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IMPACT OF CHOLESTEROL, VITAMIN E SUPPLEMENTATION AND DIETARY FATTY ACIDS ON TESTIS FUNCTION IN HIGH-FAT DIET FED RATS

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Key words: cholesterol, vitamin E, 17beta-hydroxysteroid dehydrogenase, rat, testosterone, testes

The aim of the study was to determine whether consumption of diets containing different fat (rapeseed oil or lard-rich), supplemented with a high level of cholesterol (3% w/w) and/or vitamin E (500 mg/kg of diet) for 6 weeks influenced 17beta-hydroxysteroid dehydrogenase (17β-HSD) type 3 activity (measured *in vitro*), testosterone (Tt) content in testes and plasma testosterone (Tp) concentration (measured by ELISA and RIA, respectively) in male rats.

17β-HSD activity was shown to depend on dietary fat type. Supplementation of vitamin E influenced Tt value, whereas none of the investigated dietary factors had effects on Tp. Significant differences between the investigated factors were observed only for groups fed rapeseed oil-rich diets with marked increases of Tt and Tp in rats fed vitamin E-rich diet.

INTRODUCTION

Several experiments showed that dietary fat composition and dietary fatty acids can modulate the concentrations of steroids in plasma. O'Donnell [2001] showed that a high-fat diet caused a decrease in total and free testosterone levels in blood. Other observations demonstrated that feeding period and dietary fat type affected testicular steroidogenesis and androgen plasma concentration [Gromadzka-Ostrowska *et al.,* 2002; Gromadzka-Ostrowska, 2006]. It seems that monounsaturated fatty acids (MUFAs) stimulate androgen secretion in contrast to saturated fatty acids (SFAs) which may inhibit steroid production. Polyunsaturated fatty acids (PUFAs), *i.e.* arachidonic acid, can be responsible for changes in Leydig cell membrane fluidity and thus can modify their secretory function [Sebokova *et al.,* 1990].

As it was previously shown, high-fat and hypercholesterolemic diets influenced both plasma testosterone concentration and its tissue metabolism [Gromadzka-Ostrowska, 2006]. On the other hand, reactive oxygen species formed also during steroidogenesis [Hales, 2002] can inhibit this process in Leydig cells. Antioxidants, such as vitamin E, not only protect against lipid peroxidation but also prevent testicular oxidative stress and play a key role in testis steroid production [Sen Gupta *et al.,* 2004]. However, the underlying molecular mechanisms remain largely unknown.

In the current investigation it was determined whether high-fat diets containing different types of fatty acids and supplemented with high levels of cholesterol and/or vitamin E affected testes secretory function.

MATERIALS AND METHODS

Animals, diets and experimental design. The study was conducted on 48 male adult Wistar rats (initial body weight 250±10 g) housed individually in steel cages (22°C, humidity 50%, 12:12 L:D) with free access to food and water. All procedures were approved by the Local Animal Care and Use Committee in Warsaw.

After one week of adaptation, animals were divided into two main groups differing in the type of dietary fat source: group Rs – fed a diet containing rapeseed oil rich in MUFAs (54.6%), mainly oleic acid [18:1]; and group L – fed a diet containing lard rich in SFAs (40.8%), mainly stearic acid [16:0]. In both dietary groups animals were allotted into four subgroups: 1 – a basal diet, 2 – a diet supplemented with 3% (w/w) cholesterol, 3 – a diet supplemented with 500 mg of vitamin E/100 kg of diet, and 4 – a diet supplemented with 3% cholesterol and 500 mg of vitamin E/100 kg of diet combination. All treatments were carried out for 6 weeks.

Experimental diets were high-fat (20% w/w, 38% energy from fat), semi synthetic with 17.5% of protein. Composition of diets and their fatty acid content was estimated by gas chromatography analysis [Daniewski *et al.,* 1999]. Total food intake, fatty acid intake and final body weight were measured as well.

After 45 days of feeding, the rats were bled under pentobarbital anesthesia by cardiac puncture. The testes were dissected, immediately frozen in liquid nitrogen and stored at –80°C until the examinations. Blood plasma was stored at –23°C until assays.

