Combination of obesity and high-fat feeding diminishes sensitivity to GLP-1R agonist, Exendin-4

Frank A Duca\textsuperscript{1,2,3}, Yassine Sakar\textsuperscript{1,2}, Mihai Covasa\textsuperscript{1,2,4,5}

\textsuperscript{1}INRA, UMR 1319 Micalis, Neurobiology of Ingestive Behavior, F-78352 Jouy-en-Josas, France
\textsuperscript{2}AgroParisTech, UMR Micalis, F-78352 Jouy-en-Josas, France
\textsuperscript{3}Université Pierre-et-Marie-Curie, Paris, France
\textsuperscript{4}Western University of the Health Sciences, College of Osteopathic Medicine, Department of Basic Medical Sciences, Pomona, CA
\textsuperscript{5}University “Stefan cel Mare” Suceava, Department of Health and Human Development, Suceava, Romania

Short title: GLP-1 and obesity

Address for correspondence:
INRA, Centre de Recherche de Jouy-en-Josas, UMR 1319 MICALIS, Neurobiology of Ingestive Behavior, Domaine de Vilvert, 78350 Jouy-en-Josas, FRANCE
E-mail: mcovasa@jouy.inra.fr
Tel: +33 (0)1 34 65 27 78
Fax: +33 (0)1 34 65 24 92

Word Count: 1998
Number of Tables and Figures: 4
Abstract

Gastrointestinal mechanisms involved in the suppression of appetite are compromised in obesity. Glucagon-like peptide-1 (GLP-1) is released in response to nutrients, suppresses food intake, and has been shown to play a role in regulation of energy balance. It is not known whether obese-prone (OP) rats exhibit dysfunctional GLP-1 signaling that could contribute to decreased nutrient induced satiation and hyperphagia. Therefore, we examined the effects of exogenous IP administration of GLP-1R agonist, exendin-4 (Ex-4) on food intake in OP and obese-resistant (OR) rats during chow or high-energy/high-fat (HE/HF) feeding. All doses of Ex-4 effectively suppressed intake in both OP and OR rats on chow, however, during HE/HF-feeding, OP rats suppressed intake significantly less than OR rats at all Ex-4 doses tested. This was associated with downregulation of GLP-1R mRNA expression in the vagal nodose ganglia of OP rats. Furthermore, HE/HF-fed OP rats had significantly less plasma GLP-1 levels, decreased protein levels of GLP-1 in the intestinal epithelium, and reduced number of L-cells in the distal ileum. These results demonstrate that HE/HF-feeding coupled with OP phenotype results in reduced endogenous GLP-1 and GLP-1R activation, indicating that impaired GLP-1 signaling during obesity may exacerbate hyperphagia and weight gain.
The number of overweight and obese individuals continues to increase as new estimates predict that over 50% of the United States’ population will be obese by 2030 (1). The interaction between genetics and the environment, such as dietary and lifestyle influences, plays a major role in the development of obesity, as up to 70% of human obesity is inherited in a polygenic fashion (2,3). Therefore, the use of select inbred obesity-prone (OP) and obesity-resistant (OR) polygenetic rodent models reflecting human obesity provides a useful means of unraveling these interactions. When exposed to a hypercaloric, high-fat (HE/HF) diet, OP rats become obese, which is accompanied by increased caloric intake (4) possibly from impaired post-ingestive intestinal feedback signaling (5,6). Recently, we showed that diet-induced obese rats fed HF-diet are less sensitive to the suppressive effects of lipid gastric loads, and this was associated with decreased expression of several gut peptides (5). Similarly, obese humans exhibit decreased levels of gut peptides, such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY (7). Therefore, it is plausible that HE/HF-feeding in those susceptible to obesity leads to alterations in intestinal peptide signaling, resulting in increased energy intake and subsequent weight gain.

