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Comparative secretome analysis of rat stomach under different nutritional status

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ABSTRACT

Obesity is a major public health threat for many industrialised countries. Bariatric surgery is the most effective treatment against obesity, suggesting that gut derived signals are crucial for energy balance regulation. Several descriptive studies have proven the presence of gastric endogenous systems that modulate energy homeostasis; however, these systems and the interactions between them are still not well known. In the present study, we show for the first time the comparative 2-DE gastric secretome analysis under different nutritional status. We have identified 38 differently secreted proteins by comparing stomach secretomes from tissue explant cultures of rats under feeding, fasting and re-feeding conditions. Among the proteins identified, glyceraldehyde-3-phosphate dehydrogenase was found to be more abundant in gastric secretome and plasma after re-feeding, and downregulated in obesity. Additionally, two calponin-1 species were decreased in feeding state, and other were modulated by nutritional and metabolic

Abbreviations: KRH, Krebs–Ringer–HEPES; NDSB, Dimethylbenzylammonium propane sulfonate; FWHM, Full Width at Half Maximum; GPS, Global Proteomic Server; CI, Confidence Interval.

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conditions. These and other secreted proteins identified in this work may be considered as potential gastrokines implicated in food intake regulation.

Biological significance
The present work has an important impact in the field of obesity, especially in the regulation of body weight maintenance by the stomach. Nowadays, the most effective treatment in the fight against obesity is bariatric surgery, which suggests that stomach derived signals might be crucial for the regulation of the energy homeostasis. However, until now, the knowledge about the gastrokines and its mechanism of action has been poorly elucidated. In the present work, we had updated a previously validated explant secretion model for proteomic studies; this analysis allowed us, for the first time, to study the gastric secretome without interferences from other organs. We had identified 38 differently secreted proteins comparing *ex vivo* cultured stomachs from rats under feeding, fasting and re-feeding regimes. The results in the present article provide novel targets to study the role of the stomach in body weight and appetite regulation, and suggest new potential therapeutic targets for treating obesity.

1. Introduction

Nowadays obesity is a major public health issue for many developed and emerging nations around the world. The most significant health consequences include type 2 diabetes (T2D), cardiovascular disease and cancer [1]. Although it is known that obesity is a multifactorial disease which may be explained by genetic alterations, the current obesity epidemic is mainly caused by environmental factors such as lifestyle, diet and lack of physical activity [2]. In the recent years advances in basic research have improved our understanding of body weight regulation at a molecular level generating new approaches for controlling obesity.

Existing pharmacological treatments are not very successful; being bariatric surgery the most effective method to reduce body weight in morbidly obese patients. It has been showed that the decrease of appetite and body weight after surgery is associated with the variation of gastrointestinal hormones production [3,4]. This fact indicates that signals derived from the gastrointestinal tract are crucial for the energy homeostasis regulation. Under this context, the isolation of the orexigenic peptide ghrelin from the stomach represented a step forward in the caloric intake and body weight regulation research [5]. Since then, many studies have proven the presence of gastric systems that modulate energy homeostasis such as the NUCB2/nesfatin-1 and the endocannabinoid system [6–8]. Furthermore, it has been described that mechanic and chemical gastric sensors are able to detect the presence of nutrients reporting to the brain the nutritional state of the organism [9]. Thus, the identification of proteins secreted by the stomach can be very useful to elucidate the gut–brain signalling pathways and their role in energy balance as they may reveal sources of novel therapeutic targets.

Under this context, our group has established an *ex vivo* model for studying direct gastric secretion based on tissue explant cultures [10]. The relevance of this model is based on validation data showing that food-mediated changes in plasma ghrelin levels are due to variations in ghrelin release by the stomach [8,10].

The development of high throughput technologies such as proteomics has opened new opportunities for elucidating the molecular mechanisms of complex diseases. In obesity research, the current proteomic based studies have been focused in cell lines and in adipose tissue samples and secretomes [11]; and to a lesser extent in plasma, liver, hypothalamus and muscle tissues [12,13]. Proteome variations under different nutritional status have been studied in metabolism-related organs such as brain, pituitary, liver or intestine [14–16], and biological fluids as plasma and saliva [17]. However, the use of proteomics for the study of gastric secretome has not advanced as in other tissues due to intrinsic stomach technical obstacles [18]. In accordance, there are few studies performed in human gastric fluid proteome [18] and gastric tumour cell lines [19,20]; that are focused on the biomarker discovery for gastric cancer and gastritis.

In the present study, the previously established model of gastric tissue explant culture developed by our group [10,21] has been used to assess the direct stomach secretion without the interference from other organs. We show for the first time a comparative analysis between gastric secretomes obtained from cultivated tissues of rats subjected to control *ad libitum* feeding, fasting or re-feeding. The 2-DE analysis led to the discovery of differentially secreted gastrokines between the three nutritional conditions; among then, GAPDH and calponin-1 were validated in the analysed animals and also in obese animals. The proteins identified by mass spectrometry (MS) that were classified as secreted by signalling peptide or by non-classical secreted pathway, include interesting proteins that will require further analysis.

2. Materials and methods

2.1. Ethics statement

The authors of this manuscript declare that all the procedures carried out with animal models in this study were performed under the process with code: 15005AE/10/FUN01/FIS02/LSC1 according with the institutional guidelines and the European Union standards for the care and use of experimental animals (Real Decreto 1201/2005, October 10th, about the animals used