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Long-term intake of a high prebiotic fiber diet but not high protein reduces metabolic risk after a high fat challenge and uniquely alters gut microbiota and hepatic gene expression☆

Dolan C. Saha[a], *Raylene A. Reimer*[a,b,*]

[a] *Faculty of Kinesiology, University of Calgary, Calgary, AB, Canada T2N 1N4*
[b] *Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada T2N 4N1*

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ABSTRACT

A mismatch between early developmental diet and adulthood may increase obesity risk. Our objective was to determine the effects of re-matching rats to their weaning diets high in protein or fiber after transient high-fat/high-sucrose challenge in adulthood. We hypothesize that a long-term high fiber diet will be associated with a gut microbiota and hepatic gene expression reflective of reduced adiposity. Wistar rat pups were fed a control (C), high prebiotic fiber (HF), or high protein (HP) diet from 3–15 weeks of age; a high-fat/high-sucrose diet from 15–21 weeks; their respective C, HF, or HP diets from 21–25 weeks. Gut microbiota of cecal contents and hepatic gene expression were measured when rats were terminated at 25 weeks of age. HF rats had higher total bacteria, bifidobacteria and *Bacteroides/Prevotella* spp than C and HP at 25 weeks ($P < 0.05$). Firmicutes, especially *Clostridium leptum*, decreased in HF compared to C and HP ($P < .05$). The ratio of Firmicutes:Bacteroidetes was markedly lower in HF versus C and HP at 25 weeks ($P < .05$). HF decreased hepatic cholesterol content compared to HP and C at 25 weeks. HF and HP increased 3-hydroxy-3-methylglutaryl-CoA reductase mRNA and decreased lecithin-cholesterol acyltransferase mRNA compared to C ($P < .05$). In conclusion, re-matching rats to a HF but not HP diet attenuated the typical increase in Firmicutes:Bacteroidetes ratio associated with consumption of a high fat diet. Lower hepatic cholesterol with long-term HF diet intake may be related to alterations in gut microbiota and hepatic lipid metabolism.

1. Introduction

The rising rates of obesity worldwide are precipitating an increase in associated adverse metabolic diseases, particularly cardiovascular diseases and type 2 diabetes [1,2]. Large variations in diet composition between critical stages of development and adulthood may be one contributing factor to the increased prevalence of obesity [3,4]. It has been

Abbreviations: AMPK, AMP-activated protein kinase; C, control; CYP, cholesterol 7-α-hydroxylase; DXA, dual energy x-ray absorptiometry; FAS, fatty acid synthase; HF, high prebiotic fiber; HFHS, high fat/high sucrose; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HP, high protein; qPCR, quantitative polymerase chain reaction; SREBP-1c, sterol regulatory element-binding protein 1c.

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* *Corresponding author.* Faculties of Kinesiology and Medicine, University of Calgary, Calgary, AB, Canada, T2N 1N4. Tel.: +1 403 220 8218; fax: +1 403 284 3553.

E-mail addresses: dcsaha@ucalgary.ca (D.C. Saha), reimer@ucalgary.ca (R.A. Reimer).

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suggested that the greater the divergence in the environmental signals between early developmental and later phases of life, the higher the risk of metabolic perturbations in adulthood [5]. Whether or not reducing the divergence in nutritional signals between early and later life could minimize adverse metabolic outcomes, particularly in response to a transient high fat diet, warrants further investigation.

Studies have shown that exposure to a maternal high protein diet during both pregnancy and lactation results in early changes in gene expression involved in glucose and lipid metabolism in offspring [6] and increased adiposity following a high fat diet in adulthood [7]. A high-protein/low carbohydrate diet fed to dams during lactation but not during pregnancy also predisposes offspring to higher obesity risk [8]. Similar to exposure to a maternal high protein diet, we have also shown that consumption of a high protein diet directly by offspring during early postnatal life predisposes rats to increased fat mass and hyperglycemia when exposed to a high fat challenge in adulthood [9]. However, when rats were rematched to a high protein diet following the high fat diet, some of the negative effects on adiposity and glucose tolerance were attenuated [10]. While the glycemic response and percent body fat of the high protein fed rats reverted to levels seen in control rats, these responses were still significantly higher than that seen in rats rematched to a high prebiotic fiber diet [10], which appeared to provide the greatest protection from dietary divergence.

