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Differential regulation of hepatic gene expression by starvation versus refeeding following a high-sucrose or high-fat diet

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Abstract

Objectives: The objective of this work was to determine the effects of starvation versus refeeding following a high-sucrose diet (HS) or high-fat diet (HF) on fatty acid metabolism in mice.

Methods: The mice were fed an AIN-76 control diet (CD), a modified HS, or an HF. The three dietary groups were subdivided into three groups each: those fed experimental diets for 12 wk, mice starved for 48 h after 12 wk on an experimental diet, and those with the same starvation treatment but with 72 h of refeeding after starvation, respectively.

Results: Serum total cholesterol levels of CD and HF groups decreased and then increased under starvation and refeeding states, respectively. Refeeding HS and HF increased serum levels of low-density lipoprotein (LDL) cholesterol compared with refeeding of the CD group. Starvation significantly increased hepatic levels of total cholesterol in the HS and HF groups compared with the CD group. Hepatic acyl coenzyme A (CoA) synthetase (ACS) levels in the CD and HS groups but not the HF group increased and then decreased under starved and refed states, respectively; an opposite regulation was observed in the HF group. Levels of hepatic acetyl-CoA carboxylase (ACC) in the HS and HF groups were significantly increased by refeeding. Hepatic levels of carnitine palmitoyltransferase-I mRNA were significantly enhanced by starvation and refeeding in the HS group but decreased in CD and then increased in the HF group.

Conclusions: Changes in dietary energy nutrients, fasting, and refeeding affect hepatic ACS, CPT-I, and ACC mRNA expression, and these results will serve to enhance our understanding of the molecular mechanisms underlying regulation of fatty acid metabolism.

Keywords: Acyl coenzyme synthetase; Acetyl coenzyme A carboxylase; Carnitine palmitoyltransferase-I; High-sucrose diet; High-fat diet

Introduction

Formation of fatty acyl coenzyme A (CoA) from fatty acid, adenosine triphosphate, and CoA catalyzed by acyl-CoA synthetase (ACS) is the first reaction in fatty acid metabolism. ACS plays a critical role in lipid synthesis of triacylglycerols (TGs), phospholipids, cholesterol esters, and β-oxidation of fatty acid. ACS, a member of the luciferase gene family [1], is located on microsomes, outer mitochondrial membranes, and peroxisomes in rat liver [2]. ACS mRNA is abundant in liver, adipose tissue, heart, and skeletal muscle [1,3]. Acetyl-CoA carboxylase (ACC), the rate-limiting enzyme in fatty acid synthesis, catalyzes the carboxylation of acetyl-CoA to malonyl-CoA. Malonyl-CoA synthesized by ACC is a critical material in the regulation of lipid metabolism in the oxidation and synthesis of fatty acids by providing activated acetyl groups for fatty acid synthesis and inhibiting carnitine palmitoyltransferase-I (CPT-I) and therefore β-oxidation [4,5]. CPT-I, which is located on the outer mitochondrial membrane, is

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the rate-limiting enzyme for fatty acid oxidation. This enzyme catalyzes the formation of acyl-carnitine from acyl-CoA, the first step of the transport of long-chain fatty acids from the cytosol into the mitochondrial matrix for β-oxidation [6,7].

Metabolic abnormalities of fatty acyl-CoA, which disturb the regulation of shuttling between oxidation and biosynthesis, and dysregulations of malonyl-CoA are linked to obesity [8]. In these specific conditions, fatty acyl-CoA is inappropriately esterified, leading to accumulation of TGs in adipose tissue, muscle, liver, and pancreas [9]. Increased TG accumulation is positively associated with insulin resistance and hyperlipidemia [9–11].

