

Improvement of Glucose Tolerance and Hepatic Insulin Sensitivity by Oligofructose Requires a Functional Glucagon-Like Peptide 1 Receptor

Patrice D. Cani,^{1,2} Claude Knauf,¹ Miguel A. Iglesias,¹ Daniel J. Drucker,³ Nathalie M. Delzenne,² and Rémy Burcelin¹

Nondigestible fermentable dietary fibers such as oligofructose (OFS) exert an antidiabetic effect and increase the secretion of glucagon-like peptide 1 (GLP-1). To determine the importance of GLP-1 receptor-dependent mechanisms for the actions of OFS, we studied high-fat-fed diabetic mice treated with OFS for 4 weeks in the presence or absence of the GLP-1 receptor antagonist exendin 9-39 (Ex-9). OFS improved glucose tolerance, fasting blood glucose, glucose-stimulated insulin secretion, and insulin-sensitive hepatic glucose production and reduced body weight gain. Ex-9 totally prevented the beneficial effects of OFS. Furthermore, GLP-1 receptor knockout mice (GLP-1R^{-/-}) were completely insensitive to the antidiabetic actions of OFS. At the molecular level, the effects of OFS on endogenous glucose production correlated with changes of hepatic IRS (insulin receptor substrate)-2 and Akt phosphorylation in an Ex-9-dependent manner. As inflammation is associated with diabetes and obesity, we quantified nuclear factor- κ B and inhibitor of κ B kinase β in the liver. The activity of both intracellular inflammatory effectors was reduced by OFS but, importantly, this effect could not be reverted by Ex-9. In summary, our data show that the antidiabetic actions of OFS require a functional GLP-1 receptor. These findings highlight the therapeutic potential of enhancing endogenous GLP-1 secretion for the treatment of type 2 diabetes. *Diabetes* 55:1484–1490, 2006

The recent growing occurrence of the metabolic diseases diabetes and obesity in Westernized countries is associated with changes in feeding habits, where fat constitutes an increasing percentage of total caloric intake (1). The central and early pathological consequences of high-fat feeding includes the

development of insulin resistance (2,3) and β -cell dysfunction. The mechanisms through which these functions are impaired are not completely identified but could be related to inflammation (4,5), lipotoxicity (2,6), and chronic hyperinsulinemia (7,8) primarily affecting the liver (9) and the muscles (10). Therapeutic strategies for reversing these defects involve pharmacological or nutritional approaches. Whereas the former aims at targeting tissues and molecules directly involved in insulin action or secretion, nutritional approaches aim at stimulating endogenous physiological functions that secondarily regulate energy homeostasis.

Glucagon-like peptide 1 (GLP-1) amide (7-36) is an enteroendocrine-derived peptide secreted in response to nutrient ingestion. GLP-1 stimulates insulin secretion in a glucose-dependent manner (11). Clinical trials showed that continuous GLP-1 treatment for 6 weeks improved glycemic control and body weight and reduced food intake (12). Consequently, therapeutic strategies aimed at stimulating GLP-1 secretion represent alternative approaches to pharmacological approaches delivering exogenous GLP-1.

Human studies have demonstrated that oligofructose (OFS), a nondigestible fermentable carbohydrate, lowers plasma lipid concentrations (13), increases plasma levels of GLP-1 (14), promotes satiety (15), and decreases glycemia in moderately hyperglycemic patients (16). Similarly, rats fed OFS have a reduced food intake and an elevated level of portal GLP-1 in association with increased intestinal proglucagon gene expression (17,18). However, whether the therapeutic effect of OFS is dependent on, or only associated with, enhanced levels of GLP-1 has not been determined. To ascertain the role of endogenous GLP-1 as a mediator of OFS action, high-fat-fed (HF) diabetic mice were fed OFS in the presence or absence of an intraperitoneal infusion of the GLP-1 receptor antagonist exendin 9-39 (Ex-9) for 4 weeks. Furthermore, the antidiabetic actions of OFS were studied in GLP-1 receptor knockout mice (19). Our studies demonstrate an essential role for GLP-1 receptor-dependent mechanisms as critical transducers of OFS action.

RESEARCH DESIGN AND METHODS

Eleven-week-old male C57BL/6J mice (Janvier, Le Genest Saint Isle, France) or GLP-1 receptor (GLP-1R) ^{-/-} mice (in a C57BL/6J background, from our colony) were housed in a controlled environment (inverted 12-h daylight cycle, lights off at 10:00 A.M.) with free access to food and water in groups of five mice per cage at 22°C. All animal experimental procedures have been approved by the local ethical committee of the Rangueil hospital. Mice were fed a high-fat diet for 28 days (UAR, Epinay sur Orge, France). The energy content was 72% fat (corn oil and lard), 28% protein, and <1% carbohydrates

From ¹Unité Mixte de Recherche (UMR), Centre National De La Recherche Scientifique, University Paul Sabatier, Toulouse, France; the ²Unit of Pharmacokinetics, Metabolism, Nutrition and Toxicology, Université catholique de Louvain, Brussels, Belgium; and the ³Banting and Best Diabetes Centre, Toronto General Hospital, University of Toronto, Canada.

Address correspondence and reprint requests to Prof. Rémy Burcelin, PhD, CNRS-UMR 5018, Hôpital Rangueil, 1 BP 84225, Toulouse 31432 cedex 4, France. E-mail: burcelin@toulouse.inserm.fr. Or Prof. Nathalie M. Delzenne, Phn, PhD, Université catholique de Louvain, PMNT 73/69, Av. E. Mounier, 73/69, 1200 Brussels, Belgium. E-mail: delzenne@pmnt.ucl.ac.be.

Received for publication 18 October 2005 and accepted in revised form 23 January 2006.

ELISA, enzyme-linked immunosorbent assay; GLP-1, glucagon-like peptide 1; IKK, inhibitor of κ B kinase; IRS, insulin receptor substrate; OFS, oligofructose; NF- κ B, nuclear factor- κ B.

