

## Original Article

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

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Non-digestible fermentable dietary fibers such as oligofructose (OFS) exert an anti-diabetic 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<sup>-/-</sup>) were completely insensitive to the anti-diabetic 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- $\kappa$ B and IKK- $\beta$  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 anti-diabetic 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* 55:000–000, 2006

**T**he 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  $\beta$ -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) affecting primarily 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 for 4 weeks. Furthermore, the anti-diabetic 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) <sup>-/-</sup> mice (in a C57BL/6J background, from our colony) were housed in a controlled environment (inverted 12-h daylight cycle, lights off at 10 A.M.) with free access to food and water in groups of 5 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

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ELISA, enzyme-linked immunosorbent assay; GLP-1, glucagon-like peptide 1; IRS, insulin receptor substrate; OFS, oligofructose; NF- $\kappa$ B, nuclear factor- $\kappa$ B.

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TABLE 1  
Plasma, pancreatic, and intestinal hormone characteristics

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

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

(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 (personal observation).

#### Surgical procedures

**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 Exendin 9–39 (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, 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-hours' 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 D-(3H)3-glucose (Perkin Elmer, Boston, MA) was infused at rate of  $30 \text{ } \mu\text{Ci} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  to ensure a detectable plasma D-(3H)3-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% (w/v) glucose. Plasma glucose concentrations and D-(3H)3-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) (DPP-IV inhibitor; Linco Research, St Charles, MO); after centrifugation, plasma was stored at  $-80^{\circ}\text{C}$ .

**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 hours' fasted mice received an intraperitoneal injection of glucose (1g/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  $-80^{\circ}\text{C}$ . 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 preoxi-

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), 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 hours) 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; Linco Research), as previously described (17,18). Plasma B-hydroxybutyrate was assessed from 5 μl of plasma sampled in post-absorptive conditions using a colorimetric method (Stanbio, Boerne).

**Pancreatic insulin content.** Pancreata were harvested, weighed, and immediately frozen into liquid nitrogen and kept at  $-80^{\circ}\text{C}$  before assay. The day of the assay, pancreata were homogenized at  $+4^{\circ}\text{C}$  into ethanol/acid (HCl 0.15 mmol, ethanol 75% v/v water), kept at  $4^{\circ}\text{C}$  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 observed in GLP-1R<sup>-/-</sup> mice. The intestinal content of proglucagon mRNA was increased by OFS treatment (Table 1), and OFS also significantly increased the portal

