Pergamon

0960-0760(94)E0009-A

J. Steroid Biochem. Molec. Biol. Vol. 49, No. 1, pp. 101–105, 1994

0960-0760/94 $7.00 + 0.00

Metabolic Profiles of Steroids in Urine of Alcoholics After Withdrawal

Tomas Cronholm,[1]* Stefan Borg,[2] Margareta Viestam-Rains[1] and Jan Sjövall[1]

[1]*Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm and*
[2]*Department of Psychiatry at St Görans Hospital, Karolinska Institutet, 112 81 Stockholm, Sweden*

The metabolic profiles of steroids in urine were analyzed in 13 male alcoholics during long-term abstinence, in most cases exceeding 3 months. The ratios of 5β- to 5α-reduced steroid metabolites (etiocholanolone/androsterone and tetrahydrocortisol/allotetrahydrocortisol) were initially elevated but decreased slowly following withdrawal. The half-life of this normalization exceeded 3 weeks. The change was most marked in patients with signs of liver injury, and may reflect a relative decrease of the activity of hepatic 5α-reductase. The ratio between cortisol metabolites carrying a 11β-hydroxy and an 11-oxo group was elevated in the patients and showed no tendency to normalize. This might reflect a decrease in the peripheral inactivation of cortisol.

J. Steroid Biochem. Molec. Biol., Vol. 49, No. 1, pp. 101–105, 1994

INTRODUCTION

Alcoholics may develop a pseudo-Cushing syndrome [1–5], and male patients often show signs of feminization and testicular hypofunction [6]. It is not clear if these disorders are due to effects of ethanol on hormone production and its regulation, on hormone interaction with target tissues or on peripheral metabolism of the steroids. Examples of all these mechanisms have been found, particularly in animal experiments [6]. In humans, ethanol oxidation induces acute redox changes in the metabolism of androgens and estrogens [7], and chronic ethanol abuse decreases the activity of steroid 5α-reductase [E.C. 1.3.1.22] in the liver [8]. A previous study of steroid profiles in blood and urine in alcoholics with signs of mild liver injury revealed both delayed and long-lasting changes of steroid metabolism [9]. Among the latter was an increase of the ratio between 11β-hydroxy- and 11-oxosteroids that did not normalize during the first month after withdrawal. Oxidation of the 11β-hydroxy group to an 11-oxo group is an important reaction in the peripheral inactivation of glucocorticoids [10] and it is possible that the observed change may reflect a defect of this reaction in alcoholics. The present investigation was performed in order to evaluate the persistence of the changes over a longer period and to relate them to signs of liver injury. The analyses also made it possible to study whether or not the reported decrease in the activity of 5α-reductase in livers of alcoholics is reflected in the metabolic profiles of steroids *in vivo*.

EXPERIMENTAL

Patients

The subjects were 13 male patients, who participated in an outpatient treatment of alcohol abuse. All patients fulfilled clinical criteria for alcohol dependence [11]. After withdrawal treatment as inpatients they visited the clinic three times a week. They did not receive any drugs during treatment. The goal of the treatment was total abstinence. Relapses were determined by interviews and by determination of carbohydrate-deficient transferrin in serum [12] and the ratio of 5-hydroxytryptophol to 5-hydroxyindolacetic acid in urine [13]. The latter ratio shows an immediate increase after ethanol intake, while the levels of carbohydrate-deficient transferrin increases with a half-life of about 2 weeks [12]. Urine was collected in the morning when the patients came to the clinic, and creatinine and steroid profiles were analyzed at variable intervals. The details on urine collection and data on the patients are given in Table 1. Eleven of the patients were followed for more than 3 months, and 4 were followed for more than a year.

*Correspondence to T. Cronholm.
Received 9 July 1993; accepted 23 Dec. 1993.

