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Basic nutritional investigation

Refeeding with glucose rather than fructose elicits greater hepatic inflammatory gene expression in mice

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ABSTRACT

Objective: We previously reported that refeeding after a 48-h fast, used as a study model of starvation and refeeding, promotes acute liver inflammatory gene expression, which is at least partly mediated by toll-like receptor 2 (TLR2). We also previously demonstrated that dietary carbohydrates play critical roles in this process. The aim of this study was to compare the outcomes of refeeding with different carbohydrate sources.

Methods: Mice were fasted for 46 h and then refed with 1.5% (w/w) agar gel containing 19% carbohydrate (sources: α-cornstarch, glucose, sucrose, or fructose). The liver expression of inflammatory and other specific genes was then sequentially measured for the first 14 h after refeeding initiation.

Results: Fasting for 46 h up-regulated the liver expression of endogenous ligands for TLRs (*HspA5*, *Hsp90 aa1*, and *Hspd1*). Refeeding with agar gel containing α-cornstarch or glucose increased the liver expression of *Tlr2*, proinflammatory genes (*Cxcl2*, *Cxcl10*, *Cxcl1*, *Nfkb1*, *Nfkb2*, *RelB*, *Sectm1α*, *Il1β*), stress response genes (*Atf3*, *Asns*, *Gadd45 a*, *Perk*, *Inhbe*), detoxification genes (*Hmox1*, *Gsta1*, *Abca8b*), genes involved in tissue regeneration (*Gdf15*, *Krt23*, *Myc*, *Tnfrsf12a*, *Mthfd2*), and genes involved in tumor suppression (*p53*, *Txnrd1*, *Btg2*). This refeeding also moderately but significantly elevated the serum levels of alanine aminotransferase. These effects were attenuated in mice refed with agar gel containing sucrose or fructose.

Conclusion: Dietary glucose, rather than fructose, plays a critical role in refeeding-induced acute liver inflammatory gene expression and moderate hepatocyte destruction. Further studies are recommended regarding the role of these effects in liver inflammation and, consequently, liver dysfunction.

Introduction

Acute starvation and refeeding is an important health problem that often occurs during natural disasters or wars. A previous study demonstrated that refeeding with a standard diet after a 48-h fast, a study model of acute starvation and refeeding, induces acute liver inflammatory gene expression, which is at least partly mediated by toll-like receptor 2 (TLR2), and that dietary carbohydrates play critical roles in this process [1]. Furthermore, it was previously reported that refeeding after a 48-h fast modestly but significantly elevates the serum levels of alanine aminotransferase (ALT, an index of hepatocyte destruction) [1]. Numerous studies have indicated that inflammatory responses are strongly implicated in the development and progression of multiple diseases [2–4]. Additionally, several epidemiologic studies have reported that an increase in serum ALT levels is associated with the long-term development of multiple metabolic disorders and that this also applied to

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individuals with elevated serum ALT levels within the normal range [5,6].

In the past few decades, fructose ingestion has dramatically increased due to higher worldwide consumption of sugars [7]. It has been found that fructose is a highly lipogenic carbohydrate and that long-term high-fructose diets induce chronic low-grade inflammation, dyslipidemia, insulin resistance, and hypertension [8]. Thus, dietary fructose has received increasing attention as a critical mediator of several diseases. This raises an interesting question regarding the effects of different types of carbohydrate sources on refeeding-induced inflammation. In the present study, we investigated the effects of refeeding experimental mice four different carbohydrate sources (α-cornstarch, glucose, sucrose, or fructose) on the liver expression of genes encoding endogenous ligands for TLRs and on genes involved in pro- and anti-inflammatory responses, stress responses, detoxification, tissue regeneration and carcinogenesis, and tumor suppression. We also compared the serum ALT levels in these mice.

Materials and methods

Diets

Casein, α-cornstarch, sucrose, cellulose powder, AIN-76 mineral mixture [9], AIN-76 vitamin mixture [9], and choline bitartrate were purchased from Oriental Yeast (Tokyo, Japan). DL-methionine, D-glucose, D-fructose, and agar were obtained from Wako Pure Chemical Ind. (Osaka, Japan). Soybean oil was supplied by Nisshin Oillio (Tokyo, Japan). Purified powder standard diet for acclimatization was prepared in our laboratory using food-grade ingredients. This standard diet consisted of 60.075% α-cornstarch, 10% sucrose, 15% casein, 0.225% DL-methionine, 5% soybean oil, 1% AIN-76 vitamin mixture, 3.5% AIN-76 mineral mixture, 0.2% choline bitartate, and 5% cellulose (w/w). Test diets (agar gel containing one of four different carbohydrate sources) were prepared by mixing 23.4 g of the test carbohydrate sources (α-cornstarch, glucose, sucrose, or fructose) with 100 mL of boiling water containing 1.5% (w/w) agar. After cooling, the test agar gel diets were cut into small pieces, and stored at 4°C until use.

