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Decrease in Serum Dehydroepiandrosterone Level after Fenofibrate Treatment in Males with Hyperlipidemia

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This article is dedicated to Prof. MUDr. Vratislav Schreiber, DrSc. on the occasion of his 80th birthday

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The influence of steroid hormones on plasma lipids and lipoproteins was confirmed by many studies. On the other hand, the effect of plasma lipids on metabolism of steroid hormones has so far not been examined. The objective of this research project was to determine (1) the levels of cortisol, testosterone, estradiol, dehydroepiandrosterone (DHEA), its sulfate (DHEAS), 7-hydroxylated DHEA, and SHBG in men suffering from mixed hyperlipidemia (HPL) (n=23, age 46.1±7.9 years) in comparison with healthy male volunteers (n=17, age 45.1±15.6 years); (2) whether therapy with fenofibrate influences the levels of the above mentioned steroids and SHBG; (3) what are the correlations between lipids and steroids in healthy males and HPL patients before and after therapy. Compared to controls, untreated patients had significantly higher estradiol and free testosterone index (IFT) levels ($p<0.0003$ and $p<0.02$, respectively) and significantly lower SHBG ($p<0.02$). Due to fenofibrate therapy, a significant decrease of TC, TG, and DHEA levels occurred (mean decrease: 14 %, 52 % and 21 %, respectively). Triglycerides correlated negatively with testosterone and SHBG in healthy subjects. HDL-C correlated positively and consequently, atherogenic index correlated negatively with 7-hydroxylated epimers of DHEA in treated patients. This is the first study dealing with the influence of fenofibrate administration on the steroid levels. Taking together, the most important is the finding of decrease DHEA levels after fenofibrate therapy. It could be explained, at least in part, by the effect of the fenofibrateon on the biosynthesis of DHEA and its regulation.

Key words
Dehydroepiandrosterone • Estradiol • Fenofibrate • Hyperlipidemia • SHBG

Introduction

Increased serum levels of lipids and lipoproteins are known risk factors in the genesis of cardiovascular diseases (CHD) (Stamler *et al.* 1986). On the contrary, some sex steroids and their precursors have been reported to exert a protective role in this field (Alexandersen *et al.* 1996, Poršová-Dutoit *et al.* 2000, Celec and Stárka 2003). The mechanism of steroid intervention in atherogenic processes has not been fully explained but a number of

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possibilities exist (Poršová-Dutoit *et al.* 2000). It has also been hypothesized that sex hormones including adrenal androgens may influence certain enzymes participating in the metabolism of HDL-cholesterol (HDL-C) and triglycerides (TG) or may influence lipolysis (Haffner and Valdez 1995, Poršová-Dutoit *et al.* 2000). There are two ways of investigating the relations between steroids and lipids: (1) the influence of administered steroids in pharmacological doses on the lipid levels (Šulcová *et al.* 2000) (2) the comparison of steroid and lipid levels under physiological and pathological conditions (Poršová- Dutoit *et al.* 2000).

The positive effect of estrogen administration to postmenopausal women on lipid and lipoprotein levels has been sufficiently described (Godsland 2001, Araujo *et al.* 2002). Testosterone administration to hypogonadal and elderly men led to a drop in the levels of total cholesterol (TC) and LDL-cholesterol (LDL-C); whereas HDL-cholesterol (HDL-C) remained unchanged (Zgliczynski *et al.* 1996). On the contrary, in men to whom a combination of androgens and gestagens was administered for male contraception, a drop in HDL-C levels in the serum was observed (Gonzalo *et al.* 2002).

Less clear is the relationship of endogenous steroids to the lipid spectrum under physiological and pathological conditions. Sex hormones do not significantly influence the regulation of the serum concentrations of LDL-C and triglycerides during male puberty (Wickman *et al.* 2002). However, males suffering from CHD displayed significantly lower levels of dehydroepiandrosterone sulfate (DHEAS) than healthy controls of the same age (Mitchell *et al*. 1994, Alexandersen *et al.* 1996). The normalization of the levels of dehydroepiandrosterone (DHEA) and its sulfate in men is usually associated with the improvement of cardiovascular risk factors (Haffner and Valdez 1995, Alexandersen *et al.*1996).

