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EFFECT OF HIGH-FAT DIETS WITH DIFFERENT FATTY ACID COMPOSITION ON HEPATIC 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 1 ACTIVITY IN RATS

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Recent studies suggest a key role for tissue aberrant expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and for tissue glucocorticoid excess in dysregulation of metabolism observed in diabetes and other metabolic disorders linked to central obesity. In an experiment carried out in 50 adult Wistar rats fed on diets containing sunflower oil, rapeseed oil, lard or cod liver oil at two levels (20 and 40% w/w), we were the first to show a beneficial inhibitory effect of all fats but one (sunflower oil) on hepatic 11β-HSD1 activity, given the moderately high consumption of fat (*i.e.* 20%).

INTRODUCTION

Glucocorticoid action on target tissues, among others on adipose tissue, muscles and brain, is not necessarily determined by plasma steroid levels, the extent of binding to plasma proteins and the expression of intracellular glucocorticoid receptors, but rather by their intracellular metabolism and by the availability of active forms for hormone-receptor interaction. At the prereceptor level glucocorticoids are crucially modulated by the glucocorticoid metabolizing 11β-hydroxysteroid dehydrogenases (11β-HSD), which interconvert active hormones, cortisol in humans, corticosterone in rodents, and inert 11-keto metabolites (cortisone, 11-dehydrocorticosterone, respectively) [Jamieson *et al.*, 2000].

Among target tissues, liver is the major site of glucocorticoid metabolism [Williams *et al.*, 2000] and is known to express high levels of low-affinity glucocorticoid receptors [Walker *et al*., 1995]. Within the liver, glucocorticoid functions are critically and exclusively determined by the type 1 isoenzyme of 11β-HSD (11β-HSD1), which *in vivo* acts predominantly as an oxo-reductase [Jamieson *et al.*, 2000], generating active glucocorticoids and ensuring their adequate exposure to receptors.

Relatively little is known about mechanisms of 11β-HSD1 expression regulation in liver. Factors that alter 11β-HSD1 synthesis and activity include adrenal and gonadal steroids, insulin, thyroid hormones, GH, leptin, TNFα and agonists of peroxisome proliferator–activated receptors (PPARs) [Seckl & Walker, 2001; Liu *et al.*, 2003]. 11β-HSD1 is a highly transcriptionally regulated gene, recently, however, several studies have evidenced post-transcriptional modulation of 11β-HSD1 activity through the cooperation between 11β-HSD1 and hexose-6-phosphate dehydrogenase [Bujalska *et al*., 2005].

To our knowledge, nutritional regulation of hepatic 11β-HSD1 has only been touched upon. Drake *et al.* [2005] showed a transient attenuation of hepatic 11β--HSD1 activity in rats fed for three weeks on a high-fat diet. However, neither acute intake of high amounts of fat in rats [Drake *et al*., 2005], nor chronic high-fat feeding in rats [Drake *et al.*, 2005] and mice [Morton *et al.*, 2004] affected significantly the enzyme expression and activity in liver. Similarly, in humans, after single lipid infusion no changes were observed in hepatic 11β-HSD1 activity [Mai *et al*., 2005].

Recent studies in humans and rodents with the over-expressed or null 11β-HSD1 gene suggest a key role for tissue aberrant expression of this izoenzyme and for tissue rather than plasma glucocorticoid excess in dysregulation of metabolism observed in diabetes and other metabolic disorders linked to central obesity [Masuzaki *et al*., 2001]. On the other hand, a growing body of evidence indicate that the modulation of 11β-HSD1 activity may offer a novel therapeutic approach to treating these diseases [Livingstone & Walker, 2003].

Against this background and with a potent role of fatty acids (FAs) as regulators of gene transcription in mind, we investigated further the regulation of hepatic 11β-HSD1 under high-fat diets. Specifically, we have assessed the effects of dietary fat level (40 *vs*. 20%) and fat source and composition (sunflower oil, lard, rapeseed oil and cod liver oil) on 11β-HSD1 oxo-reductase activity in liver.

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MATERIALS AND METHODS

The study protocol was reviewed and approved by the Third Local Animal Care and Use Committee in Warsaw.

