Effects of dietary fibres on weight gain, carbohydrate metabolism, and gastric ghrelin gene expression in mice fed a high-fat diet

Zhong Q. Wang, Aamir R. Zuberi, Xian H. Zhang, Jacalyn Macgowan, Jianhua Qin, Xin Ye, Leslie Son, Qinglin Wu, Kun Lian, William T. Cefalu

Abstract

Dietary fibres, such as sugarcane fibre (SCF), psyllium (PSY), and cellulose (CEL), have been shown to have substantial health benefits. We sought to compare the metabolic effects of these dietary fibres—SCF, PSY, and CEL—on body weight, carbohydrate metabolism, and stomach ghrelin gene expression in a high-fat diet–fed mouse model. Thirty-six male mice (C57BL/6) were randomly divided into 4 groups that consumed high-fat diet alone (HFD) or high-fat diet containing 10% SCF, PSY, and CEL, respectively. After baseline measurements were assessed for body weight, plasma insulin, glucose, leptin, and glucagon-like peptide 1 (GLP-1), animals were treated for 12 weeks. Parameters were reevaluated at the end of study. Whereas there was no difference at the baseline, body weight gains in the PSY and SCF groups were significantly lower than in the CEL group at the end of study. No difference in body weight was observed between the PSY and SCF animals. Body composition analysis demonstrated that fat mass in the SCF group was considerably lower than in the CEL and HFD groups. In addition, fasting plasma glucose and insulin and areas under the curve of intraperitoneal glucose tolerance test were also significantly lower in the SCF and PSY groups than in the CEL and HFD groups. Moreover, fasting plasma concentrations of leptin were significantly lower and GLP-1 level was 2-fold higher in the SCF and PSY mice than in the HFD and CEL mice. Ghrelin messenger RNA levels of stomach in the SCF group were significantly lower than in the CEL and HFD groups as well. These results suggest differences in response to dietary fibre intake in this animal model because high-fat diets incorporating dietary fibres such as SCF and PSY appeared to attenuate weight gain, enhance insulin sensitivity, and modulate leptin and GLP-1 secretion and gastric ghrelin gene expression.

1. Introduction

Numerous studies have examined the effects of macronutrients, that is, dietary fat, protein, and carbohydrates, on energy intake; but studies assessing the role of dietary fibre on this process are more limited [1]. Fiber is not considered an essential nutrient, but may play a role in modulation of energy intake and, in this regard, has been suggested to lower risk for developing obesity [2]. Dietary fibres, that is, the indigestible portion of plant foods, can be broadly classified as being either “soluble” or “insoluble” and “fermentable” or “nonfermentable.” Chemically, dietary fiber consists of nonstarch polysaccharides and several plant components such as cellulose, lignin, waxes, chitins, pectins, β-glucans, inulin, and oligosaccharides. These fiber components have unique chemical structures and characteristic physical properties, for example, bulk/volume, viscosity, waterholding capacity, adsorption/binding, or fermentability, which determine their subsequent physiologic behavior.

The American Dietetic Association recommends a minimum of 20 to 35 g/d for a healthy adult [3], whereas the average American diet barely contains half this amount, for example, 10 to 15 g daily [4]. There have been reports demonstrating significant relationships between lower intake of fiber and obesity, as suggested by epidemiologic and cross-sectional studies [5-7]. As such, increased intake of dietary fiber may offer additional health benefits to obese and diabetic patients. For example, dietary fiber supplementation...
was shown to significantly improve carbohydrate metabolism and insulin sensitivity in overweight and obese women [8]. In addition, a high intake of dietary fiber, particularly of the soluble type, improved glycemic control, decreased hyperinsulinemia, and lowered plasma lipid concentrations in patients with type 2 diabetes mellitus [9]. These benefits of increased dietary fiber intake were also observed in long term studies of rats [10,11]. Other reports suggest additional benefits to human health in delaying the emergence of some types of colon cancers and in regulating glucose and lipid absorption across the gut [12].

