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Increased Liver Enzymes in Adult Women with Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency

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Abstract. The aims were assessing liver function tests (LFT) in women with congenital adrenal hyperplasia (CAH) on glucocorticoids. Sixty-one women with genetically verified CAH due to 21-hydroxylase deficiency, aged 18-63 years were compared to 61 controls. Serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT), anthropometry and fat mass (dual energy X-ray absorptiometry) were measured. ALT and GGT were higher in the entire patient group (p=0.01and 0.002); AST, GGT and ALP in patients ≥30 years (p=0.007-0.045); all LFT in salt-wasting (p<0.001-0.042); GGT in simple virilizing (p=0.008); ALT, GGT and ALP in Null/Null genotype (p=0.018-0.040); ALT and GGT in I2splice genotype (p<0.001 and 0.011). Using a recently proposed cut-off level for ALT (>0.317 μkat/L), 54% of patients vs 23% of controls had elevated levels (p=0.028). In patients, GGT and ALP correlated with waist circumference and with total body and trunk fat (r=0.274-0.406, p=0.001-0.043). However, ALT, GGT and ALP were increased even in non-obese patients (waist circumference ≤88 cm and body mass index <30 kg/m^2) (p=0.012-0.045) mainly attributed to the patients ≥30 years who also demonstrated elevated insulin levels and HOMA-indices. In conclusion, compared with controls, women with CAH have higher LFT, in particular patients ≥30 years and those with severe forms, probably reflecting a higher lifetime glucocorticoid exposure. LFT were positively correlated to measurements of body fat. These women might have increased frequency of NAFLD. The finding of higher LFT also in non-obese patients suggests that not only central obesity but also glucocorticoids per se may influence.

Key words: Liver function tests, Non-alcoholic fatty liver disease, Congenital adrenal hyperplasia, Glucocorticoids

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CLASSIC congenital adrenal hyperplasia (CAH), most commonly due to 21-hydroxylase deficiency, results in cortisol deficiency, which stimulates ACTH secretion that increases the secretion of steroid precursors with androgenic effects [1]. These individuals are usually diagnosed at birth or in early childhood. Glucocorticoids are the cornerstones of CAH treatment suppressing the excessive androgen secretion and supplementing the cortisol deficiency [1]. The glucocorticoid doses needed to suppress adrenal androgens in CAH are often supraphysiological and bring a risk for obesity, hyperinsulinemia and gestational diabetes [2]. In the milder non-classic (NC) form glucocorticoids are not mandatory but can be used if the disease is symptomatic [1].

Polycystic ovary syndrome (PCOS) is characterized by insulin resistance, irregular menstrual cycles, androgen excess and polycystic ovaries. It can clini-

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cally be indistinguishable from NC CAH and the latter is sometimes misdiagnosed as PCOS if not proper laboratory testing is performed [3]. Increased frequency of elevated aminotransferase activity and signs of non-alcoholic fatty liver disease (NAFLD) have recently been reported in PCOS [4, 5].

NAFLD may lead to cirrhosis, hepatocellular cancer and liver-related death. In the majority of cases, however, NAFLD is slowly progressive with liver-related morbidity and mortality occurring in only a minority (5-20% and 3-9%, respectively) [6]. Importantly, NAFLD is associated with increased risk of overall death compared to the general population (standard mortality ratio 1.34), with diabetes and cirrhosis being risk factors for death. Liver disease is the third leading cause of death among NAFLD patients, compared to the 13th leading cause among the general population [6].

The aims of this study were to investigate if adult females with CAH demonstrated any elevation of liver function tests (LFT) compared with age and sex-matched controls; and if there were any differences between younger vs older patients and between different genotypes and phenotypes. We also wanted to explore if differences in LFT were found in non-obese patients vs non-obese controls.

Materials and Methods

Subjects

Recruitment and characteristics of patients and controls have been reported previously [2, 3, 7-9]. Briefly, the patient-group comprised 61 women with CAH (median age 30, range 18-63 years). Diagnoses were verified by review of original paediatric and adult records including genital examinations, laboratory reports of adrenal steroids and mutation analyses of the *CYP21A2* gene [10]. The majority were classic CAH (salt-wasting [SW] n=27, and simple virilizing [SV] n=28). The four most common genotypes were Null/Null (n=13), I2splice (n=15), I172N (n=25) and V281L (n=4).