Providing there is a connection between endogenous steroids and lipids, it would be interesting to know whether and to what extent the fibrate treatment of hyperlipidemias might influence the levels of certain steroids, particularly sex hormones and DHEA or DHEAS.

The aim of the present study was to determine 1) the levels of selected steroids and SHBG in untreated males suffering from hyperlipidemia as compared to levels in healthy control males; 2) whether and in what manner a change of lipid levels due to fenofibrate treatment may influence steroid and SHBG levels; and 3) whether healthy males and patients suffering from hyperlipidemia in the Czech population show statistically significant correlations between lipids and steroids or SHBG, and whether such correlations are influenced by fenofibrate treatment.

Subjects and Methods

The subjects of the study were 23 males aged 29-60 years suffering from mixed hyperlipidemia (HPL) (total cholesterol >5.0 mmol/l, triglycerides >2.3 mmol/l). Their BMI amounted to 28.8±4.0 kg/m^2 (median 27.8). The patients were treated with fenofibrate (Lipanthyl 200M administered once daily, 200 mg) for a period of 12 weeks.

The control group consisted of 17 healthy males aged 20-70 years who had signed a consent form agreeing to participate in the study. Their levels of the examined lipids and steroids were within the limits of current standards, and their BMI amounted to 26.1±3.4 kg/m^2 (median 25.5). They were neither on regular medication nor had health risks except for the higher age in some of them. Blood samples were obtained after an overnight fast and sera for all analyses were kept at –20 ^{o}C until assayed.

Determination of lipids

The levels of total cholesterol, triglycerides and HDL-cholesterol were measured enzymatically using kits from Roche Diagnostic. The levels of LDL-cholesterol were calculated by Friedewald's formula. The atherogenic index was calculated as TC/HDL-C ratio.

Determination of steroids and SHBG

Serum DHEA, DHEAS and estradiol were determined by RIA and SHBG IRMA methods using kits from Immunotech (France and Czech Republic). The other steroids (cortisol, total testosterone, 7α-hydroxy-DHEA and 7β-hydroxy-DHEA) were assessed by the RIA method, using antisera and radioactive tracers prepared by the Laboratory Department of Steroid Hormones, Institute of Endocrinology, Prague (Bičíková *et al.* 1988, Hampl 1994, Lapčík *et al.* 1998, 1999). The free testosterone index (IFT) was calculated as a ratio of testosterone / SHBG.

Statistical analyses

The differences between hyperlipidemic patients and controls were evaluated using Mann-Whitney's robust test, whereas the differences between the pretreatment and post-treatment results were evaluated using non-parametric Wilcoxon's paired test. Due to data asymmetry and heteroscedasticity, serial correlations were used.