As previously reported, diets consumed that are high in insoluble fiber may aid in glycemic control [13,14]. However, there is a paucity of data comparing diets consisting of preferentially soluble vs insoluble fiber on specific parameters. In addition, there are reports that fiber from specific plants, that is, bagasse from sugarcane, may affect carbohydrate and lipid metabolism [15]. Based on these reports, we sought to determine the effect of diets containing various dietary fiber contents (soluble vs insoluble) on weight gain and the parameters assessing carbohydrate metabolism. Specifically, we compared diets containing primarily insoluble fiber (purified cellulose or soluble fiber), that is, psyllium, and compared those with diets primarily containing fiber from sugarcane. In addition to assessing weight and clinical measures of carbohydrate metabolism, we sought to determine if specific mechanisms were altered with the varying dietary regimen. As such, we evaluated specific biochemical markers of leptin, glucagon-like peptide 1 (GLP-1), and gastric gene expression between the various dietary regimens.

2. Study design and methods

2.1. Study design

Thirty-six male 4-week-old C57BL/6 mice were obtained from the Jackson Laboratory (Bar Harbor, ME). After arrival, the animals were housed one per cage with ad libitum access to rodent chow and water for a 2-week acclimation period under specific pathogen-free conditions and 12-hour light-dark cycle. Animals were then randomly divided into 4 treatment groups that consisted of high-fat diet alone (HFD), high-fat diet containing 10% (wt/wt) cellulose (CEL), high-fat diet containing 10% (wt/wt) psyllium (PSY), or high-fat diet containing 10% (wt/wt) sugarcane fiber (SCF) for 12 weeks. High-fat diet was purchased from Research Diets (D-12331, New Brunswick, NJ) and contained 58% of energy from fat. The specific high-fat diet used has been well documented to induce obesity and insulin resistance in the animal model [16]. The components and energy density of these diets are demonstrated in Table 1.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>HFD</th>
<th>10% CEL</th>
<th>10% PSY</th>
<th>10% SCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/kg)</td>
<td>558.5</td>
<td>503.7</td>
<td>505.8</td>
<td>5132.7</td>
</tr>
<tr>
<td>Energy from fat (%)</td>
<td>58</td>
<td>52.2</td>
<td>52.2</td>
<td>52.2</td>
</tr>
</tbody>
</table>

Plasma, stomach, and other tissues were quickly put into liquid nitrogen container and stored at −80°C for later analysis. The Institutional Animal Care and Use Committee of Pennington Biomedical Research Center approved all animal protocols.

2.2. Source of dietary fibers

Psyllium husk powder was obtained from Source Naturals (Scotts Valley, CA). Dietary fiber cellulose powder was obtained from NutriCology (Hayward, CA). Sugarcane fiber was obtained and purified under the direction of Dr Lian at the Center for Advanced Microstructures and Devices at Louisiana State University. A proprietary method has been used to reduce sugarcane bagasse fiber to micrometer- and nanometer-sized particles (the ratio can be changed by changing the processing parameters). Bagasse fiber was cooled to cryogenic temperature; and then the bagasse was mechanically pulverized into small particles, from a few nanometers to hundreds of micrometers. During the pulverization process, chemical oxidation of bagasse was prevented by the cooler temperature. During the whole processing, no chemical and no other artificial preservative was used. This processing technology maintains the integrity of the constituents of original bagasse, which usually contains 46% cellulose, 24.5% hemicellulose, 19.95% lignin, 3.45% fats and waxes, 2.4% ash, 2.0% silicon, pulsing 1.70% other substances of weight.

2.3. Blood chemistry and hormone analysis

After 4 hours of fasting, blood samples were collected from orbital sinus of unconscious mice induced by inhalation of CO2. Plasma glucose level was measured by a colorimetric hexokinase glucose assay (Sigma Diagnostics, St Louis, MO). Plasma insulin level was determined by ultrasensitive rat insulin enzyme-linked immunosorbent assay (ELISA) kit from Crystal Chem (Downers Grove, IL). Plasma leptin was
determined by using Mouse Serum Adipokine LINCOplex Kit (catalog no. MADPK-71K, Linco Research, St Charles, MO), and plasma GLP-1 concentration was measured by GLP-1 (active) ELISA kit (catalog no. EGLP-35k; Linco Research) according to the manufacturer’s instructions. All assays were done in duplicate.

