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Original Research

GLP-1R and IL-6 expression in the gastrointestinal tract of a murine model of metabolic syndrome

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Abstract

Background and aims: Glucose homeostasis is a critical cornerstone in both health and disease. It is described to be regulated by the balance of insulin and glucagon secretion but, this bi-hormonal perspective is long overdue since glucose homeostasis is now known to be a multi-hormonal process. Metabolic syndrome (MetS) is a cluster of metabolic features that includes impaired glucose metabolism and obesity. Obesity promotes a low chronic inflammation state due to the release of bioactive molecules like cytokines, ultimately contributing to cardiovascular disease, diabetes, and cancer. The gastrointestinal tract (GIT), namely the stomach and intestine, has a vital role not only in the food uptake and absorption but also in the production of incretin hormones, such as GLP-1. We aim to evaluate the GLP-1 receptor (GLP-1R) in the GIT of a MetS animal model and to assess whether it correlates with inflammatory levels. **Material and method:** The expression of GLP-1R and interleukin-6 (IL-6) was evaluated in the stomach and intestine of mice subjected to normal diet (ND) and high fat diet (HFD) by immunohistochemistry. **Results:** We observed that HFD fed animals presented lower levels of GLP-1R in the stomach and intestine when compared with animals fed with ND. Concomitantly, these mice expressed increased levels of IL-6. **Conclusions:** GLP-1R expression is inversely correlated with the expression of the pro-inflammatory cytokine IL-6.

Keywords: metabolic syndrome, inflammation, incretins, gastrointestinal tract, GLP-1R, IL-6.

Background and aims

Impaired glucose metabolism, alongside obesity, build up an abnormal cluster, which characterizes the metabolic syndrome (MetS). This complex and heterogeneous constellation of risk factors including obesity, glucose intolerance, hypertension, dyslipidemia, and insulin resistance can result in non-communicable diseases highly prevalent nowadays, such as cardiovascular diseases, type 2 diabetes and certain types of cancer [1]. Glucose regulation is a keystone in several disorders including MetS. The glucoregulatory system is a multi-organ network that includes the gastrointestinal tract (GIT), adipose tissue (AT), pancreas, kidney, liver, and brain. GIT, namely the stomach and intestine, has a vital function in food digestion and absorption. Cells from the peripherally intestinal ileum (L- and K-cells) release incretins such

as glucagon-like protein 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), that play an important role in glucose metabolism [2].

GLP-1 is an antiobesogenic hormone, produced through proglucagon cleavage, that enhances glucose-stimulated insulin secretion of beta cells through GLP-1 Receptor (GLP-1R), and decreases glucagon production in alpha cells of the pancreas, but GLP-1 functions go beyond its influence on the pancreas. In addition to the role in glucose metabolism, GLP-1 also regulates the upper gastrointestinal tract, gastric motility, acid secretion [3, 4], increases satiety in gut-brain axis coordination, promotes thermogenesis in AT [5], exerts anti-inflammatory effects [6], among others [7]. GLP-1 and GLP-1R analogs have been widely explored as a therapeutical target in obesity and diabetes with broad acceptance [5]. However, much is still to uncover regarding GLP-1 and metabolic diseases, such as obesity or diabetes. Studies from both animal and human research reveal inconsistent results as for the secretory response of GLP-1 to the meal or glucose intake in obesity and diabetes [8].

In addition to its function of lipid accumulation, AT is an active endocrine organ constituted by several types of cells that exerts different functions and produces a variety of hormones, growth factors and cytokines [9, 10]. AT promotes chronic low-grade inflammation observed in obesity and MetS. Increased expression of proinflammatory adipokines associated with circulating free fatty acids and reactive oxygen species improve the chemo attraction of immune cells, such as macrophages, which further produce proinflammatory mediators [11–14].

IL-6 is a pleiotropic cytokine known for its essential role in immune system regulation, acting in the stimulation of B cells and in antibody production [15]. IL-6 is one of the most popular mediators in the low chronic inflammation status observed in obesity [16], though IL-6 is also an active regulator in neural, metabolic, angiogenic and regenerative mechanisms [17]. Previous studies already demonstrated an association between GLP-1 and IL-6[18–20].

It was observed, primarily in mice and then in humans, that IL-6 improved glucose metabolism via upregulation of GLP-1 in patients with type 2 diabetes or obesity [18]. Moreover, pancreatic alpha cells (proglucagon producers) are a target of IL-6 and the overexpression of IL-6 promotes alpha cell proliferation and inhibits apoptosis, which results in improved beta-cell function and insulin secretion through GLP-1 production [21].

To help uncover the relation between IL-6 and GLP-1 in MetS, we performed an *in situ* evaluation of the expression of GLP-1R and IL-6 both in the stomach and intestine.

