The Effects of Anabolic Androgenic Steroids on Serum Ubiquinone and Dolichol Levels Among Steroid Abusers

Tuomo Karila, Reijo Laaksonen, Kalle Jokelainen, Jaakko-Juhan Himberg, and Timo Seppälä

We measured serum ubiquinone and dolichol concentrations in 13 men while they abused anabolic androgenic steroids (AAS) and during the following withdrawal period. Serum total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol and triglycerides were also determined. AAS administration increased serum ubiquinone by 68% ($P < .001$) and decreased serum dolichol by 30% ($P < .002$). Both nonsterol isoprenoid levels in plasma correlated with the AAS dose, ubiquinone positively ($P < .001$) and dolichol negatively ($P < .002$). When the subjects were taking steroids, the ubiquinone to LDL ratio was 42% higher than during the withdrawal period. In conclusion, our study suggests that AAS have an influence on the by-products of the mevalonate pathway.

THE NONMEDICAL USE of anabolic androgenic steroids (AAS) by male adolescents has been on the increase in Western countries.[1] In the United States, 3% to 7% of high school male students have used or are using AAS.[2,3] It is well known that AAS can cause severe adverse effects, eg, on the liver and the reproductive system.[4] Risk factors for cardiovascular disease have also been found to be increased among steroid abusers.[5]

Androgens are produced by a metabolic pathway in which cholesterol is a key intermediate.[6] It has been demonstrated that androgens affect serum cholesterol levels by an unknown mechanism. High-density lipoprotein (HDL) cholesterol levels are particularly substantially decreased during administration of high-dose AAS.[7] It can be further hypothesized that androgens may be able to alter the levels of other isoprenoid compounds in serum and various tissues.

The present study was designed to elucidate the effect of high-dose AAS on serum lipoprotein, cholesterol, ubiquinone, and dolichol concentrations. Ubiquinones are mitochondrial coenzymes with antioxidant properties, and dolichols are intermediates in the synthesis of certain glycoproteins. These isoprenoid compounds are synthesized via the synthetic cholesterol pathway in cells and are carried by lipoproteins in the circulation.

SUBJECTS AND METHODS

Subjects and Study Design

Thirteen healthy noncompetitive male power-atheletes aged 24 to 34 years (mean, 28) volunteered for this study. Their weight and height were 94 ± 10.3 kg and 178 ± 6.4 cm (mean ± SD), respectively, accounting for the mean body mass index of 30 ± 2.6 $kg \cdot m^{-2}$. They all passed a routine medical examination, and none had a history of any chronic disease or were under any medication.

The subjects used oral and/or intramuscular AAS. The mean length of AAS administration during the study was 3 months, and the following withdrawal period was a mean of 6 months. The mean daily AAS dose used during the cycle included in the study was 1.02 ± 0.59 mg/kg (Table 1).

The subjects followed their own AAS administration schedule. For comparative purposes daily steroid dose (milligrams per day) was determined by calculating the mean daily dose during the last 10 days before a blood sample was drawn. The daily dose of an injectable steroid preparation was obtained by dividing the dose contained in each individual injection by the number of days between injections.

AAS preparations were obtained illegally on the black market by the subjects themselves. The subjects kept accurate records of the drugs and doses used during AAS administration. Most AAS used by the subjects were pharmaceutical preparations acquired from southern European pharmaceutical stores. The ingredients of various eastern European preparations were determined by mass spectrometry,[8] and all contained the appropriate steroids at 70% to 100% of the declared dose. A urine specimen was obtained simultaneously with the serum sample, and the standard screening procedure for AAS was performed.[8] There were no discrepancies between the subjects' records and the results of AAS analysis from urine.

Every second week during the abuse cycle and withdrawal period, blood samples were drawn for assay of serum ubiquinone and dolichol, total and HDL cholesterol, triglycerides, and liver aminotransferases (serum alanine and aspartate aminotransferases).

Written informed consent was obtained from all subjects, and they were frequently informed of the possible adverse effects of AAS. The Ethics Committee of the National Public Health Institute approved the study protocol.

Laboratory Methods

After blood samples were drawn, the serum was separated and aliquots were stored at −20°C and −70°C for lipid and ubiquinone/dolichol assays, respectively.

