormonal replacement therapy, consisting of either oral 17 $\beta$ -estradiol or transdermal 17 $\beta$ -estradiol, and oral norethisterone. Control subjects were studied once in the early follicular stage of the menstrual cycle. The study group consisted of 26 ( $33.2 \pm 7.9$  years) patients with Turner's syndrome and an age matched control group of 24 ( $32.7 \pm 7.6$  years) normal women. Body composition measures, apolipoprotein (apo) B and apo A-I, Lp(a), cholesterol, HDL, LDL, triglycerides, thyroxine (TT4), free thyroxine (FT4), triiodothyronine (TT3), free triiodothyronine (FT3), TSH, and leptin were determined. Apo A-I levels were higher in Turner's patients ( $P = 0.007$ ), while levels of Lp(a) were comparable in the two groups. Only when the participants were divided into groups of low ( $\leq 45$  g/l) and high ( $> 45$  g/l) Lp(a), more women with Turner's syndrome had high levels of Lp(a) than controls ( $P = 0.024$ ), while all other measures of lipid metabolism were comparable to controls. The level of TSH, FT3, and FT4 were significantly higher in Turner's patients, while TT4, TT3 and adjusted 24h energy expenditure were comparable to controls. Lp(a) ( $P = 0.005$ ), HDL ( $P = 0.045$ ) and apo A-I ( $P = 0.039$ ) decreased significantly, while there was a tendency towards a decrease in apo B ( $P = 0.063$ ) during treatment with sex hormones. In conclusion more women with Turner's syndrome than controls have high levels of apolipoprotein A-I and Lp(a), but only after dichomization, while other markers of lipid metabolism are normal. Replacement therapy with female sex hormones lowered Lp(a), HDL cholesterol and apolipoprotein A-I. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Turner Syndrome; Adults; Lipid metabolism; Lipoprotein(a); Apolipoprotein A-I; 17 $\beta$ -Estradiol; Norethisterone

## 1. Introduction

Turner's syndrome is associated with congenital malformations such as coarctation of the aorta, horse shoe kidney and pterygium colli [1], as well as less severe congenital malformations of the heart [2,3], especially in the presence of the 45,X karyotype [3–5]. There is epidemiological evidence for an increased relative risk of ischemic heart disease and vascular disease of the

brain in Turner's syndrome [6], and also in clinical studies there is evidence of increased levels of serum total cholesterol in girls [7] and in adults [8]. However, other aspects of lipid metabolism including Lp(a) levels have not been studied in patients with Turner's syndrome. Lp(a) is recognized as a key contender in the atherosclerotic process, and the serum level of Lp(a) could be a determinant of coronary heart disease in young women [9].

Most women with Turner's syndrome never produce ovarian hormones for the entire length of their lives, despite high levels of FSH and LH, and are thus in a

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state of chronic oestrogen deficiency. However, women with Turner's syndrome are commonly treated with sex hormonal replacement therapy for a prolonged period of time. In many countries it is customary to use natural oestrogens like 17 $\beta$ -estradiol in combination with a gestagen like norethisterone or medroxyprogesterone. Therefore, women with Turner's syndrome probably present the most homogeneous group of patients to be treated with female sex steroids from puberty and often beyond the normal age of menopause. In view of the epidemiological evidence for the role of oestrogen in the protection against coronary heart disease in normal women (pre- and post-menopausal alike) [10,11], it seems plausible that such long-term therapy would protect females with Turner's syndrome from coronary heart disease. It is largely unknown how this treatment affects many aspects of metabolism, including lipid metabolism. In post-menopausal women, combination therapy consisting of oestrogen and gestagen consistently show a lowering effect on circulating levels of Lp(a) [12–14]. Recent data do, however, suggest that hormonal replacement therapy does not have a role in the secondary prevention of coronary heart disease [15].

We present the detailed results of a study of the lipid metabolism in a group of adult women with Turner's syndrome, compared with an age matched group of healthy women. The women with Turner's syndrome were examined before and during replacement with female sex hormones. The first part of this study, concerning the relation between carbohydrate metabolism and cardiovascular risk factors, has previously been presented [16].

## 2. Materials and methods

### 2.1. Subjects

The study group consisted of 26 patients with the Turner's syndrome and an age matched control group of 24 normal women with presumed normal karyotype. Exclusion criteria were: untreated clinical hypo- or hyperthyreosis, former or present malignant disease, clinical liver disease, family history of thrombo-embolic diseases, extreme obesity (BMI > 40), clinical heart disease, other significant diseases, allergy towards any of the drugs used, and heavy smoking (20 cigarettes/day). All women examined were clinically euthyroid.

All subjects received oral and written information concerning the study prior to giving written informed consent. The protocol was approved by the Aarhus County Ethical Scientific Committee and the Danish National Board of Health.

