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Oral health, diabetes, and body weight

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ABSTRACT

Objectives: The effects of apical periodontitis (AP) and periodontal disease (PD) on organ weights in rats with diabetes mellitus (DM) were evaluated.
Design: Eighty male rats (*Rattus norvegicus albinus*, Wistar) were divided into eight groups of ten: normoglycemic (N), AP, PD, AP + PD, DM, DM + AP, DM + PD, and DM + AP + PD. DM was induced by streptozotocin; AP, by dental exposure to the oral environment; and PD, by periodontal ligature. Blood glucose concentration was measured at 0, 6, 15, and 35 days; body weight, measured daily; and animals were sacrificed after 30 days after induction of oral infections. Liver, kidney, pancreas, brain, heart, lungs, and gonads were each weighed. Glycemia, feed intake, organ weight, and body weight were subjected to statistical analyses ($p < 0.05$).
Results: Glycemic levels were higher in all diabetic groups after day 6, but were consistently similar in normoglycemic groups. Blood glucose was higher in DM + PD and DM + AP + PD groups than in the DM group at days 15 and 35. The feed intake was similar among all groups. Brain, heart, and gonad weights were significantly increased in DM + AP + PD. Kidney and lung weights were increased in DM, regardless of the presence of oral infections. Liver weight was reduced in AP and/or PD. Pancreas weight was reduced in DM, independent of AP or PD. Among the normoglycemic groups, there were no significant differences among organ weights.
Conclusion: Apical periodontitis and periodontal disease may potentiate the adverse effects of diabetes.

1. Introduction

The presence of oral infections has been increasingly associated with systemic and autoimmune diseases (Astolphi et al., 2013; Astolphi et al., 2015; Cintra et al., 2013; Cintra, Samuel, Azuma et al., 2014; Cintra, Samuel, Facundo et al., 2014; Cintra, da Silva Facundo et al., 2014). This relationship exists because of the spread of inflammatory mediators via the bloodstream (Cintra, Samuel, Azuma et al., 2014; Sun, Chen, Zhang, Ren, & Qin, 2010; Takano et al., 2010). The biological pathways that potentiate autoimmune diseases, such as diabetes mellitus, are the same pathways that potentiate oral infections (Akalin, Işiksal, Baltacioğlu, Renda, & Karabulut, 2008; Cintra, Samuel, Facundo et al., 2014; Sun et al., 2010; Takano et al., 2010). Thus, increased levels of blood glucose in diabetics have been associated with apical periodontitis and periodontal disease (Akalin et al., 2008; Cintra, Samuel, Facundo et al., 2014).

The deleterious effects of diabetes mellitus in some organs, such as the liver and kidneys can occur as sequelae to elevated blood glucose levels (Lee et al., 2008). However, to our knowledge, there have been no reports of changes within specific organs that directly resulted from endodontic infections. The liver is an insulin-dependent organ that plays a key role in glucose and lipid homeostasis, and which can be severely affected by diabetes (Seifter & England, 1982). It participates in the conversion, uptake, oxidation, and metabolism of free fatty acids, phospholipids, and cholesterol synthesis, as well as the secretion of certain classes of plasma lipoproteins (Greenbaum, Gumaa, & McLean, 1971). Thus, the tighter the glycemic control, the greater the degree of disturbances within the body (Greenbaum et al., 1971).

Organ weight can be a reliable indicator of some physiological changes (Lee et al., 2008). Studies have shown that diabetics tend to have increased kidney weight (Hamadi, Mansour, Hassan, Khalifi-Touhami, & Badary, 2012; Lee et al., 2008) and decreased

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liver weight. The decrease in liver weight can be attributed to a reduction in the rate of hepatic lipogenesis in diabetics (Greenbaum et al., 1971). The increased weight of the kidneys is due to renal hypertrophy. Some studies have shown that as the kidney does not require insulin for glucose uptake, there is greater accumulation of glycogen (Kumar, Sharmila Banu, & Murugesan, 2009).

There has been a lack of evidence of changes in the weights of other organs, due to diabetes and concurrent oral infections. Apical periodontitis in particular has been proven to influence the pathogenesis of many diseases (Cintra et al., 2013; Cintra, da Silva Facundo et al., 2014; Cintra, Samuel, Azuma et al., 2014; Cintra, Samuel, Facundo et al., 2014). Thus, with scientific advancement, the development of treatments based on interdisciplinary research has now become indispensable, making a holistic review of the mechanisms of health and disease necessary.

2. Materials & methods

2.1. Experimental design

Eighty male rats (*Rattus norvegicus albinus*, Wistar) with initial mean weight of 250 g were selected. The animals were housed in temperature-controlled rooms and received water and food *ad libitum*. The experimental protocol was approved by and conducted in accordance with the guidelines of the institutional ethical committee, and. in accordance with the U.K. Animals (Scientific Procedures) Act, 1986.