ACS, ACC, and CPT-I genes are affected by dietary states [12]. ACS1 mRNA levels in rat liver are increased by refeeding with a high-carbohydrate or high-fat diet after starvation [1], and hepatic ACS5 mRNA is also increased by refeeding a high-sucrose diet but decreased by a high-cholesterol diet. Refeeding a normal chow or high-sucrose diet increases hepatic ACS1 and ACS4 protein expression [13]. High-fat diets increase hepatic CPT-I activity and mRNA expression [14–16], but a high-carbohydrate diet decreases CPT-I expression and activity. Starvation and diabetes decrease ACC1 activity and refeeding a high-carbohydrate diet induces synthesis and activity of ACC1.

Leptin is the product of the *ob* gene [17] and regulates body fat mass through actions on food intake and energy expenditure [18]. Plasma leptin levels are decreased by fasting [19], and insulin have been demonstrated to increase leptin release from cultured adipocytes in vitro [20].

Despite crucial roles for ACS, ACC, and CPT-I in energy metabolism [21], very few studies [22,23] have demonstrated that dietary patterns simultaneously affect the regulation of ACS, ACC, and CPT-I expression or activity and thereby establish a controlled and coordinated molecular regulation of β-oxidation and lipogenesis. Thus, the aim of this experiment was to distinguish the effects of a high-sucrose diet versus a high-fat diet, fasting, and refeeding on transcription levels of hepatic ACS, ACC, and CPT-I, enzymes that play central roles in fatty acid metabolism. Further, serum levels of leptin, glucose, and insulin were measured to evaluate the effects of dietary patterns on lipid metabolism in mice.

Materials and methods

Animals and diets

Sixty-three male ICR mice, ages 4 wk, were purchased from Daehan Biolink Inc. (Eumsung, Korea). Mice were fed an AIN-76 control diet (CD) [21], a modified high-sucrose diet (HS) [22], or a high-fat diet (HF) [23] according to previous studies (Table 1). Each dietary group was divided into three subgroups: one group was fed a diet for 12 wk (F), another group was fed a diet for 12 wk followed by 48 h of starvation (S), and another group underwent 72 h of refeeding after starvation (R). Each mouse was housed in a polycarbonate cage in a temperature- and humidity-controlled (23 ± 1°C and 53 ± 2%) room. Animals were maintained on a light cycle (12-h light/12-h dark) with free access to diet and water. Food was removed from cages of fed mice 12 h and from those of starved mice 48 h before being killed to minimize the effect of food. Experimental protocols were approved by the institutional animal ethics committee and were conducted according to guidelines of the Korean Science Academy for the use and care of experimental animals.

Table 1
Composition of experimental diets

Ingredient	g/kg of diet		
	Control diet*	High-sucrose diet†	High-fat diet‡
Casein	200.0	200	200
DL-methionine	3	3	3
Cornstarch	150	30	—
Sucrose	500	663	500
Fiber	50	50	50
Corn oil	50	7	—
Beef tallow	—	—	200
AIN mineral mix	35	35	35
AIN vitamin mix	10	10	10
Choline bitartrate	2	2	2
Total	100.0	100.0	100.0

* From American Institute of Nutrition [21].
† From Park and Chyun [22].
‡ From Rim-Kim and Kang [23].

Sampling

Blood was collected by orbital venipuncture, coagulated, and centrifuged at 1100*g* for 15 min at 4°C, and serum was stored at −20°C until assayed. Livers were collected, immediately frozen in liquid nitrogen, and stored −80°C until analysis.

Analysis of lipids

TGs in liver were enzymatically measured with a commercial kit (Asan Pharmaceutical Co., Seoul, Korea). Serum total cholesterol (TC) was also assayed with a commercial kit (Asan Pharmaceutical Co.) based on the cholesterol oxidase method [24]. Serum low-density lipoprotein cholesterol (LDL-C) was calculated from serum levels of TG and TC. High-density lipoprotein cholesterol (HDL-C) levels were calculated as TC − LDL-C − (TG/5) according to Friedewald's formula [25].

Analysis of leptin, insulin, and glucose

Serum leptin was analyzed by using a mouse/rat leptin radioimmunoassay kit (Mediagnost, Aspenhaustr, Ger-