DOI: 10.2337/db05-1360

© 2006 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

TABLE 1
Plasma, pancreatic, and intestinal hormone characteristics

	HF-CT	HF-OFS	HF-OFS-Ex	CT GLP-1R ^{-/-}	OFS GLP-1R ^{-/-}
Fasted					
Glucose (mmol/l)	7.02 ± 0.18 ^a	5.97 ± 0.24 ^b	6.94 ± 0.17 ^a	6.52 ± 0.19	6.97 ± 0.19
Insulin (pmol/l)	73.7 ± 10.4 ^a	120.5 ± 13.5 ^b	81.5 ± 9.6 ^a	200.8 ± 25.1	235.7 ± 42.7
Fed					
Glucose (mmol/l)	8.21 ± 0.38 ^a	6.90 ± 0.29 ^b	8.35 ± 0.37 ^a	8.69 ± 0.18	9.43 ± 0.44
Insulin (pmol/l)	566.5 ± 54.7 ^a	834.7 ± 114.5 ^b	558.2 ± 87.1 ^a	309.4 ± 59.4	352.1 ± 59.5
Epididymal fat pads (g/100 g body wt)	3.15 ± 0.25 ^a	1.95 ± 0.15 ^b	2.74 ± 0.21 ^a	4.65 ± 0.8	4.62 ± 0.52
Pancreatic insulin (μg/g)	283.2 ± 30.8 ^a	358.4 ± 24.9 ^b	274.7 ± 10 ^a	237.5 ± 18.7	202.9 ± 9.6
Colonic proglucagon mRNA (RFU)	0.45 ± 0.04 ^a	0.66 ± 0.04 ^b	0.59 ± 0.02 ^b	0.44 ± 0.03 ^a	0.54 ± 0.02 ^b

Data are mean ± SE. Plasma glucose (mmol/l) and insulin (pmol/l) during the fed and fasted states in wild-type (HF) and GLP-1R^{-/-} knockout mice fed a high-fat diet and supplemented with OFS or OFS-Ex or without supplementation (CT). Epididymal fat pads weight (g/100 g body wt), pancreatic insulin content (μg/g), and colonic proglucagon mRNA (relative fluorescence units [RFU]) were also quantified in all groups. Data with different superscript letters are significantly different ($P < 0.05$) according to the post-hoc ANOVA statistical analysis convention.

(HF-control [CT]), as described (3), or a mix of high-fat diet and OFS (gift of Orafit; Tienen, Belgium) in a proportion of 90:10 (wt/wt) (HF-OFS). Mice were eating on average $5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. It is noteworthy that the extent of diabetes obtained by a high-fat carbohydrate-free diet is higher than in the presence of carbohydrates (R.B., C.K., personal observation).

Intraperitoneal catheter. Mice were anesthetized with isoflurane (Abbott, Rungis, France), and an osmotic mini-pump (Alzet Model 2004; Alza, Palo Alto, CA) was implanted subcutaneously and connected to a catheter indwelled into the intraperitoneal cavity through a 3-mm incision in the abdominal wall, as previously described (20). The minipumps were filled either with Ex-9 (Bachem, Bubendorf, Switzerland), allowing the continuous infusion of $2 \text{ pmol Ex-9} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ or NaCl (0.9%).

Femoral catheter. Under similar anesthesia, a catheter was indwelled into the femoral vein as previously described (3). The mice were then housed individually and after 2 days and showed normal feeding behavior and motor activity. The mice were allowed to recover for 4–6 days.

Glucose turnover analysis in basal and hyperinsulinemic conditions. Insulin sensitivity was assessed by the euglycemic-hyperinsulinemic clamp as described (3). Briefly, 6-h-fasted mice were infused with insulin at a rate of $4 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (physiological) for 3 h, and $\text{D}-(^3\text{H})\beta$ -glucose (Perkin Elmer, Boston, MA) was infused at rate of $30 \mu\text{Ci} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ to ensure a detectable plasma $\text{D}-(^3\text{H})\beta$ -glucose enrichment. Throughout the infusion, blood glucose was assessed from blood samples (3.5 μl) collected from the tip of the tail vein when needed using a blood glucose meter (Roche Diagnostic, Meylan, France). Euglycemia was maintained by periodically adjusting a variable infusion of 16.5% (wt/vol) glucose. Plasma glucose concentrations and $\text{D}-(^3\text{H})\beta$ -glucose specific activity were determined in 5 μl of blood sampled from the tip of the tail vein every 10 min during the last hour of the infusion, as described (3).

In a different set of mice and at completion of the feeding period, the rodents were anesthetized by intraperitoneal injection of pentobarbital (60 mg Nembutal per kg body wt; Sanofi Santé Animale Benelux, Brussels, Belgium), and portal vein blood samples were collected in EDTA tubes containing dipeptidyl peptidase intravenous inhibitor (10 μl/ml) (DPPIV inhibitor; Linc Research, St Charles, MO); after centrifugation, plasma was stored at -80°C . **Food intake assessment.** Mice were housed five per cage. Food intake was recorded twice weekly for 1 month as described (17,18). Pellets and spillage were weighed separately. The mean value for the weekly assessment was calculated.

Intraperitoneal glucose tolerance test. An intraperitoneal glucose tolerance test was performed 3 weeks after the beginning of the feeding period. Six-hour-fasted mice received an intraperitoneal injection of glucose (1 g/kg body wt). Blood glucose was determined with a glucose meter (Roche Diagnostic) on 3.5 μl of blood collected from the tip of the tail vein, 30 min before and 0, 15, 30, 60, 90, and 120 min following glucose injection.

Western blot analysis. Tissues were homogenized in lysis buffer (21), solubilized for 30 min on ice, and clarified by centrifugation. Supernatants were snap frozen in aliquots and stored at -80°C . The hepatic IRS-2 total and tyrosine-phosphorylated forms were assessed from 500 μg of total protein extracts, which were immunoprecipitated for 2 h with IRS-2 antibodies (Anti-IRS 2; Upstate, Mundolsheim, France). Immune complexes were then collected with 100 μl of 50% slurry of protein-A Sepharose (Sigma, Lyon, France), washed with lysis buffer, resolved on 10% SDS-PAGE, and transferred onto nitrocellulose. The blots were then probed with polyclonal antibodies against IRS-2 (Upstate) or by using antiphosphotyrosine horseradish proxi-

dase conjugate (Upstate). In addition, 30 μg of total protein extract were resolved on 10% SDS-PAGE and transferred onto nitrocellulose for the quantification of the total and phosphorylated forms of Akt (Ser⁴⁷³) (Cell Signaling, Saint Quentin Yvelines, France), nuclear factor-κB (NF-κB) and NF-κB-p65 (Cell signaling), inhibitor of κB kinase (IKK)-α/β, and IKK-β-P (Santa Cruz, Le Perray en Yvelines, France). The binding of the specific primary antibody was quantified by the use of a secondary antibody coupled with the horseradish peroxidase (Upstate) and revealed by chemiluminescence (Amersham, Orsay, France).