2.4. Body composition measurement

Body composition for all animals was measured by nuclear magnetic resonance [17]. Total fat mass (FM) and free fat mass (FFM) were recorded.

2.5. Assessment of carbohydrate metabolism

The effect of the diets on insulin and glucose parameters were determined with the use of an intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (IPITT) obtained at week 11 and week 12 of the study, respectively. After an overnight fast, IPGTT was performed by intraperitoneal injection of 2 g glucose (20% glucose in 0.9% NaCL) per kilogram body weight, and blood glucose was measured at the designated times as described below [18]. For IPITT, an intraperitoneal injection of human insulin (Eli Lilly, Indianapolis, IN) at dose of 0.75 U/kg body weight was administered after 4 hours of fasting. Whole blood glucose was measured from the tail vein at 0, 30, 60, 90, and 120 minutes after injections for both IPGTT and IPITT using the FreeStyle blood glucose monitoring system (TheraSense, Phoenix, AZ).

2.6. Quantitative reverse transcriptase–polymerase chain reaction procedure

Total RNA was extracted from gastric tissues using TRIzol Reagent (Invitrogen, Carlsbad, CA). The RNA analysis and quantitation were performed with RNA 6000 Nano LabChip kit (Agilent Technologies, Foster City, CA).

Amplification of mouse ghrelin was performed with the Brilliant QRT-PCR 1-step master mix kit (catalog no. 60055; Stratagene, Cedar Creek, TX), and cyclophilin B messenger RNA (mRNA) was measured by SYBR Green QPCR master kit (catalog no. 600548, Stratagene) according to the manufacturer’s protocol. After each run, a relative quantification of the amplified polymerase chain reaction product in the different samples was measured. A standard curve was used to obtain the relative concentration of the target gene, and the results were corrected according to the concentration of cyclophilin B. The results were expressed as percentage of HFD group, setting the mean of the control group at 100% and then calculating each individual value of the other 3 groups of animals studied. TaqMan primer-probe sets of mouse ghrelin (NM_01190296, catalog no. 445046) were purchased from Applied Biosystems (Foster City, CA). Primers for mouse cyclophilin B were designed by using PRIMER EXPRESS software (Applied Biosystems). The target gene primer pairs are as follows: for mouse cyclophilin (NM_011149), forward, 5′-TGGAGAGCACCAAGACACGACA-3′ and reverse, 5′-GTCGACAATGATGACATCC-TTCA-3′. These were obtained from Integrated DNA Technologies (Coralville, LA).

2.7. Statistical analysis

All data were expressed as mean ± SEM. Data were evaluated for statistical significance by 2-way analysis of variance, and \( P < .05 \) was considered significant.

3. Results

3.1. Food intake

Energy intake (in kilocalories per kilogram), normalized by body weight, was not shown to differ among groups (Fig. 1A). The average energy intake in all groups expressed per unit body weight was reduced by about 35% at the end of the study when compared with baseline (Fig. 1A).

3.2. Body weight and body composition

There was no difference in body weight or body composition between the 4 groups at baseline. Beginning at week 3, the body weights of the SCF and the PSY groups...
were observed to be lower than that of the CEL group ($P < .01$ and $P < .05$, respectively); and this trend continued up to the end of study (Fig. 1B). At the end of study, the net body weight gain (mean ± SEM) was $12.4 ± 1.03$ g for the SCF group, $14.38 ± 0.88$ g for HFD alone, $14.4 ± 1.6$ g for the PSY group, and $16.7 ± 1.3$ g for the CEL groups. The net body gains in the SCF, HFD, and PSY groups were significantly less than that in the CEL group ($P < .01$, $P < .05$, and $P < .05$, respectively). There were no significant differences of body weight among the SCF, HFD, and PSY animals. Body composition analysis showed that the FM of the SCF and PSY groups was significantly lower than that of the CEL group ($P < .05$), but there were no significant differences between the HFD and PSY groups (Fig. 2A). The FFM in all groups was not significantly different (Fig. 2B), except for the CEL group at week 8 ($P < .05$), when compared with the HFD group.

