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THE EFFECT OF ESSENTIAL FATTY ACID DEFICIENCY ON HEPATIC BILE SALT SULPHOTRANSFERASE IN RATS

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Summary—Hepatic bile salt sulphotransferase (BSS) activity and the contents of unconjugated oestradiol-17β (E2) and conjugated oestrone (cE1) in liver tissue was significantly lower in young essential fatty acid (EFA) deficient female rats than in female control rats. No corresponding differences were found between male EFA deficient and control rats. A significant sex difference, with higher values in females, was found for BSS activity and E2 and cE1 contents in control rats but not in EFA deficient rats. The decrease in hepatic BSS activity in female rats caused by EFA deficiency may be mediated via a decreased estrogenic action on the liver.

INTRODUCTION

Sulphoconjugation is considered an important pathway in increasing the excretion of toxic bile acids [1]. Essential fatty acid (EFA) deficiency not only affects lipid metabolism but also endocrine functions [2]. It is known that adolescent and mature female rats have higher activity of bile salt sulphotransferase (BSS) than male rats, and this sex difference is related to oestrogen levels [3]. In order to clarify the influence of EFA deficiency on hepatic BSS we measured the glycolithocholate sulphotransferase activities in late adolescent EFA deficient rats and age and sex matched controls.

EXPERIMENTAL

Pregnant white Sprague–Dawley rats were obtained from ALAB AB (Stockholm, Sweden). Ten days before delivery and during lactation, one group of the pregnant animals as well as their offspring (EFA deficient rats) were fed a semi-synthetic diet deficient in EFA (Astra EWOS, Södertälje, Sweden). Another group of pregnant rats and their offspring (controls) received the same diet except that hydrogenated lard was replaced by soybean lipid [4]. The rats had free access to tap water and their respective diets, and were housed under conditions with controlled dark–light cycles (lights on 6 a.m. to 6 p.m.), the humidity was kept at 55–60% and the temperature at 22°C. Adolescent male and female rats, 47 days old, were used for the experiments. At that time, the EFA deficient rats showed retarded growth and slight scaliness of hindlegs and tail, symptoms characteristic of EFA deficiency. The mean weight ($\pm$SE) of the EFA deficient rats was 128 ± 8.6 g (males) and 110 ± 5.1 g (females) and of the controls 159 ± 4.3 and 137 ± 5.5 g, respectively ($P < 0.05$ and < 0.01). The triene–tetraene ratio [20:3 ($w9$)/20:4 ($w6$)], used as a biochemical criterion of EFA deficiency, has been shown to be 7 in serum phospholipids in rats of the same age in the same experimental setting in our laboratory. According to Holman [5], a ratio < 0.4 excludes EFA deficiency.

METHODOLOGY

The liver was taken out immediately after decapitation and homogenized in 4 parts of ice cold buffer [0.02 M Tris–HCl (pH 7.4) containing 0.15 M KCl, 0.01 M EDTA and 0.01 M 2-mercaptoethanol] in an Elvehjem-Potter homogenizer. The homogenate was centrifuged at 150,000 *g* for 60 min at 4°C, and the supernatant was collected and stored at −80°C until used. BSS activity was assayed in duplicate using a reaction mixture of 25 nmol glycolitocholate

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and 20 nmol 3′-phosphoadenosine-5′-phosphosulphate (Sigma Chemical Co., St Louis, MO) and 4–6 × 10^5 cpm [^{35}S]3′-phosphoadenosine-5′-phosphosulphate (New England Nuclear Corp., Boston, MA) in a total vol of 100 μl in the buffer mentioned above. The reaction was initiated by adding 50 μl cytosol diluted to contain about 50 μg of protein. Incubations were carried out at 37°C for 30 min. The reaction was terminated by boiling for 30 s and ice cooling for 5 min. The reaction mixture was diluted with 0.4 ml of ice cold 1 M ammonium hydroxide, and the [^{35}S]-labeled sulphate products were extracted with 1 ml of cold *n*-butanol by vigorous shaking for 15 s as described by Barnes *et al.* [6]. After centrifugation at 3000 *g* for 15 min, 0.9 ml of the butanol supernatant was separated and washed for 5 s with 0.45 ml of ice cold 1 M ammonium hydroxide containing 100 mM sodium phosphate buffer (pH 7.0) presaturated with butanol, final pH 10.8. Following centrifugation at 3000 *g* for 15 min, 0.5 ml of the butanol fraction was mixed with 3 ml Instagel scintillant, and counted in a LKB Wallac 1214 Rack Beta liquid scintillation counter. Protein was determined according to Peterson with bovine serum albumin as standard [7]. The enzymatic activity was expressed as pmol of formed glycolithocholate sulphate/min/mg protein. The coefficient of variation was similar within and between assays 8–9% ($n = 10$).