Plasma parameters. Plasma insulin concentration was determined at completion of the feeding period in the fed or fasted (6 h) state in 5 μl of plasma collected from tail blood using an enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Uppsala, Sweden). Plasma insulin was also determined at completion of the insulin infusions from 5 μl of plasma appropriately diluted according to the experimental conditions. GLP-1 (7-36) amide was measured either in the plasma or in tissue extracts using an ELISA kit (GLP-1 active ELISA kit; Linc Research), as previously described (17,18). Plasma β-hydroxybutyrate was assessed from 5 μl of plasma sampled in postabsorptive conditions using a colorimetric method (Stanbio, Boerne, TX).

Pancreatic insulin content. Pancreata were harvested, weighed, and immediately frozen into liquid nitrogen and kept at -80°C before assay. The day of the assay, pancreata were homogenized at $+4^\circ\text{C}$ into ethanol/acid (HCl 0.15 mmol, ethanol 75% [vol/vol] water), kept at 4°C overnight, and centrifuged (2000g). Insulin was then assayed in the supernatant using an ELISA kit (Mercodia).

Proglucagon and actin mRNA: RT-PCR. Total RNA from the colon was isolated and RT-PCR performed as previously described (17,18). Briefly, after the reverse transcriptase reaction, the quantification of the PCR products was performed using a fluorimetric method (Rediplate96PicoGreen dsDNA Quantitation-Kit; Molecular Probes, Leiden, the Netherlands). Results are presented as mean ratio of relative fluorescence unit of proglucagon mRNA/actin mRNA.

Statistical analysis. Results are presented as mean ± SE. Two-way ANOVA, one-way ANOVA followed by post hoc (Tukey's honestly significant difference) tests, or Student's *t* test were used to assess statistical significance between groups. $P < 0.05$ was regarded as statistically significant.

RESULTS

Plasma and pancreatic and intestinal hormone characteristics. High-fat feeding in rodents induces features of type 2 diabetes, as previously described (3). HF mice are insulin resistant, glucose intolerant, and have reduced glucose-stimulated insulin secretion (3). Treatment of diabetic mice with OFS reduced levels of fasted and fed glucose (Table 1) in association with increased levels of plasma insulin and pancreatic insulin content. In contrast, concomitant treatment with Ex-9 increased the concentration of glucose and reduced plasma levels of insulin and pancreatic insulin content (Table 1). It is noteworthy that Ex-9 is specific for the GLP-1 receptor and at the rate studied here, no nonspecific effect could be envisioned (as discussed in 20). Furthermore, no effect of OFS was

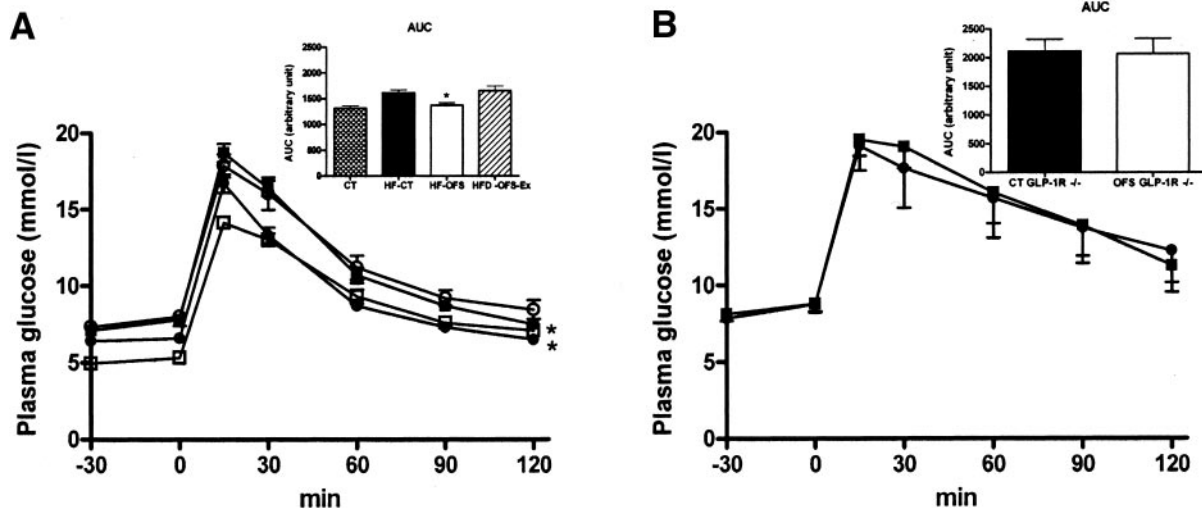


FIG. 1. Effect of high-fat diet on intraperitoneal glucose tolerance. *A*: Intraperitoneal glucose tolerance in CT (□; *n* = 6), HF-OFS (●; *n* = 10), HF-CT (■; *n* = 8), and HF-OFS-Ex-9 (○; *n* = 9) mice after a 4-week period of high-fat feeding. Area under the curve (AUC) of glucose in CT (*n* = 6), HF-OFS (*n* = 10), HF-CT (*n* = 8), and HF-OFS-Ex-9 (*n* = 9) (inserts). *B*: Intraperitoneal glucose tolerance tests in CT GLP-1R^{-/-} (*n* = 5) and OFS GLP-1R^{-/-} (*n* = 5) mice. **P* < 0.05 vs HF-CT and HF-OFS-Ex. Data are mean ± SE.

observed in GLP-1R^{-/-} mice. The intestinal content of proglucagon mRNA was increased by OFS treatment (Table 1), and OFS also significantly increased the portal plasma GLP-1 concentration: 15.6 ± 0.3 vs. 25 ± 2 pmol/l, *P* < 0.05, in control vs. OFS-treated animals, respectively. Total GLP-1 content was increased by 110% in the proximal colon of OFS-fed mice but not in the cecum or distal colon. Proximal colon: 89.6 ± 14.8 vs. 189.6 ± 19.7, *P* < 0.05; distal colon: 141.8 ± 7.9 vs. 141.0 ± 7.8; cecum: 29.7 ± 5.9 vs. 23.9 ± 5.5 pmol/g, in control and OFS-treated mice, respectively. β-Hydroxybutyrate was also assessed to determine whether differences in ketogenesis occurred. Plasma β-hydroxybutyrate concentration was slightly increased by the high-fat diet treatment (90 ± 10 vs. 121 ± 20 μmol/l in CT and HF mice, respectively). Neither the OFS nor Ex-9 treatment significantly changed the concentration (132 ± 30 vs. 117 ± 10 μmol/l, respectively).