Analysis of steroids

Steroids in 5 ml urine were extracted using a solid-phase method [14]. After enzymatic hydrolysis of conjugates and reextraction, neutral steroids were purified by passage through a lipophilic ion exchanger [15]. Steroid profiles in urine were determined by capillary gas chromatography [9] and the excretion was related to that of creatinine. The C_{19} steroids androsterone and etiocholanolone, which are major metabolites of testicular testosterone and adrenal dehydroepiandrosterone sulfate, and the C_{21} steroids tetrahydrocortisol (THF, 3α,11β,17α,21-tetrahydroxy-5β-pregnan-20-one), tetrahydrocortisone (THE, 3α,17α,21-trihydroxy-5β-pregnane-11,20-dione), allotetrahydrocortisol (aTHF, 3α,11β,17α,21-tetrahydroxy-5α-pregnan-20-one), α- and β-cortolone [3α,17α,20α(and 20β),21-tetrahydroxy-5β-pregnan-11-one], and α-cortol (5β-pregnane-3α,11β,17α,20α,21-pentol), which are major metabolites of cortisol, were quantitatively determined. β-Cortol (5β-pregnane-3α,11β,17α,20β,21-pentol) was included in the measured amount of β-cortolone [15].

The values obtained were compared, with the use of Student's *t*-test, with those reported for males in other studies using liquid chromatographic [16] or gas chromatographic [17] methods of analysis.

RESULTS

The patients were divided into two groups, one showing no signs of liver injury and another with initial elevations of the serum levels of at least two of the markers γ-glutamate transpeptidase (GT), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) (Table 1).

The rates of steroid excretion were essentially constant in all but one patient. The apparently higher steroid excretion in our patients than previously reported in controls (Table 2) may be explained by the higher cortisol production in the morning when the patient samples were collected.

The ratios between 5β- and 5α-steroids were used to evaluate the relative activities of the 5β- and 5α-reductases. Both the C_{19} steroid pair, etiocholanolone/androsterone, and the C_{21} steroid pair, THF/aTHF, were significantly shifted towards the 5β-metabolites during the period of abstinence (Table 2). The etiocholanolone/androsterone ratio was shifted from 0.46 in controls to 1.38 in patients with signs of liver injury. The corresponding values for the THF/aTHF ratio were 1.51 and 2.88, respectively. The shifts were less marked in patients without signs of liver injury. The logarithms of the ratios were plotted as in the previous study [9], and were found to decrease or remain unchanged during the first 60 days after admission (Figs 1 and 2). The decrease of the ratios was statistically significant in about half of the patients and the half-lives for the change exceeded 3 weeks in all patients. The mean values are given in Table 2.

The extent of oxidation of the 11β-hydroxy group of cortisol was evaluated by the THF/THE ratio. This ratio did not change significantly over the period of observation but it was significantly higher in patients than in controls. In contrast, the redox state of the oxygen function at C-20 was slightly but significantly less reduced in patients than in controls.

DISCUSSION

This study confirms our previous observation that alcohol abuse leads to chronic changes in the metabolism of corticosteroids and androgens [9]. The time course of these changes shows that they are not caused by the acute redox effects of alcohol, but are due to an alcohol-induced tissue damage. Two changes of the urinary steroid profiles predominate: an increase of the ratio of 5β- to 5α-reduced metabolites and an increase of the ratio of 11β-hydroxy to 11-oxo metabolites.

Table 1. Data on participating patients and collection of urine samples for steroid analysis

Patient code	Initial age (years)	Positive liver tests initially	Urine collection Period from admission (days)	Number of samples
113	34	GT, ASAT, ALAT	9–112	14
114	39	GT, ASAT, ALAT	9–215	27
115	41	GT, ALAT	13–34	4
116	58	—	14–199	15
117	30	—	6–596	24
118	42	—	13–538	18
119	52	—	10–471	17
120	27	—	10–136	10
121	39	GT, ALAT	2–370	35
123	41	GT, ASAT, ALAT	2–148	14
124	36	ASAT, ALAT	8–181	22
125	41	GT, ASAT, ALAT	1–104	10
126	40	—	9–21	3