Materials and methods

Animal experiments

This experiment followed the guidelines and recommendations of FELASA (Federation of European Laboratory Animal Science Associations) and the European Directive 2010/63/EU related to animal protection in scientific studies. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

G*Power 3 software was used to establish the minimum number of animals necessary to conduct the study [22]. The ideal number achieved was three for each group considering the diet and physiological conditions, with a significance level of 95% and an actual power of 0.80 (86%). Six C57Bl/6J mice were divided into two groups: one group with a normal diet (n=3) and a group with a high-fat diet (n=3). Animals were maintained in suitable conditions of temperature and light for 180 days and fed *ad libitum*. After sacrifice, stomach and intestine were extracted, fixed in formalin and paraffin-embedded to perform immunohistochemistry assays.

Immunohistochemistry assays

Paraffin-embedded sections of the stomach and intestine were deparaffinized and hydrated. Sections were subjected to heat antigenic recovery. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in Phosphate Buffered Saline (PBS) and non-specific binding was blocked with 10% swine serum (NSS)

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in bovine serum albumin. Sections were further incubated overnight, at 4°C in a humidified chamber, with a primary antibody. For GLP-1 receptor (GLP-1R), primary rabbit anti-GLP-1R polyclonal antibody was used (sc-66911 Santa Cruz Biotechnology, Inc., USA; 1:200 dilution) followed by the biotinylated antibody goat anti-rabbit IgG-B (sc-2040 Santa Cruz Biotechnology, Inc., USA; 1:200 dilution). For inflammation evaluation, was used rabbit polyclonal antibody anti-human IL-6 (ab6672, Abcam plc, UK; 1:600 dilution) followed by the biotinylated goat anti-rabbit antibody IgG-B (sc-2040, Santa Cruz Biotechnology, Inc., USA; 1:200 dilution). Immunoreactivity was visualized with Avidin-Biotin reaction and revelation was performed using DAB (3,3-diaminobenzidine) HRP substrate (Abcam, ab94665, UK).

Image analysis

Histological sections were analyzed under an optical microscope (Nikon Eclipse 50i, Nikon Instruments Inc.) and images captured with an attached digital microscope camera (Nikon DS-Fil, Nikon Instruments, Inc.). Ten pictures of each tissue were taken, at 200× optical amplification. Images were quantified using the color deconvolution plugin in Image J 1.49u (Wayne Rasband, National Institute of Health, USA).

Statistical analysis

It was calculated the mean ± standard error (SEM) for non-parametric tests with the Mann–Whitney test. The statistical analysis was done with GraphPad Prism software (GraphPad Software, Inc) and results were considered statistically significant when p-values <0.05.

Results

Incretin system evaluation

Immunohistochemistry of GLP-1R was performed to evaluate the incretin system in the stomach and intestine of HFD and ND mice (Figure 1). Staining was predominantly observed in the glandular area of the stomach and in the intestine mucosa. In the stomach, the HFD group presented a significant decrease ($p<0.01$) in the GLP-1R expression in HFD mice, with a mean area value of 1664±317 μm^2 in comparison with the ND group that presented a mean value of 6049±1094 μm^2. In the intestine, although not significant ($p=0.1592$), a decreased expression of GLP-1R expression in HFD mice was observed (1785±195 μm^2) when compared to the mean value obtained in the ND mice (2082±569 μm^2).

Inflammation levels evaluation

Evaluation of the inflammation levels in stomach and intestine in healthy (ND) and MetS (HFD) animals was assessed by IL-6 immunohistochemistry (Figure 2). IL-6 expression was observed in the glandular areas of stomach and in intestine mucosa and submucosa, also in the intestine, mostly in the HFD mice, punctual staining around or alongside vessels was observed.

In the stomach, HFD mice presented a significant increase in IL-6 expression ($p<0.01$) with quantification of 18,793±2026 μm^2, when compared to ND mice group with 9641±1504 μm^2. In the intestine, the HFD group also presented a significant overexpression of IL-6 ($p<0.0001$) with quantification of 7890±931 μm^2 compared to 3650±267 μm^2 in the ND group.

Discussion and conclusions

Obesity is a pathological feature of MetS along with inflammation, hypertension, insulin resistance, and diabetes, connected by a complex network of dynamic intervenients like transcription factors, hormones, and cytokines, among them GLP-1 and IL-6 are important mediators for their active participation in the associated pathways of obesity and inflammation.