Glucose tolerance. HF-CT mice exhibit glucose intolerance when compared with control mice fed regular diet, as previously described (3). In the present study, OFS-treated mice exhibited improved glucose tolerance, as evidenced by the lower area under the curve calculated from glucose

profiles (Fig. 1). Treatment with the GLP-1R antagonist Ex-9 abolished the glucose-lowering effect of OFS (Fig. 1). Conversely, glucose tolerance was not improved by OFS treatment in GLP-1R^{-/-} mice on high-fat diet (Fig. 1).

Glucose utilization and production rates. As glucose intolerance may be associated with hepatic and muscle insulin resistance (22), we studied these features in diabetic OFS-treated mice using the euglycemic-hyperinsulinemic clamp coupled with glucose tracer infusions. To assess hepatic insulin sensitivity we used an insulin infusion, maintaining physiological concentration of insulin with a range of 326 ± 18, 347 ± 45, or 357 ± 54 pmol/l in HF-CT, HF-OFS, or HF-OFS-Ex, respectively. In this condition, the glucose infusion rate required to maintain euglycemia was increased by the OFS treatment in wild-type mice but not in mice treated with Ex-9 (Fig. 2A). Furthermore, OFS-treated mice exhibited an enhanced inhibitory effect of insulin on hepatic glucose production (Fig. 2B); however, no improvement of peripheral insulin sensitivity was detected since glucose clearance rates were unchanged between groups (1.14 ± 0.34, 1.16 ± 0.12, and 1.15 ± 0.42 ml · kg⁻¹ · min⁻¹ in HF-CT, vs. HF-OFS,

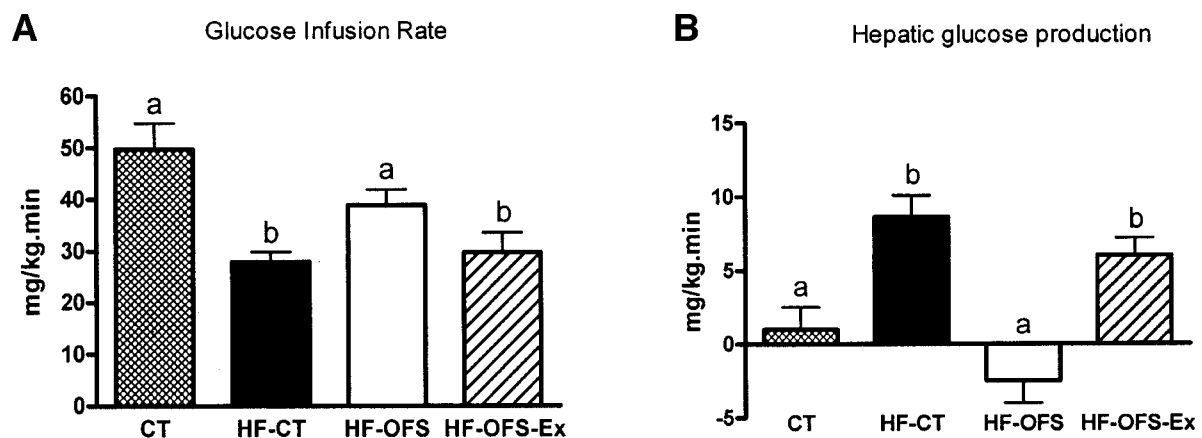


FIG. 2. GLP-1-dependent control of whole-body glucose infusion rate and hepatic glucose production. Glucose infusion rate (*A*) and hepatic glucose production (*B*) were calculated (mg · kg⁻¹ · min⁻¹) in steady-state euglycemic (5.5 mmol/l) hyperinsulinemic clamp (4 mU · kg⁻¹ · min⁻¹) in CT (*n* = 6), HF-OFS (*n* = 8) relative to HF-CT (*n* = 8), and HF-OFS-Ex-9 (*n* = 8) mice after a 4-week period of high-fat feeding. Data are mean ± SE. Data with different superscript letters are significantly different (*P* < 0.05), according to the post hoc ANOVA statistical analysis convention.

and vs. HF-OFS-Ex, respectively). To ascertain whether peripheral glucose utilization rates were unchanged in insulin-sensitive tissues, we studied insulin-stimulated glucose utilization in different muscles (gastrocnemius and vastus lateralis) and white adipose tissue. In this condition, we used a pharmacological rate of insulin infusion to ensure maximal insulin stimulation of glucose utilization by muscle and hyperinsulinemia (>5 nmol/l). Similar to the clearance data, no differences were noted in rates of glucose utilization in OFS-treated mice in the presence or absence of Ex-9 (data not shown).

Hepatic insulin signaling. OFS treatment normalized the excessive hepatic glucose production rate of diabetic mice (Fig. 2B). Similarly, insulin-stimulated phosphorylation of IRS-2 and Akt in the liver of OFS-treated mice was improved, demonstrating at a molecular level the beneficial effect of OFS (Fig. 3A and B). In contrast, Ex-9 treatment reversed the improvement in hepatic glucose production (Fig. 2B) and decreased levels of phosphorylated IRS-2 and Akt (Fig. 3A and B).

Whole-body weight gain and energy consumption. High-fat feeding increased body weight over a 4-week period (normal diet 26.4 ± 0.6 g vs. HF-CT 32.5 ± 0.8 g). However, during the 4-week treatment with OFS, body weight gain was significantly lower (Fig. 4). The reduction in body weight was totally prevented by Ex-9 treatment and not observed in OFS-treated GLP-1 receptor knockout mice (Fig. 4). It is noteworthy that the energy consumption of high-fat diet mice treated with Ex only (without OFS administration) was reduced when compared with HF-CT group (data not shown). This suggests that the pharmacological and chronic inhibition of GLP-1 receptor controls the basal (not OFS-induced) effect of GLP-1 on food intake. Furthermore, the fat pad weights were $\sim 40\%$ lower in HF-OFS mice than in HF-CT and HF-OFS-Ex mice ($P < 0.05$) (Table 1). No differences were observed between CT and OFS-GLP-1R^{-/-} mice.