The factors responsible for the observed protective versus detrimental effects of long-term diets high in fiber or protein are not well understood but may include the gut microbiota which is known to change in response to diet [11]. The intestinal microbiota appears to be one factor contributing to the development of obesity [12] and there is growing interest in identifying dietary strategies that shift the microbiome towards a lean profile. Prebiotic fiber, in addition to effects on satiety hormones and adiposity, has been shown to improve gut microbiota profiles [13]. Fermentation of prebiotic fiber by gut microbiota produces short-chain fatty acids (SCFA) [14], which in turn affects hepatic cholesterol and fatty acid metabolism [15–17]. Given that many of the actions of prebiotic fiber and its fermentative end products target metabolism in the liver, it is plausible that this may be one site mediating the protective effect we have previously demonstrated in rats consuming prebiotic fiber early in life [9,10].

Our objective, therefore, was to examine the total cumulative change in gut microbiota and hepatic gene expression that occurs in response to a temporary high energy diet challenge in the context of long-term diets high in protein or fiber. It was hypothesized that a long-term high fiber diet would be associated with a gut microbiota and hepatic gene expression profile reflective of reduced adiposity. This study is unique in that it examines the impact of transient dietary divergence in early adulthood on obesity risk and its effect on overall metabolic health involving a combination of factors influencing changes in hepatic cholesterol metabolism, body weight and gut microbiota.

2. Methods and materials

2.1. Animals and diet treatments

Ethical approval for the study was granted by the University of Calgary Health Sciences Animal Care Committee. All procedures followed the Guide for Care and Use of Laboratory Animals. Rats had free access to food and water at all times and were kept on a 12-hour light–dark cycle. Male and female Wistar rats were obtained from Charles River (Montreal, PQ, Canada) and mated in wire-bottomed cages. On the day a copulation plug was found, the dams were isolated and given free access to control diet. At birth, litters were culled to 10 pups of equal sex where possible. At 21 days of age, pups were weaned onto one of 3 experimental diets: control (C), high fiber (HF, 21% wt/wt) or high protein (HP, 40% wt/wt). Detailed experimental diet composition is provided in Table 1. The HF diet used a 1:1 ratio of the prebiotic fibers inulin (Orafti HP) and oligofructose (Orafti® P95, Beneo GmbH, Mannheim, Germany). The rats consumed the experimental diets until 15 weeks of age when they were switched to an ad libitum high fat/high sucrose (HFHS) diet for 6 weeks to perturb metabolism. The HFHS diet provided 40% of energy from fat and 45% from sucrose and contained (g/100 g): casein (20.0), sucrose (49.9), soybean oil (10.0), lard (10.0), Alphacel (5.0), AIN-93 M mineral mix (3.5), AIN-93 vitamin mix (1.0), DL-methionine (0.3), choline bitartrate (0.25) (Dyets Inc, Bethlehem, PA, USA). After 6 weeks of HFHS feeding, the rats reverted back to their respective C, HF, or HP diet for an additional 4 weeks to represent a rematched dietary phase. Body weight was measured weekly throughout the experiment and has been previously published [10]. After the end of the rematching period at 25 weeks of age, the rats were lightly anaesthetized with isoflurane and body composition was measured using dual energy x-ray absorptiometry (DXA) with software for small animal analysis (Hologic QDR 4500, Hologic, Inc, Bedford, MA).

Table 1 – Experimental diet composition

Composition (g/kg)	Control [a]	High protein	High fiber
Cornstarch	397.5	197.5	262
Casein	200	400	173
Dextrinized cornstarch	132	132	114
Sucrose	100	100	87
Soybean oil	70	70	61
Alphacel	50	50	43
AIN-93-MX	35	35	30
AIN-93-VX	10	10	9
L-Cystine	3	3	2.8
Choline bitartrate	2.5	2.5	2.2
Inulin/oligofructose [b]	0	0	216

[a] Based on AIN-93G purified diet. The control and high protein diets provide 3.76 kcal/g and the high fiber diet provides 3.30 kcal/g due to the reduced energy content of the inulin and oligofructose.
[b] Inulin supplied as Orafti HP and oligofructose as Orafti P95 in a 1:1 blend by weight (Beneo GmbH).