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Plasma hormone concentration. Plasma testosterone (Tp) concentration expressed as nmol/L was measured by the RIA method using the Orion Diagnostica test, Finland. The cross-reactivity of the antiserum with other steroid hormones was <0.1%, inter- and intra-assay coefficient precision was 7.0% and 7.5%, respectively.

17beta hydroxysteroid dehydrogenase type 3 activity in testes. The activity of the steroidogenic enzyme 17beta hydroxysteroid dehydrogenase type 3 (17β-HSD3) was assayed as described by Payne & Youngblood [1995]. Conversion of ^{3}H-androstendione (androst-4-ene-3,17-dione) [^{3}H-A] to ^{3}H-testosterone [^{3}H-T] in testis microsome fraction took place during 120 min of incubation at 37°C (0.05 mol/L phosphate buffer, pH=7.4, 5 mmol/L NADP (Sigma Aldrich), 0.07 mCi androstendione [74.0 Ci/mmol, NEN Life Science Product Inc., USA]). Reaction products were separated by thin layer chromatography. The enzyme activity was expressed as percent of conversion ^{3}H-A to ^{3}H-T per 100 µg of testis microsome fraction protein concentration measured using Bradford reagent (Sigma, Aldrich)

Testosterone concentration in testes. Testes cytosol testosterone content (Tt) was measured using DSL-10-4000 Testosterone EIA test (DSL, Inc., USA) using polystyrene micro titer wells with goat anti-rabbit IgG, wavelength measurement at 450 nm. The cross-reactivity of the anti-androgen serum was 100% with testosterone and 6.6% with 5α-dihydrotestosterone. The inter- and intra-assay coefficient precision was 4.9% and 6.8%, respectively. Tt was expressed as ng per 100 µg of testis cytosol fraction protein concentration measured using Bradford reagent (Sigma, Aldrich).

Statistical analysis. Statistical analysis (multifactor variance ANOVA and simple regression) preceded by the post-hoc least difference Fisher's test was performed using Statgraphics Plus v. 4.1. Difference was considered as statistically significant at $p \leq 0.05$. All data are expressed as means ± SEM.

RESULTS

Final body weights, food and fatty acids intake

Final body weight (FBW) depended on dietary fat type and vitamin E supplementation (ANOVA $p \leq 0.0002$ and $p \leq 0.01$, respectively) and was significantly lower in rats fed Rs diets (289.79±8.02 g) than in rats reared on diets containing lard (332.12±6.38 g). In Rs subgroups, FBW was higher in rats fed diets supplemented with vitamin E and enriched with both cholesterol and vitamin E.

Total food and dietary fat intake also depended on dietary fat type and vitamin E supplementation (ANOVA $p \leq 0.0001$ and $p \leq 0.02$, respectively for both parameters). Total food, total fat, SFA intakes as well as FBW did not differ significantly between L groups.

17beta hydroxysteroid dehydrogenase type 3 activity in testes

As shown by ANOVA analysis, 17β-HSD3 activity differed significantly between dietary groups ($p \leq 0.05$) with higher values in rats fed diets containing rapeseed oil. Cholesterol or vitamin E dietary supplementation in Rs and L groups had no influence on this parameter (Figure 1).

Testosterone concentration in testes

The diet containing rapeseed oil with cholesterol (Rs2) had no effect on Tt, whereas supplementation of vitamin E significantly elevated T level in rat male gonads (ANOVA $p \leq 0.03$). No significant differences in L groups were found. Rats fed Rs1 diet had lower Tt value than those fed the L1 diet (Figure 2).

