Alpha-amylase expression in pAncreas of an animal model of undernutrition

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Abstract

The pancreas is both an exocrine and an endocrine organ. The exocrine pancreas plays a key role in digestive process by producing important digestive enzymes, among which amylase, which is exocytosed in response to ingestion of carbohydrates. Several studies have reported that malnutrition, which includes both overnutrition and undernutrition, leads to functional impairment of several major organs, including pancreas. In the present study, we investigated whether the exocrine pancreas activity and morphology is affected by undernutrition in mice. A light microscopic morphometry and immunohistochemical expression of α-amylase were undertaken. The area and perimeter of pancreatic acini in mice of the control and experimental group were compared, and no significant differences (P<0.05) were found. Likewise, no obvious difference in α-amylase immunostaining in acini of control and undernourished group of mice was found. In conclusion, our results suggest this level of undernutrition and/or with this duration did not induce morphological changes or the expression of α-amylase in pancreas. Keywords: Mice, exocrine pancreas, α-amylase, undernutrition.

INTRODUCTION

Today the world faces a double burden of malnutrition that includes both undernutrition and overweight, especially in developing countries. Failing of essential nutrients as a consequence of undernutrition is the leading cause of an increased vulnerability to diseases, mortality and poor child development [1,2,3]. Several studies have reported that undernutrition causes dysfunction and morphological changes of the exocrine pancreas [4,5], so it is important to study its effects in humans and animal models. The aim of the present study was to evaluate the effects of undernutrition on histology and expression of α-amylase in the pancreas of mice.

MATERIAL AND METHODS

BALB/c female mice were divided into 2 groups: control (N = 6) and undernutrition (N = 6). Undernutrition was induced by administering 60-65% of mean food weight consumed by the animals from control group in the previous day. After 2 weeks, animals were euthanized and pancreas removed and processed by routine histological techniques. Sections were stained with H&E and immunohistochemistry was performed using the labeled-(strept) avidin-biotin (LAB-SA) UltraVision Detection System kit (Thermo Scientific, USA, ref TP-015-HD). Prior to incubation with the anti-α-amylase antibody (Santa Cruz, sc-46657, 1:500, 4°C, overnight) the sections were heated for antigen retrieval, in citrate buffer (pH 6.0), at 98°C, for 20 minutes. As a negative control, a pancreas section with the primary antibody replaced by PBS was used. Slides were observed under light microscope at 250X magnification, and images acquired by a digital camera. Perimeter and area of acini (at least 50/animal) were assessed using SigmaScan Pro 5.0 software. Data were statistically analysed through nonparametric tests, using SPSS 16 software and differences were considered significant for P <0.05. Immunostaining was evaluated qualitatively according to the following scale: 0 (negative); 1 (weak); 2 (moderate); 3 (strong).

RESULTS

Significant differences in body weight was found between the control group and the experimental group at the end of the experiment, in which the group subjected to undernutrition showed lower body weight when compared to the control group (Table 1). No histopathological changes were observed and no significant differences were observed in the areas and perimeters of pancreatic acini between the animals of both groups (Table 2). Regarding the expression of α-amylase no obvious differences between the two groups were observed.
DISCUSSION AND CONCLUSIONS
The results of this study clearly demonstrate a significant decrease in body weight of mice subjected to undernutrition, when compared to those of control group, which agrees with a previous work [6]. Several authors observed in parotid, an organ with some histological similarities, a compensatory acinar hypertrophy, in undernourished individuals [7,8], as well for salivary glands from the mice of the present study [9]. In our experiment, the absence of significant changes in pancreatic acini morphology suggest an physiological adjustment to counteract the effects of nutritional deficiencies. No obvious difference in the α-amylase expression in pancreatic acini was found between the control and undernourished mice group. However some papers have reported significant differences in the levels of serum α-amylase in undernourished individuals, verifying its decline [4,5]. Probably the immunohistochemical technique does not have the adequate sensitivity, which may have been enhanced by dilution (1:500) used. The lack of evidence in relation to histologic parameters suggests in the future: increasing the sample size (animals and number of analysed acini), extending the duration of the experiment, to determine the relative pancreas weight and the serum levels of α-amylase and to evaluate the acini ultrastructural changes.

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