### 2.2. Design

All patients were receiving female hormone replacement therapy prior to the study. This therapy was discontinued 4 months before the study (basal examination of Turner's patients,  $T_B$ ). Following the initial evaluation, patients were randomised to one of two regimens of hormone substitution for 6 months (treatment examination of Turner's patients,  $T_T$ ): Oral hormone substitution consisting of 2 mg 17 $\beta$ -estradiol/day from day 1 to 12 of the menstrual cycle, 2 mg 17 $\beta$ -estradiol/day and 1 mg norethisterone acetate/day from day 13 to 22, and 1 mg 17 $\beta$ -estradiol/day from day 23 to 28 (Trisekvens<sup>®</sup>, Novo Nordisk, Bagsvaerd, Denmark), or transdermal oestrogen substitution consisting of approximately 50 mg 17 $\beta$ -estradiol/55 kg per day for 28 days (Estraderm<sup>®</sup>, Ciba-Geigy, Denmark) and 1 mg norethisterone (Noretisteron Dak<sup>®</sup>, Nycomed DAK, Copenhagen, Denmark) administered orally from day 13 to 22. A total of 11 subjects were randomly allocated to the group receiving transdermal oestrogen, and 15 subjects to the group receiving oral oestrogen. Preliminary analysis did not indicate significant differences between the two treatment groups in changes of any of the variables considered, except TT4 (see below) and subsequently the two groups were pooled in all statistical computations.

All patients were studied twice with a 6-month interval, whereas all controls were evaluated once. Control subjects were studied in the early follicular stage (day 5–10) of the menstrual cycle; Turner's subjects were studied on day 5–10 of the hormone replacement therapy cycle whilst taking oestrogen alone.

### 2.3. Methods

Subjects were admitted at 08:00 h after an overnight fast (10–12 h). After an initial bed rest of at least 45 min, resistance and impedance were measured and fat mass (FM), fat free mass (FFM) and total body water (TBW) were determined, employing bioelectrical impedance (Animeter, HTS-Engineering APS, Odense, Denmark) [17]. Body mass index was calculated as weight (kg) divided by height ( $m$ ) squared, and the waist-to-hip ( $W/H$ ) ratio was determined in the supine position. Indirect calorimetry (Deltatrac Metabolic Monitor, Datex, Helsinki, Finland) with a ventilated hood at 40 l/min was performed; energy expenditure (EE), respiratory quotient (RQ), and 24 h excretion of urea in urine were measured; and glucose, protein and lipid oxidation were calculated [18].

### 2.4. Assays

Apolipoprotein (apo) B and apo A-I concentrations were measured by radio-immuno-assays (Pharmacia,

Sweden). All samples were analysed in duplicate. Sample pairs with an intra-assay variation above 10% were reanalysed. Lipoprotein(a) (Lp(a)) concentrations were measured by a commercial two-site immuno-radiometric (IRMA) assay, as described in detail previously [19]. Our laboratory participated in an international Lp(a) standardization program, the results of which indicated that the variation between laboratories using the IRMA method is small. Our intra-assay and inter-assay coefficients of variation were 2.2 and 3.1%, respectively. Linear regression analysis indicated that the commonly used threshold value for Lp(a) of 30 mg/dl, as measured by ELISA [20] corresponded to a concentration of about 45 mg/dl, as measured by the IRMA technique. We, therefore, used a threshold level of 45 mg/dl to categorize Lp(a) data in two groups: low or high concentration of Lp(a). Cholesterol, high density lipoprotein (HDL) and triglycerides were determined on a COBAS INTEGRA analyser (Roche, Hvidovre, Denmark). Low density lipoprotein (LDL) was calculated according to Friedewald's equation [21]. Serum thyroxine (TT4), free thyroxine (FT4), triiodothyronine (TT3), reverse triiodothyronine (rTT3) and free triiodothyronine (FT3) were measured by radio-immunoassays (RIA) [22]. Serum thyroid stimulating hormone (TSH) was measured by a commercial chemi-luminescent immuno-assay (Diagnostic Products Corporation, Llanberis, UK), and leptin by a commercial RIA (Linco, Inc., St. Louis, MO, USA).

### 2.5. Statistical analysis

All calculations were done with SPSS statistical software for Windows version 7.5 (SPSS Inc, Chicago, IL, USA). Data were examined by Student's two-tailed unpaired and paired *t*-tests or the Mann–Whitney or Wilcoxon two-tailed test when appropriate. Multiple

backwards stepwise linear regression, Pearson product moment correlation and Spearman correlation were used to examine the relationships among different variables. Stepwise linear regression identified FFM, fat-mass and TT3 as independent predictors of energy expenditure, and subsequent analysis of covariance was used to calculate adjusted rates of total energy expenditure, as this method allows for the removal of the linear effects of covariates on the dependent variable [23,24]. The approach eliminates problems arising from not having a correlation of  $r = 1.0$  between the dependent variable and independent variables and the intercept between any two variables is different from zero. Results are expressed as mean  $\pm$  standard deviation (SD) or as geometric mean  $\times / \div$  antilog SD (range) (Lp(a), triglyceride, TSH and FT4). Statistical significance was assumed for *P* less than 5%.