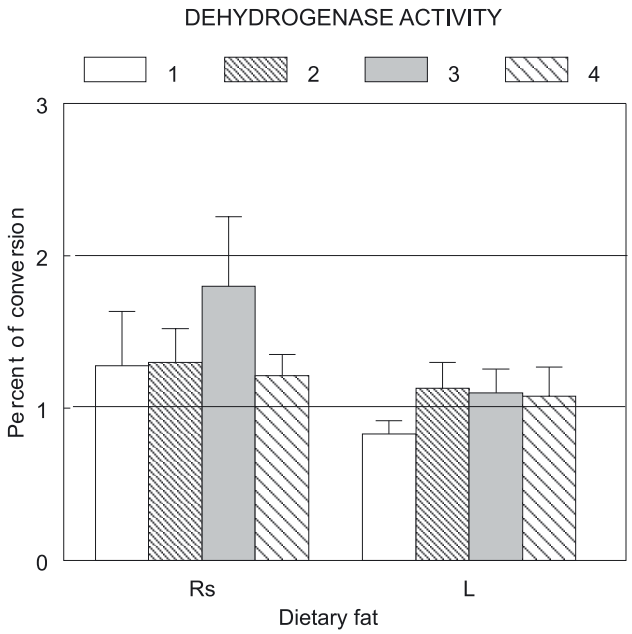

FIGURE 1. 17β-hydroxysteroid dehydrogenase activity in testes microsome in rats fed high-fat (20% w/w) diets containing rapeseed oil (Rs) or lard (L) as a fat source for 6 weeks (mean ± SEM).

Numbers in the legend above figure indicate: 1 – basal diet; 2 – diet supplemented with 3% (w/w) of cholesterol; 3 – diet supplemented with vitamin E (500 mg/kg of diet); 4 – diet supplemented both with 3% cholesterol and vitamin E (500 mg/kg of diet).

Plasma testosterone concentration

Tp in animals fed rapeseed oil-rich diets showed the same difference pattern as for Tt. Supplementation with vitamin E alone or together with cholesterol raised Tp in groups Rs3 and Rs4 *vs.* groups Rs1 and Rs2 ($p \leq 0.001$). In all L groups no significant differences were observed, but as for Tt, Rs1 animals had lower Tp in comparison to L1 rats (Figure 3).

A highly positive correlation was found for Tp and Tt ($r=0.83$; $p \leq 0.00001$).

DISCUSSION

The present study was undertaken to explore the effects of dietary cholesterol and/or vitamin E supplementation on testicular 17beta-HDS activities and on testis and plasma

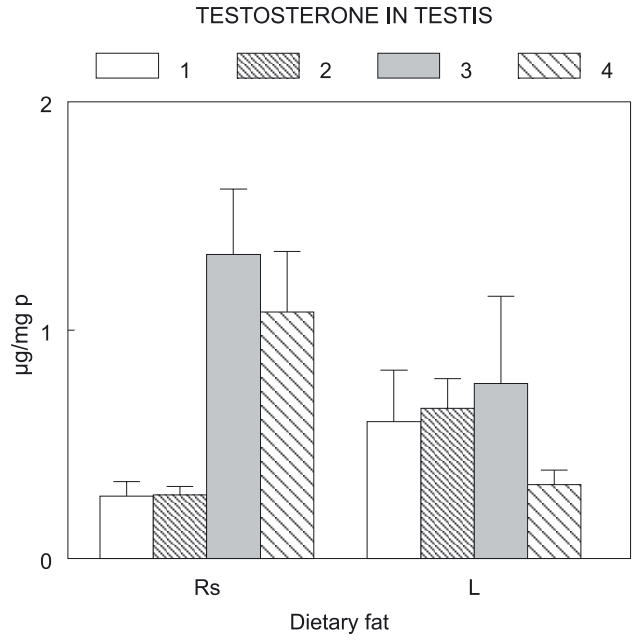

FIGURE 2. Testosterone level in testis cytosol (Tt) in rats fed high--fat (20% w/w) diets containing rapeseed oil (Rs) or lard (L) as a fat source for 6 weeks (mean ± SEM).

Legends and letters as in Fig. 1.