Male Wistar rats (n=50 with initial body weight of 240--260 g) were individually housed in stable environmental conditions [temperature 22˚C, air humidity 50-60% and 12 h (06.00-18.00) light: (18.00-06.00) dark cycle] and were given free access to food and water throughout the study.

After 2-week adaptation period, the animals were randomly assigned to eight experimental groups (n=6-7) fed on diets differing in the fat content (20% and 40% by weight) and source (sunflower oil S, rapeseed oil R, cod liver oil F or lard L) for 3 weeks. All experimental diets contained 20% of protein by weight. At the end of the experiment, the rats were subjected to a 12-h food deprivation, anesthetized intraperitoneally with thiopental, and sacrificed *via* exsanguination by cardiac puncture. Thereafter, the liver was dissected from each animal, weighted, immediately frozen in liquid nitrogen, and stored at -80˚C for enzyme activity analysis. Plasma was stored at -23˚C for hormone determination.

Plasma corticosterone was measured using Rat Corticosterone RIA test (DSL, USA). The 11β-hydroxysteroid dehydrogenase type 1 activity was measured by the modified method described by Jamieson *et al.* [1995] in microsomal fraction of liver. Reaction of conversion of [^{3}H]corticosterone into [^{3}H]11β-dehydrocorticosterone was carried out during 2-h incubation at 37˚C. Both of radioactive compounds were extracted and divided by thin layer chromatography method, and their radioactivity was measured in the β-counter.

Plasma corticosterone concentration's data were analysed by two-way analysis of variance (ANOVA) with fat type and content as the discriminating factors, and by Fisher's least significant difference post hock test. 11β-hydroxysteroid dehydrogenase activity data were analysed by Kruskal-Wallis test. All statistical analyses were performed using the computer program STATGRAPHICS® Plus 4.0.

RESULTS

Fat type and content in diets significantly influenced plasma corticosterone concentration (ANOVA $p<0.0005$ and $p<0.0008$, respectively). Plasma corticosterone concentration in rats fed on diets containing 20% of all fat sources was higher than in rats reared on diets containing 40% of fat ($p<0.001$). Within the 20%-fat-groups, plasma hormone concentration was the highest in rats consuming the F diet ($p<0.0005$). Moreover, plasma Cs was higher in rats fed on the diet containing 20% of R than in animals reared on the diet containing 20% of S ($p<0.0005$) (Table 1).

Type 1 11β-hydroxysteroid dehydrogenase activity in rats fed on diets containing 40% was higher than in rats fed on diets containing 20% of fat ($p<0.05$). The effect of fat type on 11β-HSD1 was significant for 20%-fat-groups only, with the enzyme activity being higher in rats consuming S diet than in rats consuming 20% of R, F and L ($p<0.05$) (Table 1).

DISCUSSION

The current work is the first to demonstrate that dietary manipulation through long-term feeding on high-fat diets with different fatty acid composition, *i.e.* rich in either long chain saturated fatty acids (FAs) (lard), or *n*-6 polyunsaturated FAs (sunflower oil and rapeseed oil), or *n*-3 polyunsaturated FAs (mainly cod liver oil) represents a powerful mechanism regulating hepatic 11β-HSD1 activity in rats. The principal finding of our study is the up-regulation of hepatic 11β-HSD1 activity in rats under sunflower oil-rich diet and its down-regulation in rats fed on other experimental 20%-fat diets.

We cannot compare our results as to the effect of fat type on 11β-HSD1 activity due to the lack of respective research. We can only speculate on the possible mechanisms of the suppressive impact of dietary fats and certain FAs on hepatic 11β-HSD1 activity.

As 11β-HSD1 gene is regulated through activation of PPARs, particular FAs could influence 11β-HSD1 activity through modulation of gene expression. Long chain FAs bind to PPARs at physiological concentrations to directly up- or down-regulate transcription process of several genes. The suppressive effect of FAs is restricted to PUFAs; C18:3, *n*-3 is more potent than C18:2, *n*-6 and the products of Δ-6 desaturation are 2-4 times more potent suppressors than their FA parent compounds [Niot *et al.*, 1997]. In this respect, however, our results are quite striking. FAs could indirectly account for the differences in the activity of 11β-HSD1 observed here through inhibition of binding of glucocorticoids, potent up-regulators of 11β-HSD1 gene, to their receptors. The suppressive effect of particular FAs is a function of increasing dose, degree of unsaturation and chain length [Valette *et al*., 1991]. Another possible explanation could be the indirect regulatory effect of FAs on the secretion of hormonal regulators of 11β-HSD1 gene transcription.

