Short-Chain Fructooligosaccharides Influence Insulin Sensitivity and Gene Expression of Fat Tissue in Obese Dogs

Frédérique Respondek,1,3 Kelly S. Swanson,4,5 Katherine R. Belsito,4 Brittany M. Vester,4 Anne Wagner,3 Louis Istasse,6 and Marianne Diez6

Dietary fibers may modulate insulin resistance and glucose homeostasis in dogs. Their efficacy is, however, dependent on their origin, physical properties, and fermentability in the large bowel. Eight healthy Beagle dogs were fed a commercial diet at twice their maintenance requirements until they became obese. They were then maintained in the obese state and used in a cross-over design study to evaluate the effects of short-chain fructooligosaccharide (scFOS) supplementation (1% wt:wt dry matter in the diet). The euglycemic hyperinsulinemic clamp technique was performed before and after fattening and at the end of each 6-wk cross-over period. Fat tissue biopsies were taken in food-deprived and postprandial phases to measure mRNA abundance of genes involved with fatty acid, glucose metabolism, or inflammation. Insulin resistance appeared progressively with fattening and the rate of glucose infusion during euglycemic clamp was lower (P < 0.05) at the end of the fattening period (7.39 mg·kg⁻¹·min⁻¹) than at baseline (21.21 mg·kg⁻¹·min⁻¹). In stable obese dogs, scFOS increased (P < 0.05) the rate of glucose infusion compared with control (7.77 vs. 4.72 mg·kg⁻¹·min⁻¹). Plasma insulin and triglyceride concentrations were greater in obese than in lean dogs but were not altered by scFOS. Whereas mRNA was not affected in food-deprived dogs, scFOS increased uncoupling protein 2 (1P = 0.05) and tended to increase carnitine palmitoyl transferase 1 adipose mRNA levels during the postprandial period (P = 0.09). Adding 1% scFOS to the diet of obese dogs decreases insulin resistance and appears to modulate the transcription of genes involved in fatty acid or glucose metabolism. J. Nutr. 138: 1712–1718, 2008.

Introduction

Weight gain and obesity are closely associated with insulin resistance and chronic diseases like type 2 diabetes and metabolic syndrome (1). Several ways whereby adipose tissue may influence glucose homeostasis and insulin resistance have been suggested, one being that excessive fat storage results in increased triacylglycerol storage in peripheral tissues, which induces insulin resistance. Adipose tissue is also a highly active endocrine organ that plays a pivotal role in energy homeostasis and, thus, obesity incidence. In addition to nonesterified fatty acids released for oxidative fuel during fasting, adipocytes release a variety of adipokines, including leptin and tumor necrosis factor-α. Many of these adipokines, which are proinflammatory mediators and (or) influence insulin sensitivity, have been demonstrated to increase with obesity (2).

The diet, especially the amount of dietary fat, qualitative composition of dietary fat, and type of dietary carbohydrates, are important causative factors for peripheral insulin resistance (3,4). Epidemiological studies have proven that the reduction of type 2 diabetes is correlated with a greater consumption of dietary fibers; however, their efficacy differs according to their origin, physical properties, and fermentability in the lower part of the gut (5). Furthermore, the respective contributions of the reduction of glycemic index or the colonic fermentation of fibers are difficult to assess separately with, for example, soluble viscous fibers. Nonviscous β-fructan fibers, considered to be prebiotics, are known to be selectively fermented and allow specific changes in the growth and (or) activity in the gastrointestinal microflora that confer benefits upon host health (6). As they do not influence glycemic response, studying their effect on insulin sensitivity can highlight the effects of colonic fermentation on the regulation of insulin sensitivity. An example of β-fructans, short-chain fructo-oligosaccharides (scFOS),7 has been shown to alter glucose and lipid metabolism in humans (7,8) and animals (9,10). For example, feeding a mix of fibers

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1 K. S. Swanson, K. R. Belsito, B. M. Vester, L. Istasse, and M. Diez, no conflicts of interest.
2 Supplemental Table 1 is available with the online posting of this paper at jn.nutrition.org.
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including scFOS has already demonstrated improved glucose homeostasis in healthy dogs (9,11).