Cholesterol levels in whole serum and in the HDL fraction and serum triglyceride levels were measured using commercial kits from Boehringer-Mannheim Diagnostica (Mannheim, Germany). The HDL fraction was obtained by the Mg^{2+}/dextran sulfate precipitation method,[9] and low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation.[10]

Ubiquinone determinations were performed according to the method reported by Laaksonen et al[11] with high-performance liquid chromatography (HPLC). The samples were extracted with *n*-propanol, purified on a C-18 column, and chromatographed as quinones using coenzyme Q_9 as an internal standard. The lowest level of detection for plasma samples was 0.1 $mg \cdot L^{-1}$, and

From the National Public Health Institute, Laboratory of Pharmacology and Toxicology, Helsinki; the Institute of Biomedicine, Department of Pharmacology and Toxicology, the Department of Clinical Pharmacology, and the Research Unit of Alcohol Diseases, Department of Internal Medicine, University of Helsinki, Helsinki, Finland.

Submitted July 27, 1995; accepted December 4, 1995.

Address reprint requests to Tuomo Karila, B Med, National Public Health Institute, Mannerheimintie 166 F, 00300 Helsinki, Finland.

0026-0495/96/4507-0009$03.00/0

Table 1. AAS Abuse Data

Parameter	Mean ± SD
Steroid use during the study (d)	97 ± 38
Withdrawal duration (d)	181 ± 88
Cumulative dose (g)	10.4 ± 8.8
Daily dose/mass (mg · d^{-1} · kg^{-1})	1.02 ± 0.59
Lifetime use (yr)	5 ± 4

intraday and interday coefficients of variation were 7% and 11%, respectively.

Coenzyme Q_{10} exists in oxidized (ubiquinone) and reduced (ubiquinol) form in circulation and within human tissues. This redoxequilibrium is unstable and can be altered while processing the samples. Therefore, all samples were oxidized before analysis, and the results are expressed as ubiquinone.

The dolichols were analyzed by a HPLC method.[12] A reversed-phase ODS column was used with a gradient elution program running from 50% isopropanol plus 50% methanol to 80% isopropanol plus 20% methanol for 20 minutes. UV detection was performed at 210 nm. Heneicosaprenol (Sigma Chemical, St Louis, MO) was used as an internal standard. Serum levels of dolichols were expressed as the sum of the three homologs of 18, 19, and 20 isoprene units.

Statistical Analysis

Wilcoxon's paired test was used for evaluation of the significance of differences. Probabilities not greater than .05 were regarded as statistically significant. Spearman's correlation coefficients were calculated to assess any relationship between daily steroid dose and serum ubiquinone, dolichol, cholesterol, HDL, and LDL. Statistical analyses were performed using Systat[13] software, and results are expressed as the mean ± SD.

RESULTS

LDL cholesterol increased by 18% ($P < .005$) and HDL cholesterol decreased by 58% ($P < .001$) during AAS use versus the withdrawal period (Table 2).

AAS administration increased serum ubiquinone concentrations by 68% ($P < .001$). The mean concentrations during AAS administration and following the withdrawal period were 1.91 and 1.14 mg/L, respectively. Serum ubiquinone concentration correlated positively with daily steroid dose ($P < .001$; Fig 1a). There was a significant correlation between ubiquinone and LDL cholesterol concentrations ($P < .001$; Fig 2a). When the subjects were taking steroids, the ubiquinone to LDL ratio was 42% higher than during the withdrawal period.