OFS reduces hepatic phosphorylation of IKK- β and NF κ -B independently of GLP-1 action. Obesity and insulin resistance are features associated with a proinflammatory status (23). Insulin resistance affects the liver and could lead to steatosis; hence, we quantified proteins involved in hepatic inflammation, namely IKK- β and NF κ -B in the liver (24–26). The level of phosphorylated IKK- β and NF κ -B measured in the fasted state was reduced by the OFS treatment when compared with diabetic mice treated with the high-fat diet alone (Fig. 5). GLP-1 receptor blockade with Ex-9 did not prevent the effect of OFS on phosphorylated IKK- β and NF κ -B. This suggests that OFS may exert indirect anti-inflammatory actions independent of mechanisms requiring a functional GLP-1 receptor.

DISCUSSION

The present data demonstrate that OFS treatment reduces the impact of high-fat feeding on the occurrence of diabetes and obesity in a GLP-1R-dependent manner. Nutritional strategies represent alternatives to pharmaceutical approaches for reducing hyperglycemia and body weight. Human studies demonstrate that OFS lowers plasma lipid concentrations (13) and decreases glycemia in moderately hyperglycemic patients (16). The regulatory mechanisms were previously associated with increased plasma concentrations of GLP-1 (14). Similarly, in animal models of diabetes we previously reported that OFS treatment in-

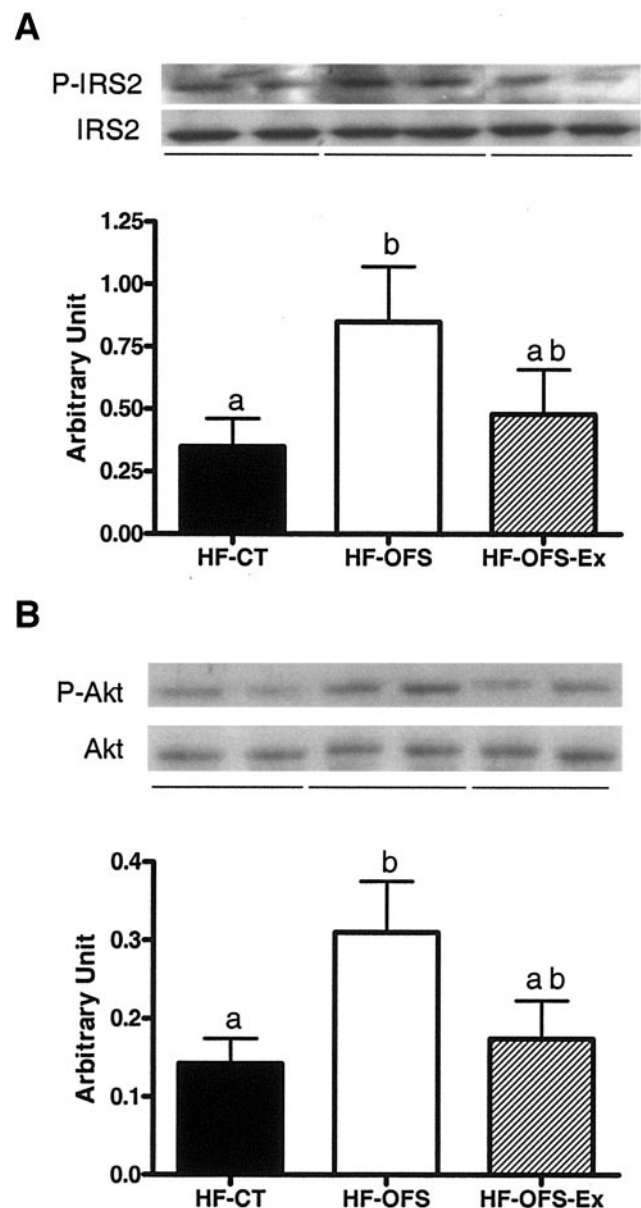


FIG. 3. Representative Western blot of IRS-2 (A) and Akt (B) at completion of hyperinsulinemic clamps. Total and phosphorylated form of IRS-2 (A) and Akt (B) in the liver of mice at completion of a 3-h euglycemic-hyperinsulinemic clamp. Arbitrary units represent the phosphorylated (P)-to-total form ratio for each protein in HF-OFS ($n = 8$), HF-CT ($n = 8$), and HF-OFS-Ex-9 ($n = 8$) mice after a 4-week period of high-fat feeding. Data are mean \pm SE. Data with different superscript letters are significantly different ($P < 0.05$), according to the post hoc ANOVA statistical analysis convention.

creased levels of proglucagon mRNA transcripts in the proximal colon and enhanced concentrations of GLP-1 in the hepatoportal vein (17,18).

We now extend these findings by demonstrating that a 4-week OFS treatment reduced the development of glucose intolerance and both fasted and fed hyperglycemia following high-fat feeding. Although the dietary treatment used (high-fat, carbohydrate-free diet) was not standard, the OFS treatment was accompanied by an increased plasma insulin concentration, in association with higher levels of colonic proglucagon mRNA, GLP-1 content, and circulating levels of GLP-1. Furthermore, the antidiabetic effects of OFS were largely dependent on GLP-1 action, as