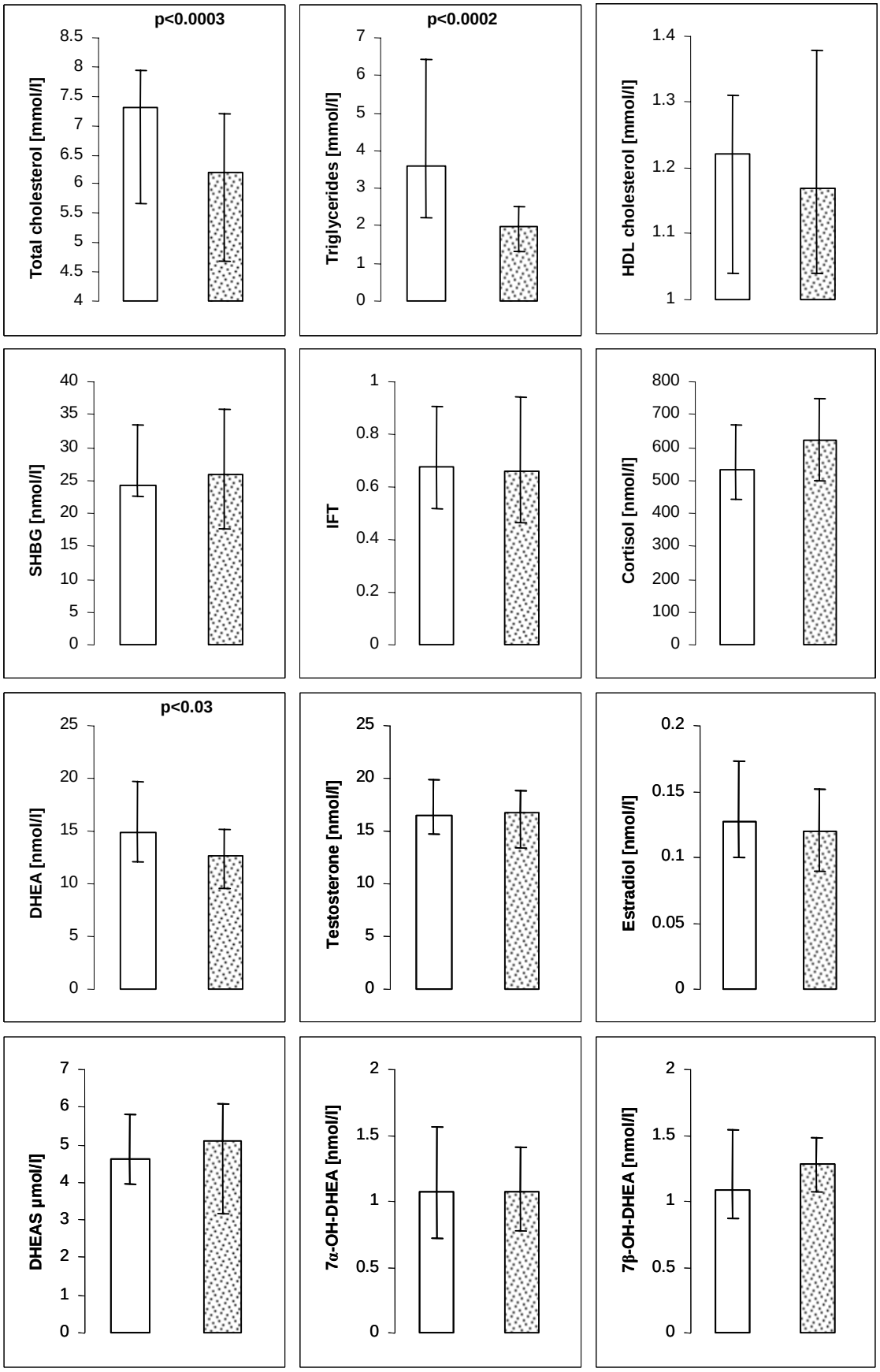

Fig. 1. Levels of lipids, selected steroids, and SHBG in patients suffering from hyperlipidemia treated with fenofibrate. Empty columns = prior to therapy, dotted columns = after therapy. Columns displaying error bars show group medians with quartiles. Wilcoxon robust pair test was used for statistical evaluation of differences.

Table 1. Differences between hyperlipidemic patients (n=23) and controls (n=17), before and after treatment.

	Age (years)	TC (mmol/l)	TG (mmol/l)	HDL-C (mmol/l)	Cortisol (nmol/l)	Testosterone (nmol/l)	Estradiol (nmol/l)
Patients							
before treatment	46.1±7.9	7.08±1.50	4.48±3.19	1.24±0.31	546±146	16.8±3.7	0.130±0.046
vs. controls	*NS*	***p<0.000001***	***p<0.0000001***	*NS*	*NS*	*NS*	***p<0.00003***
after treatment	46.1±7.9	6.10±1.52	2.17±1.09	1.21±0.27	612±146	17.3±5.4	0.121±0.041
vs. controls	*NS*	***p<0.003***	***p<0.0003***	*NS*	*NS*	*NS*	***p<0.0005***
Controls	45.1±15.6	4.77±0.67	1.27±0.37	1.27±0.22	562±92	17.9±4.9	0.079±0.020