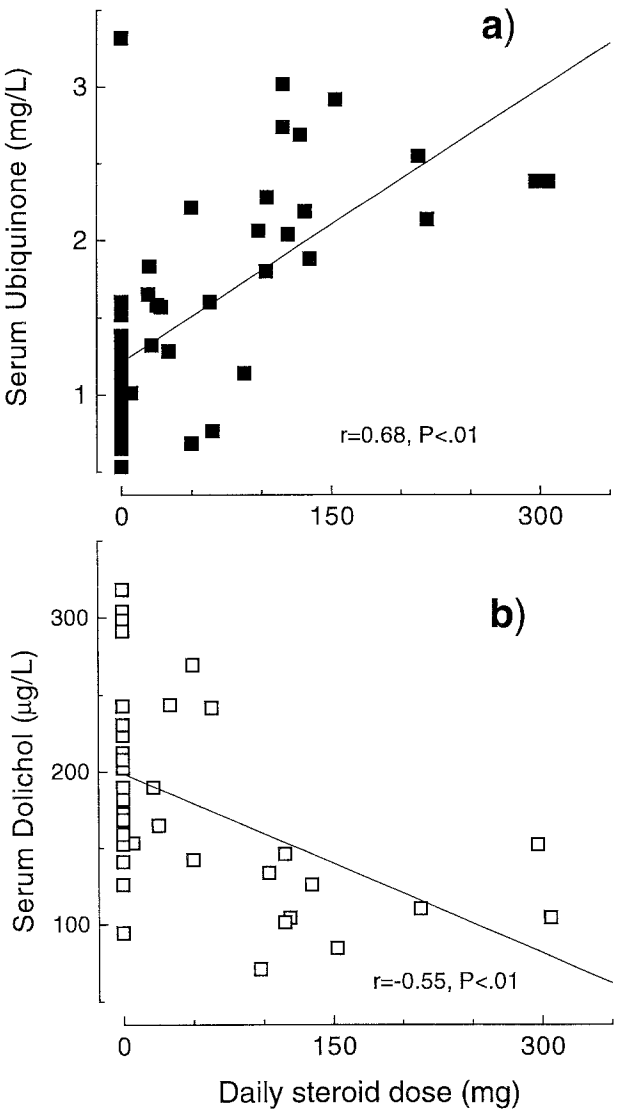

Fig 1. Correlation of daily AAS dose with serum ubiquinone (a) and dolichol (b) concentrations. Samples from the withdrawal period are indicated as a daily steroid dose equal to 0. Spearman's correlation coefficients and *P* values are shown (n = 54 for ubiquinone and n = 38 for dolichol assays).

Table 2. Serum Lipoprotein, Ubiquinone, and Dolichol Concentrations During Steroid Administration and Withdrawal Period (mean ± SD)

Parameter	On Steroids	Off Steroids
Cholesterol (mmol · L^{-1})		
Total	3.58 ± 0.58	3.84 ± 0.55‡
LDL	2.60 ± 0.64	2.20 ± 0.51*
HDL	0.48 ± 0.36	1.02 ± 0.38†
Triglycerides (mmol · L^{-1})	1.09 ± 0.47	1.36 ± 0.66‡
Ubiquinone (mg · L^{-1})	1.91 ± 0.64	1.14 ± 0.51†
Total dolichol (µg · L^{-1})	143.38 ± 54.05	205.26 ± 59.97*

NOTE. Significance of difference determined by Wilcoxon's paired test.

*$P < .01$.

†$P < .001$.

‡Nonsignificant.

Serum dolichol concentrations decreased significantly during administration of AAS (by a mean of 30%, $P < .002$; Table 2). Daily steroid dose correlated negatively with serum dolichol concentration ($P < .002$; Fig 1b). On the other hand, there was a positive significant correlation between serum dolichol and HDL cholesterol ($P < .001$; Fig 2f). There were no alterations in HPLC profiles of the dolichol fractions.

DISCUSSION

Our findings confirm that AAS result in profound alterations of serum HDL and LDL cholesterol concentra-