Table 2. Excretion of steroids in urine of alcoholics

Parameter	Patients with signs of liver injury[a]	Patients without signs of liver injury	Controls
Concentration of $C_{19}O_2$ steroids (mmol/mol creatinine)	1.87 ± 0.35 (7)	1.84 ± 0.71 (5)	1.44 ± 0.54 (33)[b]
Concentration of C_{21} steroids, (mmol/mol creatinine)	2.22 ± 0.49 (7)[d]	3.06 ± 0.69 (5)[c]	1.75 ± 0.40 (33)[b]
Log of etiocholanolone/androsterone ratio[f]	0.141 ± 0.135 (6)[d]	0.136 ± 0.139 (4)[e]	−0.335 ± 0.292 (9)[g]
Decrease of log (etiocholanolone/androsterone), per 100 days[f]	0.41 ± 0.30 (6)	0.50 ± 0.32 (4)	—
Log (THF/aTHF)[f]	0.460 ± 0.211 (6)[d]	0.273 ± 0.120 (4)	0.179 ± 0.165 (10)[g]
Decrease of log(THF/aTHF), per 100 days[f]	0.73 ± 0.65 (6)	0.38 ± 0.30 (4)	—
Mean log(THF/THE)	−0.140 ± 0.011 (7)[c]	−0.167 ± 0.112 (6)[c]	−0.408 ± 0.048 (10)[g]
Mean log(20-hydroxy-/20-oxo-5β-steroids)	−0.365 ± 0.060 (7)[e]	−0.450 ± 0.090 (6)[d]	−0.298 ± 0.064 (10)[g]

Values are mean ± SD (number of individuals in parentheses).
[a]Patients with positive liver tests initially (see Table 1).
[b]Calculated from data in Ref. [16].
[c]$P < 0.001$, [d]$P < 0.01$ and [e]$P < 0.05$ in t-tests of significant difference from value obtained with controls.
[f]Calculated from the linear regression of log values determined in samples collected during the first 60 days after admission.
[g]Calculated from data in Ref [17].

The NADPH-dependent reduction of the 4,5-double bond in steroid hormones to yield 5α- and 5β-reduced metabolites can occur in many tissues but the liver is considered to be the major site for these reactions. *In vitro* measurements have shown the hepatic 5α-reductase activity to be decreased in alcoholics [8]. Our study shows that the proportion of 5α-reduced C_{19}- and C_{21}-steroid metabolites is decreased in urine, and indicates that the lowered activity of hepatic 5α-reductase is reflected in the metabolism of steroids *in vivo*. This conclusion was also drawn in a preliminary report on the cortisol metabolites in alcoholic patients [18]. In the present study the etiocholanolone/androsterone ratio was initially increased to the same extent as the THF/aTHF ratio (5β/5α-reduced cortisol metabolites). The two ratios then decreased slowly after withdrawal. The half-life of this normalization process was more than 3 weeks. The parallel changes of the two 5α/5β-steroid couples suggest that the same enzyme catalyzes the reduction of the 4,5-double bond both in C_{19} and C_{21} steroids. This conclusion is supported by the positive correlation between the etiocholanolone/androsterone and the THF/aTHF ratios in healthy men and women [19].

The pathophysiological significance of an altered relation between 5α- and 5β-reduction remains to be established. The 5α-reduction of testosterone leads to 5α-dihydrotestosterone, which is an active androgen in certain target tissues. Most women have a higher ratio of 5β- to 5α-reduced metabolites in urine than men [19], indicating that the increased ratio might reflect feminization in alcoholics. However, the 5α-reductases in the liver and target tissues are probably different enzymes [20], and the results therefore do not allow any conclusions regarding the mechanism behind feminization.

The oxidation to cortisone is an important reaction for the inactivation of cortisol in several target tissues [10, 21]. The reaction is catalyzed by 11β-hydroxysteroid dehydrogenase, which exists in different forms which catalyze oxidation and reduction at different rates [10, 22–25]. The distribution of the different forms is tissue-specific [10, 21, 22, 26]. Failure to oxidatively inactivate cortisol in the kidney leads to a syndrome of apparent mineralocorticoid excess [10, 21, 27]; these patients have elevated ratios of 11β-hydroxy- to 11-oxosteroids, similar to those seen in Cushing's syndrome [28].

It is possible that the increased THF/THE ratio in the alcoholics reflects a decreased oxidative inactivation of cortisol. In the liver this could contribute to the fat accumulation [29]. Diminished oxidation in the kidney and testis could conceivably lead to hypertension and decreased testosterone production, conditions which are common in alcoholics [6, 10, 21]. An important question is whether the THF/THE ratio in urine only reflects the state of oxidoreduction in the liver. The ratio was higher in our previous study of patients with liver injury who also had an increased ratio of 20α-hydroxy- to 20-oxosteroids [9]. In contrast, Edwards and Stewart reported normal ratios of cortisol to cortisone-metabolites in 6 hypertensive alcoholics [18]. The

differences between the studies may be due to differences in the degree of liver injury. The metabolism of cortisol to THE is decreased and that to 20α-hydroxysteroids is increased in patients with alcoholic cirrhosis [30]. The reduction of prednisone to prednisolone is also impaired [31, 32]. These results support an abnormality in the function of 11β-hydroxysteroid dehydrogenase in liver cirrhosis and could explain why the THF/THE ratio was not normalized in our patients.