	DHEAS (µmol/l)	DHEA (nmol/l)	7α-OH-DHEA (nmol/l)	7β-OH-DHEA (nmol/l)	SHBG (nmol/l)	IFT
Patients						
before treatment	5.0±1.8	16.91±8.89	1.28±0.75	1.17±0.43	26.6±14.9	0.781±0.382
vs. controls	*NS*	***(p=0.07)***	*NS*	*NS*	***p<0.02***	***p<0.02***
after treatment	5.0±1.9	13.41±5.02	1.23±0.64	1.28±0.43	28.5±14.1	0.717±0.360
vs. controls	*NS*	***p<0.007***	*NS*	*NS*	*NS*	***(p=0.09)***
Controls	5.0±2.3	23.03±10.88	1.37±0.69	1.31±0.62	39.6±21.2	0.522±0.182

Data are means ± S.D.

Results

Lipid and steroid levels in untreated patients suffering from hyperlipidemia compared to control males

Table 1 shows the levels of total cholesterol, triglycerides, HDL-C, cortisol, testosterone, estradiol, DHEAS, DHEA, 7-hydroxylated metabolites of DHEA (7α-hydroxy-DHEA and 7β-hydroxy-DHEA) and SHBG in hyperlipidemic men before and after therapy, and in control males. The statistical evaluation of differences in all examined parameters between patients and controls indicated significantly increased levels of total cholesterol, triglycerides, estradiol and IFT and, conversely, decreased levels of SHBG in pretreated patients as compared to the controls. Such differences were negligible for HDL-C, cortisol, total testosterone, DHEAS and 7-hydroxylated DHEA. Similarly, the difference in unconjugated DHEA between the patients before treatment and the controls was not statistically significant, even though the average levels, median and upper percentile were markedly lower in patients than in the controls.

Post-treatment levels of lipids and steroids in patients suffering from hyperlipidemia compared to control males

Table 1 further shows that in spite of the significant drop in total cholesterol and triglycerides levels, their levels remained significantly higher in comparison with the controls even after treatment. The estradiol levels in patients also remained significantly elevated. On the contrary, the difference in SHBG and IFT was no longer significant. On the other hand, the significant drop in unconjugated DHEA in patients subjected to treatment (Fig. 1) also showed a statistically significant difference in DHEA between the treated patients and control males (Table 1).

Changes of lipids, steroids and SHBG in HPL patients under the influence of fenofibrate treatment

Fenofibrate treatment induced a very significant decrease of total cholesterol and triglycerides as well as of unconjugated DHEA. Neither the slight decrease of HDL-C and estradiol levels nor the slight increase of cortisol, DHEAS and 7β-hydroxy-DHEA levels were significant.

The levels of testosterone, 7α-hydroxy-DHEA, SHBG and IFT did not undergo any change (Fig. 1).

Correlation between the levels of lipids and steroids

Only the negative correlations between TG and testosterone (correl. coeff. –0.551, $p<0.022$) and SHBG (correl. coeff. –0.560, $p<0.019$) was significant in healthy control males. No significant correlations were found in untreated HPL patients between the determined lipids, steroids and SHBG.

In treated patients, HDL-C correlated positively with two 7-hydroxylated DHEA epimers (7α: correl. coeff. 0.468, $p<0.024$; 7β: correl. coeff. 0.451, $p<0.031$). Inversely, these epimers correlated negatively with the atherogenic index (TC/HDL-C) in the following way: 7α-OH-DHEA: correl. coeff. –0.455, $p<0.029$; 7β-OH-DHEA: correl. coeff. –0.607, $p<0.002$.

Discussion

It has been postulated that in connection with CHD and with the increase of the respective risk factors, hyperestrogenemia and hypotestosteronemia were observed in men (Phillips *et al.* 1994). In our patients suffering from mixed hyperlipidemia (HPL), we detected significantly higher estradiol levels than in control males with a normal lipid spectrum but equal testosterone levels. However, in HPL patients there was a higher index of free testosterone due to the significantly lower SHBG level. We have consequently confirmed hyperestrogenemia in cases of CHD risk, whereas hypotestosteronemia was not confirmed.

