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Journal of Nutritional Biochemistry 24 (2013) 1314–1323

Journal of Nutritional Biochemistry

Refeeding with a standard diet after a 48-h fast elicits an inflammatory response in the mouse liver☆

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Received 11 May 2012; received in revised form 21 September 2012; accepted 15 October 2012

Abstract

Unhealthy eating behaviors increase the risk of metabolic diseases, but the underlying mechanisms are not fully elucidated. Because inflammation contributes to the pathogenesis of metabolic diseases, it is important to understand the effects of unhealthy eating on the inflammatory state. The objective of our present study was to address the effects of a fasting–refeeding regime, a model of irregular eating, on the hepatic inflammatory responses in mouse. The animals were fasted for 48 h and then refed either a standard or low-carbohydrate/high-fat diet. Inflammatory gene expression in the liver was then sequentially measured for the first 17 h after initiation of refeeding. To assess the roles of dietary carbohydrates and toll-like receptor 2 (TLR2) in the refeeding-induced inflammatory changes, gene expression levels in mice refed only carbohydrates (α-corn starch and sucrose) at different doses and in TLR2-deficient mice refed a standard diet were also analyzed. Refeeding with a standard diet increased the liver expression of *Tlr2*, proinflammatory mediators (*Cxcl10*, *Cxcl1*, *Cxcl2*, *Icam-1*) and negative regulators of TLR-signaling (*A20* and *Atf3*). These increases were attenuated in mice refed a low-carbohydrate/high-fat diet. Refeeding only α-corn starch and sucrose also increased the expression of these inflammatory pathway genes depending on the doses. TLR2 deficiency significantly attenuated the refeeding-induced increase in the liver expression of *Cxcl10*, *Cxcl1*, *Icam-1* and *A20*. These findings suggest that an irregular eating behavior can elicit a liver inflammatory response, which is at least partly mediated by TLR2, and that dietary carbohydrates play critical roles in this process.

Keywords: Carbohydrate; Endogenous ligand; Fasting; Inflammatory mediator; Irregular eating; Refeeding; Toll-like receptor 2

1. Introduction

Considerable evidence has accumulated to indicate that unhealthy eating behaviors including overeating, skipping meals and picky eating increase the risk of cardiovascular and metabolic diseases such as atherosclerosis and type 2 diabetes [1,2]. However, the underlying mechanism of action is not fully understood. Although inflammation is important for the innate and adaptive immune systems, inappropriate inflammatory responses are strongly implicated in the development and progression of these diseases [3–5]. Hence, elucidating the effects of unhealthy eating behaviors on the inflammatory state will be an important aspect to clarify their pathogenic mechanisms.

Table 1
Composition of test diets

	Standard diet (g/kg)	Low-carbohydrate/ high-fat diet (g/kg)	100%-carbohydrate diet (g/kg carbohydrate source)[a]
α-Corn starch	600.75	380.75	875
Sucrose	100	100	125
Casein	150	150	0
DL-Methionine	2.25	2.25	0
Soybean oil	50	80	0
Palm oil	0	80	0
Mineral mixture[b]	35	35	0
Vitamin mixture[b]	10	10	0
Choline bitartrate	2	2	0
Cellulose	50	160	0

[a] This diet was prepared by mixing α-corn starch and sucrose in a 7:1 ratio with boiling water.
[b] AIN-76 mineral and vitamin mixtures [20].

Abbreviations: A20, tumor necrosis factor, alpha-induced protein 3; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Atf3, activating transcription factor 3; Cxcl, chemokine (C-X-C motif) ligand; Gapdh, glyceraldehydes-3-phosphate dehydrogenase; Glut2, glucose transporter 2; Grp94, glucose-regulated protein 94; Hmgb1, high mobility group box 1; Hspd1, heat shock protein 1; Icam-1, intercellular adhesion molecule-1; NF-κB, nuclear factor κB; TLR, toll-like receptor; TNF-α, tumor necrosis factor-α.

☆ This study was supported in part by a Grant-in Aid for Science Research (no. 21580135) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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http://dx.doi.org/10.1016/j.jnutbio.2012.10.006

Table 2
Primer sequences (5'→3')

Gene	Acc. number	Forward	Reverse
GAPDH	M32599	TGCACCACCAACTGCTTAG	GGATGCAGGGATGATGTTC
Glut2	M23383	ACGGTCTTCACGTTGGTCTC	GAAGGCCACAAAGCCAAATA
Tlr2	NM_011905	TGGCTCAAATCCTGGTTGAC	GAGAAGGGCACAGCAGACTC
Tlr4	NM_021297	AACATGGCAGTTTCTGAGCA	GAGGATTGTCCTCCCATTCC
Hspd1	BC018545	GAACTGCCTTACTGGATGCTG	ACATGCCGCCTCCCATAC
Grp94	BC010445	GAGGGTCCTGTGGGTGTTG	CTGTCCTTGAGCCTTCTCG
Hmgb1	BC111468	GGCTGACAAGGCTCGTTATG	CCAATGGATAAGCCAGGATG
Cxcl1	BC132502	GCCTATCGCCAATGAGCTG	GAACCAAGGGAGCTTCAGG
Cxcl2	BC119512	AGTGAACTGCGCTGTCAATG	GCCCTTGAGAGTGGCTATGA
Cxcl10	NM_021274	GGTCTGAGTGGGACTCAAGG	GTGGCAATGATCTCAACACG
Icam-1	NM_010493	GTGATCCCTGGGCCTGGTG	GGAAACGAATACGCGGTGATGG
Tnf-α	BC137720	ACGTGGAACTGGCAGAAGAG	GAGGCCATTTGGGAACTTCT
A20	BC060221	ATGGAGTGCCAGCACCTAAG	GCCAAAGTGATCACAAGCAG
Atf3	BC019946	GCCAGGTCTCTGCCTCAG	GGGTCTGTTGTTGACGGTAA