GLP-1 is a potent incretin and plays a physiological role in satiation, as administration of GLP-1 and GLP-1R agonists (exendin-4 and liraglutide) reduce food intake, while blockade of GLP-1R increases intake and attenuates nutrient-induced satiety (8). Several studies have implicated GLP-1 in the pathogenesis of obesity (7). For example, obese humans exhibit blunted post-prandial GLP-1 release (9), while weight loss following bariatric surgery results in increased plasma GLP-1 (10). In the rat, HF-feeding causes decreased circulating GLP-1 and attenuated anorexigenic response to GLP-1 and exendin-4 (Ex-4) (11). However, no study has addressed the role of GLP-1 in the obese-prone animal model encompassing the gene and environment interaction that closely resembles human obesity, allowing us to distinguish
between the effects of the phenotype from those of the diet. Therefore, in this study, we first examined the effect of GLP-1R agonist, Ex-4, on food intake in OP and OR rats maintained on either chow or HE/HF-diet. Second, to determine if changes in sensitivity to Ex-4 are the result of alterations in vagal afferent receptors, we evaluated GLP-1R mRNA expression in the nodose ganglia. Third, we assessed intestinal peptide protein expression and circulating levels of GLP-1, and quantified GLP-1 expressing enteroendocrine cells (EECs) in the distal ileum to determine if HE/HF-feeding leads to decreases in endogenous GLP-1 in OP rats.

**Research Design and Methods**

**Animals**

Twenty OP and OR male rats (n=10 per phenotype, Charles River, MA) were housed individually in a temperature controlled vivarium with 12:12 h light/dark cycle (lights on at 0700). Except where otherwise noted, rats had ad libitum access to standard rat chow (SDS Diets, 3.1kcal/g, Essex, UK). All experiments were carried out in accordance with the European Guidelines for the Care and Use of Laboratory Animals.

**Feeding responses to Ex-4**

Eight-week-old OP and OR rats (287.1 ± 3g and 223.3 ± 3g respectively) were separated into two groups (n=5 per phenotype and diet) and placed on either chow or HE/HF-diet (Research Diets, NJ, D12334B, 4.2 kcal/g) for 5 weeks before testing. Following 16 hr fast (17:00-9:00), rats were given either Ex-4 (American Peptides, Sunnyvale, CA) or saline vehicle. Food intake was measured at 1, 3, and 24 h post injection. Ex-4 (0.625, 1.25, 2.5, 5 µg/kg, IP) was administered in random order, at least twice, with each dose bracketed by vehicle injection, with a minimum of 72hr elapsing between injections.
**Tissue Collection**

Four weeks after Ex-4 tests, animals were sacrificed, and proximal intestinal epithelial cells and bilateral nodose ganglia were collected and processed for quantitative Real-Time PCR and western blotting (5). Three cm sections of the distal ileum were also removed for confocal immunofluorescence. Plasma was extracted from vena-cava blood and total and active GLP-1 were measured with ELISA (Millipore). Fat pads (retroperitoneal, visceral, epididymal) were removed, weighed, and adiposity index calculated (total fat/ body weight*100).

**Immunofluorescence**

Distal ileum was fixed, and 4-µm paraffin-cut sections were incubated with rabbit polyclonal antibody raised against GLP-1 (1:200, Abcam, France ab22625) followed by donkey anti-rabbit IgG H&L (DyLight 488) secondary antibody (1:200, Abcam, ab96891). Images were acquired with LSM510 confocal microscope (LSM Image Browser) and GLP-1 containing EECs were quantified by counting both total villi and GLP-1 positive cells throughout the entire length of the section (over 20, non-overlapping microscopic areas) for each animal.

**Statistical Analyses**

All statistics were computed with GraphPad Prism 5 (La Jolla, CA), and Statistical Analysis Software (SAS, version 9.1.3 Cary, N.C.). Bi-weekly average body weights and 24h food intake were analyzed with rmANOVA and *post hoc* Bonferroni adjustment. For all Ex-4 tests, raw food intake as well as percent suppression of food intake was analyzed by three-way (phenotype, treatment, diet) rmANOVA with *post hoc* Bonferroni adjustment. Fat pads, plasma GLP-1, qPCR, western blots, and GLP-1 cell counts were all analyzed by two-way ANOVA (diet x phenotype) with Bonferroni post-hoc tests. Significance was considered at $\alpha < 0.05$ for all tests.
Results

Body weight, adiposity and 24-h food intake

OP rats were significantly heavier than OR rats two weeks after HE/HF-feeding (P<0.0001) (Fig. 1A), and their chow-fed counterparts after 6 weeks of HE/HF-feeding, (P<0.0001). OP rats consumed more calories from HE/HF diet than OR (P=0.0057) or OP fed chow rats (P<0.001) (Fig. 1B). In addition, OP rats had significantly larger adiposity index than OR rats fed the HE/HF-diet (P<0.0001) (Fig. 1C), with no difference during chow-feeding.