Animals and experimental design

Specific pathogen-free, 6-wk-old female BALB/c mice were obtained from Charles River Japan (Atsugi, Japan) and maintained on a purified powder standard diet with water ad libitum. To familiarize the mice with an agar gel diet, the purified powder standard diet was used initially (prepared by mixing 15 g of purified powder standard diet and 100 mL boiling water containing 1.5% agar). This diet was given four times (each for 6 h) during the acclimatization period of 10 d (Fig. 1). After this, the mice were deprived of food for 46 h but were allowed free access to water and then immediately administered the test agar gel diet containing one of four test carbohydrate sources ad libitum for 14 h. The animals were sacrificed at 0, 3, 5, 8, 11, and 14 h after refeeding had commenced, and their blood and livers were harvested. Mice that were fasted for 46 h and then refed with galactose showed a significantly smaller food intake than mice that had been refed with other carbohydrate sources. These galactose-refed animals were thus excluded from our analysis.

All animal housing, handling, and sample collection procedures conformed to the policies and recommendations of the Laboratory Animal Care Advisory Committee of Chiba University (Chiba, Japan).

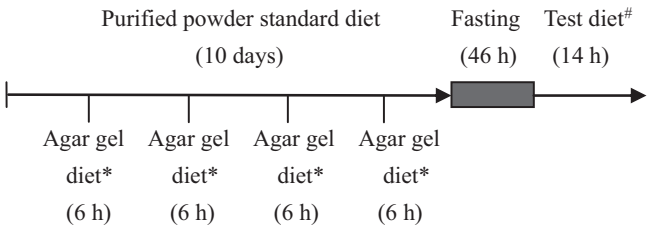

Fig. 1. The feeding and fasting protocol. *Agar gel containing the purified powder standard diet. #Agar gel containing one of four different carbohydrate sources.

Measurement of glucose, insulin, nonesterified fatty acid, and triacylglycerol levels and ALT and aspartate aminotransferase activities in the serum

Blood was collected from the mice and allowed to clot for 1 h at room temperature. Serum was then separated by centrifugation at 1500*g* for 20 min at 4°C and stored at −80°C until use. The serum glucose, triacylglycerol (TG), nonesterified fatty acid (NEFA), ALT and aspartate aminotransferase (AST) levels were measured using commercially available kits (i.e., glucose CII-test, TG E-test, NEFA C and transaminase CII-test [Wako Pure Chemical Ind.]). The serum insulin levels were measured using an ultra-sensitive mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Tokyo, Japan). All assays were performed in duplicate, and the data averages were statistically analyzed.

RNA extraction and quantitative real-time RT-PCR

Total RNA was isolated from mouse livers using an RNeasy extraction kit (Qiagen, Santa Clarita, CA), and quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR) was performed with an ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) as described previously [10]. Primer sequences are listed in Supplementary Table 1.

Interleukin-1β and tumor necrosis factor-α assay

Mouse livers were homogenized in ice-cold phosphate-buffered saline (10%, v/v) with a glass handheld homogenizer and centrifuged at 6000*g* for 30 min at 4°C to pellet the debris. Interleukin (IL)-1β and tumor necrosis factor (TNF)-α levels were determined in sample supernatants using an ELISA kit (R&D systems, Minneapolis, MN, USA).

Statistical analysis

Data are presented as the mean ± SD of representative measurements. Statistical comparisons between measurements taken 46 h before (prefasting, normal levels) and at 0, 3, 5, 8, 11, or 14 h after commencement of refeeding were made using unpaired Student's *t* tests. Differences in mice refed on different carbohydrate sources were analyzed using one-way analysis of variance and the Tukey's b test for post hoc analysis. Differences were considered significant at $P < 0.05$. All statistical analyses were performed using SPSS software (SPSS Inc., Tokyo, Japan).

Results

Metabolic parameters in mice fasted for 46 h and refed using different carbohydrate sources

No significant differences were observed in the food intake among the four test groups (Fig. 2A). Fasting for 46 h resulted in a decrease in body weight from 21.37 ± 0.87 g (mean ± SD; before fasting; n = 16 per group) to 18.31 ± 0.82 g. The liver weights recovered to normal prefasting levels by 5 h after refeeding in all four test groups (Fig. 2B). In the α-cornstarch- or glucose-refed groups, serum glucose and insulin levels rapidly and markedly increased over the normal prefasting levels (Fig. 2C, D). Increases in serum glucose levels were significantly attenuated, and serum insulin levels were not elevated in the sucrose- or fructose-refed groups. The TG and NEFA serum levels were lower than or the same as the normal prefasting levels during the 14-h period after the initiation of refeeding in all four test groups (Fig. 2E, F).

Effects of refeeding with different carbohydrate sources on the hepatic mRNA expression of pro- and anti-inflammatory genes

The liver *Tlr2* expression levels significantly increased over the normal prefasting levels in the α-cornstarch- and glucose-refed groups and in the sucrose-refed group at 5 to 8 h and 14 h after refeeding, respectively (Fig. 3A). These increases were not observed in the fructose-refed groups. The liver *Tlr4* expression levels decreased below the normal prefasting levels in all four test groups (Fig. 3B). *Cxcl2*, *Cxcl10.* and *Cxcl1* are target genes of the transcription factor nuclear factor (NF)-κB and are