The obese dog has been suggested as a relevant model for obesity of the normal human population, contrary to rodents, which may represent a more extreme obesity in humans (12). As in humans, the consumption of a high-fat diet in dogs induces a rapid increase of visceral adipose tissue and a later increase of subcutaneous adipose tissue. The accumulation of fat in muscle or other tissues generally observed in rodent models is only observed in extreme obesity in human subjects (12). Furthermore obesity is also linked to hypertriglyceridemia and insulin resistance in dogs (13,14). Among the above-cited adipokines, tumor necrosis factor-α has been reported to increase with canine obesity (15). However, adipose tissue gene expression profiles have not been extensively studied in the obese canine model and have not been used to evaluate prebiotic supplementation.

The main objective of this study was to assess the effects of scFOS on insulin sensitivity in obese dogs through the utilization of the euglycemic-hyperinsulinemic clamp technique. Analysis of several gene transcripts (mRNA) in peripheral adipose tissue was also performed in an attempt to identify mechanisms by which scFOS feeding affects glucose and lipid metabolism.

**Subjects and Methods**

**Dogs and feeding.** Eight Beagle dogs (4 neutered males, 4 neutered females; mean age = 6.5 y at baseline) were used. Dogs were pair-housed in cages (2 × 1.5 m) with plastic slatted floors and had access to outdoor paddock for 3–4 h per day. All dogs were vaccinated and dewormed before the study. Body weight (BW) and body condition scores were controlled before the study. The experimental protocol was approved by the University Ethical Committee of the University of Liege, Belgium prior to experimentation (N°442).

Dogs were fed once a day at 0900 with a dry food formulated (Premium Adult Croc, Affinity) to meet maintenance requirements (Table 1). Energy contribution from protein, fat, and carbohydrates was 24%, 32%, and 44%, respectively. To induce obesity and insulin resistance, dogs received twice the level of the NRC (16) recommendations for maintenance. Once obese, the amount of food was adjusted weekly to maintain a stable BW. Dogs had unlimited access to fresh water at all times.

**Experimental design.** The experiment consisted of 2 phases: the fattening phase followed by the treatment phase. The fattening phase lasted 14 wk and allowed dogs to gain ~50% of their original BW. The treatment phase was designed as a cross-over to assess the efficiency of scFOS (degree of polymerization, 3–5, Profeed, Beghin-Meiji) supplementation (1% w/w of dry matter top-touched on the food) on glucose homeostasis and adipocyte metabolism. Periods of the cross-over lasted 6 wk and were separated by a 3-wk washout period.

**Evaluation of insulin sensitivity.** Periperal insulin sensitivity was assessed by a euglycemic-hyperinsulinemic clamp after an overnight food-deprived period at the beginning of the study, after the fattening period, and after the 6-wk cross-over study. The detailed protocol is described in Baillache et al. (13). Briefly, a catheter (Surflash IV catheter 20G 1 1/4, Terumo) was placed into the cephalic vein for infusion of insulin and glucose successively and another one (Vyon Leader heart catheter, 20G, 8 cm) in the jugular vein for blood sampling. Blood was sampled every 5 min during the first hour, then every 10 min until the end of the clamp, which lasted 180 min. Insulin sensitivity index (ISI) indeed was calculated as: 

\[
\text{ISI} = \frac{\text{glucose infusion rate (mg/kg }\times \text{min}^{-1})/\text{mean plasma insulin (mU/L) at steady state}}{22.5 (18)}.
\]

**Measurement of blood analytes.** Glycemia was controlled by using a glucometer (Accu-chek Active, Roche Diagnostics). Insulinemia was also controlled from the jugular vein after an overnight food-deprived period and between 100 and 180 min during the clamp. These samples were taken into heparinized tubes stored at 4°C and centrifuged (3000 × g; 20 min). Plasma was then stored at −20°C until analysis. Plasma insulin was measured by using radioimmuno kits (Biosource INS-IRMA, Biosource Europe). Plasma triglyceride and cholesterol concentrations were assessed by colorimetric methods (Kit EliTech Diagnostics and kit Ecoline S, Diagnostic System, respectively). The homeostasis model of insulin resistance (HOMA-IR) was also calculated from basal glycemia (G0; mmol/L) and basal insulinemia (I0; mU/L) as the following HOMA-IR = (G0 × I0)/22.5.