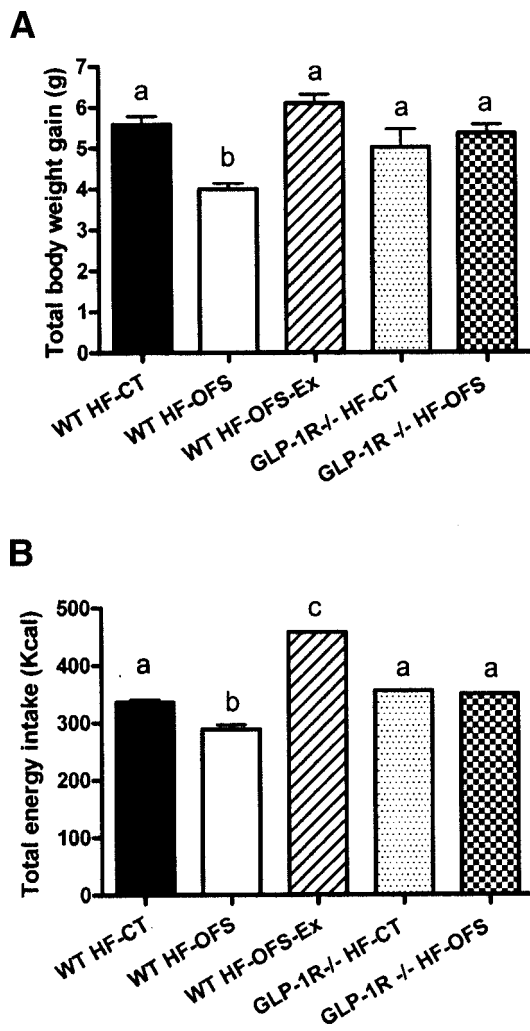


FIG. 4. Total body weight and energy intake. Total body weight gain (A) and total cumulative energy intake (B) in diabetic (HF), wild-type (WT), or GLP-1 receptor knockout (GLP-1R^{-/-}) ($n = 5/\text{group}$) mice treated for 28 days with ($n = 10$) or without (CT; $n = 8$) OFS or OFS plus Ex-9 (Ex; $n = 9$). Data are mean \pm SE. Data with different superscript letters are significantly different ($P < 0.05$), according to the post hoc ANOVA statistical analysis convention.

disruption of GLP-1R function, by infusing Ex-9, prevented the majority of beneficial effects observed following OFS treatment. We further confirmed the importance of GLP-1R-dependent pathways for the actions of OFS using GLP-1R^{-/-} mice fed a high-fat diet. GLP-1R^{-/-} mice display fasting hyperglycemia, glucose intolerance, and reduced plasma insulin levels in response to an oral glucose challenge (19). However, glucose intolerance was not observed in response to an intravenous glucose challenge, showing the importance of GLP-1 as an incretin effector. Surprisingly, GLP-1R^{-/-} mice gained similar weight to wild-type mice under high-fat diet (27). This would suggest that GLP-1 is not mainly involved in the control of food intake or that adaptive mechanisms occurred following the mutation. The latter hypothesis has been proposed, and the authors showed that the lack of GLP-1 receptor was compensated by upregulating both glucose-dependent insulinotropic polypeptide plasma levels and action of glucose-dependent insulinotropic polypeptide on pancreatic islets (28,29). No beneficial effects of OFS treatment were observed in GLP-1R^{-/-} mice. Hence, using two independent experimental approaches, we demonstrate

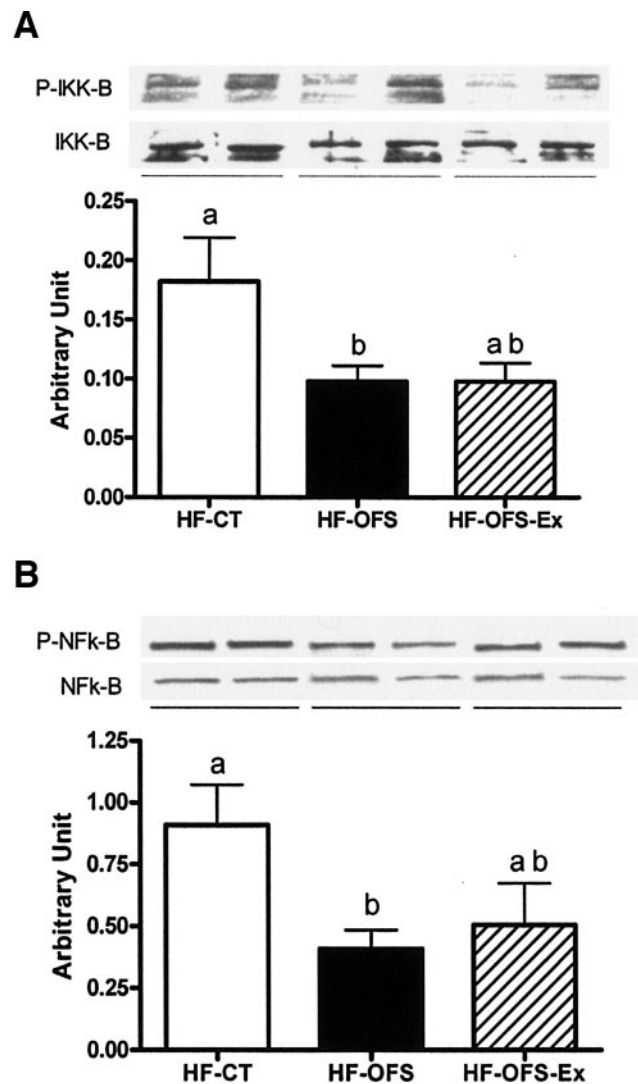


FIG. 5. Representative Western blot of IKK- β (A) and NF- κ B (B) at basal level. Total and phosphorylated form of IKK- β (A) and NF- κ B (B) in the liver of mice. Arbitrary units represent the phosphorylated (P)-to-total form ratio for each protein in HF-OFS ($n = 8$), HF-CT ($n = 8$), and HF-OFS-Ex-9 ($n = 8$) mice after a 4-week period of high-fat feeding. Data are mean \pm SE. Data with different superscript letters are significantly different ($P < 0.05$), according to the post hoc ANOVA statistical analysis convention.

that the antidiabetic effect of OFS requires a functional GLP-1 receptor.

To ascertain the mechanism whereby OFS improves glucose tolerance, we further assessed insulin action following OFS administration. Four weeks of OFS treatment did not improve insulin-stimulated whole-body or individual tissue glucose utilization. However, hepatic insulin resistance was reverted by OFS treatment. At the molecular level, the liver content of phosphorylated IRS2 and Akt was increased by OFS, features prevented by Ex-9 infusion. These results are consistent with a model whereby OFS feeding increases the colonic content and release of GLP-1, leading to stimulation of insulin secretion and reduction of hepatic glucose production, fasting glycemia, and glucose intolerance.