## 3. Results

### 3.1. Untreated Turner's patients versus controls

In Table 1 the relevant anthropometric data of Turner's patients and of controls are given. We made no attempt to match Turner's patients and controls on BMI, while age matching was performed. Turner's women have a characteristic anthropometry and body composition [25], and although BMI was slightly higher in Turner's patients than in the control group, FFM was significantly higher in controls (Table 1).

Apolipoprotein A-I was higher in Turner's patients ( $P = 0.007$ ). Levels of Lp(a) were comparable in the two groups; but when the participants were divided into groups of low ( $\leq 45$  g/l) and high ( $> 45$  g/l) Lp(a), more women with Turner's syndrome had high levels of Lp(a) than controls ( $\chi^2 = 5.128$ ;  $P = 0.024$ ).

Table 1  
Number and age of participants and mean  $\pm$  SD levels of anthropometric data in Turner's patients at baseline and during sex hormone replacement, and in controls

	Turner's ( $T_B$ )	Turner's ( $T_T$ )	Control	$P$ ( $T_B$ vs $T_T$ ) <sup>a</sup>	$P$ ( $T_B$ vs $C$ ) <sup>b</sup>
Number	26		24		
Age	33.2 $\pm$ 7.9		32.7 $\pm$ 7.8		0.8
Height (cm)	147.1 $\pm$ 6.8		168.4 $\pm$ 6.1		0.0005
Weight (kg)	57.7 $\pm$ 11.7	57.8 $\pm$ 11.8	67.9 $\pm$ 14.4	0.6	0.009
BMI (kg/m <sup>2</sup> )	26.6 $\pm$ 4.6	26.7 $\pm$ 4.8	23.8 $\pm$ 4.2	0.6	0.04
FFM (kg)	38.2 $\pm$ 6.2	39.1 $\pm$ 6.4	48.5 $\pm$ 6.8	0.02	0.0005
FM (kg)	19.7 $\pm$ 6.4	18.9 $\pm$ 6.3	19.4 $\pm$ 8.4	0.008	0.9
<i>W/H</i>	0.88 $\pm$ 0.09	0.88 $\pm$ 0.09	0.78 $\pm$ 0.06	0.4	0.0005
EE (kcal/24 h)	1290 $\pm$ 168	1270 $\pm$ 158	1425 $\pm$ 37	0.3	0.009
Adjusted EE (kcal/24 h) <sup>c</sup>	1340 $\pm$ 30		1369 $\pm$ 31		0.6

<sup>a</sup> Paired *t*-test.

<sup>b</sup> Independent *t*-test.

<sup>c</sup> Adjusted for fat free mass, fat mass and TT3 (mean  $\pm$  SE); for further information, see statistical methods.

Table 2  
Mean  $\pm$  SD (geometric mean  $\times / \div$  antilog SD (range) of triglycerides and lipoprotein (a) levels of measures of lipid metabolism in Turner's patients at baseline and during sex hormone, and in controls

	Turner's ( $T_B$ )	Turner's ( $T_T$ )	Control	$P$ ( $T_B$ vs $T_T$ ) <sup>a</sup>	$P$ ( $T_B$ vs $C$ ) <sup>b</sup>
Total cholesterol	4.9 $\pm$ 1.2	4.7 $\pm$ 0.8	4.7 $\pm$ 0.9	0.4	0.4
HDL cholesterol	1.6 $\pm$ 0.6	1.4 $\pm$ 0.3	1.5 $\pm$ 0.4	0.045	0.5
Triglycerides	0.8 $\times / \div$ 1.8 (0.2–2.0)	0.8 $\times / \div$ 1.7 (0.4–2.7)	0.9 $\times / \div$ 1.5 (0.5–2.0)	0.8 <sup>c</sup>	0.8 <sup>d</sup>
LDL cholesterol	3.0 $\pm$ 1.2	2.9 $\pm$ 0.7	2.7 $\pm$ 0.8	0.7	0.4
Apolipoprotein A-I (g/l)	1.77 $\pm$ 0.41	1.59 $\pm$ 0.31	1.50 $\pm$ 0.26	0.039	0.007
Apolipoprotein B (g/l)	0.91 $\pm$ 0.23	0.87 $\pm$ 0.21	0.82 $\pm$ 0.21	0.063	0.1
Lipoprotein (a) (g/l)	7.9 $\times / \div$ 4.2 (1.5–127.7)	6.4 $\times / \div$ 4.2 (1.7–106.9)	10.2 $\times / \div$ 2.4 (1.7–43.7)	0.005 <sup>c</sup>	0.2 <sup>d</sup>
Lp(a) coding <sup>e</sup>	21/5	23/3	24/0	0.4 <sup>f</sup>	0.024 <sup>g</sup>

<sup>a</sup> Paired *t*-test.

<sup>b</sup> Independent *t*-test.

<sup>c</sup> Wilcoxon two-tailed test.

<sup>d</sup> Mann–Whitney rank sum test.

<sup>e</sup> Coding was performed to divide the populations into two, with a normal or high level of Lp(a) (for further information see materials and methods).