### 3.3. Glucose and insulin levels

Fasting glucose levels were significantly lower in the PSY and SCF groups than those in the CEL and HFD groups beginning at 8 weeks and continuing up to the end of study (Fig. 3A). Fasting plasma insulin was much lower in the PSY and SCF groups and that in the CEL group from week 4 and was maintained to the end of the study ($P < .05$ and $P < .01$, respectively). However, insulin concentration in the CEL group was significantly higher than that in the HFD group from week 4 to week 12. Insulin level was substantially lower in the SCF group than in the HFD group, and there was no difference between the PSY and HFD groups (Fig. 3B).
The IPGTT data showed glucose concentrations were much lower in the PSY and SCF groups than in the control and CEL groups \((P < .01 \text{ and } P < .001, \text{ respectively; Fig. 4A})\). Area under the curve for glucose during the IPGTT was 945 ± 115 mg/dL in HFD, 1101 ± 36 mg/dL in CEL, 724 ± 39 mg/dL in PSY, and 667 ± 24 mg/dL in SCF. The IPITT results in these groups showed a similar trend (Fig. 4B).

### 3.4. Stomach ghrelin gene expression analysis

Stomach ghrelin mRNA levels were not statistically different between the HFD and CEL groups. However, ghrelin gene expression in the PSY and the SCF animals were significantly lower than that in the HFD and CEL animals \((P < .05 \text{ and } P < .001, \text{ respectively})\) as shown in Fig. 5.

### 3.5. Effect of high-fat diet and dietary fiber supplementation on plasma leptin concentration in mice

At baseline, there was no difference in plasma leptin level among all groups. At week 12, leptin levels increased from basal 358 ± 38 to 3871 ± 279 pg/mL in the HFD, 286 ± 64 to 5054 ± 370 pg/mL in the CEL, 309 ± 42 to 1097 ± 256 pg/mL in the SCF. Plasma leptin concentrations were significantly lower in the SCF and PSY groups than in the CEL and HFD groups \((P < .001; \text{ Fig. 6})\). However, leptin level at week 12 in the CEL group was greatly higher than that in the HFD group \((P < .05)\).

### 3.6. High-fat diet and dietary fiber affect plasma GLP-1 level

There was no difference in fasting plasma GLP-1 concentrations between groups at week 0. After 12 weeks of feeding, GLP-1 levels slightly decreased in the HFD and CEL groups \((-4.5\% \text{ and } -8.9\%, \text{ respectively})\) and significantly increased in the PSY and SCF groups \((+85\% \text{ and } +87.7\%, \text{ respectively; } P < .001)\) (Fig. 7).

### 4. Discussion

We demonstrated that diets containing identical fiber content (10% content in the case of this study) but differing in quantity of soluble vs insoluble fiber may have different effects on body weight gain and carbohydrate metabolism. Specifically, the data suggest that high-fat diets containing a larger percentage of soluble fiber, such as provided in the diet with sugarcane fiber or psyllium, resulted in lower glucose and insulin levels in this animal model. Specifically, fasting plasma glucose and insulin levels during the study were observed to be significantly lower in the SCF and PSY groups than in the CEL groups. The mechanism is not precisely known, but a contributing factor may be altering the rate of glucose absorption in the gut. Dietary fiber, particularly soluble fiber found in barley and oats, may slow digestion and absorption of carbohydrates and hence lower blood glucose and insulin levels [19]. The body composition analysis also revealed that diets incorporating these 2 fibers, as opposed to cellulose, appeared to attenuate weight gain from ingestion of a high-fat diet. The effectiveness of the
sugar cane fiber of lower fasting glucose in this study confirmed the early reported results in streptozotocin-induced diabetic rats fed a diet containing 5% fiber (bagasse). Plasma glucagon levels were decreased in bagasse and significantly increased in the control animals, whereas plasma insulin levels were not changed in these groups [20]. However, in that study, body weight gain was greater for the sugarcane fiber as opposed to the results observed in this study.