All patients received glucocorticoids: prednisolone (n=30, mean dose 6.3±0.32 mg), hydrocortisone (n=17, mean dose 33.3±2.1 mg), cortisone acetate (n=5, mean dose 40±2.5 mg), dexamethasone (n=7, mean dose 0.55±0.08 mg), and a combination of two glucocorticoids (n=2). Fludrocortisone was taken by 50 patients (82%).

Female controls born on the same date as the patients with CAH were recruited from local population registries. The only preset exclusion criteria were glucocorticoid therapy, malignancy and severe mental or psychiatric disturbance with inability to consent to the study. The controls were examined within one year from their age-matched patients.

There was no evidence of excessive alcohol intake in either patients or controls and there were no reports of liver disease including hepatitis B or C. In one patient with normal LFT two liver hemangiomas were previously diagnosed incidentally and judged to be without clinical relevance.

The study was approved by the research ethics committees of the Karolinska Institute, Stockholm and the Göteborg University, Göteborg, Sweden. All participants gave written informed consent.

Study protocol

Patients and controls were examined as outpatients at the Department of Endocrinology, Metabolism and Diabetes, Karolinska University Hospital, Stockholm (n=50) or the Department of Obstetrics and Gynaecology, Sahlgrenska University Hospital, Göteborg (n=11), Sweden.

A medical history was obtained from all patients and controls. In addition, all participants answered questionnaires on social situation and previous and present health. A physical examination was performed including weight, height, waist and hip circumference [2]. Body mass index (BMI) was calculated dividing total body weight with height^2 (kg/m^2). Obesity was defined as BMI $\geq$30 kg/m^2 together with a waist circumference >88 cm as these are the cut-off levels where the risk for type 2 diabetes, hypertension and cardiovascular disease increases markedly [11]. Individuals below these two cut-off levels were considered non-obese. Blood samples were collected in the morning after an overnight fast.

Biochemical assays

The liver function tests, serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT) were analysed on SYNCHRON

LX Systems (Beckman Coulter Inc, Fullerton, California, USA). The reference limits for females given by the manufacturer were for ALP: <3.8 μkat/L; ALT and AST: <0.60 μkat/L; and GGT: <0.80 μkat/L. According to Prati *et al.* [12] ALT >0.317 μkat/L (19 U/L) in women should be considered abnormal. Serum cholesterol, triglycerides and HDL were measured on SYNCHRON LX Systems (Beckman Coulter Inc, Fullerton, California, USA). LDL concentration was calculated as suggested by Friedewald *et al*. [13]. Serum insulin was analyzed by fluoroimmunoassay (AutoDelfia, Wallac Inc, Turku, Finland). RIA method was used for the determination of serum IGF-I [14]. When IGF-I levels were expressed as SD scores (SDS), they were calculated from the regression line of values in 448 healthy subjects, aged 20-96 years [15]. Glucose was measured using routine assay. The HOMA-index [insulin/($22.5e^{-In\ glu}$)] was calculated as an estimation of insulin resistance using a single fasting sample of insulin and glucose [16]. It has been suggested that a HOMA-index ≥2.77 indicates insulin resistance [17].

Dual energy X-ray absorptiometry (DXA)

Total and regional body fat, lean body mass, and bone mineral density (BMD) in total body, lumbar spine and neck were estimated by DXA in 57 patients and 60 control subjects using a Lunar Model DPX-L or Prodigy equipment (Lunar Radiation, Madison, WI, USA) using a standard procedure previously described [18]. The two instruments were calibrated. Three patients were assessed using Hologic QDR 4500 (Hologic Inc., Waltham, MA, USA) and were excluded from the statistical analysis. To adjust for the height difference between patients and controls, fat and lean body mass were divided by height^2 (kg/m^2).

Glucocorticoid supplementation

The present doses of glucocorticoids were converted to hydrocortisone equivalents using: anti-inflammatory equivalents (30 mg hydrocortisone=37.5 mg cortisone acetate=7.5 mg prednisolone=0.75 mg dexamethasone) [19]; and growth-retarding equivalents (30 mg hydrocortisone=37.5 mg cortisone acetate=6 mg prednisolone=0.375 mg dexamethasone) [20]. Thereafter, hydrocortisone equivalents were reported in mg per body surface (mg/m^2).