Our results revealed a decrease in the GLP-1R in the HFD group in both stomach and intestine, which is in accordance with the majority of studies [8, 23]. For instance, a study

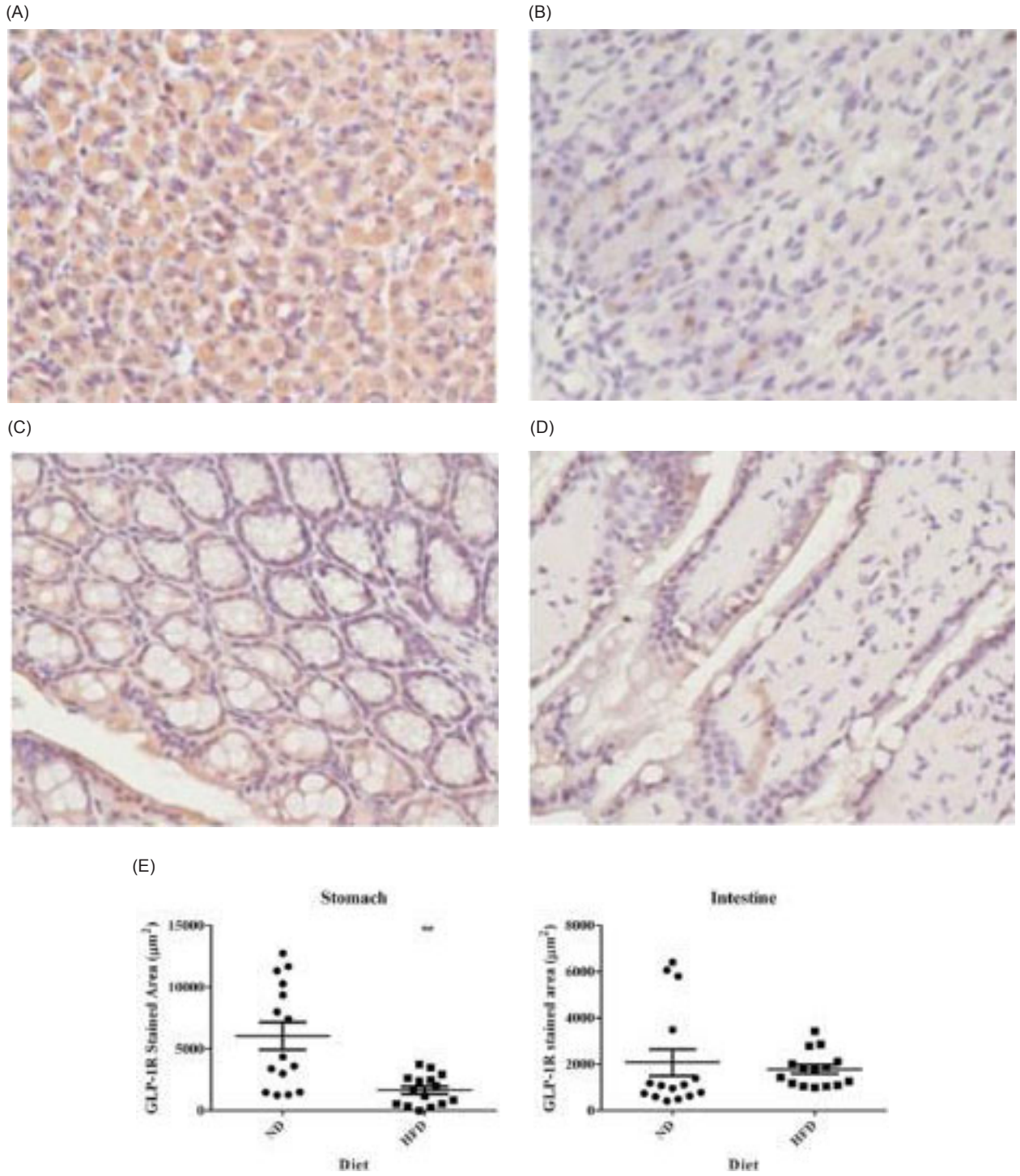

Figure 1: Representative images of GLP-1R immunohistochemistry. Sections of stomach in ND mice (A) and HFD mice (B) and intestine of ND mice (C) and HFD mice (D) (200×); Quantification of GLP-1R (F) **p<0.01. The values are represented as the mean ± SEM.

performed in gastric glands of patients with impaired glucose metabolism found a decrease in GLP-1R expression when compared to healthy individuals [23]. Our findings regarding IL-6 were also in accordance with the literature and from the studies performed *in situ* in stomach [24] and intestine [25]. We observed a significant increase of IL-6 in both stomach and intestine. Furthermore, our results show that the expression of GLP-1R and IL-6 are to some extent co-localized, so we hypothesized they might be related and inversely correlated.