<sup>f</sup>  $\chi^2 = 0.591$ ;

<sup>g</sup>  $\chi^2 = 5.128$ .

There were no differences in the TSH levels among those Turner's with normal Lp(a) and those with high Lp(a) levels (results not shown). The other lipid variables did not differ significantly among groups (Table 2), and we found no significant association between the lipid variables, and thyroid hormones or energy expenditure.

TSH ( $2.5 \times / \div 3.7$  vs  $1.1 \times / \div 1.7$  mU/l,  $P = 0.04$ ) was significantly higher in Turner's patients compared with controls, while TT3 ( $1.9 \pm 0.3$  vs  $1.7 \pm 0.2$  nmol/l,  $P = 0.06$ ), TT4 ( $130 \pm 14$  vs  $124 \pm 15$  nmol/l,  $P = 0.3$ ), and *r*TT3 ( $0.23 \pm 0.05$  vs  $0.26 \pm 0.06$  nmol/l,  $P = 0.2$ ) did not differ between groups. On the contrary FT3 ( $6.2 \pm 1.4$  vs  $5.3 \pm 1.2$  pmol/l,  $P = 0.03$ ) and FT4 ( $21.4 \times / \div 1.3$  (13–37) vs  $19.1 \times / \div 1.3$  (13–44) pmol/l,  $P = 0.04$ ) were slightly, but significantly higher in Turner's patients.

By multiple linear regression FFM, FM, and TT3 were all independent explanatory variables of energy expenditure (multiple  $r = 0.835$ ,  $P < 0.0005$ ), while status (Turner's syndrome or control) was not a significant variable. Energy expenditure (24 h) adjusted for FFM, fatmass and TT3, was comparable between Turner's syndrome women and controls (Table 1). Serum leptin was not different in the two groups ( $11.3 \times / \div 1.8$  vs  $8.9 \times / \div 1.9$  mg/l,  $P = 0.4$ ). Leptin correlated closely to energy expenditure in both Turner's and controls, but did not contribute significantly in the multiple regression model. Most of the variance in energy expenditure was accounted for by FFM (61%,  $P < 0.0005$ ), and FM (6%,  $P = 0.006$ ). An additional 3% of the variance in energy expenditure was accounted for by TT3 ( $P = 0.06$ ). There were no differences in glucose, lipid and protein oxidation (results not shown).

### 3.2. Treatment versus washout phase in Turner's syndrome

Lp(a) decreased significantly with 17%, and HDL cholesterol and apolipoprotein A-I also decreased, while apolipoprotein B tended to decrease (Table 2). A decrease in Lp(a) was noted in almost all individuals and seen both in the low and high concentration area of Lp(a) (Fig. 1a, b). The changes in Lp(a) correlated significantly and inversely with the small changes in TT3 (Fig. 1c).

Serum TSH, TT3, FT3, FT4, leptin and 24 h energy expenditure were all unchanged by sex hormone replacement, while TT4 increased significantly in the orally treated group ( $P = 0.01$ ) and decreased significantly in the transdermally treated group ( $P = 0.04$ ). Thus the route of administration of  $17\beta$ -estradiol had a significant impact on TT4 ( $\Delta$ TT4:  $-4.8 \pm 6.6$  (transdermal treatment) vs  $12.3 \pm 16.1$  nmol/l (oral treatment),  $P = 0.003$ ). As expected, *r*TT3 ( $0.23 \pm 0.04$  vs  $0.29 \pm 0.07$ ,  $P < 0.0005$ ) increased significantly during treatment.

## 4. Discussion

Compensated hypothyroidism and insufficiently treated hypothyroidism are associated with elevated LDL cholesterol, apolipoprotein B levels, and coronary artery disease [26–28]. Since hypothyroidism is a major clinical problem in Turner's syndrome, elevated lipids may help explain part of the increased risk of cardiovascular disease in Turner's syndrome found recently in a register-based study [6]. This increased morbidity was not related to the common congenital malformations asso-

ciated with Turner's syndrome [6]. Chronic oestrogen deficiency, known to affect most adult women with Turner's syndrome, is likely to be associated with the increased cardiovascular morbidity. However, in the present study there were no indications of imminent hypothyreosis and in accordance with this, and despite unfavourable changes in the body composition and distribution of fatmass, most markers of lipid metabolism were comparable to an age-matched, somewhat leaner, group of women without any clinical