Toll-like receptors (TLRs) are one of the key pattern recognition receptor families and elicit signaling pathways that regulate inflammatory responses [6]. It has been demonstrated that endogenous ligands, as well as the pathogen-associated molecular pattern of invading microbes, activate the TLR-mediated signaling pathway and elicit consequent inflammatory responses [7,8]. Recent studies have shown that interaction between TLRs and their endogenous ligands contributes to the pathogenesis of insulin resistance, diabetes and atherosclerosis via the regulation of inflammatory responses [9–11]. Expression of TLR2 and TLR4 and their endogenous ligands, such as heat shock protein (HSP) 60, HSP70 and high mobility group box 1 (HMGB1), was elevated in type 1 and type 2 diabetic subjects [12,13]. TLR2 deficiency resulted in attenuation of proinflammatory state of diabetes and amelioration of insulin resistance induced by a high-fat diet [14–16].

In our present study, we investigated the effects of refeeding a standard or a low-carbohydrate/high-fat diet on the liver expression of the TLRs, their endogenous ligands, proinflammatory mediators which are synthesized via TLR signaling pathways [11,17–19] and negative regulators of inflammatory responses. In addition, we examined the inflammatory effects of refeeding using a diet with a 100% carbohydrate energy source to obtain a clearer understanding of the role of carbohydrates in this phenomenon. Furthermore, we compared the extent of liver inflammatory responses in wild-type and TLR2-deficient mice refed the standard diet to investigate the importance of TLR2 in the mechanisms underlying refeeding-induced inflammation.

2. Materials and methods

2.1. Diets

Casein, α-corn starch, sucrose, cellulose powder, AIN-76 mineral mixture [20], AIN-76 vitamin mixture [20] and choline bitartrate were purchased from Oriental Yeast (Tokyo, Japan). DL-Methionine was obtained from Wako Pure Chemical Ind. (Osaka, Japan). Soybean oil was supplied by Nisshin Oillio (Tokyo, Japan), and palm oil was supplied by NOF (Tokyo, Japan). Purified rodent powder diets were prepared in our laboratory using food-grade ingredients. The composition of these test diets is shown in Table 1. The two dietary groups examined in the current study could be differentiated by their α-corn starch weight percentages, i.e., 60% (standard diet) or 38% (low-carbohydrate/high-fat diet). α-Corn starch was exchanged isoenergetically with fat.

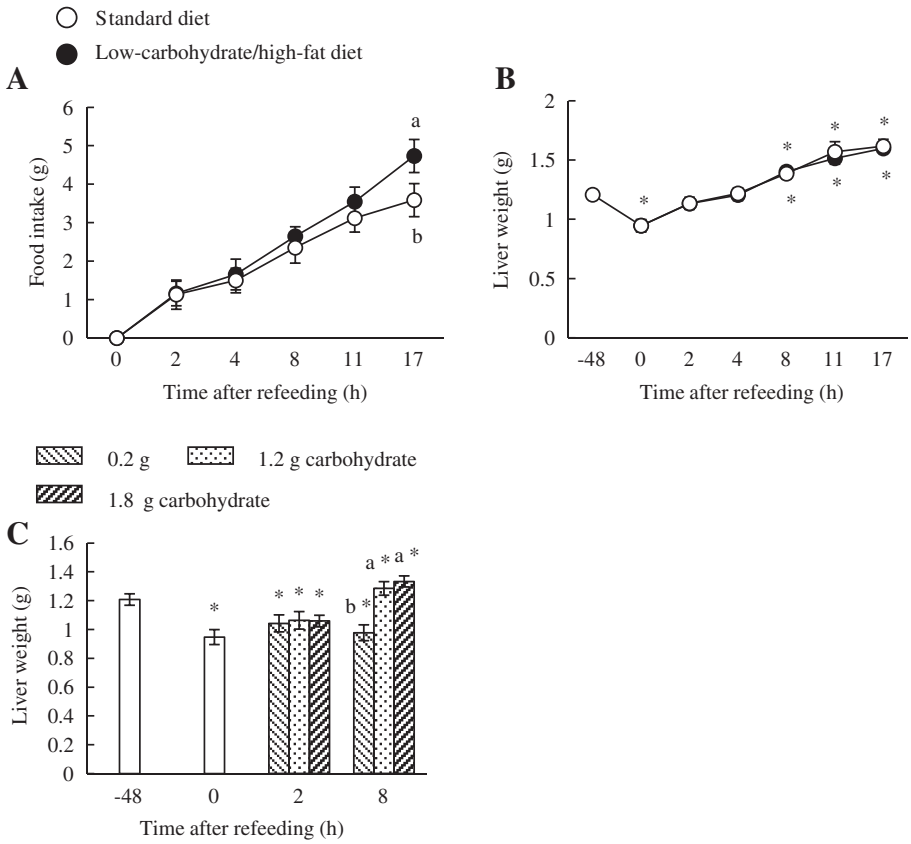

Fig. 1. (A) Food intake and (B) liver weights in mice fasted for 48 h and refed a standard or low-carbohydrate/high-fat diet. (C) Liver weights in mice fasted for 48 h and refed with a 100% carbohydrate energy diet at one of three dosages (0.2, 1.2 or 1.8 g carbohydrate per mouse). The values represent the mean±S.D. (n=5–7 per test group at each time point). −-48 h, prefasting; 0 h, end of fasting for 48 h (start of refeeding). [a,b]Mean values with different superscripts are significantly different for the same time point (P<.05). *Mean values were significantly different from the normal (prefasting) levels (P<.05).