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Basic nutritional investigation

Withdrawal from high-carbohydrate, high-saturated-fat diet changes saturated fat distribution and improves hepatic low-density-lipoprotein receptor expression to ameliorate metabolic syndrome in rats

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ABSTRACT

Objective: The "lipid hypothesis" determined that saturated fatty acid (SFA) raises low-density lipoprotein cholesterol, thereby increasing the risk for metabolic syndrome (MetS). The aim of this study was to investigate the effect of subchronic withdrawal from a high-carbohydrate, high-saturated fat (HCHF) diet during MetS with reference to changes in deleterious SFA (C12:0, lauric acid; C14:0, myristic acid; C16:0, palmitic acid; C18:0, stearic acid) distribution in liver, white adipose tissue (WAT), and feces.

Methods: MetS induced by prolonged feeding of an HCHF diet in Wistar albino rat is used as a model of human MetS. The MetS-induced rats were withdrawn from the HCHF diet and changed to a basal diet for final 4 wk of the total experimental duration of 16 wk. SFA distribution in target tissues and hepatic low-density lipoprotein receptor (LDLr) expression were analyzed.

Results: Analyses of changes in SFA concentration of target tissues indicate that C16:0 and C18:0 reduced in WAT and liver after withdrawal of the HCHF diet. There was a significant ($P < 0.001$) decrease in fecal C12:0 with HCHF feeding, which significantly ($P < 0.01$) increased after withdrawal of this diet. Also, an improvement in expression of hepatic LDLr was observed after withdrawal of HCHF diet.

Conclusion: The prolonged consumption of an HCHF diet leads to increased SFA accumulation in liver and WAT, decreased SFA excretion, and reduced hepatic LDLr expression during MetS, which is prominently reversed after subchronic withdrawal of the HCHF diet. This can contribute to better understanding of the metabolic fate of dietary SFA during MetS and may apply to the potential reversal of complications by the simple approach of nutritional modification.

Introduction

Metabolic syndrome (MetS) is the clustering of interrelated risk factors that include hyperglycemia, hypertension, dyslipidemia, central adiposity, and nonalcoholic fatty liver disease [1]. The global burden of MetS and its consequences are alarmingly rising, producing enormous loss of life in both developed and developing nations [2]. However, most of these burdens are preventable as they are primarily due to suboptimal lifestyle, including excessive calorie intake and an unbalanced diet [3]. Hence, it is of major public health importance to identify and target dietary interventions that can contribute to the prevention of MetS.

A significant role has been attributed to the quality, rather than quantity of dietary fat in the development and progression of cardiometabolic complications [4]. Studies report that MetS-associated lipid and lipoprotein metabolism abnormalities are

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inherent to lipogenesis and lipolysis in the liver and white adipose tissue (WAT) and this can be attributed to the amount and quality of dietary fat [5]. Also, it is accepted that dietary saturated fatty acids (SFAs) are detrimental to health [6] and a high intake of SFA, specifically lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0), is positively associated with increased levels of blood cholesterol and high cardiovascular mortality rate [7,8]. This increase in cholesterol level has been attributed to a decrease in low-density lipoprotein receptor (LDLr) activity [9].

Although studies indicate the effect of various fatty acids (FAs) on disease conditions of MetS; its effect on tissue SFA distribution with a dietary modification by withdrawal from a high-carbohydrate, high-saturated fat (HCHF) diet and replacement with a standard diet still demands investigation. We recently demonstrated that the replacement of an HCHF diet with a standard rodent diet initiates stages of natural recovery in the pathophysiology of target organs during the pathogenesis of MetS in HCHF diet–induced MetS in rats, the same model used in the present study [10]. In view of this, it has been hypothesized in this study that the same dietary intervention may induce beneficial changes on the complications of MetS in terms of tissue SFA distribution. Hence, the main focus of this study was to determine the responses in tissue SFA profiles of MetS-induced rats after a dietary intervention that replaced an HCHF diet with a standard rodent diet. To determine the effect of this dietary intervention, we investigated SFA profiles in WAT and liver with special reference to the highly deleterious SFAs, C12:0, C14:0, C16:0, and C18:0 and analyzed hepatic LDLr expression. Also, an attempt was made to investigate the fecal SFA profile to analyze the changes in metabolic fate of dietary SFA during this dietary intervention.

Methods

Animals and diets

The Institutional Animal Ethics Committee approved all experimental protocols (1706/GO/C/13/CPCSEA). MetS was induced in rats by feeding them an HCHF diet, following a previously described method [10]. Male Wistar rats (9 to 10 wk old, weighing 100–130 g; N = 24) were obtained from the animal housing facility of Institute of Advanced Study in Science and Technology. The rats were randomly divided into three experimental groups (n = 8/group): basal diet (B), HCHF diet (H), and HCHF-to-basal diet (H-to-B). Groups B and H continued on their respective diets for 16 wk; rats in group H-to-B received an HCHF diet for 12 wk and then were switched to a basal diet for an additional 4 wk (i.e., weeks 13–16). The HCHF diet consisted of fructose, sweetened condensed milk, mutton fat (MF), powdered rodent feed, Hubble Mendel and Wakeman salt mixture, and water. HCHF diet–fed animals were supplemented with 25% fructose in drinking water [10], whereas rats in group B were given normal drinking water. The basal diet contained standard rodent feed obtained from Nutrilab, Kolkata, India. The dietary components and SFA composition of group specific diets are described in Table 1. Animals were housed in individual cages in an ambient temperature of 27 ± 3°C and relative humidity 50 ± 5% with a 12-h light/dark cycle. All animals were given ad libitum access to food and water. Animals were sacrificed at the end of week 16, and blood, tissue, and fecal samples were collected and appropriately stored for later analysis. This study was done together with our previous study on the pathophysiology of MetS to allow future interstudy comparisons and therefore shares the same data for body weight, fasting blood glucose (FBG), and lipid profile [10].