Statistics

Results are presented as mean±SEM if not otherwise stated. Comparisons between groups were performed by unpaired t test when data were normally distributed. Otherwise, the Mann-Whitney rank-sum test was used, and in these cases median and range were stated. When 2 x 2 frequency table calculations were performed, Chi square with Yates correction was used or if the expected frequency were small (<5), Fisher's exact test. Spearman's correlation and multiple regression analysis were applied to assess correlation between continuous variables. Statistical significance was set at $p<0.05$. Statistical analyses were performed using SigmaStat for Windows (Jandel Scientific, Erkarath, Germany).

Results

Liver function tests, glucose and insulin in women with CAH and controls

ALT and GGT were significantly elevated in patients compared to controls, whereas the elevation of ALP almost reached significance (p=0.052). AST was similar in patients and controls (Table 1). The majority had LFT within the given normal range and a few subjects had modest elevations. In patients, elevations were found of AST (n=1, 0.62 μkat/L), ALT (n=3, highest value 0.64 μkat/L), GGT (n=3, highest value 2.8 μkat/L), and ALP (n=3, highest value 4.1 μkat/L). One 31-year-old woman had elevations of AST, ALT and GGT. In controls, elevations were found in ALT (n=2, highest value 1.1 μkat/L), GGT (n=1, 1.2 μkat/L), and ALP (n=3, highest value 6.0 μkat/L). In all, 7 women vs 6 controls had at least one of the LFT elevated. However, if the stricter upper reference limit suggested by Prati *et al*. [12] for ALT was used, 54% (33/61) women with CAH vs 33% (20/61) controls demonstrated elevated ALT (p=0.028).

Elevations compared to controls were mainly seen in the 34 patients ≥30 years (AST, GGT, and ALP: p=0.035, 0.007 and 0.045). Only one patient and two controls < 30 years of age had LFT elevations. Moreover, compared with controls, patients with SW demonstrated elevation of all four LFT, women with SV had only increased GGT and no differences were found in the six NC patients (Table 1). A similar pat-

Table 1. Liver function tests in adult females divided into different clinical phenotypes of congenital adrenal hyperplasia due to 21-hydroxylase deficiency and age- and sex-matched controls (mean±SEM or median and range)

	Patients (n=61)	Controls (n=61)	SW (n=27)	Controls (n=27)	SV (n=28)	Controls (n=28)	NC (n=6)	Controls (n=6)
S-AST (μkat/L)	0.39±0.01	0.37±0.01	0.39±0.015*	0.35±0.013	0.38±0.014	0.37±0.019	0.45±0.034	0.44±0.036
S-ALT (μkat/L)	0.32 (0.14-0.64)**	0.26 (0.10-1.1)	0.37±0.025***	0.27±0.015	0.28 (0.14-0.44)	0.25 (0.10-1.1)	0.39±0.14	0.39±0.14
Elevated[a] (n)	33*	20	17**	6	10	10	4	4
S-ALP (μkat/L)	2.5 (0.60-4.1)[b]	2.2 (0.70-6.0)	2.67±0.11*	2.26±0.14	2.25 (0.60-4.1)	2.15 (0.70-6.0)	2.67±0.33	2.53±0.33
S-GGT (μkat/L)	0.30 (0.10-2.8)**	0.20 (0.10-1.2)	0.30 (0.20-2.8)*	0.20 (0.10-0.60)	0.35 (0.10-1.5)**	0.20 (0.10-0.50)	0.30±0.058	0.48±0.15

a, ALT > 0.317 μkat/L (19 U/L). *, p<0.05; **, p<0.01; ***, p<0.001; b, p=0.052; all compared to their control.

Table 2. Liver function tests in adult female patients with different genotypes of congenital adrenal hyperplasia due to 21-hydroxylase deficiency and age- and sex-matched controls (mean±SEM or median and range)

	Null/Null (n=13)	Controls (n=13)	I2splice (n=15)	Controls (n=15)	I172N (n=25)	Controls (n=25)
S-AST (μkat/L)	0.40±0.28	0.36±0.019	0.41 (0.16-0.48)[b]	0.37 (0.24-0.40)	0.37±0.013	0.37±0.021
S-ALT (μkat/L)	0.39±0.043*	0.28±0.023	0.34±0.024***	0.22±0.021	0.28 (0.18-0.44)	0.29 (0.10-1.1)
Elevated[a] (n)	8	4	10**	2	9	10
S-ALP (μkat/L)	2.84±0.16*	2.28±0.16	2.34±0.16	2.03±0.21	2.33±0.17	2.42±0.25
S-GGT (μkat/L)	0.30 (0.20-2.8)*	0.20 (0.10-0.60)	0.30 (0.20-0.50)*	0.20 (0.10-0.50)	0.40 (0.10-1.5)[c]	0.20 (0.20-0.60)

a, ALT > 0.317 μkat/L (19 U/L). *, p<0.05; **, p<0.01; ***, p<0.001; b, p=0.069; c, p=0.11; all compared to their controls.