We and others have previously shown that extra pancreatic effects of GLP-1 contribute to the regulation of glucose homeostasis (30–32). The blockade of the hepatoportal glucose sensor by a continuous infusion of Ex-9

into the corresponding vein prevented the regulatory role of this glucose sensor on glucose metabolism (33). This was further confirmed in dogs (34) but remains contradictory (35,36), depending on the model studied. Balkan et al. (37) showed that portal GLP-1 increased insulin secretion through a mechanism that requires the autonomic nervous system to transmit the glucose signal to the pancreas. Similarly, we recently showed that GLP-1 in the brain controls glucose-dependent insulin secretion (20), further supporting the important role of extrapancreatic GLP-1 actions for the control of glucose homeostasis. However, mRNA concentration of the proglucagon gene was quantified by RT-PCR in hypothalami from HF-CT- and HF-OFS-treated mice. No differences were noted between groups. This data could suggest that OFS has no effect on brain GLP-1 but does not rule out that OFS could control GLP-1 secretory pathway.

The available evidence suggests that GLP-1 released from intestinal L-cells may interact with afferent sensory nerve fibers arising from the nodose ganglions, which send impulses to the nucleus of the solitary tract and onwards to the hypothalamus, which may be transmitted to the pancreas (38). Consequently, releasing GLP-1 directly into the portal vein may represent an important feature of the mechanisms related to OFS-improved glucose homeostasis. OFS treatment by stimulating GLP-1 secretion or the use of dipeptidyl peptidase-IV inhibitors by preventing GLP-1 degradation (39) represent strategies for potentiating the action of GLP-1 released into the hepatportal circulation.

Intriguingly, the reduction of body weight gain by OFS treatment was prevented by functional inactivation of the GLP-1 receptor. This result could be due to the satiating effects of endogenous GLP-1 (40–42) since in our model energy consumption was reduced by OFS treatment and increased by Ex-9 infusion. Furthermore and consistent with our findings, body weight gain and food intake were increased in normal rats receiving repeated intracerebroventricular administration of Ex-9 (43).

To gain some insights on putative intracellular factors involved in the reversal of hepatic insulin resistance during the OFS treatment, we quantified markers of inflammation IKK- β and NF- κ B in the liver, since inflammation has been recently suggested as a link between obesity and insulin resistance (23,24). Intriguingly, the improved hepatic insulin sensitivity observed in HF-OFS mice was associated with a twofold reduction of levels of phosphorylated IKK- β and NF- κ B. This data further confirm that OFS may reduce the hepatic inflammatory status of the diabetic mice, as we previously described (44), thus improving insulin sensitivity. Importantly, the lowering of the inflammatory mediators IKK- β and NF- κ B in the liver was not prevented by Ex-9 treatment as insulin sensitivity was. This discrepancy remains unexplained.

In conclusion, OFS attenuates the development of diabetes and obesity induced by high-fat feeding in mice through a mechanism requiring a functional GLP-1 receptor. We further demonstrate that an important physiological mechanism responsible for the OFS-mediated improvement in glucose tolerance was an increase in hepatic insulin sensitivity. In the light of our recent data (20), we could speculate that some of the effect of OFS might be dependant on GLP-1 in the brain. Whether OFS may generate similar GLP-1-dependent beneficial effects in humans without unanticipated undesirable side effects remains to be determined.

ACKNOWLEDGMENTS

P.D.C. is a teaching assistant (Université catholique de Louvain) and was supported by grants from the Fond National de la Recherche Scientifique (FNRS) as “Crédit pour Bref Séjour à l'étranger” and FSR from the Université catholique de Louvain. This work was also supported by grants from the ATIP Centre National De La Recherche Scientifique program (to R.B.) and by grants from the Canada Research Chairs Program, the Canadian Diabetes Association, and the Juvenile Diabetes Research Foundation (to D.J.D.).

The authors would like to acknowledge the excellent technical assistance of Nicole Maton, Elodie Bernard, and André Pech.

REFERENCES

- Parillo M, Riccardi G: Diet composition and the risk of type 2 diabetes: epidemiological and clinical evidence. *Br J Nutr* 92:7–19, 2004
- Boden G, Shulman GI: Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. *Eur J Clin Invest* 32 (Suppl. 3):14–23, 2002
- Burcelin R, Crivelli V, Dacosta A, Roy-Tirelli A, Thorens B: Heterogeneous metabolic adaptation of C57BL/6J mice to high-fat diet. *Am J Physiol Endocrinol Metab* 282:E834–E842, 2002
- Perseghin G, Petersen K, Shulman GI: Cellular mechanism of insulin resistance: potential links with inflammation. *Int J Obes Relat Metab Disord* 27 (Suppl. 3):S6–S11, 2003
- Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H: Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112:1821–1830, 2003
- Hegarty BD, Furler SM, Ye J, Cooney GJ, Kraegen EW: The role of intramuscular lipid in insulin resistance. *Acta Physiol Scand* 178:373–383, 2003
- Bonner G: Hyperinsulinemia, insulin resistance, and hypertension. *J Cardiovasc Pharmacol* 24 (Suppl. 2):S39–S49, 1994
- Koopmans SJ, Ohman L, Haywood JR, Mandarino LJ, DeFronzo RA: Seven days of euglycemic hyperinsulinemia induces insulin resistance for glucose metabolism but not hypertension, elevated catecholamine levels, or increased sodium retention in conscious normal rats. *Diabetes* 46:1572–1578, 1997
- Yki-Jarvinen H: Fat in the liver and insulin resistance. *Ann Med* 37:347–356, 2005
- Raz I, Eldor R, Cernea S, Shafrir E: Diabetes: insulin resistance and derangements in lipid metabolism: cure through intervention in fat transport and storage. *Diabetes Metab Res Rev* 21:3–14, 2005
- Holst JJ, Gromada J: Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *Am J Physiol Endocrinol Metab* 287:E199–E206, 2004
- Zander M, Madsbad S, Madsen JL, Holst JJ: Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 359:824–830, 2002
- Delzenne NM, Williams CM: Prebiotics and lipid metabolism. *Curr Opin Lipidol* 13:61–67, 2002
- Piche T, des Varannes SB, Sacher-Huvelin S, Holst JJ, Cuber JC, Galmiche JP: Colonic fermentation influences lower esophageal sphincter function in gastroesophageal reflux disease. *Gastroenterology* 124:894–902, 2003
- Cani PD, Joly E, Horsmans Y, Delzenne NM: Oligofructose promotes satiety in healthy human: a pilot study. *Eur J Clin Nutr* 2005 [Epub ahead of print]
- Yamashita K, Itakura M, Kawai K: Effects of fructo-oligosaccharides on blood glucose and serum lipids in diabetics subjects. *Nutr Res* 4:961–966, 1984
- Cani PD, Neyrinck AM, Maton N, Delzenne NM: Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like peptide-1. *Obes Res* 13:1000–1007, 2005
- Cani PD, Daubioul CA, Reusens B, Remacle C, Catillon G, Delzenne NM: Involvement of endogenous glucagon-like peptide-1(7-36) amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats. *J Endocrinol* 185:457–465, 2005
- Scrocchi LA, Brown TJ, McClusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ: Glucose intolerance but normal satiety in mice with a null