In addition to the weight and carbohydrate parameters, several biochemical parameters, such as ghrelin and GLP-1 levels, were altered in the diets containing primarily sugarcane fiber or psyllium. The reasons for evaluating these parameters were based on the hypothesis that dietary modifications may modulate these regulatory systems. Ghrelin is an endogenous ligand for the growth hormone secretagogue receptor (GHSR). Accumulating evidence has suggested that ghrelin may play a role in signaling and reversing states of energy insufficiency. Ghrelin levels rise after food deprivation, and ghrelin administration stimulates feeding and increases body weight and adiposity [21,22]. Glucagon-like peptide 1 is secreted from enteroendocrine L cells, which are localized in the distal ileum and colon [23]. Glucagon-like peptide 1 acts through a specific G-protein–coupled receptor to potently stimulate glucose-dependent insulin secretion [24]. Glucagon-like peptide 1 further reduces hyperglycemia through inhibition of both glucagon secretion and gastric emptying [25-27]. Our data therefore suggest that diets varying in dietary fiber content may potentially modulate these systems.

It has been suggested that high-fiber foods may increase gastric distention and promote sensations of fullness [28]; but clearly, if that is a contributing factor, that does not explain the differences in weight gain observed with diets containing equal amount of fiber but differing in the amount of insoluble vs soluble fiber as evaluated in this study. The degree of fermentation has been suggested to influence the effects resulting from ingestion of dietary fiber. Dietary fiber, once ingested, is fermented by bacteria in the colon, producing short-chain fatty acids (SCFAs) including acetate, propionate, and butyrate and gases. Butyrate may be an important source of energy for the cells of the colon. Wheat bran and oat bran have been reported to produce higher proportions of butyrate [29]. Furthermore, the physical form of the grain or seed determines whether dietary fiber is fermented. For example, lignin and phenolic acids in the cell walls of the grain kernel may restrict access to bacteria and hence limit fermentation of dietary fiber. It has been demonstrated that the molecular weight of guar gum affects SCFA profile in model intestinal fermentation [30].

Observations suggest that ghrelin-responsive pathways are an important component of coordinated body weight control. For example, when fed a high-fat diet, both female and male GHSR-null mice eat less food, store less of their consumed calories, preferentially use fat as an energy substrate, and accumulate less body weight and adiposity than control mice [31]. Similar effects on body weight and adiposity were also observed in female, but not male, GHSR-null mice fed standard chow. Moreover, it suggests that ghrelin signaling is required for the development of the full phenotype of diet-induced obesity [32]. Our data suggested that high-fat diets containing 10% of either the sugarcane fiber or psyllium significantly lowered stomach ghrelin mRNA levels when compared with high-fat diets alone or high-fat diets containing 10% cellulose. The mechanism by which this may occur is not known.

Leptin resistance is defined as decreased sensitivity to the anorexigenic or weight loss effects of leptin. Rodents with diet-induced obesity [33] and most obese humans are resistant to the effects of leptin [34]. Obesity in humans is associated with hyperleptinemia, and increased adiposity is believed to be linked to the development of leptin resistance [35]. Leptin is reported to induce hepatic leptin resistance in diet-induced obesity by impairing the activation of phosphatidylinositol 3-kinase [36]. Other reports suggest that high-fat diets may induce leptin resistance [37]. Long-term consumption of the high-fat diet increased fat cell size and plasma leptin concentration. Leptin is reported to be released from adipose tissue 60 to 120 minutes postprandially [38]. However, it has been demonstrated that leptin is also synthesized by the gastric chief cells and that the release of gastric leptin occurs 15 minutes after refeeding in fasted rat [39]. A high-fat sucrose diet resulted in hyperleptinemia and hyperinsulinemia before adipocyte size was observed to increase [40]. It was interesting to note in this study that high-fat diets containing primarily sugarcane and psyllium resulted in lower plasma leptin concentrations when compared with high-fat diet alone or high-fat diet containing 10% cellulose. An interesting question regarding the mechanism, therefore, would be whether the processing of the dietary fiber, that is, degree of fermentation and production of SCFAs, had a direct effect on leptin production as suggested by other reports [41] or whether an unrelated mechanism was responsible. Currently, this is not precisely known.