Table 3. Liver function tests, age and different measurements of the glucose metabolism in adult women with congenital adrenal hyperplasia due to 21-hydroxylase and controls with a waist circumference ≤88 cm and a body mass index <30 kg/m^2; also divided into the groups <30 and ≥30 years of age (mean±SEM or median and range)

	Patients (n=44)	Controls (n=41)	Patients <30yrs (n=23)	Controls <30yrs (n=19)	Patients ≥30yrs (n=21)	Controls ≥30yrs (n=22)
Age (yrs)	28.5(18-57)	31(19-63)	24.2±0.7	24.4±0.8	35(30-57)	35.5(30-63)
BMI (kg/m^2)	22.6±0.38*	21.5±0.29	21.9±0.61	21.0±0.42	23.3±0.37*	21.9±0.40
Waist (cm)	76.1±0.71	75.6±0.85	73.9±0.91	74.5±1.29	78.6±0.84	76.8±1.09
Total fat mass (kg)	17.5±0.82	19.1±1.21	16.4±1.27	20.3±2.33	18.6±0.95	18.0±1.04
Total fat mass (kg/m^2)	6.8±0.32	6.9±0.47	6.4±0.51	6.4±0.38	7.3±0.33	6.6±0.40
Trunk fat mass (kg)	7.5±0.42	7.9±0.38	6.8±0.63	7.9±0.48	8.3±0.51	7.9±0.59
Lean mass (kg)	37.8±0.57[b]	39.2±0.61	37.9±0.70	38.9±0.96	37.6±0.94	39.2±0.79
Lean mass (kg/m^2)	14.7±0.19**	14.0±0.15	14.6±0.27**	13.7±0.18	14.8±0.26	14.4±0.70
S-AST (μkat/L)	0.40 (0.30-0.53)	0.39 (0.22-0.54)	0.40±0.012	0.42±0.020	0.38 (0.30-0.48)	0.35 (0.22-0.48)
S-ALT (μkat/L)	0.32 (0.14-0.63)*	0.25 (0.12-0.89)	0.33±0.027	0.30±0.039	0.31±0.016	0.28±0.025
Elevated[a] (n)	22	14	11	7	11	7
S-ALP (μkat/L)	2.45 (0.60-4.10)*	1.90 (0.70-6.0)	2.33±0.14	2.10±0.25	2.50 (0.60-4.10)**	1.80 (0.70-6.0)
S-GGT (μkat/L)	0.30 (0.10-0.7)*	0.20 (0.10-1.2)	0.30 (0.10-0.70)	0.20 (0.10-1.20)	0.30 (0.10-0.50)[c]	0.20 (0.10-0.50)
fP-Glucose (mmol/L)	4.70±0.09*	4.98±0.07	4.61±0.09*	4.86±0.07	4.81±0.16	5.08±0.11
fS-Insulin (mU/L)	7.43±0.53[d]	6.23±0.38	7.96±0.79	7.68±0.58	6.83±0.68*	4.99±0.34
HOMA-index	1.56±0.11	1.38±0.09	1.64±0.16	1.67±0.14	1.48±0.16*	1.16±0.09

a, ALT >0.317 μkat/L (19 U/L). *, p<0.05; **, p<0.01; ***, p<0.001; b, p=0.089; c, p=0.084; d, p=0.072; all compared to controls.

tern was found when comparing the three most common genotypes (Table 2).

Compared to controls, women with CAH had lower fasting glucose levels (4.8 mmol/L [3.7-7.3] vs 5.0 [4.2-6.4]; p=0.004), similar fasting insulin (7.8 mU/L [1.6-29] vs 6.6 mU/L [2.4-21]; p=0.085), and HOMA-index (1.7 [0.37-6.2] vs 1.4 [0.51-6.0]; p=NS). Thirteen patients and 6 controls had HOMA-index ≥2.77 (p=0.134). Patients ≥ 30 years of age showed a trend to increased HOMA-index (1.7 [0.37-6.2] vs 1.3 [0.51-6.0]; p=0.086) which could not be demonstrated in individuals <30 years of age (1.7±0.16 vs 1.8±0.13; p=NS).