While the factors determining the relative rates of oxidation and reduction at C-11 are not fully understood [10, 21], the degree of glycosylation of the enzyme may be important. Inhibition of glycosylation leads to a relative decrease in oxidizing activity [25]. This is of interest since prolonged ethanol consumption leads to decreased glycosylation of transferrin [12]. A similar decrease of the glycosylation of 11β-hydroxysteroid dehydrogenase could conceivably lead to the observed increase of the THF/THE ratio in alcoholics.

The THF/THE and 5β-/5α-steroid ratios are potential parameters for monitoring of alcoholics. The THF/THE ratio is not normalized after several months of abstinence whereas the 5β-/5α-steroid ratio decreases slowly toward normal. The results indicate that the hepatic and adrenocorticoid functions recover only very slowly during abstinence.

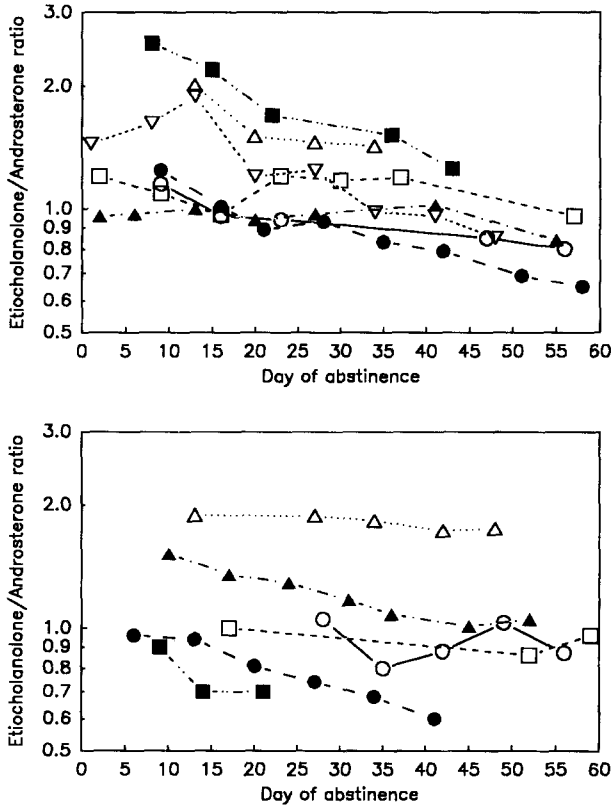

Fig. 1. Ratios between etiocholanolone and androsterone in urine collected during the first 60 days after admission from alcoholics with (upper panel) or without (lower panel) initial signs of liver affection (see Table 1). Subjects shown in upper panel: 113 (○), 114 (●), 115 (△), 121 (▲), 123 (□), 124 (■) and 125 (▽) and in lower panel: 116 (○), 117 (●), 118 (△), 119 (▲), 120 (□) and 126 (■).

