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Beneficial effects versus toxicity of medium-chain triacylglycerols in rats with NASH☆

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***Background/Aims*: Replacing long-chain triacylglycerols (LCT) with medium-chain triacylglycerols (MCT) reduces alcohol-induced liver injury. Because of the similarity of the pathogenesis of alcohol-induced liver damage and non-alcoholic steatohepatitis (NASH), our aim was to assess whether MCT is also beneficial in NASH.**

***Methods*: We used a rat NASH model in which corn oil (35% of total calories) was isocalorically replaced with MCT.**

***Results*: Partial replacement of LCT did not ameliorate hepatic fat accumulation, 4-hydroxynonenal, collagen type I and its mRNA but it increased TNF-α and its mRNA ($p < 0.001$). However, in rats given the high-fat diet restricted to 2/3 of the amount they were consuming, these adverse effects decreased, with and without MCT including less liver steatosis and lower triacylglycerols, but without beneficial effects of MCT. When 70% of the fat calories were replaced with MCT with no LCT remaining in the diet, no steatosis developed and hepatic TNF-α was low. When all MCT were given with carbohydrates (instead of LCT) hepatic TNF-α also decreased ($p < 0.001$).**

***Conclusions*: MCT are not hepatotoxic, provided the diet contains no significant amount of LCT. Total replacement of dietary LCT with MCT fed *ad libitum* is beneficial whereas partial replacement becomes hepatotoxic, unless the dietary intake is restricted.**

***Keywords*: Long-chain triacylglycerols; Medium-chain triacylglycerols; Non-alcoholic steatohepatitis; TNF-α**

1. Introduction

Obesity is frequently associated with non-alcoholic fatty liver disease, including non-alcoholic steatohepatitis (NASH) [1–3]. A new experimental rat model of NASH [1] has been useful in studying pathogenetic factors of obesity and therapeutic approaches. This rat model of NASH has all the metabolic features of the clinical condition, including induction of CYP2E1 which causes oxidative stress associated with increased TNF-α and the flexibility of a liquid diet in which all components can be tested with different amounts to find the best combination and the one most applicable to a clinical setting [1]. It has also been shown that hepatic CYP2E1 is significantly increased in obese patients with type 2 diabetes [4] and in patients with NASH [5,6]. A possible mechanism for this increase could be insulin resistance and increased ketogenesis, common features of these conditions.

We showed that replacement of long-chain triacylglycerols (LCT) (42% of total dietary calories) with

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medium-chain triacylglycerols (MCT) reduced alcohol-induced liver injury [7], an effect confirmed later and attributed to improvement of lipid peroxidation [8].

MCT in amounts up to 40% of energy requirements are given to patients for prevention of obesity [9]. Since obese patients are prone to develop NASH, it would be of importance to determine whether in these subjects MCT aggravate their pathology.

Research showed that replacement of LCT with MCT leads to increased energy expenditure and satiety which lowers body weight gain [10]. Since the pathogenesis of alcohol-induced liver damage and NASH is similar [1], we used our rat model of NASH to determine whether MCT might also be beneficial in the latter condition. In fact, our aim was to define a safe therapeutic use of MCT while minimizing the possibility of its toxicity.

2. Materials and methods

2.1. Animals and diets

Forty-eight male Sprague–Dawley rats [CRL:CD (SD)], purchased from Charles River Laboratories (Wilmington, MA), were individually housed and fed *ad libitum* for 21 days three different high fat (HF) and one control diet (Fig. 1). (1) LCT ($n = 12$): a HF diet consisting mainly of LCT (purchased from Dyets Inc., Bethlehem, PA) with 70% of energy derived from fat [1]; (2) LCT + MCT ($n = 12$): a HF diet in which corn oil (35% of total calories) was isocalorically replaced with MCT purchased from Novartis (St. Paul, MN). These two diets were given either *ad libitum* or restricted to 2/3 of the quantity spontaneously consumed; (3) MCT ($n = 6$): HF diet in which LCT was totally replaced by 70% MCT; (4) MCT^{dex} ($n = 6$) control diet containing 47% carbohydrate with MCT (35% of calories) replacing LCT. All diets contained 3% safflower oil as a source of essential fatty acids. These protocols were approved by the Medical Center Institutional Animal Care and Use Committee, accredited by AAALAC.