The regulatory effect of GLP-1 on energy intake has been well studied. For example, central GLP-1 administration reduces food intake in rodents, whereas peripheral administration of GLP-1 promotes satiety and decreases body weight in humans [23-27]. Our observations suggesting that dietary fiber may modulate GLP-1 levels are intriguing; but once again, the mechanism is not known. However, modulation of GLP-1 may again be secondary to gut processing of the fiber. For example, it has been reported that the SCFA butyrate can increase gene expression of proglucagon (the gene encoding GLP-1) in a dose-dependent manner in vitro [42]. Elevated GLP-1 may also result in putative satiety signals [43]. It has also been suggested that leptin stimulates GLP-1 secretion from enteroendocrine L cells in vitro and in vivo [40]. The C57BL/6 mice on the high-fat diet (45%) for 8 weeks became obese; developed glucose intolerance, hyperinsulinemia, and hyperleptinemia; and were leptin resistant. Mice
on the high-fat diet also had 2-fold lower basal plasma GLP-1 and a diminished GLP-1 response to oral glucose in comparison with low-fat–fed mice. Other reports suggest that leptin resistance in obese C57BL/6 mice is associated with impaired secretion of GLP-1 [44]. The increase in leptin observed in high-fat diet–fed animal models was associated with elevated plasma insulin levels and was closely correlated with fat cell hypertrophy, but was unrelated to energy intake or markers of energy expenditure [2,45]. Finally, other reports suggest that peripheral GLP-1 plays a role in the regulation of macronutrient selection as well as food intake in rats [46].

An interesting question regarding the results of the study is whether the viscosity of the sugarcane fiber was responsible for the observations. Each fiber was provided as part of the diet, so the specific viscosity as it pertains to what occurred in the gastrointestinal environment is not known. In addition, we did not assess viscosity of the fibers individually. It may well be that the viscosity of sugarcane fiber is higher than cellulose and may be lower than psyllium. We did measure the percentage of soluble fiber in sugarcane fiber, and it was observed to be higher than cellulose and lower than psyllium (Table 1). Viscosity of the diet and fiber may delay gastric emptying. However, it is not generally agreed that the distention may reduce gastric mRNA ghrelin concentration and reduce small intestinal GLP-1 secretion. For example, it is reported that distention and chemosensitization of the stomach are insufficient to induce a ghrelin response, suggesting that postgastric feedback is required. This is suggested to be regulated through insulin [47,48]. It is also reported that fermentable fibers such as fructans, which are rapidly and extensively fermented in the proximal part of the colon, may potentially modulate GLP-1 [7-36], amide, and ghrelin [49].

In summary, our data have demonstrated that mice fed a high-fat diet containing dietary fibers in the form of either sugarcane fiber or psyllium had effects to improve glucose levels, lower insulin, and attenuate weight gain in a model of dietary-induced obesity when compared with high-fat diet alone or high-fat diet supplemented with cellulose. The data revealed that the characteristics of the fiber, that is, soluble vs insoluble, clearly played a role. The mechanism by which this occurred, specifically the mechanism by which lowered GLP-1 was induced and gene expression of ghrelin was modulated, is not currently known. Although the mechanism is not precisely known, the data suggest that the fiber intake may inhibit high-fat diet–induced leptin secretion and gastric ghrelin gene expression, as well as elevate GLP-1 concentration. The study of potential cellular signaling mechanisms induced by altering intake of dietary fibers is currently the focus of ongoing studies.

Acknowledgments

The authors thank Dr Jennifer Rood, Jamie Tuminello, Zhiqia Gao, and Jun Zhou for excellent technical assistance. We also thank Nicole Mestayer for assistance in manuscript preparation. The study was supported in part by pilot funding from the Nutrition and Chronic Disease Division and American Sugar Cane League awarded to ZQW, by National Institutes of Health grant P50AT002776-01 from the National Center for Complementary and Alternative Medicine and the Office of Dietary Supplements, and by grant DK 064071 to ARZ.

References