**Adipose tissue sampling and analysis.** Adipose tissue samples were collected from 4 dogs on the same day after an overnight food-deprived period. Anesthesia was induced using i.v. buprenorphine (0.3 g/L; Temgesic) and i.v. propofol (3–4 g/L; Diprivan, Zeneca Pharmaceuticals). General anesthesia was maintained with isoflurane. Adipose tissue samples were collected under aseptic conditions. After clipping and scrubbing of the ventral abdomen, a 2-cm skin incision was made on the linea alba, between the umbilicus and the pubis, and 1 sample (2 g; 1–1.5 cm³) of subcutaneous fat (unfed state) was harvested using blunt dissection. The skin incision was closed using a subcuticular pattern (PDS 4–0, Ethicon) and anesthesia was discontinued. Three hours later, the dogs were allowed to eat their whole meal. One hour postfeeding, dogs were anesthetized and prepared using the same protocol and another fat sample was harvested just caudal to the first collection site.

Once collected, adipose tissue samples were placed into sterile cryovials, immediately flash-frozen in liquid nitrogen, and stored at −80°C. Total cellular RNA was isolated from adipose tissue using Trizol (Invitrogen). The concentration and purity of RNA was determined using a ND-1000 spectrophotometer (Nanodrop Technologies). Conversion of RNA to cDNA was conducted using methods described by the ABI cDNA Archive kit (Applied Biosystems). Isolated cDNA was amplified using quantitative RT-PCR on an ABI PRISM 7900HT Sequence Detection System. The main objective of this study was to assess the effects of scFOS on insulin sensitivity in obese dogs through the utilization of the euglycemic-hyperinsulinemic clamp technique. Analysis of several gene transcripts (mRNA) in peripheral adipose tissue was also performed in an attempt to identify mechanisms by which scFOS feeding affects glucose and lipid metabolism.

**TABLE 1** Analyzed nutrients composition of the basal diet as fed to dogs

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/kg</th>
</tr>
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<tbody>
<tr>
<td>Cereals and derivatives</td>
<td>406.0</td>
</tr>
<tr>
<td>Meat and derivatives</td>
<td>397.0</td>
</tr>
<tr>
<td>Vegetable protein</td>
<td>88.0</td>
</tr>
<tr>
<td>Fish oil</td>
<td>72.0</td>
</tr>
<tr>
<td>Dehydrated egg</td>
<td>8.8</td>
</tr>
<tr>
<td>Yeast</td>
<td>8.8</td>
</tr>
<tr>
<td>Mineral premix¹</td>
<td>15.0</td>
</tr>
<tr>
<td>Vitamin premix²</td>
<td>4.4</td>
</tr>
<tr>
<td>Energy, kJ/g</td>
<td>17.5</td>
</tr>
<tr>
<td>Dry matter</td>
<td>927</td>
</tr>
<tr>
<td>Protein</td>
<td>29.4</td>
</tr>
<tr>
<td>Fat</td>
<td>18.6</td>
</tr>
<tr>
<td>Ash</td>
<td>6.2</td>
</tr>
<tr>
<td>NFC²</td>
<td>44.6</td>
</tr>
<tr>
<td>Starch</td>
<td>33.8</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>1.2</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.5</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1.0</td>
</tr>
</tbody>
</table>

¹ Provided per kg of diet: Cl (as KCl), 6.7 g; K (as KCl), 6.5 g; iron (as FeSO₄), 236 mg; Zn (as ZnSO₄), 169 mg; Cu (as CuSO₄), 15.66 mg; Mn (as MnSO₄), 56 mg; I (as KI), 1.97 mg; and Se (as Na₂SeO₃), 0.31 mg.

² Provided per kg of diet: vitamin A as retinyl acetate, 9.28 mg; cholecalciferol, 45 μg; vitamin E as α-tocopherol acetate, 64.88 mg; thiamin, 48 ppm; riboflavin, 23 ppm; pyridoxine, 14 mg; vitamin B-12, 144 mg; vitamin C, 500 mg; niacin, 191 mg; pantothenic acid, 45 mg; folic acid, 5.89 mg; and biotin, 0.14 mg.

3 NFE, Nitrogen-free extract.
system (Applied Biosystems). Gene-specific primers were used designed using Primer Express 2.0 Software (Perkin Elmer) (listed in Supplemental Table 1). The PCR mixture contained 1 ng of genomic DNA from adipose tissue per μL, 15 pmol of each primer, and 5 μL SYBR Green. The final volume was adjusted to 10 μL using sterile deionized water. The 18S ribosomal RNA gene was used as an internal standard. Data analyses were conducted with sequence detection system software (Applied Biosystems).