It has also been demonstrated that DHEAS levels are commonly decreased in cases of CHD risk (Mitchell *et al.* 1994, Alexandersen *et al.* 1996). While we observed no difference between DHEAS levels in HPL males and the control group, the concentration of unconjugated DHEA was markedly lower in our HPL patients than in the controls. This pretreatment difference did not attain statistical significance; but this can be explained by a large individual differences in DHEA levels in adult males (Šulcová *et al.* 1997) in general and in both compared groups (Table 1).

Until now, nothing was known about the direct effect of fibrates on the steroid spectrum. The influence of varying lipid concentrations on the level of steroids under experimental conditions was described by Tanaka *et al.* (2001). Dietary-induced hypercholesterolemia caused a significant decrease of both the serum and testicular testosterone in rats (Tanaka *et al.* 2001). The significant decrease in the levels of total cholesterol and triglycerides after fenofibrate administration was not, in our study, accompanied by an increase in testosterone levels as could have been expected. Furthermore, no significant drop in the increased estradiol level occurred, i.e. no relative strengthening of androgenicity had occurred. The only significant change in the studied segment of the steroid spectrum was the unexpected decrease of the level of unconjugated DHEA which had already been lower prior to the therapy (though only slightly below the significance limit) in HPL patients as compared to the controls. This runs against the tendency of estradiol and SHBG whose levels shifted upon treatment slightly towards normal values. We have no sure explanation for this behavior of DHEA. It could well be the effect of direct intervention of fenofibrate in DHEA biosynthesis or metabolism by influencing the activity of the respective enzymes. In case of biosynthesis it could involve inhibition of the steroid C17,20 lyase (its precursor being 17-hydroxypregnenolone) or of sulphatase (DHEAS precursor). The slight but non-significant post-treatment increase in DHEAS levels argues for a possible inhibition of sulphatase. Inversely, the process of DHEA degradation could be explained as activation of 7-hydroxylases converting DHEA to 7α/7β-hydroxy-DHEA. 7-hydroxylated DHEA is sometimes considered to be a possible intermediate product of positive (especially immunomodulatory, immunoprotective or some other) effects of DHEA (Hampl *et al.* 1997). We did not observe any change of 7α-OH-DHEA level in our patients due to therapy. While the 7β-OH-DHEA level had increased, but this increase was not statistically significant. The positive correlation between HDL-C and the levels of its two epimers and, consequently, the negative correlation between 7α/7β-hydroxy-DHEA and the atherogenic index might suggest a possible positive role of 7-hydroxylated DHEA in CHD risk.

A negative correlation between SHBG and triglycerides was described in men (Stefanick *et al.* 1987, Haffner *et al.* 1993). This corresponds to the negative correlation also reported in this work between TG, testosterone, and SHBG in healthy control males.

Okamoto (1996) observed a negative correlation between DHEAS and LDL-C and inversely a positive correlation between DHEAS and HDL-C level. On the other hand, Hautanen *et al.* (1994) found a negative relation between DHEAS and HDL-C and a positive relation between DHEAS levels and triglycerides. By administering dehydroepiandrosterone to elderly men for a period of four

months, Arlt *et al.* (2001) achieved an increase of endogenous DHEA and DHEAS levels to values typical for young males but did not observe any influence on serum lipids. We have not observed any significant correlation between the DHEAS or DHEA levels and lipids either in healthy men or in HPL patients. This confirms the suspicion of some authors who consider the correlation of DHEA, DHEAS and lipids questionable, since both a negative and positive correlation, as well as no correlation between these steroids and atherogenic lipids have been found (Haffner *et al.* 1993).

Abbreviations

C	cholesterol
CHD	cardiovascular diseases
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
HDL	high density lipoprotein
HPL	hyperlipidemia
IFT	free testosterone index
LDL	low density lipoprotein
SHBG	sex hormone binding globulin
TC	total cholesterol
TG	triglycerides

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