Tissue concentrations of unconjugated oestradiol-17β (E2) and conjugated oestrone (cE1) were analysed after extraction of the oestrogens from liver tissue with methanol (four consecutive overnight soakings), defatting of the extract by partition between 80% ethanol and *n*-hexane and separation of conjugated and unconjugated oestrogens by partition between physiological saline and diethyl ether. The ether extract was evaporated to dryness, dissolved in E2-free serum (E2 assay kit zero calibrator) and analysed for E2 using a commercial direct radioimmunoassay kit (Coat-a-Count, Diagnostic Products Corp, Los Angeles, CA). The saline phase was analysed for cE1 according to Carlström and Sköldefors [8]. This method includes enzymatic hydrolysis of the conjugates, ether extraction and radioimmunoassay. The concentrations were expressed as pmol of oestrogen per kg of wet weight. Coefficients of variation within and between assays were for unconjugated E2 6 and 10%, and for cE1, 7 and 9%, respectively.

Table 1. Hepatic BSS activity (pmol of glycolithocholate sulphate formed per min and per mg protein) and hepatic tissue content of E2 and cE1 (both in pmol/kg wet weight) in EFA deficient and control rats (mean ± SD)

	EFA deficient		Controls	
	Female	Male	Female	Male
n	5	5	5	4
BSS	30.8 ± 5.7	25.2 ± 4.3	65.2 ± 6.5^{c**}	26.3 ± 3.4
E2	118 ± 56	174 ± 61	370 ± 121^{b*}	148 ± 57
cE1	492 ± 305	372 ± 179	900 ± 162***	403 ± 51

EFA deficient vs controls: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.
Female vs male: $^{*}P < 0.05$; $^{**}P < 0.001$.

Student's *t*-test for unpaired observations was used for statistical analysis.

RESULTS AND DISCUSSION

Hepatic BSS activity and oestrogen content are given in Table 1. BSS activity and tissue oestrogen concentrations were significantly lower in female EFA deficient rats than in female controls. While female control rats had higher BSS activity and tissue oestrogen content than male controls, no such sex difference was present in the EFA deficient rats.

Sulphoconjugation markedly increases the water solubility of bile salts, resulting in lower hepatic toxicity and higher renal excretion [9]. It has been demonstrated that the sex difference in hepatic BSS activity is due to different isoenzymes. Activity of BSS I is enhanced by oestrogen, and contributes 90% of total sulphotransferase capacity in postpubertal female rats but only 30% in males. BSS II activity is similar in both sexes and not influenced by oestrogen [3].

EFA deficiency delays sex maturation in the female rats. This delay appears to be due to reduced prostaglandin E2 synthesis and suppressed gonadotropin secretion [10]. A triene tetraene ratio >6 is considered to hamper prostaglandin production [11], which might be the case in our series. Although oestrogen treatment of postpubertal male rats enhances the activity of BSS I, postpubertal female rats are not affected by oestrogen treatment because the activity is maximally enhanced by endogenous oestrogen [12]. Our results showed that both oestrogen levels and BSS activity were low in the EFA deficient female rats. This was not only due to a delayed puberty since the activity was similarly low in adult female EFA deficient rats ($n = 5$), mean value being 43.6 ± 5.02 vs 74.17 ± 9.05 pmol/min/mg protein in adult female control rats ($n = 5$) (K. Obinata *et al.*, unpublished observation). A diminished

oestrogen production caused by EFA deficiency, may thus reduce the activity of BSS I in the female rats. This putative effect of oestrogens may be direct or mediated via pituitary factors [13]. If there is an oestrogen effect, oestrogen treatment may be effective in enhancing BSS activity in the EFA deficient rats.

The results might have implications for the medical treatment of patients with cholestasis, since EFA deficiency has been reported in infants with cholestasis [14] as well as a lower percentage of sulphated urinary bile salt conjugates [15, 16]. However, in these studies females and males were not separated.

It has also been reported that EFA deficiency occurs in patients with cystic fibrosis (CF). Signs and symptoms in CF, e.g. liver steatosis, pancreatic dysfunction and lung changes are similar to findings in animals with EFA deficiency [17]. This study would indicate the need of investigations of metabolic changes in the liver of patients with advanced CF, especially since female patients with CF are generally said to have a shorter life expectancy, to which liver disorder might contribute.

CONCLUSION

The hepatic glycolithocholate sulphotransferase activity was reduced in female EFA deficient rats compared to control animals ($P < 0.01$). Since the E2 level in the liver of the female EFA deficient rats was also lower than in the control female rats, ($P < 0.01$), the results suggest that EFA deficiency suppresses synthesis of estrogen and that that is associated with an inhibited activity of hepatic BSS in the female rats.

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