**Statistical analysis.** Blood analyte data were analyzed by using the mixed procedure of SAS with repeated measures (SAS Proc. Mixed, SAS system release 8.2., SAS Institute). Relative calculated levels of mRNA in adipose tissue were analyzed by using the mixed procedure of SAS, testing the effects of treatment (scFOS vs. control), feeding state (food-deprived vs. fed), and the interaction of these terms. The significance level was set at \( P < 0.05 \) and \( 0.05 < P < 0.10 \) was considered to be a trend.

**Results**

**BW.** At baseline, dogs weighed 12.8 ± 1.3 kg. Dogs received 1081 ± 58 kJ/kg BW\(^{0.75}\) during the 14-wk fattening period and reached a BW of 19.2 ± 2.3 kg, which represents a 50 ± 13% increase. After the fattening period, dogs were fed 783 ± 67 kJ/kg BW\(^{0.75}\) to maintain stable BW until the end of the study.

**Insulin sensitivity.** The glucose infusion rate during the euglycemic hyperinsulinemic clamp was markedly lower \( (P < 0.05) \) in obese compared with lean dogs (Table 2). The plateau in the insulin concentration clamp was lower during the \( (P < 0.05) \) at the start of the study (i.e. in lean dogs) than at the end of the fattening period. Both the ISI and HOMA-IR were affected \( (P < 0.05) \) by obesity in dogs.

The rate of glucose infusion during the clamp was higher \( (P < 0.05) \) in obese dogs supplemented with scFOS than in those fed the control diet (Table 2). The plateau in the insulin concentration was not modified. The ISI tended to be greater in dogs supplemented with scFOS \( (P = 0.07) \). The HOMA-IR decreased \( (P < 0.05) \) in dogs supplemented with scFOS compared with the obese dogs fed the control diet (Table 2).

**Plasma analytes.** Plasma glucose measured after overnight food deprivation was not influenced either by adiposity or scFOS supplementation (Table 2). The plasma insulin concentration was higher \( (P < 0.05) \) in obese than in lean dogs but was not affected by scFOS consumption. Plasma cholesterol (Table 3) was not changed during the study, but plasma triglyceride concentrations were higher \( (P < 0.05) \) at the end of each cross-over period than at baseline and at the end of the fattening period \( (P < 0.05) \). Plasma cholesterol or triglyceride concentrations measured after overnight food deprivation were not affected by scFOS (Table 3).

**Gene expression.** Due to RNA degradation, adipose samples from only 2 lean dogs were viable. Thus, we did not compare these data between lean and obese states. All mRNA samples were of acceptable quality from dogs in the obese state eating the control or scFOS treatments and were included in the analyses. In the obese state, feeding tended to increase \( (P = 0.09) \) hormone-sensitive lipase mRNA abundance, regardless of dietary treatment (Fig. 1). Dogs fed scFOS had increased \( (P = 0.05) \) uncoupling protein-2 (UCP-2) mRNA and tended to have greater \( (P = 0.09) \) carnitine palmitoyltransferase-1 (CPT-1) mRNA abundance (Fig. 1).

**Discussion**

The higher HOMA-IR and lower ISI in obese than in lean dogs confirmed that insulin resistance was associated with obesity induced in 14 wk by feeding a hyper-caloric diet with a standard range of fat contribution to the energy intake. The lower rate of glucose infusion during the euglycemic hyperinsulinemic clamp illustrates the peripheral insulin resistance in obese compared with lean dogs. Several authors have reported the development of peripheral insulin resistance with obesity in dogs (13,14,19). Our results confirm that increased adiposity seems more important than the type of diet to induce insulin resistance in dogs. Indeed, our hyper-energetic diet had similar effects as those reported with hyper-energetic diets rich in fructose (20) or fat (14,21) or a isocaloric diet enriched in fat (22).