Sensitivity to Ex-4

Ex-4 produced a significant reduction in 1h and 3h food intake in rats maintained on chow irrespective of phenotype (P <0.05). However, while the lowest doses of Ex-4 (0.625 and 1.25µg/kg) suppressed intake significantly in OR rats fed the HE/HF-diet, it failed to decrease 1h and 3h food intake in OP rats maintained on the same diet (P>0.05) (Table 1). The two highest doses of Ex-4 (2.5 and 5.0 µg/kg) reduced food intake at 1, 3 and 24h compared to saline and this effect was significant for both diets and phenotypes. Furthermore, percent suppression of food intake at 1h and 3h was significantly lower in OP compared to OR rats fed HE/HF diet for all Ex-4 doses tested (P<0.05). However, at 24h, there were no significant differences in percent suppression between OP and OR rats fed either chow or HE/HF-diet.

GLP-1 protein and mRNA expression in intestinal epithelium

There was a significant decrease in GLP-1 protein, but not mRNA, expression in HE/HF-fed OP rats compared to HE/HF-fed OR rats and chow-fed OP rats (P<0.05 for both) (Fig. 2A).
**GLP-1R mRNA expression in the nodose ganglia**

HE/HF-feeding resulted in significant downregulation of nodose ganglia GLP-1R mRNA in OP, but not OR, rats (P<0.05) (Fig. 2B). There were no significant differences in GLP-1R mRNA expression between phenotypes when rats were maintained on chow.

**Circulating GLP-1**

Total and active GLP-1 plasma levels were significantly decreased in HE/HF-fed OP rats compared to both HE/HF-fed OR rats (total: P<0.01; active: P<0.001) and chow-fed OP rats (total: P<0.05; active: P<0.01) (Fig. 2C-D). There was no significant difference in circulating GLP-1 between phenotypes when rats were maintained on chow, and there was no effect of the diet on GLP-1 in OR rats.

**Immunofluorescence**

GLP-1 was present in cells lining the villi of the distal ileum (Fig. 3A). The number of GLP-1 containing L-cells was similar between strains during chow-feeding, however, OP rats fed HE/HF-diet exhibited significantly less GLP-1 positive EECs than OR HE/HF-fed rats (P<0.0001) and OP rats fed standard chow (P<0.001) (Fig. 3B).

**Discussion**

Our results demonstrate that high-fat feeding in animals prone to obesity results in impaired GLP-1 satiation signaling. Specifically, OP animals maintained on HE/HF-diet exhibit decreased sensitivity to the satiating effects of Ex-4, a GLP-1R agonist, compared to OR rats fed a similar diet or their counterparts fed chow. Furthermore, and similar to previous studies, we found that during HE/HF-feeding, OP rats gained significantly more weight, had increased body
adiposity, and consumed more calories during 24h compared to OR rats (4,5). Increased energy intake in HE/HF-fed OP rat is a function of increased meal size (4), indicating an inability of OP rats to effectively suppress appetite and calorie intake following a meal. Indeed, diet-induced obese rats exhibit decreased sensitivity to intraintestinal lipid-induced satiation compared to diet-resistant rats, an effect associated with diminished GLP-1 protein expression in the intestinal epithelium (5). Fat is a potent GLP-1 secretagogue (12), and GLP-1 is thought to contribute to meal-induced satiation (8) by acting in a vagal-dependent manner (13). Therefore, we hypothesized that decreased responsiveness to lipids in OP rats may result from defective GLP-1 signaling during HE/HF-feeding, however, whether this phenomenon is an effect of HF-feeding, or of the ensued obesity or both, is not known. Here, we found that chow-fed OP and OR rats suppress food intake equally following all doses of Ex-4. In contrast, during HE/HF feeding, Ex-4 either failed to inhibit intake or was less efficacious in suppressing intake in OP compared to OR rats suggesting that obesity and HF-feeding interact to aggravate the deficits in GLP-1 signaling.

GLP-1 exerts its anorectic effect most likely in a paracrine-like fashion on vagal afferent terminals [see (13) for review]. Therefore, the decrease in short-term responsiveness to Ex-4 during HE/HF-feeding in OP rats is likely a result of reduced peripheral vagal afferent activation. Indeed, HF-fed animals exhibit reductions in vagal afferent sensitivity and receptor expression (14,15), and likewise, GLP-1R mRNA in the nodose ganglia was decreased in HE/HF-fed OP rats in the current study. Reduced vagal responsiveness to Ex-4 in OP rats may also be due to dysregulation between GLP-1 and leptin signaling, as vagal afferents contain both leptin and GLP-1 receptors (5,16) and leptin enhances the response to GLP-1 (17). Indeed, leptin resistance occurs rapidly in vagal afferents of obese rats fed HF-diet (18), and diminished vagal
response to leptin may impair the ability of GLP-1 to activate vagal afferents, as has been demonstrated for CCK (19). Although the synergistic effect of leptin and GLP-1 is not vagally mediated (17), it is possible that leptin resistance in OP rats contributes to reduced responsiveness to Ex-4 via post-vagal afferent activation on downstream CNS neurons (20).