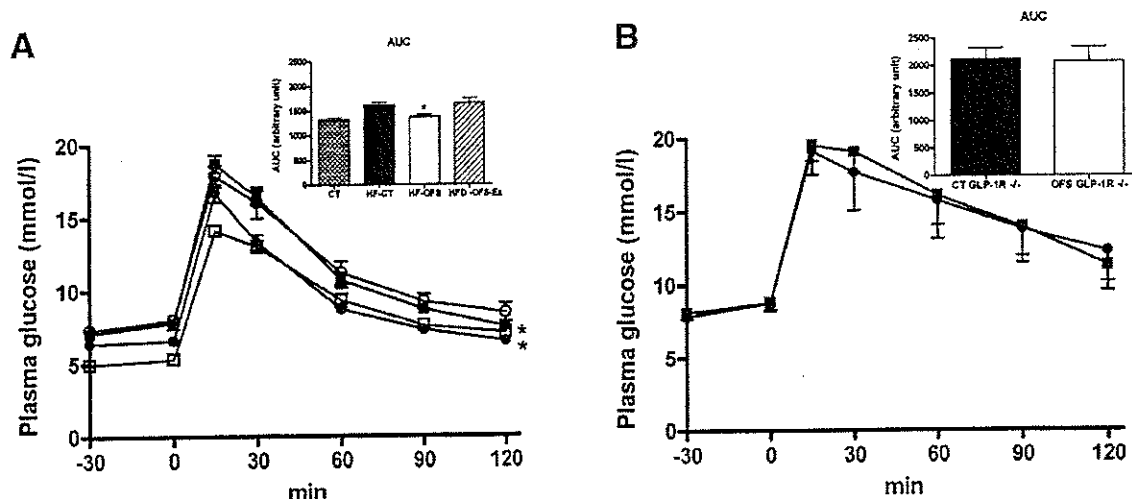


FIG. 1. Effect of high-fat diet on intraperitoneal glucose tolerance. A: Intraperitoneal glucose tolerance in CT ( $\square$ ;  $n = 6$ ), HF-OFS ( $\bullet$ ;  $n = 10$ ), HF-CT ( $\blacksquare$ ;  $n = 8$ ), and HF-OFS-Ex-9 ( $\circ$ ;  $n = 9$ ) mice after a 4-week period of high-fat feeding. Area 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  $\pm$  SE.

plasma GLP-1 concentration:  $15.6 \pm 0.3$  vs.  $25 \pm 2$  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:  $89.6 \pm 14.8$  vs.  $189.6 \pm 19.7$ ,  $P < 0.05$ ; distal colon:  $141.8 \pm 7.9$  vs.  $141.0 \pm 7.8$ ; cecum:  $29.7 \pm 5.9$  vs.  $23.9 \pm 5.5$  pmol/g, in control and OFS-treated mice, respectively. B hydroxybutyrate was also assessed to determine whether differences in ketogenesis occurred. Plasma B-hydroxybutyrate concentration was slightly increased by the high-fat diet treatment ( $90 \pm 10$  vs.  $121 \pm 20$   $\mu$ mol/l in CT and HF mice, respectively). Neither the OFS or Ex-9 treatments significantly changed the concentration ( $132 \pm 30$  vs.  $117 \pm 10$   $\mu$ mol/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 a glucose infusion maintaining physiological concentration of insulin with a range of  $326 \pm 18$ ,  $347 \pm 45$ , or  $357 \pm 54$  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 ( $1.14 \pm 0.34$ ,  $1.16 \pm 0.12$ , and  $1.15 \pm 0.42$  ml  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$  in HF-CT, vs. HF-OFS, and vs. HF-OFS-Ex, respectively). To ascertain whether

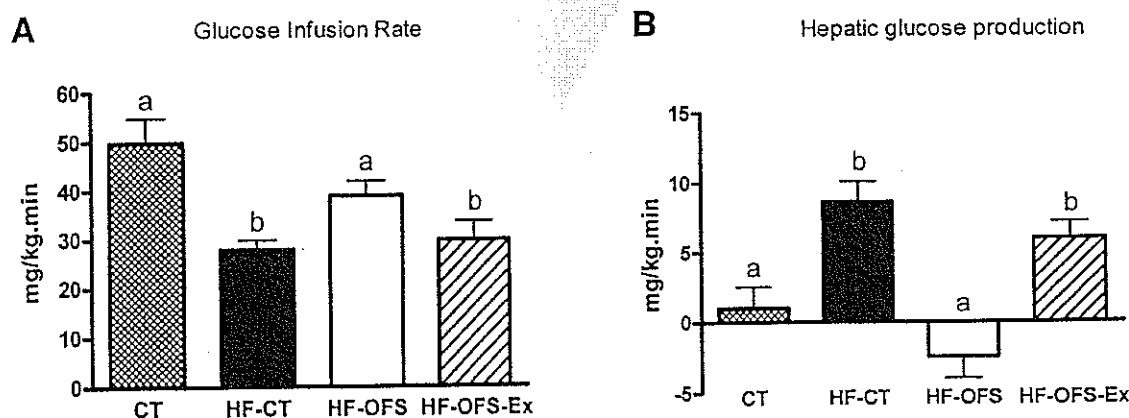


FIG. 2. GLP-1-dependent control of whole-body glucose infusion rate and hepatic glucose production. Glucose infusion rate (A) and hepatic glucose production (B) were calculated ( $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) in steady-state euglycemic ( $5.5$  mmol/l) hyperinsulinemic clamp ( $4\text{mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) 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  $\pm$  S.E.M. Data with different superscript letters are significantly different  $P < 0.05$ , according to the post hoc ANOVA statistical analysis convention.