Similar to previous observations in obese dogs, glycemia measured after an overnight food-deprived period was maintained with fasting, whereas plasma insulin and triglycerides increased \( (13,21,23) \). Higher secretion by β-cell or lower extraction of insulin by the liver can explain hyperinsulinemia. Recent work demonstrated a temporal pattern of both mechanisms contributing to hyperinsulinemia, starting with a higher insulin secretion followed by a lower hepatic extraction (24). The increase in triglyceride concentration took a longer time to appear than the hyperinsulinemia, because it was not significant after the initial 14 wk but only after at least 20 wk. This might explain why some authors did not report increased triglycerides after 6 wk of feeding a hyper-caloric, high-fat diet (14).

**Table 2** Plasma glucose and insulin concentrations, the glucose infusion rate, the plateau of the insulin concentration, the ISI, and the HOMA-IR during the hyperinsulinemic euglycemic clamp in lean and obese dogs supplemented or not with scFOS

<table>
<thead>
<tr>
<th></th>
<th>Fattening period</th>
<th>Stable obesity period</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Prefattening</td>
<td>Postfattening</td>
</tr>
<tr>
<td>glucose, mmol/L</td>
<td>4.88 ± 0.06</td>
<td>4.88 ± 0.06</td>
</tr>
<tr>
<td>insulin, pmol/L</td>
<td>69 ± 7</td>
<td>135 ± 13*</td>
</tr>
<tr>
<td>glucose infusion, mg kg(^{-1}) min(^{-1})</td>
<td>21.21 ± 2.86</td>
<td>7.39 ± 1.51*</td>
</tr>
<tr>
<td>Plateau insulin, pmol/L</td>
<td>596 ± 83</td>
<td>849 ± 90*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.14 ± 0.49</td>
<td>4.28 ± 1.08*</td>
</tr>
<tr>
<td>ISI(^2)</td>
<td>0.260 ± 0.043</td>
<td>0.068 ± 0.030*</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SD; \( n = 8 \) (fattening period) or 7 (stable obesity period). \(^*\) Different from prefattening, \( P < 0.05 \); \(^{**}\) different from control diet, \( P < 0.05 \).

\(^{2}\) ISI was defined as the mean plasma glucose infusion rate (mg kg\(^{-1}\) min\(^{-1}\)) divided by the mean plasma insulin concentration (mU/L) at steady state.

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Dietary supplementation with scFOS reduced the basal insulin resistance evaluated through the calculation of the HOMA-IR in obese dogs. This was confirmed by better peripheral insulin sensitivity in a stimulated situation as illustrated by a higher rate of glucose infusion with scFOS than with the control diet during the euglycemic hyperinsulinemic clamp and a higher ISI value. The effect remains moderate compared with the reduction of ISI induced by obesity.

However, this result confirms the effects of β-fructans to improve glucose homeostasis during an oral glucose tolerance test when used in association with other fibers in dogs (11) or included at 10% (wt:wt) in the diet of normal or genetically obese rats (25,26). In hypercholesterolemic humans, a 2-mo supplementation with 10 g/d of scFOS decreased the postprandial insulin response, whereas glycemia was not affected, which also suggests improved insulin sensitivity (27).

Previous studies demonstrated that scFOS could lower blood cholesterol in dogs (9,28). This was not observed in our study and might be explained by the relatively low dosage of scFOS used or by the initial concentration of cholesterol, which was not modified by fattening and remained in normal physiological range for Beagle dogs. During another study with healthy Beagle dogs, a dose effect between the dietary fiber content and the reduction of blood cholesterol level was observed. Whereas a diet containing 10% fiber (2% sugar beet pulp, 8% oligofructose) lowered blood cholesterol level was observed. Whereas a diet containing 10% fiber (2% sugar beet pulp, 8% oligofructose) lowered blood cholesterol, a reduction in blood cholesterol was not significant with 5% fiber (9). In hypercholesterolemic Collie dogs, the effect of 3% of scFOS on cholesterol reduction was transient and variable with a trend for lower triglycerides (28). Blood triglycerides were also reduced by β-fructans in rodents when incorporated at 10% in the diet (29–31) or in humans fed doses between 10 and 20 g/d (27,32). However, the effect seems to be dependent on the type of basal diet. Whereas a supplementation with β-fructans reduced the concentration of triglycerides in the case of a high-fructose diet, there was no effect with a starch-based diet (29). Our results might also suggest that scFOS are not efficient to reduce triglycerides in advanced stages of hypertriglyceridemia.