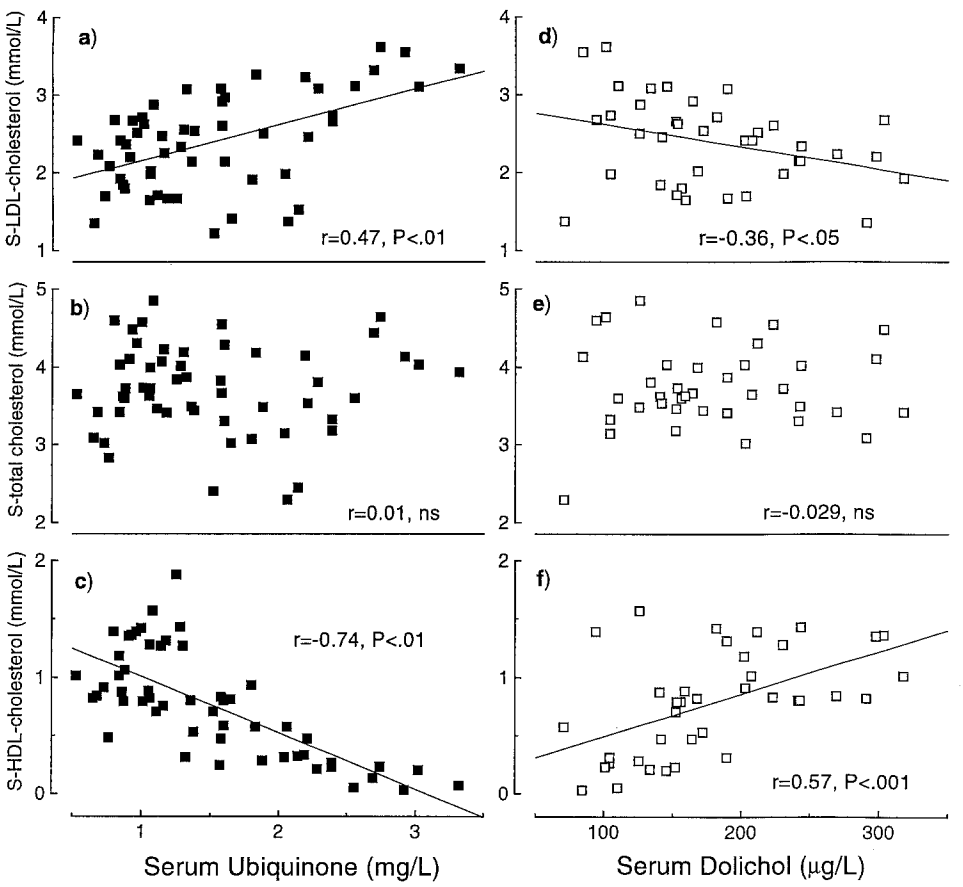

Fig 2. Correlation of serum ubiquinone (■) and dolichol (□) with serum LDL cholesterol (a and d), total cholesterol (b and e), and HDL cholesterol (c and f) in subjects using AAS. Spearman's correlation coefficients and *P* values are shown (n = 54 for ubiquinone and n = 38 for dolichol assays).

tions.[14] We also measured serum levels of two nonsterol isoprenoid compounds, ubiquinone and dolichol, which are by-products of the synthetic pathway of cholesterol[15] and are also influenced by AAS. Other compounds, although not analyzed, that are bound to lipoproteins may also show altered serum levels, or their transportation to the periphery could be interfered with during AAS administration.

Ubiquinone acts as a lipid-soluble electron carrier in the electron transport chain of the mitochondrium,[15] but its reduced form, ubiquinol, has a powerful antioxidant effect on LDL, and it can therefore be hypothesized that it may protect against atherosclerosis.[16] We observed a significant increase in serum ubiquinone during AAS administration, from which it can be assumed that the AAS-induced atherogenic lipoprotein profile could be partly countered by an increase in ubiquinone level.

Ubiquinone is mainly carried in the circulation by LDL,[11] but when AAS were used, the ubiquinone to LDL ratio increased. In previous studies, this ratio has been invariable in all conditions investigated.[17,18]

Dolichols are α-saturated polyisoprenoid alcohols synthesized in microsomes and stored in lysosomes,[19] and the liver reportedly has an important role in regulating blood dolichol supply.[20] In the phosphorylated form, dolichols function in the biosynthesis of glycoproteins[19] and also modify biological membrane fluidity, stability, and permeability, and the process of fusion.[21,22] Dolichols are transported in the circulation mainly by HDL.[23]

In a manner dissimilar to that observed for ubiquinone, serum dolichol concentration decreased concomitantly with HDL concentration, and the serum dolichol to HDL ratio remained practically unchanged.

The increase in ubiquinone without a similar concomitant LDL increment could indicate increased isoprenoid production in liver cells, some of which have been shown to control cell growth.[24] Further studies have shown that isoprenylation of growth-regulating proteins produces a potential signaling factor for cellular proliferation.[25,26] Altered production of isoprenoid derivatives by AAS could lead to interference with the cellular proliferation mechanism. On the other hand, it has been shown that AAS increase the prevalence of benign hepatocellular adenomas.[27,28]

It is concluded that AAS have an influence on the by-products of the mevalonate pathway, at least serum ubiquinone and dolichol levels. These drugs may also have an effect on other sterols. AAS could mediate anabolic properties or cause undesirable side effects by altering the levels of these sterols.