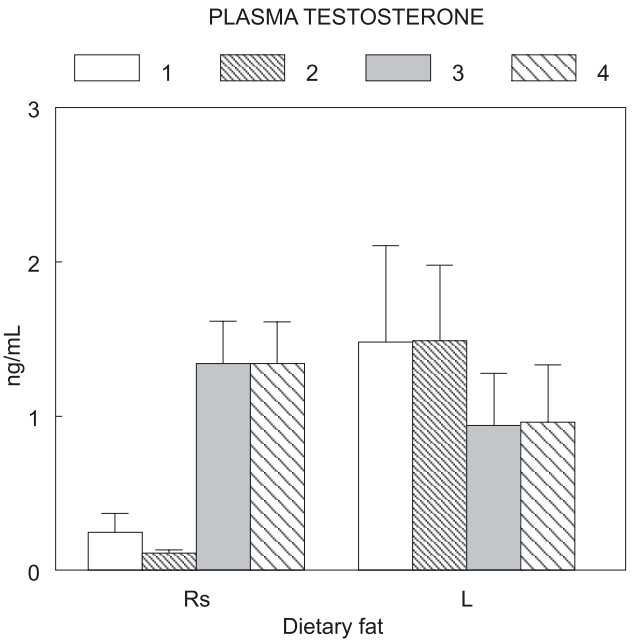

FIGURE 3. Testosterone plasma concentration (Tp) in rats fed high--fat (20% w/w) diets containing rapeseed oil (Rs) or lard (L) as a fat source for 6 weeks (mean ± SEM).

Legends and letters as in Fig. 1.

testosterone concentrations in mature male rats fed MUFA or SFA-rich diets. The results of our experiment suggest that 3% of dietary cholesterol is not associated with HDS activity differences as well as testes and plasma testosterone concentrations in both dietary groups, whereas a significant increase in plasma and testes testosterone levels as a result of alpha-tocopherol co-administration or only vitamin E supply in groups fed MUFAs rich diet was observed.

Previous studies suggested that oxidant-induced damage, *e.g.* caused by lipid peroxidation and reactive oxygen species may play a role in the reduced ability of rat Leydig cells to produce testosterone [Murugesan *et al.,* 2005]. Mori *et al.* [1980] found that testosterone production rate did not differ when rats were fed on a cholesterol-rich diet for a longer period.

The present investigation clearly indicates that vitamin E supplementation of the diet changes testosterone secretion and its gonadal accumulation of testosterone without any changes in steroidogenesis on HDS step. As found by Sen Gupta *et al.* [2004] supplementation with vitamin E reduced testicular reactive oxygen species and restored normal testicular function probably by co-regulating StAR gene expression in steroid production. Dietary supplementation of rats with vitamin E for a period of 429 days caused significant changes in steroidogenesis by affecting early steps of cholesterol transformation [Barella *et al.,* 2004]. Also Chen *et al.* [2005] reported *in vivo* and *in vitro* studies showing a protective vitamin E effect on steroidogenesis in Leyding cell.

It was previously observed that α-tocopherol applied in men caused a decrease in serum androstenedione and testosterone levels [Hartman *et al.,* 2001]. Similarly, Hartman *et al.* [1999] affirmed that androstenedione, testosterone and sex hormone-binding globulin were significantly inversely associated with serum α-tocopherol. In our work we found a contrary effect of vitamin E supplementation inducing an increase in testicular testosterone concentration in rats fed high-fat diets rich in MUFAs and also a little rise (tendency) in 17-betaHSD3 activity in opposition to the effected high-fat diets rich in SFAs. It can be linked with mitochondrial and endoplasmic localization of α-tocopherol [Bjorneboe *et al.,* 1990] and with antioxidant function of vitamin E to protect the membrane lipids against free radical attack [Wang *et al.,* 2000]. It seems that basal diets with SFAs presented in lard in opposition to dietary MUFAs (rapeseed oil) may also increase testosterone level in testes.

CONCLUSIONS

1. High-fat diets containing different types of fatty acids and supplemented with high levels of vitamin E affect testes function.
2. Secretory function of male gonads is influenced mainly by vitamin E addition to diet containing rapeseed oil.

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DZIAŁANIE SUPLEMENTACJI CHOLESTEROLEM I/LUB WITAMINĄ E ORAZ KWASÓW TŁUSZCZOWYCH DIETY NA FUNKCJE GONAD SZCZURÓW KARMIONYCH WYSOKOTŁUSZCZOWYMI DIETAMI

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Celem badań było określenie wpływu spożycia przez 6 tygodni diet zawierających olej rzepakowy (RS) lub smalec (L) z dodatkiem lub bez 3% cholesterolu i/lub witaminy E (500 mg/kg diety) na aktywność 17β-dehydrogenazy steroidowej (7β-HDS) i zawartość testosteronu w gonadach (Tt) oraz stężenie tego steroidu w osoczu krwi (Tp) szczurów.Aktywność enzymu zależała od rodzaju tłuszczu diety i była wyższa w grupach RS. Suplementacja witaminą E spowodowała wzrost wartości Tt i Tp tylko w grupach Rs.