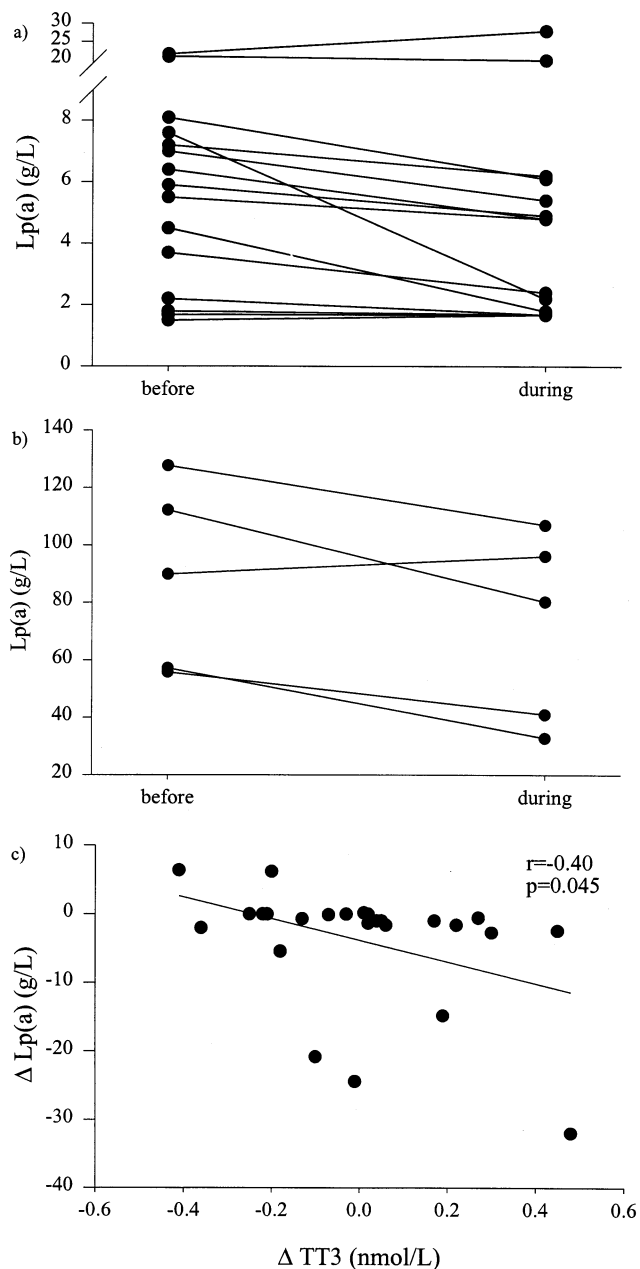


Fig. 1. (a) Individuals with Turner's syndrome with normal levels of Lp(a) before and during sex hormone replacement therapy. (b) Individuals with Turner's syndrome with high levels of Lp(a) before and during sex hormone replacement therapy. (c)  $\Delta$ Lp(a) versus  $\Delta$ TT3 in women with Turner's syndrome.

disease. The HDL associated apolipoprotein A-I levels were increased in Turner's patients in the untreated state. This could be attributable to either increased synthesis or decreased degradation of apolipoprotein A-I. In ovariectomized and hysterectomized baboons oestrogen increases apolipoprotein A-I production [29] and the same is seen in pre-menopausal women [30] in comparison with the untreated state. Thus, it is probably most likely that the increase in apolipoprotein A-I is caused by decreased degradation of the HDL particles. Apolipoprotein A-I may confer protection against ischemic heart disease. The finding of elevated levels of apolipoprotein A-I is surprising, since HDL and apolipoprotein A-I are inversely associated with atherosclerosis. Since increased morbidity from atherosclerosis has been documented in Turner's syndrome, this finding emphasizes that other factors further the development of atherosclerosis in Turner's syndrome, stressing that atherosclerosis has to be viewed as a multifactorial disease where numerous factors are involved and have to be identified. Studying Lp(a) as a continuous variable, showed there was no difference between Turner's patients and controls. Only after categorisation of Lp(a), more women with Turner's syndrome had high levels of Lp(a). Lp(a) is important in atherosclerosis, high Lp(a) is also associated with coronary heart disease in young women [9]. Levels of Lp(a) are higher in post-menopausal compared with pre-menopausal women, and the rise in Lp(a) has been shown to be associated with changes in hormonal status [31]. Treatment with oestrogen causes a substantial reduction in Lp(a) levels in males with prostate cancer [32], as in post-menopausal women [33], likewise, treatment with a combination of oestrogen and gestagen causes significant reductions in serum levels of Lp(a) [12–14]. Other studies have indicated that the effect of Lp(a) is modulated by the concomitant level of LDL cholesterol [34]. In the present study Lp(a), apolipoprotein A-I, and HDL decreased significantly, and apolipoprotein B tended to decrease during treatment with female sex hormones. There was no difference in the response in these lipid parameters due to the route of administration of  $17\beta$ -estradiol, i.e. oral or transdermal. However, this could be due to the small number of patients in each group, since previous studies have shown a more pronounced reduction in Lp(a) in post-menopausal women after oral  $17\beta$ -estradiol administration, as well as differential effects on HDL and LDL [13,35,36]. The reduction in Lp(a) is in line with previous large scale clinical trials, showing that estradiol decreases Lp(a), while gestagen does not affect it [37]. Adults with Turner's syndrome without menstruation have undetectable levels of  $17\beta$ -estradiol and low levels of oestrone and oestronesulphate (unpublished observations). Thus, the present data could be interpreted as the 'pure' effect of  $17\beta$ -estradiol on Lp(a) in a model