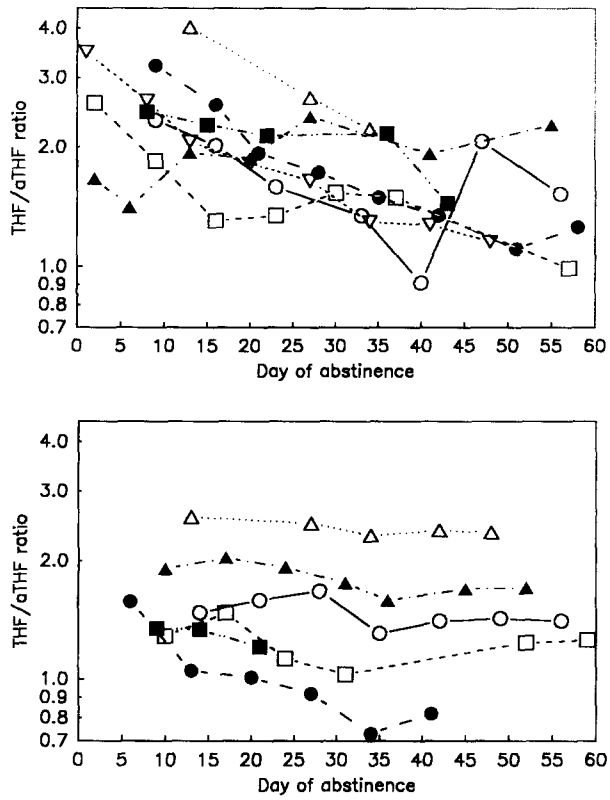

Fig. 2. Ratios between THF and aTHF in urine collected during the first 60 days after admission from alcoholics with (upper panel) or without (lower panel) initial signs of liver affection (see Table 1). For explanation of symbols see legend to Fig. 1.

Acknowledgements—This work was supported by grants from the Swedish Medical Research Council (No. 2189), the Swedish Alcohol Research Fund, Petrus and Augusta Hedlunds Stiftelse and Karolinska Institutet.

REFERENCES

1. Smals A. G., Kloppenborg P. W., Njo K. T., Knoben J. M. and Ruland C. M.: Alcohol-induced Cushingoid syndrome. *Lancet* ii (1976) 1298.
2. Rees L. H., Besser G. M., Jeffcoate W. J., Goldie D. J. and Marks V.: Alcohol-induced pseudo-Cushing's syndrome. *Lancet* i (1977) 726–728.
3. Irwin M. R., Risch S. C., Brown S. A., Howard T. L., Smith T. L., Knapp S. and Schuckit M. A.: Urinary free cortisol excretion in depressed alcoholic patients. *Biol. Psychiat.* 24 (1988) 713–716.
4. Buydens-Branchey L., Branchey M., Worner T. M., Zucker D., Aramsombatdee E. and Lieber C. S.: Increase in tryptophan oxygenase activity in alcoholic patients. *Alcohol. Clin. Exp. Res.* 12 (1988) 163–167.
5. Hiramatsu R. and Nisula B. C.: Effect of alcohol on the interaction of cortisol with plasma proteins, glucocorticoid receptors and erythrocytes. *J. Steroid Biochem.* 33 (1989) 65–70.
6. Van Thiel D. H., Gavaler J. S., Rosenblum E. and Tarter R. E.: Ethanol, its metabolism and hepatotoxicity as well as its gonadal effects: Effects of sex. *Pharmac. Ther.* 41 (1989) 27–48.
7. Andersson S., Cronholm T. and Sjövall J.: Redox effects of ethanol on steroid metabolism. *Alcohol. Clin. Exp. Res.* 10 (1986) 55S–63S.