In mice, scFOS has been demonstrated to modify gene expression in the intestine (33). The relatively low dose that we used in the present study might explain to some extent why only 2 genes were differently expressed. A recent study showed a markedly reduced expression of PPARγ in visceral adipose tissue associated with obesity, but the difference was not significant in the subcutaneous adipose tissue (15). Thus, measurement of gene expression at the level of the visceral adipose tissue might have been more appropriate. CPT-1 is the “gate keeper” of free fatty acid oxidation, which takes place in the cellular mitochondria. Although their physiological role is controversial, uncoupling proteins have been suggested to help preserve the normal function of mitochondria for substrate oxidation (34). Thus, upregulation of CPT-1 and UCP-2 in adipocytes would be considered beneficial and may have contributed to the increased insulin sensitivity observed in scFOS-fed dogs of the current experiment.

The effects of scFOS feeding on UCP-2 and CPT-1 expression in the current study are similar to that of bezafibrate administration (35). Fibrates are hypolipidemic agents that function by activating PPAR. Bezafibrate, which has been shown to decrease fat deposition and improve glucose tolerance in diabetics, increased CPT-1 expression by 4.5-fold and UCP-2 expression by 1.5-fold in primary adipocyte cultures (35). Although the expression of PPARγ was not altered in the current experiment, the responses of UCP-2 and CPT-1 may be important mechanisms by which scFOS feeding improved insulin sensitivity. Several factors may have been responsible for changes in UCP-2 and CPT-1 expression, including circulating leptin and SCFA. Although blood or tissue leptin levels were not measured in this experiment, leptin infusion has been shown to increase UCP-2 expression in white adipose tissue (36). In adipocyte culture, leptin administration has also been shown to increase FFA oxidation by upregulating CPT-1 mRNA (37).

Because 1% scFOS is not likely to modify the physical properties of the feed (e.g. viscosity), we suggest that the effect of scFOS on insulin sensitivity was mediated by its action on the intestinal microbiota. Furthermore, as illustrated by elevated insulin levels in patients suffering from ulcerative colitis or resection of the colon, microbial fermentation occurring within the colon affects insulin sensitivity (38). There is also accumulative evidence that the gut microbiota can regulate fat storage (39,40). Colonic fermentative by-products, primarily SCFA, may have direct or indirect effects on adipocyte metabolism. High fermentative activity in the colon may increase circulatory SCFA concentrations and thus have a direct impact on adipose metabolism.

Acetate feeding in rats has been shown to not only inhibit glycolysis in liver and skeletal muscle tissue but also to increase

### TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Prefattening</th>
<th>Postfattening</th>
<th>Control diet</th>
<th>scFOS diet</th>
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<tbody>
<tr>
<td><strong>Cholesterol, mmol/L</strong></td>
<td>4.8 ± 1.2</td>
<td>5.1 ± 0.9</td>
<td>5.4 ± 0.7</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td><strong>Triglycerides, mmol/L</strong></td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>1.4 ± 0.6*</td>
<td>1.5 ± 0.5*</td>
</tr>
</tbody>
</table>

* Values are means ± SD; n = 7. *Different between the scFOS and control diets, P < 0.05.

![FIGURE 1](https://academic.oup.com/jn/article-abstract/138/9/1712/4750842)
fatty acid oxidation in hepatic tissue (41). If adipose tissue responds in a similar manner, this may be one mechanism by which scFOS feeding led to upregulated UCP-2 and CPT-1 expression. Supplementation of scFOS to insulin-resistant rats has also resulted in reduced activity of hepatic fatty acid synthase, reduced FFA concentrations, and increased serum acetate concentrations (42). β-Fructans have also been shown to increase whole-body acetate turnover in healthy dogs when fed at a level of 3% in the diet (43).

G-protein–coupled receptors have been suggested as targets of SCFA in adipose tissue, which affect adipogenesis, adipocyte differentiation, and fatty acid metabolism (38). SCFA may also influence adipose tissue via indirect mechanisms, including the actions of glucagon-like peptid e1 (GLP-1). GLP-1 production increases with high colonic microbial activity, preferentially in the proximal region, and has been associated with increased insulin response (44). In rats, β-fructans enhanced the secretion of GLP-1 in the proximal colon and shorter chain molecules were more effective than longer chains (45). The link between colonic microflora and insulin resistance following the consumption of a high-fat diet might also imply bacterial lipopolysaccharides or the modulation of the ifat gene at the level of the gut epithelium as recently proposed (40,46).