Physiological parameters

Food and water intake was monitored daily and body weights measured weekly. Body length (nose to anus) was measured every fourth week using a standard measuring tape. Body mass index (BMI) was calculated as body weight (g)/[body length (cm)]^2. Feed efficiency was calculated as mean body weight gain (g)/daily energy intake (kJ) [11]. FBG was measured every week in tail vein blood by using glucometer (Accu-Check Active Roche, Germany).

Table 1
Dietary composition and fatty acid profile of HCHF and basal diets

Constituent (g/kg)	Diets	
	Basal	HCHF
Powdered rat feed*	1000	155
Mutton lard	–	200
Fructose	–	175
Sweetened condensed milk	–	395
Hubble, Mendel and Wakeman salt mixture	–	25
Water	–	50
Gross energy (kJ/g)	16.47	20.72
Saturated fatty acid, g/100 g of total recovered fatty acid (n = 3)		
C12:0	0.00 ± 0.000	0.00 ± 0.000
C14:0	0.11 ± 0.002	0.07 ± 0.002
C16:0	0.23 ± 0.001	20.9 ± 0.17
C18:0	0.00 ± 0.000	19.6 ± 0.88

HCHF, high-carbohydrate, high-saturated fat
For the dietary fatty acid compositions, each value is a mean ± SEM. Number of repetitive experiments are indicated within parenthesis
* Meat-free rat feed (Nutrilab) contains the following in (percentage per 100 g of diet): nitrogen-free extract (consisting of carbohydrates, sugars, and starches) 51.65, crude protein 21.36, crude fat 10.63, total ash 7.41, moisture 6.32, crude fiber 2.63, calcium 1.75, phosphorous 1.1, water 0.23.

Metabolic parameters

Activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and concentrations of total cholesterol (TC), triacylglycerols (TG), and high-density lipoprotein cholesterol (HDL-C) were determined at week 16. All measurements were done in serum using enzymatic kits (Accurex Biomedical Pvt. Ltd., Thane, India). Low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) were calculated using Friedwald's formula [12].

Fatty acid analysis

The extraction of lipids from diet and biological samples was done by following the method of manual solvent extraction using 2:1 chloroform/methanol mixture with 0.1% butylated hydroxytoluene as an antioxidant. One gram of WAT, liver, and feces were separately macerated in 20 mL of chloroform/methanol solvent. This was mixed in a shaker for 40 min and then centrifuged at 2500*g* for 5 min. The extracting solvent was pooled by repeating the extraction procedure twice and subsequently washed with double-distilled water to remove all polar materials. Chloroform was evaporated in a rotary evaporator and samples obtained were esterified to form FA methyl esters (FAME) by following ISO boron trifluoride method with slight modification [13]. Methanol was first added to break down TGs into FA and convert it to FAMEs through acid-based catalyzed transesterification in the presence of methanol and boron trifluoride. One mL of sample and 0.5 M methanolic sodium hydroxide were added to a GC vial and heated in an 80°C water bath for 15 min until the fat droplets reacted with the reagents and disappeared. Subsequently, 1 mL of boron fluoride in methanol (10% w/w) was added to the mixture and heated for an additional 15 min at 80°C. Liquid–liquid extraction was performed by the addition of 2 mL of saturated sodium chloride solution and 1 mL of hexane to extract the FAMEs. Two clear and distinctive layers were observed. Of these, the top hexane layer was used for gas chromatography mass spectrophotometer (GC/MS) analysis [14]. FAMEs were analyzed on a fused-silica capillary column (30 m × 0.25 × 0.25 μm) by a GC2010 system coupled with a QP2010 mass spectrophotometer detector (Shimadzu, Kyoto, Japan). The injection and the detector temperatures were set at 280°C and 320°C, respectively. The column temperature was set at 40°C for 2 min and raised at 6°C/min to 320°C. Carrier gas (helium) was passed at 49 kPa, with a constant linear velocity of 15 cm/s. A sample of 1 μL was injected with a split ratio of 50. The total program time was 49.67 min. Quantization and identification of the FA in all samples was based on a linear calibration equation obtained from individual FA standards for C12:0, C14:0, C16:0, and C18:0 purchased from Sigma.

Protein extraction and immunoblotting

Total protein was extracted from liver tissue using lysis buffer (20 mM Tris-HCl, pH 7.5) with 1 mM phenylmethanesulfonyl fluoride and 1% protease inhibitor cocktail (Sigma, St. Louis, MO, USA). Lysates were homogenized with a 23-gauge syringe needle. Protein concentration was measured, and 40 μg of