Reduced sensitivity to Ex-4 by HE/HF-feeding and obesity was seen at 1h and 3h post injection, whereas 24hr suppression of food intake remains similar between OP and OR rats on both diets tested. As Ex-4 has a much longer half-life than endogenous GLP-1 (21), and can cross the blood-brain barrier (22), it is likely that long-term reduction of food intake following Ex-4 is mediated by central GLP-1R populations, possibly in the NTS or paraventricular hypothalamus (13). This long-term sensitivity to Ex-4 during obesity is consistent with previous reports (23,24).

The decrease in endogenous GLP-1 protein levels may further contribute to impaired satiation signaling in OP animals, ultimately exacerbating hyperphagia and weight gain. This agrees with our previous work, albeit in a different rat model, which showed that diet-induced obese rats exhibit decreased responsiveness to intestinal lipid, which was associated with reduced GLP-1 protein expression in intestinal mucosa (5). Furthermore, plasma levels of GLP-1 were decreased in OP rats, only during HE/HF-feeding, and were likely a consequence of decreased intestinal GLP-1 protein and L-cell numbers in the distal ileum. Reductions in active GLP-1 levels were likely secondary to reduced total GLP-1 levels observed, and not due to enhanced DPP-4 activity, which is similar to the finding in obese humans (25).

Taken together, it is likely that decreased endogenous GLP-1 signaling, through both decreased nutrient-induced GLP-1 release and decreased vagal sensitivity to GLP-1, results in
reduced sensitivity to luminal nutrients in diet-induced obese rats (5). The observed effects are only present during HE/HF-feeding and obesity, indicating an interaction of the diet and the genetic make-up of OP rats. In conclusion, our studies demonstrate an overall impairment in the ability of GLP-1 to reduce meal size that may contribute to overconsumption and excess weight gain following HF-feeding in the obese, thus perpetuating obesity.
Acknowledgements
The study was supported by INRA through a scientific package awarded to M. C.

No potential conflicts of interest relevant to this article were reported.

F.A.D and M.C. designed the study, researched data, and wrote the manuscript. Y.S. researched data and reviewed the article.

M.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
References


22. Kastin AJ, Akerstrom V: Entry of exendin-4 into brain is rapid but may be limited at high doses. *Int J Obes Relat Metab Disord* 27:313-318, 2003


Table 1. Food intake in OP and OR rats following Ex-4 administration

<table>
<thead>
<tr>
<th>Dose</th>
<th>CHOW</th>
<th>HE/HF</th>
<th>CHOW</th>
<th>HE/HF</th>
<th>CHOW</th>
<th>HE/HF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>OP</td>
<td>OR</td>
<td>OP</td>
<td>OR</td>
<td>OP</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>3 h</td>
<td>24 h</td>
<td>1 h</td>
<td>3 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>7.3 ± 0.3</td>
<td>6.9 ± 0.4</td>
<td>9.1 ± 0.4</td>
<td>11.1 ± 0.5</td>
<td>8.9 ± 0.4</td>
<td>9.7 ± 0.4</td>
</tr>
<tr>
<td>0.625 µg/kg</td>
<td>3.1 ± 0.3**</td>
<td>4.9 ± 0.5*</td>
<td>3.1 ± 0.2**</td>
<td>7.1 ± 0.4</td>
<td>5.1 ± 0.6*</td>
<td>6.8 ± 0.3**</td>
</tr>
<tr>
<td>1.25 µg/kg</td>
<td>2.3 ± 0.3***</td>
<td>4.1 ± 0.5**</td>
<td>2.4 ± 0.4**</td>
<td>6.2 ± 0.5</td>
<td>3.8 ± 0.6**</td>
<td>6.3 ± 0.7</td>
</tr>
<tr>
<td>2.5 µg/kg</td>
<td>1.4 ± 0.3***</td>
<td>3.2 ± 0.5***</td>
<td>0.7 ± 0.9*</td>
<td>3.8 ± 0.5***</td>
<td>4.4 ± 0.8*</td>
<td>6.0 ± 0.3***</td>
</tr>
<tr>
<td>5.0 µg/kg</td>
<td>0.1 ± 0.1***</td>
<td>2.1 ± 0.5***</td>
<td>0.2 ± 0.1***</td>
<td>3.3 ± 0.9*</td>
<td>1.0 ± 0.5***</td>
<td>4.2 ± 0.5***</td>
</tr>
</tbody>
</table>