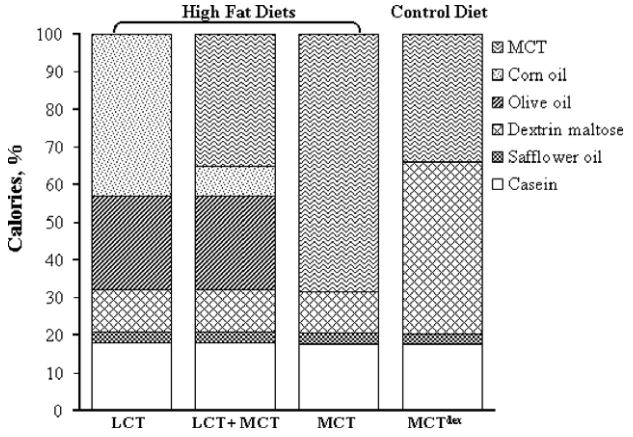

Fig. 1. Composition of the liquid diets. The LCT diet is the same HF diet previously published in the experimental rat model of NASH. When MCT was the only source of fat (diets MCT and MCT^{dex}) the LCT was isocalorically replaced by dextrin-maltose. All diets contained 3% of safflower oil as a source of essential fatty acids. All rats were fed for three weeks *ad libitum*. The LCT and LCT + MCT were also restricted to 2/3 of calories.

2.2. Procedures

The animals were anesthetized with pentobarbital. After exsanguinations blood and liver were collected. Subcellular fractions (microsomes, cytosol) were obtained as described before [11] with some modifications [12]. Other liver aliquots, including a sterile one (mRNA), were obtained and stored at −80 °C for other measurements.

2.3. Morphology and immunohistochemistry of proliferating cell nuclear antigen (PCNA)

Liver tissue was fixed in neutral-buffered formalin solution, embedded in paraffin and 4-μm thick liver sections were stained for routine morphology and immunohistochemistry with monoclonal anti-PCNA antibodies (DAKO, Carpinteria, CA) according to Isayama et al. [13]. PCNA positively stained cells were counted under the light microscope in 40 randomly selected fields at X40 objective.

2.4. Measurement of lipids and lipid peroxidation

Liver and plasma lipids (triacylglycerols) were measured according to the WAKO L Type TGH kit (Richmond, VA). Hepatic 4-hydroxynonenal (4-HNE) was determined by GC/MS spectrometry as published [14,15]. A Hewlett-Packard (Wilmington, DE) 5890 gas chromatograph and a VG TRIO-1000 (VG Masslab, Manchester, UK) mass spectrometer with negative chemical ionization technique were used. 4-HNE was derivatized to pentafluorobenzyloxime trimethylsilyl ethers and separated on a 15-m Dura Bond 5-ms column (0.25 mm inner diameter, 0.25 μm film thickness; J&W Scientific, Folsom, CA). 4-HNE was monitored at amu 152, and the internal standard, $[^2H]_3$4-HNE, at amu 155 obtained from Sigma Chemical Co. (St. Louis, MO).

2.5. Western blots for CYP2E1 protein level

To assess CYP2E1 expression microsomal proteins were subjected to sodium dodecyl sulfate–polyacrylamide 10% gel electrophoresis [16]. After transfer to a nitrocellulose membrane, CYP2E1 was revealed with rabbit polyclonal anti-hamster CYP2E1 lgG (produced in our laboratory). Microsomes with known amounts of CYP2E1 were used as control. Bands were quantified by densitometry using Evaluation Image Analysis Systems MCID (Imaging Research Inc., St. Catharines, Ontario, Canada).

2.6. TNF-α and collagen type I protein expression

Snap-frozen rat liver tissues were homogenized on ice in a lysis buffer (50 mM Tris, pH 7.6, 0.25% Triton X-100, 0.15 M NaCl, 10 mM $CaCl_2$) containing serine- and thiolprotease inhibitors (PMSF 0.1 mM, leupeptin 10 μM, pepstatin A 10 μM, aprotinin 25 μg/ml, iodoacetamide 0.1 mM). The homogenates were centrifuged at 4 °C (14,000*g*) for 30 min to remove cell debris and protein aggregates. The protein concentration was measured with the BCA protein assay kit (Pierce, Rockland, IL). Fifty microlitres of the obtained supernatant was used to determine the concentration of TNF-α by ELISA using the Quantikine Rat ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Collagen type I was measured by ELISA performed in our laboratory [17] according to a modification of Rennard et al. [18].

2.7. Extraction of mRNA

Liver total RNA was isolated by phenol chloroform extraction and further enriched using RNeasy mini columns with digestion of RNAse-free DNAse (Qiagen).