with undetectable levels of 17 $\beta$ -estradiol. Likewise estradiol increases HDL cholesterol and this effect is opposed by the addition of gestagen [37]. Supposedly the reduction in Lp(a), and apolipoprotein B can be ascribed to the effect of 17 $\beta$ -estradiol, while the untoward decrease in apolipoprotein A-I and HDL may be ascribed to the gestagen component. The changes in Lp(a) during sex steroid replacement therapy were correlated to small changes in TT3, supporting the contention that thyroid hormones are independent regulators of the Lp(a) concentration, with low levels in the hyperthyroid state and high levels in the hypothyroid state [38,39]. It should be noted that the level of TT3 was unchanged by sex hormone treatment in the group of women with Turner's syndrome as a whole. Whether the observed lipid profile in the untreated state in Turner's women can explain the increased cardiovascular morbidity and mortality in Turner's syndrome [6,40] is probably doubtful. However, since it is becoming clear that treatment with oestrogen not only confers cardioprotectivity during a lowering of harmful circulating lipids, but also through direct antioxidant effects [41], a change in the vascular reactivity [42] and its interaction with vascular smooth muscle [43,44], part of the increased cardiovascular morbidity and mortality in Turner's syndrome could still be explained by non-use of oestrogen.

In the present study the level of TSH was higher in healthy Turner's patients compared with controls, along with a high level of TT3 in Turner's patients, while the level of TT4 was comparable with controls. Free hormone levels were slightly, but significantly, higher in women with Turner's syndrome. We do not have any ready explanation for this slight elevation of free thyroid hormones. Thus, despite the increased TSH levels, this group of women can not be characterised as having subclinical hypothyroidism, since one would expect normal or decreased levels of thyroid hormone concentrations [9,45,46]. We did see divergent effects of the mode of administration of oestrogen on TT4, with an increase during treatment with oral oestrogen. This effect was possibly mediated by divergent effects of the treatment regimens on the level of thyroxine-binding globulin (TBG), where orally administered oestrogen especially increases TBG.

Energy expenditure (24 h) was higher in controls. However, differences in body composition variables accounted for the major part of this variation (FFM and FM, 70%), in line with previous reports of FFM as the major determinant of 24-h energy expenditure [47,48], and a minor part of the variation was explained by the level of TT3 [49]. The finding of similar levels of 24-h energy expenditure when adjusting for relevant predictive variables makes it highly unlikely that the group of women with Turner's syndrome are on the brink of clinical hypothyroidism. Interestingly, levels of

leptin were similar in Turner's and controls, despite rather large differences in body composition between the two groups. The percentage of fat was increased in the Turner's patients, while the total amount of body fat in kilograms was similar to controls. Serum leptin levels are tightly correlated to the percentage of body fat, and are higher in women compared with men [50]. These findings may suggest that women with Turner's syndrome are in a state of hypoleptinemia, since one would expect a higher level of leptin with the observed percentage of body fat. Speculatively, this could be viewed as impaired feedback from the adipose tissue and could perhaps help explain why many women with Turner's syndrome have a relatively elevated BMI with increased body fat [25,51,52].

In conclusion, women with Turner's syndrome have high levels of apo A-I. Levels of Lp(a) were normal, and only after categorisation did more women with Turner's syndrome have high levels of Lp(a). However, most markers of lipid metabolism are normal compared with age-matched control women. Replacement therapy with female sex hormones reduced levels of Lp(a) and exerted small opposing effects on the other markers of lipid metabolism; the route of administration of 17 $\beta$ -estradiol did not influence the different markers of lipid metabolism. Moreover, some adult women with Turner's syndrome have elevated levels of TSH, but normal levels of peripheral thyroid hormones.

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### References

- [1] Lippe B. Turner's syndrome. *Endocrinol Metab Clin North Am* 1991;20:121–52.
- [2] Dawson FKL, Wright AM, Bakker B, Pitlick PT, Rosenfeld RG. Cardiovascular evaluation in Turner's syndrome: utility of MR imaging. *Austral Radiol* 1992;36:204–9.
- [3] Gotzsche CO, Krag Olsen B, Nielsen J, Sorensen KE, Kristensen BO. Prevalence of cardiovascular malformations and association with karyotypes in Turner's syndrome. *Arch Dis Child* 1994;71:433–6.
- [4] Berdahl LD, Wenstrom KD, Hanson JW. Web neck anomaly and its association with congenital heart disease. *Am J Med Genet* 1995;56:304–7.