TABLE 1. 11β-Hydroxysteroid dehydrogenase activity in the microsomal fraction of liver and plasma corticosterone concentration in rats fed on diets containing 20% or 40% (by weight) of fat (sunflower oil S, rapeseed oil R, fish oil F or lard L) for 3 weeks.

Fat	Dose	11β-Hydroxysteroid dehydrogenase activity[1]; median	Plasma corticosterone concentration (ng/mL); mean±SE
S	20%	1.93^a	224.11 ±22.25^c
	40%	1.58^a	89.14 ±22.73^d
R	20%	0.71^b	345.37 ±10.38^b
	40%	1.81^a	111.94 ±27.71^d
F	20%	0.85^b	430.97 ±28.39^a
	40%	2.24^a	130.42 ±35.69^d
L	20%	0.43^b	291.88 ±47.56^{b,c}
	40%	2.03^a	126.07 ±31.40^d

[1][percentage of conversion of corticosterone into 11β-dehydrocorticosterone per mg microsomal fraction of liver protein determined by the method of Lowry's *et al.* [1951]]; ^{a,b,c,d} – different letters indicate significant differences between groups ($p\leq0.05$)

In addition, we cannot exclude post-transcriptional modulation of hepatic 11β-HSD1 activity in our study, since in the only, to our knowledge, respective study, the transient diminution in 11β-HSD1 activity observed in rats reared for 3 weeks on a high fat diet was not accompanied by any changes in hepatic 11β-HSD1 mRNA [Drake *et al.*, 2005].

The inverse relationship between hepatic 11β-HSD1 activity and circulating corticosterone concentration shown here seems to reinforce previous observations by Kotelevtsev *et al*. [1997] that diminished 11β-HSD1 activity and the resultant diminution in 11-dehydrocorticosterone to corticosterone conversion prevents regeneration of active glucocorticoid in the periphery and increases metabolic clearance rate for cortisol. As a consequence, the hypothalamus-pituitary--adrenal cortex axis is ativated through the negative feedback mechanism and corticosterone secretion is increased to maintain normal circulating glucocorticoid concentrations.

CONCLUSIONS

To sum up, the type 1 11β-HSD is now established as a crucial pre-receptor signalling pathway for the glucocorticoid hormone paracrine/autocrine action at peripheral tissue level including liver, where glucocorticoids act as a potent regulator of metabolic processes. As shown recently, 11β--HSD1 activity correlates with measures of adiposity and insulin resistance in humans [Lindsay *et al.*, 2003]. Therefore, the nutritional regulation of this enzyme, evidenced here in rats reared on high-fat diets, might, if confirmed in long-term experiments and replicated in human beings, have profound positive ramifications for the metabolic profile of an organism and might represent a clinically germane protective mechanism offsetting the deletorious metabolic consequences of obesity.

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WPŁYW DIET WYSOKOTŁUSZCZOWYCH O RÓŻNYM SKŁADZIE KWASÓW TŁUSZCZOWYCH NA AKTYWNOŚĆ WĄTROBOWEJ DEHYDROGENAZY 11β-HYDROKSYSTERYDOWEJ TYPU 1 U SZCZURÓW

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Badania ostatnich lat wskazują na rolę zwiększonej aktywności dehydrogenazy 11β-hydroksysterydowej typu 1 (11β-HSD1) i zwiększonego stężenia aktywnych glukokortykoidów w tkankach obwodowych, m.in. w wątrobie, w patogenezie zaburzeń metabolicznych towarzyszących otyłości. W doświadczeniu przeprowadzonym na 50 dorosłych szczurach Wistar żywionych dietami zawierającymi olej słonecznikowy, olej rzepakowy, tran lub smalec na dwóch poziomach (20 i 40% wagowo) wykazano po raz pierwszy korzystny, obniżający aktywność 11β-HSD1 w wątrobie, efekt badanych tłuszczów (z wyjątkiem oleju słonecznikowego) spożywanych w diecie zawierającej 20% tłuszczu.