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  $26.4 \pm 0.6$  g vs. HF-CT  $32.5 \pm 0.8$  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 about 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<sup>-/-</sup> mice.

**OFS reduces hepatic phosphorylation of IKK- $\beta$  and NF- $\kappa$ -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- $\beta$  and NF- $\kappa$ -B in the liver (24–26). The level of phosphorylated IKK- $\beta$  and NF- $\kappa$ -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- $\beta$  and NF- $\kappa$ -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 increased levels of proglucagon mRNA transcripts in the

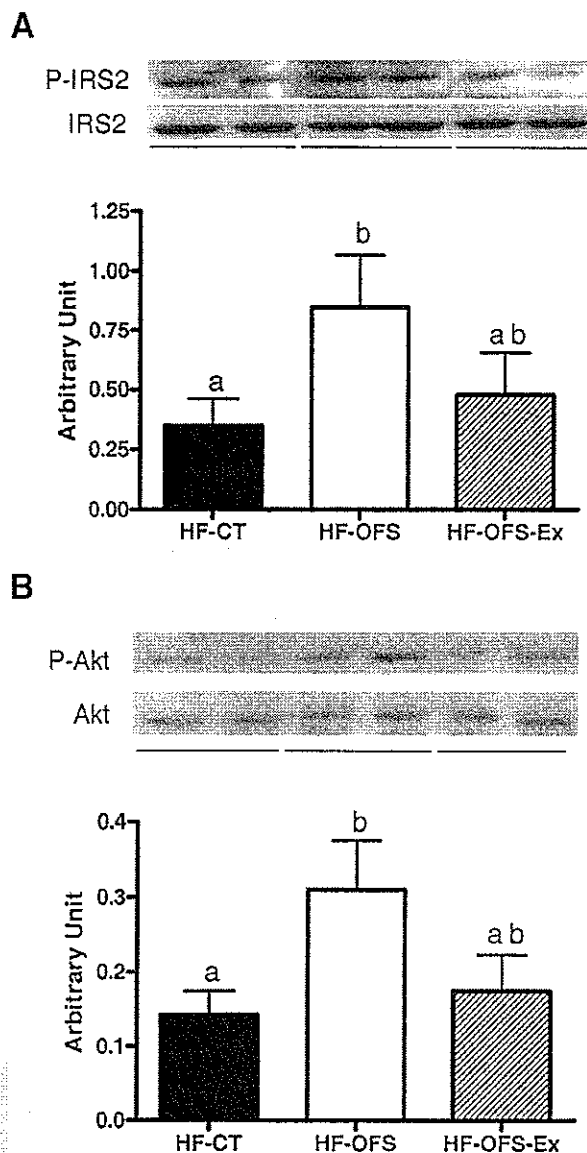


FIG. 3. Representative Western blot of IRS-2 (A) and Akt (B) at completion of hyperinsulinemic clamps. Total and phosphorylated form of IRS-2 (A) and Akt (B) in the liver of mice at completion of a 3-h euglycemic-hyperinsulinemic clamp. Arbitrary units represent the phosphorylated (P-) over total-form 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.

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 disruption of GLP-1R function, by infusing Ex-9, prevented