Indeed, previous studies already established a possible relation between GLP-1R and

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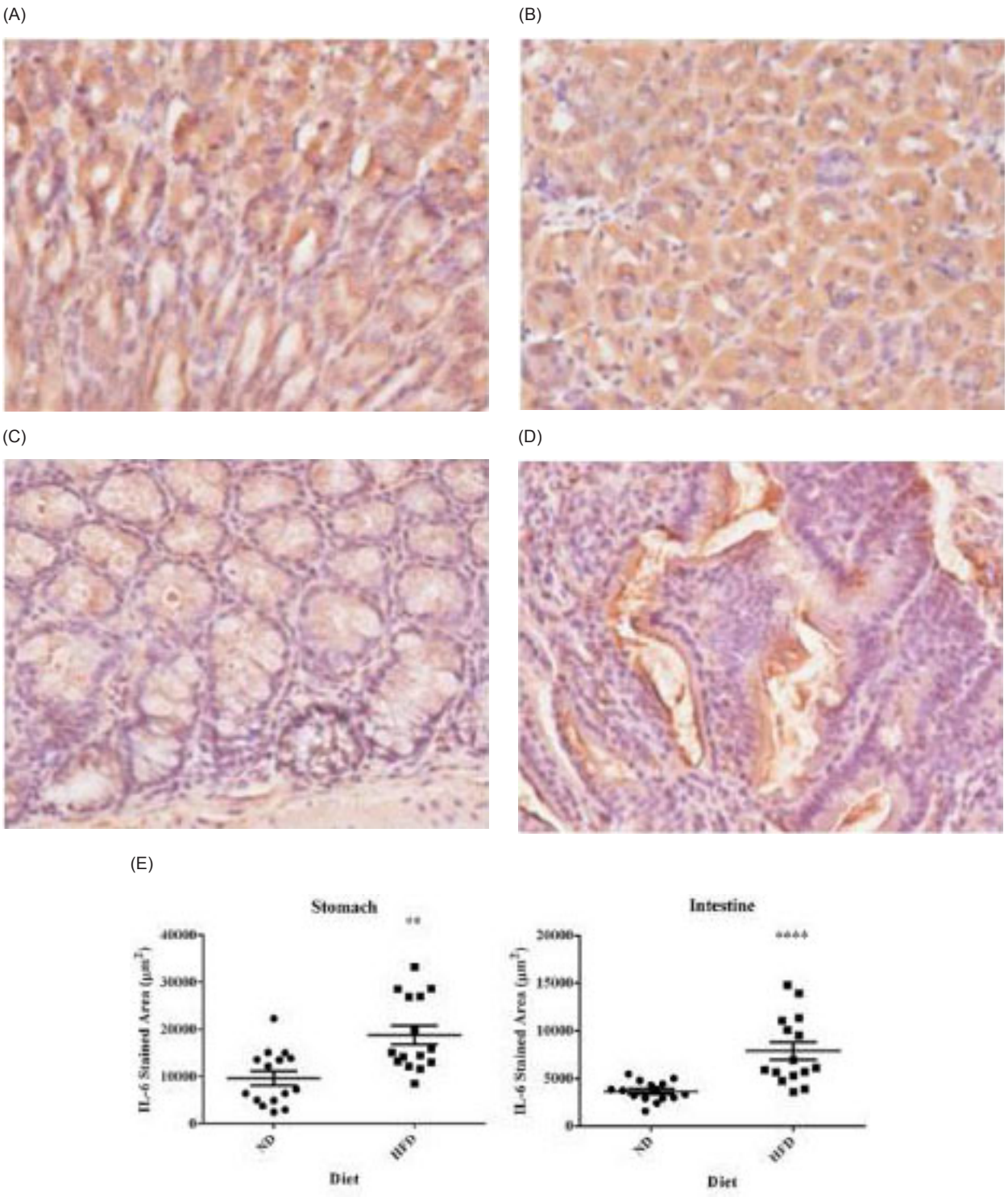

Figure 2: Representative images of IL-6 immunohistochemistry. Sections of stomach in ND mice (A) and HFD mice (B) and intestine of ND mice (C) and HFD mice (D) (200×); Quantification of IL-6 (F) **p<0.01; ****p<0.0001. The values are represented as the mean±SEM.

IL-6 via the GLP-1 potential in engaging inflammation [6]. Through the observation of GLP-1 based therapies, data suggests that GLP-1 not only shows anti-inflammatory effects in pancreatic cells (with the glycemic decrease in diabetic subjects) but also in the liver, kidney, lung, testis, skin, and vessels, lowering the levels of inflammatory cytokines and promoting the infiltration of immune cells [6]. As concerns for animal studies, RedIL6 strain mice were used to evaluate the interactions of IL-6 and GLP-1 in the central amygdala, since previous results showed that

IL-6 in a downstream moderator of GLP-1R in the brain [26]. Results disclosed that, in the central amygdala, 40% of cells expressing GLP-1R, also expressed IL-6 in a co-localized manner [27].

Our work proposes that the relation between IL-6 and GLP-1 is also occurring in the stomach and in the intestine. Further studies are needed to better understand the pathway cascade on which IL-6 and GLP-1R are connected and to what extent could they become potential therapeutical targets in MetS.

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Conflict of interest

The authors declare no conflict of interest.

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