Given that dogs in the current study fed scFOS had increased insulin sensitivity, we hypothesized that glucose transporter 4 (GLUT4) and insulin receptor substrate 2 (IRS2) mRNA would be greater in adipose tissue. The lack of response of these 2 genes is of interest and requires further study. Several possible explanations for the lack of GLUT4 and IRS2 mRNA response in the current experiment exist, including: 1) the collection and analysis of subcutaneous adipose tissue but not visceral adipose tissue or skeletal muscle; 2) measurement of IRS2 mRNA, but not that of IRS1; and 3) the measurement of mRNA, but not protein concentration, translocation to the plasma membrane (GLUT4), or phosphorylation (IRS2).

A likely reason for observing no differences in GLUT4 and IRS2 mRNA abundance is the site of adipose collection. GLUT4 protein translocation and mRNA of visceral adipose tissue and skeletal muscle have been shown to be responsive to changes in insulin sensitivity in past experiments studying obese dogs and humans. GLUT4 mRNA response among adipose tissue depots, however, has been variable. For example, GLUT4 protein in adipocyte plasma membranes decreased in obese compared with lean dogs (47). Similarly, skeletal muscle GLUT4 expression was reported to be inversely related to insulin resistance in morbidly obese people (48). Gayet et al. (21) reported decreased (>50%) GLUT4 mRNA in visceral adipose and skeletal muscle tissues of obese insulin-resistant dogs compared with lean control dogs but did not observe differences in subcutaneous adipose samples. These same researchers later reported increased insulin sensitivity and GLUT4 mRNA abundance in visceral and subcutaneous adipose tissues in dogs supplemented with green tea extract (49). Because skeletal muscle accounts for ~80% of insulin-mediated glucose uptake (50), it is possible that increased insulin sensitivity in dogs fed scFOS was due to changes in skeletal muscle and (or) visceral adipose tissue without great differences being detected in subcutaneous adipose tissue.

To our knowledge, mRNA abundance of IRS1 or IRS2 has not been measured in dogs previously. Because sequence information for the IRS1 gene was not available for measurement via RT-PCR during the current experiment, only IRS2 mRNA was measured. The roles of IRS1 and IRS2 in skeletal muscle and adipose tissues have been the focus of many rodent and human experiments. Although in muscle cells, IRS1 is thought to have a predominant role in GLUT4 translocation and glucose uptake, it appears that both IRS1 and IRS2 participate in the regulation of glucose uptake in fat cells [reviewed by Thirone et al. (51)]. In adipose tissue, however, body fat mass appears to affect IRS protein function differently. In healthy subjects, IRS1 is the main docking protein for phosphatidylinositol 3-kinase in adipocytes. However, IRS1 has been demonstrated to be markedly reduced in noninsulin-dependent diabetic patients, with the unchanged IRS2 protein becoming the primary docking protein (52). Thus, in the current experiment, it is possible that IRS1 mRNA and (or) protein was responsive to insulin sensitivity without observing differences in IRS2. Once sequence data becomes available for IRS1, further research is required to characterize the roles of these IRS proteins in adipose tissue.

Finally, measurement of GLUT4 and IRS2 mRNA abundance, without the measurement of protein concentration, protein translocation to the plasma membrane (GLUT4), or protein phosphorylation (IRS2), was performed in the current experiment. Insulin-stimulated glucose uptake via increased GLUT4 translocation and IRS1 phosphorylation have been demonstrated in canine adipocytes (53) and skeletal muscle cells (54). Because mRNA concentrations are not always indicative of protein concentrations or protein modifications, however, it is possible that dogs fed scFOS had increased GLUT4 translocation and IRS1 and (or) IRS2 phosphorylation in skeletal muscle and adipose tissues without detecting differences in mRNA. Further research, incorporating the use of these assays, is crucial for our understanding of glucose metabolism in adipose tissue and how it may be affected by dietary manipulation, including large intestinal microbial fermentation.

In conclusion, even though the mechanism of action remains unclear and the dose effect relationship would need to be further evaluated, the effects of scFOS on insulin sensitivity in the obese dog model are promising for application in people with the risk of metabolic syndrome.

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Literature Cited

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