Food intake in grams (± SEM) after Ex-4 treatment. OP and OR rats fed chow suppressed 1 h and 3 h intake significantly from baseline following all Ex-4 doses tested. When rats were fed HE/HF-diet, all doses of Ex-4 decreased 1 h and 3 h food intake significantly from baseline in OR rats whereas OP rats failed to decrease intake at the two lowest doses. The two highest doses of Ex-4 decreased food intake in both groups, compared to saline. *P<0.05, **P<0.001, ***P<0.0001 from saline treatment within each group.
Figure Legends

Fig. 1. Body weights (A), 24-h caloric intake (B), and adiposity index (C) in OP and OR animals fed HE/HF or chow-diet. (A) OP chow (black circles) and OP HE/HF (black boxes) animals weighed more than respective OR chow (white circles) and OR HE/HF (white boxes) rats after two weeks on diet, and OP rats on HE/HF-diet weighed more than chow-fed OP rats after 6-wk. (B) HE/HF-fed OP rats consumed significantly more calories per day than HE/HF-fed OR and chow-fed OP rats. (C) HE/HF-fed OP rats had a significantly greater adiposity index compared to both HE/HF-fed OR and chow-fed OP animals. Data are expressed as means ± SEM, * denotes significant difference from OR rats within diet condition, *P < 0.05, **P < 0.01, ***P < 0.0001. † denotes significant difference from chow-fed diet condition within phenotype, ††P < 0.01, †††P < 0.0001.

Fig 2. Proximal intestinal epithelial cell expression of GLP-1 protein (A), mRNA transcript of GLP-1R in the nodose ganglia (B), and total- (C) and active- (D) GLP-1 plasma concentrations after brief (5-h) fast in OP and OR rats. OP rats had decreased GLP-1 protein expression during HE/HF-feeding compared to HE/HF-fed OR and chow-fed OP rats (A). HE/HF-fed OP rats have decreased gene expression of GLP-1R compared to chow-fed OP rats (B). HE/HF-fed OP rats have decreased circulating total (C) and active (D) GLP-1 compared to both HE/HF-fed OR and chow-fed OP rats. Data are expressed as means ± SEM, * denotes significant difference from OR rats within diet condition. *P < 0.05, **P < 0.01, ***P < 0.0001. † denotes significant difference from chow-fed diet condition within phenotype, †P < 0.05, ††P < 0.01.

Fig 3. Representative images (A) and total count (B) of GLP-1 containing enteroendocrine cells in distal ileum of OP and OR rats. (A) i. 40x zoom image of chow-fed OR rat villus with
stained nuclei \textit{(left)}, stained with GLP-1 antibody \textit{(middle)}, and combined nuclei and GLP-1 staining \textit{(right)}. \textit{ii.} 3x digital zoom of above images. \textit{(B)} HE/HF-feeding led to decreased number of L-cells per villi in the distal ileum of OP rats compared to both chow-fed OP and HE/HF-fed OR rats. Data are expressed as means ± SEM, * denotes significant difference from OR rats within diet condition, **P < 0.0001. † denotes significant difference from chow-fed diet condition within phenotype, ††P < 0.01.
Fig. 1

A

Weeks on Diet

Weight (g)

- OR CHOW
- OR HE/HF
- OP CHOW
- OP HE/HF

B

24-h intake (kcal)

- OR
- OP

C

Adiposity index

- OR
- OP

Diabetes

†††

***

**
Fig. 2

A

GLP-1 / β-Actin

CHOW OR OP OR HE/HF OR OP

B

Relative to OR Chow

CHOW OR OP OR HE/HF OR OP

C

Total GLP-1 (pM)

CHOW OR OP OR HE/HF OR OP

D

active-GLP-1 (pM)

CHOW OR OP OR HE/HF OR OP
Diabetes

**Fig. 3**

**A**

(i)

(ii)

**B**

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>OP</th>
<th>OR</th>
<th>OP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHOW</strong></td>
<td>0.4</td>
<td>0.3</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>HE/HF</strong></td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

GLP-1 cell per villi

[Bars with asterisks indicating statistical significance: *****, † †]