Liver function tests, glucose and insulin in a non-obese subgroup

In order to minimize the effect of body fat on liver function tests the 44 patients and 41 controls who were not obese (BMI <30 and waist circumference ≤88 cm) were analysed separately (Table 3). Neither waist circumference nor DXA measurement of total body fat and trunk fat mass were different when comparing all the non-obese patients with the non-obese controls nor when non-obese patients and non-obese controls <30 and ≥30 years of age were analysed separately. Corrected for the difference in body height between patients and controls, fat mass (kg/m^2) was still similar whereas lean body mass (kg/m^2) was higher in the patients, although insignificantly in the older group of women.

These non-obese patients demonstrated similar elevations of ALT, GGT and ALP compared to controls as was seen in the entire group of 61 patients and controls. When dividing the non-obese group into <30 and ≥30 years of age, significant elevation of ALP and a trend to higher GGT compared to controls were only found in the older group of patients (Table 3).

Younger non-obese women with CAH had slightly lower fasting glucose levels while older demonstrated elevated fasting insulin levels compared to controls. HOMA-index was higher in the older non-obese patients compared to non-obese controls (Table 3). Only four patients and one control had insulin resistance if the suggested cut-off of 2.77 was used (p=NS).

Medication

The glucocorticoid doses were similar in younger and older patients when comparing hydrocortisone equivalents (growth-retarding equivalents: 21.5±1.3 vs 20.1±1.0 mg/m^2 [p=NS]) and when comparing patients with SW, SV and NC (growth-retarding equivalents: 22.1±1.1 vs 19.5±1.1 vs 20.2±3.4 mg/m^2 [p=NS]). Patients with the Null/Null mutation tended to have higher doses than those with I2splice and the doses were higher than in those with the I172N mutation (growth-retarding equivalents: 24.3±1.4 vs 20.0±1.3 vs 19.5±1.1 mg/m^2 [p=0.053 and 0.007]). No difference was found between I2splice and I172N. Hydrocortisone anti-inflammatory equivalents in mg/m^2 were similar in all groups [partly presented in Ref 7]. No correlations were found between LFT and the present glucocorticoid dose expressed as hydrocortisone equivalents (growth-retarding or anti-inflammatory equivalents) per body surface.

Three patients used antihypertensive medications and two used lipid lowering medication. These five patients were between 35 and 63 years of age and were obese. One non-obese patient used metformin in an effort to conceive as she demonstrated polycystic ovaries [3]. None of the controls used any of the above mentioned medications.

Relationships between biochemical assays and other variables

In both patients and controls, positive correlations were found between concentrations of AST, ALT and GGT (r=0.40-0.58, p=0.014 – <0.001) but not between ALP and the other LFT.

Correlations were calculated between LFT and indices of body fat (BMI, waist circumference, total body fat, trunk fat), glucose, insulin, HOMA-index, IGF-I (SDS), and lipids in patients and controls.

In patients, GGT correlated with indices of total body fat and trunk fat, the highest correlation being with waist circumference (r=0.406, p=0.001) which also correlated with ALP (r=0.312, p=0.014).

In patients, ALT and ALP levels correlated with glucose (r=0.242, p=0.006 and r=0.29, p=0.022) and in controls, ALT correlated with insulin (r=0.35, p=0.006).

In patients, GGT correlated negatively with IGF-I (SDS) (r=-0.29, p=0.025), and a similar trend was

found with AST (r=-0.272, p=0.059). In controls, ALT was negatively correlated with IGF-I (SDS) (r= -0.291, p=0.023).

Multiple linear regression analysis of patients with GGT and BMI, waist circumference and IGF-I (SDS) demonstrated a stronger correlation (r=0.504, p<0.001). A similar correlation was found using ALP and BMI, waist circumference and glucose (r=0.419, p=0.011). In both analyses waist circumference was the strongest and only significant explaining variable.

A negative association between ALP and HDL cholesterol was found in the patient cohort (r=-0.26, p=0.043).

There was no association between LFT and bone mineral density (data not shown).