- [5] Lacro RV, Jones KL, Benirschke K. Coarctation of the aorta in Turner's syndrome: a pathologic study of fetuses with nuchal cystic hygromas, hydrops fetalis, and female genitalia. *Pediatrics* 1988;81:445–51.
- [6] Gravholt CH, Juul S, Naeraa RW, Hansen J. Morbidity in Turner's syndrome. *J Clin Epidemiol* 1998;51:147–58.
- [7] Ross JL, Feuille P, Long LM, Kowal K, Kushner H, Cutler GBJ. Lipid abnormalities in Turner's syndrome. *J Pediatr* 1995;126:242–5.
- [8] Garden AS, Diver MJ, Fraser WD. Undiagnosed morbidity in adult women with Turner's syndrome. *Clin Endocrinol Oxf* 1996;45:589–93.
- [9] Orth Gomer K, Mittleman MA, Schenck Gustafsson K, Wamala SP, Eriksson M, Belkic K, Kirkeeide R, Svane B, Ryden L. Lipoprotein(a) as a determinant of coronary heart disease in young women. *Circulation* 1997;95:329–34.
- [10] Barrett Connor E, Bush TL. Oestrogen and coronary heart disease in women. *J Am Med Assoc* 1991;265:1861–7.
- [11] Grady D, Rubin SM, Petitti DB, Fox CS, Black D, Ettinger B, Ernster VL, Cummings SR. Hormone therapy to prevent disease and prolong life in post-menopausal women. *Ann Intern Med* 1992;117:1016–37.
- [12] Kim CJ, Jang HC, Cho DH, Min YK. Effects of hormone replacement therapy on lipoprotein(a) and lipids in post-menopausal women. *Arterioscler Thromb* 1994;14:275–81.
- [13] Taskinen MR, Puolakka J, Pyörälä T, Luotola H, Bjaoran M, Kaarianen J, Lahdenpera S, Ehnholm C. Hormone replacement therapy lowers plasma Lp(a) concentrations. Comparison of cyclic transdermal and continuous oestrogen–progestin regimens. *Arterioscler Thromb Vasc Biol* 1996;16:1215–21.
- [14] Darling GM, Johns JA, McCloud PI, Davis SR. Oestrogen and progestin compared with simvastatin for hypercholesterolemia in post-menopausal women. *New Engl J Med* 1997;337:595–601.
- [15] Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E. Randomised trial of oestrogen plus progestin for secondary prevention of coronary heart disease in post-menopausal women. Heart and oestrogen/progestin replacement study (HERS) research group. *J Am Med Assoc* 1998;280:605–13.
- [16] Gravholt CH, Naeraa RW, Nyholm B, Gerdes U, Christiansen E, Schmitz O, Christiansen JS. Glucose metabolism, lipid metabolism, and cardiovascular risk factors in adult Turner's syndrome: the impact of sex hormone replacement. *Diabetes Care* 1998;21:1062–70.
- [17] Heitmann BL. Prediction of body water and fat in adult Danes from measurement of electrical impedance: a validation study. *Int J Obes* 1990;14:789–802.
- [18] Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* 1983;55:628–34.
- [19] Klausen IC, Gerdes LU, Schmidt EB, Dyerberg J, Faergeman O. Differences in apolipoprotein (a) polymorphism in west Greenland Eskimos and Caucasian Danes. *Hum Genet* 1992;89:384–8.
- [20] Sandholzer C, Saha N, Kark JD, Rees A, Jaross W, Dieplinger H, Hoppichler F, Boerwinkle E, Utermann G. Apo(a) isoforms predict risk for coronary heart disease. A study in six populations. *Arterioscler Thromb* 1992;12:1214–26.
- [21] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
- [22] Weeke J, Orskov H. Ultrasensitive radioimmunoassay for direct determination of free triiodothyronine concentration in serum. *Scand J Clin Lab Invest* 1975;35:237–44.
- [23] Toth MJ, Goran MI, Ades PA, Howard DB, Poehlman ET. Examination of data normalization procedures for expressing peak VO<sub>2</sub> data. *J Appl Physiol* 1993;75:2288–92.
- [24] Poehlman ET, Toth MJ. Mathematical ratios lead to spurious conclusions regarding age- and sex-related differences in resting metabolic rate. *Am J Clin Nutr* 1995;61:482–5.
- [25] Gravholt CH, Naeraa RW. Reference values for body proportions and body composition in adult women with Turner's syndrome. *Am J Med Genet* 1997;72:403–8.
- [26] Dean JW, Fowler PB. Exaggerated responsiveness to thyrotrophin releasing hormone: a risk factor in women with coronary artery disease. *Br Med J* 1985;290:1555–61.
- [27] Arem R, Patsch W. Lipoprotein and apolipoprotein levels in subclinical hypothyroidism. Effect of levothyroxine therapy. *Arch Intern Med* 1990;150:2097–100.
- [28] Arem R, Escalante D. Subclinical hypothyroidism: epidemiology, diagnosis and significance. In: *Advances in Internal Medicine*. St. Louis, MO: Mosby, 1996:213–50.
- [29] Kushwaha RS, Foster DM, Murthy VN, Carey KD, Bernard MG. Metabolic regulation of apoproteins of high-density lipoproteins by oestrogen and progesterone in the baboon (*Papio sp*). *Metabolism* 1990;39:544–52.
- [30] Schaefer EJ, Foster DM, Zech LA, Lindgren FT, Brewer HBJ, Levy RI. The effects of oestrogen administration on plasma lipoprotein metabolism in pre-menopausal females. *J Clin Endocrinol Metab* 1983;57:262–7.
- [31] Brown SA, Morrisett JD, Boerwinkle E, Hutchinson R, Patsch W. The relation of lipoprotein[a] concentrations and apolipoprotein[a] phenotypes with asymptomatic atherosclerosis in subjects of the atherosclerosis risk in communities (ARIC) Study. *Arterioscler Thromb* 1993;13:1558–66.
- [32] Henriksson P, Angelin B, Berglund L. Hormonal regulation of serum Lp (a) levels. Opposite effects after oestrogen treatment and orchidectomy in males with prostatic carcinoma. *J Clin Invest* 1992;89:1166–71.
- [33] Conard J, Basdevant A, Thomas JL, Ochslein E, Denis C, Guyene TT, Degrelle H. Cardiovascular risk factors and combined oestrogen–progestin replacement therapy: a placebo-controlled study with norgestrel acetate and estradiol. *Fertil Steril* 1995;64:957–62.
- [34] Maher VM, Brown BG. Lipoprotein (a) and coronary heart disease. *Curr Opin Lipidol* 1995;6:229–35.
- [35] Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnkar V, Sacks FM. Effects of post-menopausal oestrogen replacement on the concentrations and metabolism of plasma lipoproteins. *New Engl J Med* 1991;325:1196–204.
- [36] Hanggi W, Lippuner K, Riesen W, Jaeger P, Birkhauser MH. Long-term influence of different post-menopausal hormone replacement regimens on serum lipids and lipoprotein(a): a randomised study. *Br J Obstet Gynaecol* 1997;104:708–17.
- [37] Sacks FM, Gerhard M, Walsh BW. Sex hormones, lipoproteins, and vascular reactivity. *Curr Opin Lipidol* 1995;6:161–6.
- [38] Klausen IC, Hegedus L, Hansen PS, Nielsen FE, Gerdes LU, Faergeman O. Apolipoprotein(a) phenotypes and lipoprotein(a) concentrations in patients with hyperthyroidism. *J Mol Med* 1995;73:41–6.
- [39] de Bruin TW, van Barlingen H, van Linde Sibenius Trip M, van Vuurst de Vries AR, Akveld MJ, Erkelens DW. Lipoprotein(a) and apolipoprotein B plasma concentrations in hypothyroid, euthyroid, and hyperthyroid subjects. *J Clin Endocrinol Metab* 1993;76:121–6.
- [40] Naeraa RW, Gravholt CH, Hansen J, Nielsen J, Juul S. Mortality in Turner's syndrome. In: Albertsson-Wikland K, Ranke MB, editors. *Turner's Syndrome in a Life Span Perspective: Research and Clinical Aspects*. Amsterdam: Elsevier, 1995:323–3.
- [41] Sack MN, Rader DJ, Cannon RO. Oestrogen and inhibition of oxidation of low-density lipoproteins in post-menopausal women. *Lancet* 1994;343:269–70.