8. Gordon G. G., Vittek J., Ho R., Rosenthal W. S., Southren A. L. and Lieber C. S.: Effect of chronic alcohol use on hepatic testosterone 5α-A-ring reductase in the baboon and in the human being. *Gastroenterology* 77 (1979) 110–114.
9. Cronholm T., Curstedt T., Schmidt D. N. and Sjövall J.: Steroid profiles in urine and plasma of alcoholics during withdrawal. *Alcohol* 2 (1985) 677–682.
10. Monder C.: Corticosteroids, receptors, and the organ-specific functions of 11β-hydroxysteroid dehydrogenase. *FASEB J.* 5 (1991) 3047–3054.
11. American Psychiatric Association: *Diagnostic and Statistical Manual of Mental Disorders* APA, Washington DC, 3rd Edn (1980).
12. Stibler H.: Carbohydrate-deficient transferrin in serum: a new marker of potentially harmful alcohol consumption reviewed. *Clin. Chem.* 37 (1991) 2029–2037.
13. Voltaire A., Beck O. and Borg S.: Urinary 5-hydroxytryptophol: A possible marker of recent alcohol consumption. *Alcohol. Clin. Exp. Res.* 16 (1992) 281–285.
14. Shackleton C. H. L. and Whitney J. O.: Use of Sep-Pak cartridges for urinary steroid extraction: Evaluation of the method for use prior to gas chromatographic analysis. *Clin. Chim. Acta* 107 (1980) 231–243.
15. Axelson M., Sahlberg B. -L. and Sjövall J.: Analysis of profiles of conjugated steroids in urine by ion-exchange separation and gas chromatography-mass spectrometry. *J. Chromat.* 224 (1981) 355–370.
16. Vestergaard P.: Excretion of common neutral steroids in healthy subjects as estimated by multi-column chromatography. *Acta Endocr.* 88 (Suppl. 217) (1978) 157–189.
17. Setchell K. D. R., Almé B. and Sjövall J.: The multicomponent analysis of conjugates of neutral steroids in urine by lipophilic ion exchange chromatography and computerised gas chromatography-mass spectrometry. *J. Steroid Biochem.* 7 (1976) 615–629.
18. Edwards C. R. W. and Stewart P. M.: Alcohol and hypertension. *Proc. R. Coll. Phys. Edinburgh* 19 (1989) 273–279.
19. Pfaffenberger C. D. and Horning E. C.: Sex differences in human urinary steroid metabolic profiles determined by gas chromatography. *Analyt. Biochem.* 80 (1977) 329–343.
20. Jenkins E. P., Andersson S., Imperato-McGinley J., Wilson J. D. and Russell D. W.: Genetic and pharmacological evidence for more than one human steroid 5α-reductase. *J. Clin. Invest.* 89 (1992) 293–300.
21. Walker B. R. and Edwards C. R. W.: 11β-hydroxysteroid dehydrogenase and enzyme-mediated receptor protection: Life after liquorice? *Clin. Endocr.* 35 (1991) 281–289.
22. Monder C. and Shackleton C. H. L.: 11β-hydroxysteroid dehydrogenase: Fact or fancy? *Steroids* 44 (1984) 383–417.
23. Lakshmi V. and Monder C.: Purification and characterization of the corticosteroid 11β-dehydrogenase component of the rat liver 11β-hydroxysteroid dehydrogenase complex. *Endocrinology* 123 (1988) 2390–2398.
24. Agarwal A. K., Monder C., Eckstein B. and White P. C.: Cloning and expression of rat cDNA encoding corticosteroid 11β-dehydrogenase. *J. Biol. Chem.* 264 (1989) 18939–18943.
25. Agarwal A. K., Tusie-Luna M. T., Monder C. and White P. C.: Expression of 11β-hydroxysteroid dehydrogenase using recombinant vaccinia virus. *Molec. Endocr.* 4 (1990) 1827–1832.
26. Krozowski Z., Obeyesekere V., Smith R. and Mercer W.: Tissue-specific expression of an 11β-hydroxysteroid dehydrogenase with a truncated N-terminal domain. A potential mechanism for differential intracellular localization within mineralocorticoid target cells. *J. Biol. Chem.* 267 (1992) 2569–2574.
27. Monder C., Shackleton C. H. L., Bradlow H. L., New M. I., Stoner E., Iohan F. and Lakshmi V.: The syndrome of apparent mineralocorticoid excess: Its association with 11β-dehydrogenase and 5β-reductase deficiency and some consequences for corticosteroid metabolism. *J. Clin. Endocr. Metab.* 63 (1986) 550–557.
28. Sayegh J. F. and Vestergaard P.: Multi-column chromatography of urinary steroids and adrenocortical dysfunction. *Acta Endocr.* 88 (Suppl. 217) (1978) 207–214.
29. Brindley D. N.: What factors control triacylglycerol accumulation in alcohol abuse? *Biochem. Soc. Trans.* 16 (1988) 251–253.
30. Zumoff B., Bradlow H. L., Gallagher T. F. and Hellman L.: Cortisol metabolism in cirrhosis. *J. Clin. Invest.* 46 (1967) 1735–1743.
31. Madsbad S., Bjerregaard B., Henriksen J. H., Juhl E. and Kehlet H.: Impaired conversion of prednisone to prednisolone in patients with liver cirrhosis. *Gut* 21 (1980) 52–56.
32. Ui T., Mitsunaga M., Tanaka T. and Horiguchi M.: Determination of predisone and prednisolone in human serum by high-performance liquid chromatography—especially on impaired conversion of corticosteroids in patients with chronic liver disease. *J. Chromat.* 239 (1982) 711–716.