Discussion

To the best of our knowledge, this is the first study showing that women with CAH have higher liver function tests than age- and sex-matched controls. The increases were modest, but 54% of women with CAH compared to 33% of controls displayed elevation of ALT using the lower cut off limit for women suggested by Prati *et al*. [12]. This limit was based on results from a large sample of blood donors without history of liver disease, negative for hepatitis C antibodies and without evidence of alcohol and drug abuse, as well as from another sample of individuals with subclinical hepatitis C infection who volunteered for blood donation. Similar cut off limits have also been proposed by other authors [21, 22]. We did not measure serology for hepatitis in our cohort, however, the frequency of chronic hepatitis B infection or positive hepatitis C antibodies in Sweden is very low (0.2% and 0.37%, respectively)[23].

We found positive correlations between LFT and waist circumference, and with DXA measurements of trunk and total body fat. One probable reason for higher LFT in our patients compared to controls is increased liver fat storage supported by the positive associations to estimates of trunkal fat. Correlations with BMI, waist/hip ratio and hepatic steatosis in women have also been demonstrated previously [24]. In PCOS, Setji *et al.* [5] demonstrated an association with liver enzymes and low HDL, high triglycerides and high fasting insulin. In contrast, in women with CAH, most of them with insulin concentrations, HOMA-indices and lipid profiles within the normal range, we only found a weak inverse relationship between serum ALP and HDL cholesterol.

Interestingly, when comparing non-obese patients and controls (i.e BMI <30 kg/m^2 and waist circumference ≤88cm) patients still had elevated LFT. Patients and controls had similar total body and trunkal fat mass measured by DXA but higher lean body mass adjusted for height, the latter could partly explain the somewhat higher BMI. The finding of higher LFT in non-obese patients than in non-obese controls suggests that not only central obesity but also glucocorticoids *per se* influence LFT in women with CAH.

Hepatic steatosis has been demonstrated both in endogenous Cushing's syndrome [25] and in long-term pharmacological glucocorticoid therapy [26]. In CAH the glucocorticoid doses needed to suppress elevated androgens are often slightly supraphysiological. In the present cohort of women with CAH we have shown both markedly suppressed adrenal androgens and low bone mineral density, suggesting past and present overtreatment [2, 7]. Although no correlations between LFT and age or present glucocorticoid dose could be found, we consider the life-long glucocorticoid treatment as the most probable reason for higher LFT in patients compared with controls. The cumulative life-time doses, not calculated in the present study, have most likely been higher in older patients and in those having a more severe disease and elevated LFT were mainly found in the more severe salt-wasting pheno- and genotypes showing almost three times as many women with elevated ALT using the lower reference limits compared to controls. Unfortunately, no imaging or biopsy of the liver was done to confirm NAFLD, a condition which is greatly underdiagnosed using standard upper reference limits for liver enzymes as they may be normal in up to 79% of cases [27].

The women with CAH, in particular the younger group, had slightly lower fasting blood glucose levels than controls. Cortisol insufficiency in the morning is probably the reason and may have caused some underestimation of insulin resistance using HOMA-index as a marker.

We found a weak, but significant, negative correlation between LFT and the virtually normal IGF-I levels which may reflect a mild disturbance in hepatocyte function. Hepatocytes are the main source of circulating IGF-I, and low levels are seen in chronic liver

disease with an apparent correlation of IGF-I concentrations with the degree of liver dysfunction [28]. An alternative explanation could be the previously described negative relationship between trunk fat and GH secretion with low GH production leading to low IGF-I serum levels [29].

ALP comprises a family of enzymes located in liver, bone, placenta and intestine. In healthy subjects, circulating ALP is derived from bone and liver [30]. We did not measure bone or liver specific ALP, so presumably ALP came from both locations. However, there were correlations between ALP and measurements of body fat and no correlation with BMD indicating a predominant hepatic origin.

In conclusion, women with CAH due to 21-hydroxylase deficiency have raised LFT compared to age and sex-matched controls; more than half demonstrated elevated ALT using a strict upper reference limit. Only the group ≥30 years of age, the more serious phenotypes and genotypes demonstrated elevation of LFT compared to controls, probably reflecting lifetime glucocorticoid exposure. LFT were positively correlated to BMI, body fat, trunk fat and waist circumference and negatively correlated to HDL in women with CAH. Non-obese patients demonstrated also elevated LFT indicating that not only central obesity but also glucocorticoids *per se* influence LFT. Women with CAH may have increased frequency of NAFLD. It may be worth screening women with CAH with LFT, as NAFLD may lead to increased morbidity and mortality; however, more studies are needed.

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