- [42] Gilligan DM, Quyyumi AA, Cannon RO. Effects of physiological levels of oestrogen on coronary vasomotor function in postmenopausal women. *Circulation* 1994;89:2545–51.
- [43] Losordo DW, Kearney M, Kim EA, Jekanowski J, Isner JM. Variable expression of the oestrogen receptor in normal and atherosclerotic coronary arteries of pre-menopausal women. *Circulation* 1994;89:1501–10.
- [44] Selzman CH, Gaynor JS, Turner AS, Johnson SM, Horwitz LD, Whitehill TA, Harken AH. Ovarian ablation alone promotes aortic intimal hyperplasia and accumulation of fibroblast growth factor. *Circulation* 1998;98:2049–54.
- [45] Pacchiarotti A, Martino E, Bartalena L, Aghini Lombardi F, Grasso L, Buratti L, Falcone M, Pinchera A. Serum free thyroid hormones in subclinical hypothyroidism. *J Endocrinol Invest* 1986;9:315–9.
- [46] Surks MI, Ocampo E. Subclinical thyroid disease. *Am J Med* 1996;100:217–23.
- [47] Bogardus C, Lillioja S, Ravussin E, Abbott W, Zawadzki JK, Young A, Knowler WC, Jacobowitz R, Moll PP. Familial dependence of the resting metabolic rate. *New Engl J Med* 1986;315:96–100.
- [48] Welle S, Nair KS. Relationship of resting metabolic rate to body composition and protein turnover. *Am J Physiol* 1990;258:E990–8.
- [49] Astrup A, Buemann B, Toubro S, Ranneries C, Raben A. Low resting metabolic rate in subjects predisposed to obesity: a role for thyroid status. *Am J Clin Nutr* 1996;63:879–83.
- [50] Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *New Engl J Med* 1996;334:292–5.
- [51] Bosze P, Eiben OG, Gaal M, Laszlo J. Body measurements of patients with streak gonads and their bearing upon the karyotype. *Hum Genet* 1980;54:355–60.
- [52] Holl RW, Kunze D, Etzrodt H, Teller W, Heinze E. Turner's syndrome: final height, glucose tolerance, bone density and psychosocial status in 25 adult patients. *Eur J Pediatr* 1994;153:11–6.