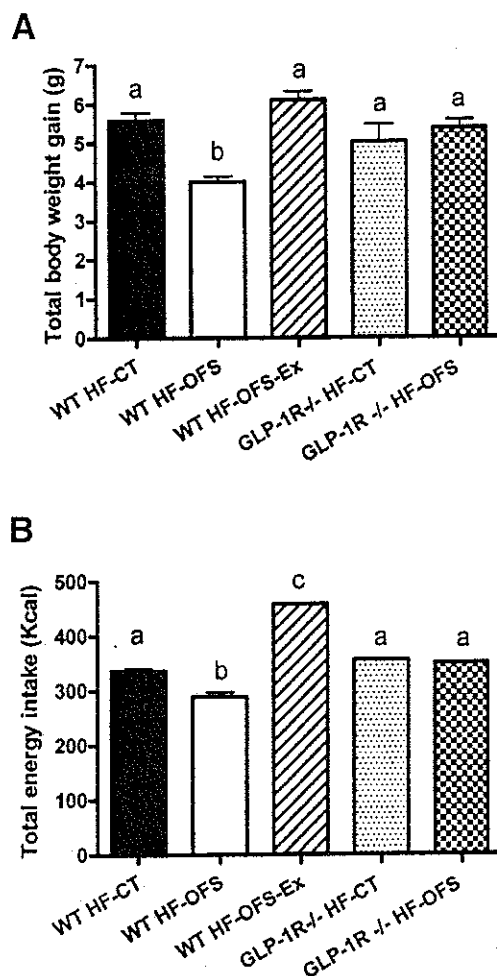


FIG. 4. Total body weight and energy intake. Total body weight gain (A) and total cumulative energy intake (B) in diabetic (HF), wild-type (WT), or GLP-1 receptor knockout (GLP-1R<sup>-/-</sup>) ( $n = 5$ /group) mice treated for 28 days with ( $n = 10$ ) or without (CT;  $n = 8$ ) OFS or OFS plus Ex-9 (Ex;  $n = 9$ ) mice. 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.

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<sup>-/-</sup> mice fed a high-fat diet. GLP-1R<sup>-/-</sup> 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<sup>-/-</sup> 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 GIP plasma levels and action of GIP on pancreatic islets (28,29). No beneficial effects of OFS treatment were observed in GLP-1R<sup>-/-</sup> mice. Hence, using two independent experimental approaches, we demonstrate that the antidiabetic effect of OFS requires a functional GLP-1 receptor.

To ascertain the mechanism whereby OFS improves

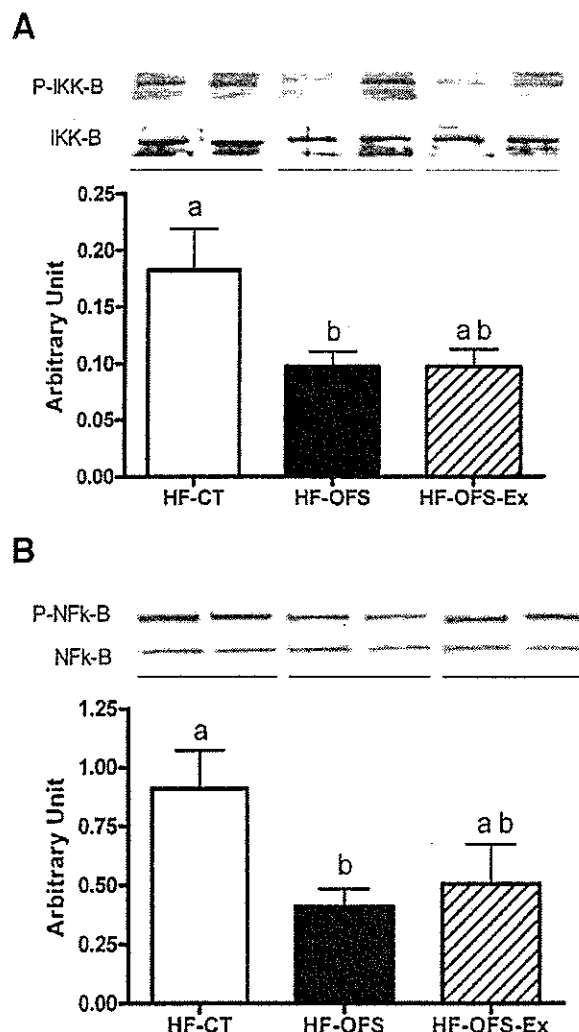


FIG. 5. Representative Western blot of IKK- $\beta$  (A) and NF- $\kappa$ B (B) at basal level. Total and phosphorylated form of IKK- $\beta$  (A) and NF- $\kappa$ B (B) in the liver of mice. Arbitrary units represent the phosphorylated (P-) over total-form ratio for each protein in HF-OFS ( $n = 8$ ), 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.

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 contradic-

tory (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 hepatoportal 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- $\beta$  and NF- $\kappa$ 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- $\beta$  and NF- $\kappa$ 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- $\beta$  and NF- $\kappa$ 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.

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