- mutation in the glucagon-like peptide 1 receptor gene. *Nat Med* 2:1254–1258, 1996
20. Knauf C, Cani PD, Perrin C, Iglesias MA, Maury J, Bernard E, Benhamed F, Gremeaux T, Drucker DJ, Kahn CR, Girard J, Tanti J, Delzenne NM, Postic C, Burcelin R: Brain glucagon-like peptide-1 increases insulin secretion and glucose insulin resistance to favor hepatic glycogen storage. *J Clin Invest* 112:3554–3563, 2005
 21. Postic C, Shiota M, Niswender KD, Jetton TL, Chen Y, Moates JM, Shelton KD, Lindner J, Cherrington AD, Magnuson MA: Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. *J Biol Chem* 274:305–315, 1999
 22. DeFronzo R, Simonson D, Ferannini E: Hepatic and peripheral insulin resistance: a common feature of type 2 (non-insulin-dependent) and type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 23:313–319, 1982
 23. Pickup JC, Crook MA: Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia* 41:1241–1248, 1998
 24. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE: Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* 11:183–190, 2005
 25. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M: IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* 11:191–198, 2005
 26. Rohl M, Pasparakis M, Baudler S, Baumgartl J, Gautam D, Huth M, De Lorenzi R, Krone W, Rajewsky K, Bruning JC: Conditional disruption of IkkappaB kinase 2 fails to prevent obesity-induced insulin resistance. *J Clin Invest* 113:474–481, 2004
 27. Scrocchi LA, Drucker DJ: Effects of aging and a high fat diet on body weight and glucose tolerance in glucagon-like peptide-1 receptor(-/-) mice. *Endocrinology* 139:3127–3132, 1998
 28. Flamez D, Van Breusegem A, Scrocchi LA, Quartier E, Pipeleers D, Drucker DJ, Schuit F: Mouse pancreatic β -cells exhibit preserved glucose competence after disruption of the glucagon-like peptide-1 receptor gene. *Diabetes* 47:646–652, 1998
 29. Pederson RA, Satkunarajah M, McIntosh CHS, Scrocchi LA, Flamez D, Schuit F, Drucker DJ, Wheeler MB: Enhanced glucose-dependent insulinotropic polypeptide secretion and insulinotropic action in glucagon-like peptide 1 receptor^{-/-} mice. *Diabetes* 47:1046–1052, 1998
 30. Vella A, Rizza RA: Extraprostatic effects of GIP and GLP-1. *Horm Metab Res* 36:830–836, 2004
 31. Burcelin R: The incretins: a link between nutrients and well-being. *Br J Nutr* 93 (Suppl. 1):S147–S156, 2005
 32. Ahren B: Sensory nerves contribute to insulin secretion by glucagon-like peptide-1 in mice. *Am J Physiol Regul Integr Comp Physiol* 286:R269–R272, 2004
 33. Burcelin R, Da Costa A, Drucker D, Thorens B: Glucose competence of the hepatoportal vein sensor requires the presence of an activated glucagon-like peptide-1 receptor. *Diabetes* 50:1720–1728, 2001
 34. Ionut V, Hucking K, Liberty IF, Bergman RN: Synergistic effect of portal glucose and glucagon-like peptide-1 to lower systemic glucose and stimulate counter-regulatory hormones. *Diabetologia* 48:967–975, 2005
 35. Dardevet D, Moore MC, Neal D, DiCostanzo CA, Snead W, Cherrington AD: Insulin-independent effects of GLP-1 on canine liver glucose metabolism: duration of infusion and involvement of hepatoportal region. *Am J Physiol Endocrinol Metab* 287:E75–E81, 2004
 36. Dardevet D, Moore MC, DiCostanzo CA, Farmer B, Neal DW, Snead W, Lautz M, Cherrington AD: Insulin secretion-independent effects of glucagon-like peptide 1 (GLP-1) on canine liver glucose metabolism do not involve portal vein GLP-1 receptors. *Am J Physiol Gastrointest Liver Physiol* 289:G806–G814, 2005
 37. Balkan B, Li X: Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. *Am J Physiol Regul Integr Comp Physiol* 279:R1449–R1454, 2000
 38. Nakabayashi H, Nishizawa M, Nakagawa A, Takeda R, Nijima A: Vagal hepatopancreatic reflex effect evoked by intraportal appearance of tGLP-1. *Am J Physiol* 271:E808–E813, 1996
 39. Deacon CF, Knudsen LB, Madsen K, Wiberg FC, Jacobsen O, Holst JJ: Dipeptidyl peptidase IV resistant analogues of glucagon-like peptide-1 which have extended metabolic stability and improved biological activity. *Diabetologia* 41:271–278, 1998
 40. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR: A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379:69–72, 1996
 41. Van Dijk G, Lindskog S, Holst JJ, Steffens AB, Ahren B: Effects of glucagon-like peptide-I on glucose turnover in rats. *Am J Physiol* 270: E1015–E1021, 1996
 42. Rodriguez, dF, Navarro M, Alvarez E, Roncero I, Chowen JA, Maestre O, Gomez R, Munoz RM, Eng J, Blazquez E: Peripheral versus central effects of glucagon-like peptide-1 receptor agonists on satiety and body weight loss in Zucker obese rats. *Metabolism* 49:709–717, 2000
 43. Meeran K, O'Shea D, Edwards CM, Turton MD, Heath MM, Gunn I, Abusnana S, Rossi M, Small CJ, Goldstone AP, Taylor GM, Sunter D, Steere J, Choi SJ, Ghatei MA, Bloom SR: Repeated intracerebroventricular administration of glucagon-like peptide-1-(7-36) amide or exendin-(9-39) alters body weight in the rat. *Endocrinology* 140:244–250, 1999
 44. Neyrinck AM, Alexiou H, Delzenne NM: Kupffer cell activity is involved in the hepatoprotective effect of dietary oligofructose in rats with endotoxic shock. *J Nutr* 134:1124–1129, 2004