REFERENCES

1. Terney R, McLain L: The use of anabolic steroids in high school students. Am J Dis Child 144:99-103, 1990

2. Windsor R, Dumitru D: Prevalence of anabolic steroid use by male and female adolescents. Med Sci Sports Exerc 21:494-497, 1989

3. Buckley WE, Yesalis CE, Friedl KE, et al: Estimated prevalence of anabolic steroid use among male high school seniors. JAMA 260:3441-3445, 1988

4. Friedl K: Reappraisal of the health risks associated with the use of high doses of oral and injectable anabolic steroids, in Anabolic Steroid Abuse, NIDA Research Monograph. Rockville, MD, National Institute on Drug Abuse, 1990

5. Wilson J: Androgen abuse by athletes. Endocr Rev 9:81-199, 1988

6. Gwynne J, Straus SJ: The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroidogenic glands. Endocr Rev 3:299-329, 1982

7. Glazer G: Atherogenic effects of anabolic steroids on serum lipid levels. A literature review. Arch Intern Med 151:1925-1933, 1991

8. Donike M, Geyer H, Gotzmann A, et al: 1988 Dope analysis, in Bellotti P, Benzi G, Ljungqist A (eds): Official Proceedings of the International Athletic Foundation World Symposium on Doping in Sport—1987. London, UK, International Athletic Foundation, 1988, pp 53-80

9. Finley PR, Schifman RB, Williams RJ, et al: Cholesterol in high density lipoprotein: Use of Mg^{2+}/dextran sulphate in its enzymatic measurement. Clin Chem 24:931-933, 1978

10. 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 18:499-502, 1972

11. Laaksonen R, Riihimäki A, Laitila J, et al: Serum and muscle tissue ubiquinone levels in healthy subjects. J Clin Lab Med 25:517-521, 1995

12. Jokelainen K, Salmela K, Humaloja K, et al: Blood dolichol in lysosomal diseases. Biochem Cell Biol 70:481-485, 1992

13. Systat: Systat for Windows: Statistics, version 5. Evanston, IL, Systat, 1992

14. Webb O, Laskarzewski P, Glueck C: Severe depression of high-density lipoprotein cholesterol levels in weight lifters and body builders by self-administered exogenous testosterone and anabolic-androgenic steroids. Metabolism 33:971-975, 1984

15. Olson RE, Rudney H: Biosynthesis of ubiquinone. Vitam Horm 40:1-43, 1983

16. Stocker R, Bowry VW, Frei B: Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does α-tocopherol. Proc Natl Acad Sci USA 88:1646-1650, 1991

17. Karlsson J, Diamant B, Folkers K: Skeletal muscle characteristics of significance for metabolism in health and disease. Adv Myochem 2:283-291, 1989

18. Karlsson J, Diamant B, Folkers K, et al: Plasma ubiquinone and cholesterol contents with and without ubiquinone treatment, in Lenaz G, Barnabe O, Rabbi A, et al (eds): Highlights in Ubiquinone Research. London, UK, Taylor & Francis, 1990, pp 296-302

19. Rip J, Rupar C, Ravi K, et al: Distribution, metabolism and function of dolichol and polyprenols. Prog Lipid Res 24:269-309, 1985

20. Marino M, Bruscalupi G, Manzi P, et al: Changes in plasma dolichol levels, transport, and hepatic delivery during rat liver regeneration. Metabolism 43:677-680, 1994

21. Lai C, Schutzbach J: Dolichol induces membrane leakage of liposomes composed of phosphatidylethanolamine and phosphatidylcholine. FEBS Lett 169:279-282, 1984

22. Valtersson C, van Duijn A, Verkleij T, et al: The influence of dolichol, dolichol esters, and dolichyl phosphate on phospholipid polymorphism and fluidity in model membranes. J Biol Chem 260:2742-2751, 1985

23. Yasugi E, Oshima M: Sequential microanalyses of free dolichol, dolichyl fatty acid ester and dolichyl phosphate levels in human serum. Biochim Biophys Acta 1211:107-113, 1994

24. Siperstain M: Role of cholesterogenesis and isoprenoid synthesis in DNA replication and cell growth. J Lipid Res 25:1462-1468, 1984

25. Santos E, Nebreda A: Structural and functional properties of ras proteins. Fed Am Soc Exp Biol J 3:2151-2163, 1989

26. Mendola C, Baker J: Lovastatin blocks N-ras oncogene-induced neuronal differentiation. Cell Growth Diff 1:499-502, 1990

27. Craig J, Peters R, Edmondson H: Atlas of Tumor Pathology, Second Series, Fascicle 26: Tumor of the Liver and Intrahepatic Bile Ducts. Washington, DC, Armed Forces Institute of Pathology, 1989, pp 36-41

28. Anthony P: Hepatoma associated with androgenic steroids. Lancet 1:685-686, 1975