2.2. Induction of diabetes mellitus

The rats were fasted overnight (14–16 h) and the fasting blood glucose level was measured (day 0) from blood samples from the tail vein using an automatic blood glucose monitoring system (Accu-Check® Performa – Roche-Diagnostics Corporation, Indianapolis, IN, USA). The rats were anesthetized with intramuscular administration of ketamine (87 mg/kg) (Francotar – Virbac do Brasil Ind. e Com. Ltda, Roseira, Brasil) and xylazine (13 mg/kg) (Rompum – Bayer S. A., São Paulo, Brazil). They were then randomly assigned to receive an intravenous injection (in the penile vein) of streptozotocin (Sigma–Aldrich Corp., St. Louis, MO, USA) dissolved in citrate buffer solution at 35 mg/kg for the experimental induction of diabetes (Cintra, Samuel, Azuma et al., 2014; Cintra, da Silva Facundo et al., 2014). For normoglycemic rats, citrate buffer solution 0.01 M, pH 4.5 was administered also in penile vein.

Six days after induction of diabetes, blood samples were collected from each animal to determine glycemic levels (day 6). Only blood glucose levels >200 mg/dL were considered in the present study (Cintra et al., 2013; Cintra, da Silva Facundo et al., 2014; Cintra, Samuel, Azuma et al., 2014). Blood glucose concentration was again measured at days 15 and 35.

2.3. Induction of oral infections

After confirmation of hyperglycemia, the animals were sedated for the induction of oral infections. For the development of apical periodontitis, the pulps of the right maxillary first molars were exposed on the mesial surface using surgical round burs (Broca Ln Long Neck – Maillefer, Dentsply Ind. e Com. Ltda, Petrópolis, Rio de Janeiro, Brazil) (Cintra et al., 2013; Cintra, da Silva Facundo et al., 2014; Cintra, Samuel, Azuma et al., 2014).

For the development of periodontal disease, sterile 4/0 silk ligatures (Ethicon, Johnson & Johnson, São Paulo, SP, Brazil) were placed around the left maxillary second molars (Cintra et al., 2013; Cintra, da Silva Facundo et al., 2014; Cintra, Samuel, Azuma et al., 2014). After the respective inductions, the following groups were assigned: normoglycemic group (N), AP, PD, AP + PD DM, DM + AP, DM + PD, DM + AP + PD.

2.4. Measurement of feed intake

Throughout the study, the animals received Labina® food (Purina Agribrands do Brasil Ltda, Paulínia, São Paulo, Brazil) and water ad libitum. The energy content of Labina® was 3.3 Kcal/g (Table 1). Consumption of food and water was measured every day from the induction of oral infections.

2.5. Measurement of body weight and organ weight

Rats were weighed (to the nearest 0.1 kg) on a digital balance (model 707; Seca, Hamburg, Germany), at the same time every day for 30 days for the duration of the study.

After 30 days, the animals were sacrificed with an anesthetic overdose of sodium thiopental (Thiopentax; Cristália, Itapira, SP, Brazil). Liver, kidney, brain, heart, lung, and gonads were resected by a trained researcher and weighed on a digital balance (model 707; Seca, Hamburg, Germany). The relative weight of each organ was evaluated according to the index calculated between organ weight (WO) and body weight (BW) of each animal, multiplied by 100 (WO/BW × 100) (Eleazu, Iroaganachi, Okafor, Ijeh, & Eleazu, 2013; Kim et al., 2006; Kumar et al., 2009).

2.6. Statistical analysis

The total values were tabulated for each experimental group and data were analyzed by a single calibrated operator in a blinded manner. Two-way analysis of variance (ANOVA) followed by the Holm-Sidak method for pairwise multiple comparisons were applied at a significance level of 5% ($p < 0.05$).

3. Results

3.1. Blood glucose level

At day 0, all rats had similar glycemic levels ($p > 0.05$). In diabetic rats, blood glucose levels were higher than they were in rats without diabetes at days 6, 15, and 30 after induction of DM ($p < 0.05$). Glycemic levels were similar among all groups without diabetes throughout the duration of the study (Table 2). Among the diabetic rats, the presence of PD and PD + AP increased blood glucose levels on days 15 and 30, when compared to the DM control. The presence of AP alone also resulted in increased blood glucose levels; however, this difference was not significant ($p > 0.05$) (Table 2).

3.2. Feed intake

Feed intake of diabetic and normoglycemic rats was not significantly different ($p > 0.05$); however, after induction of oral

Table 1
Composition of commercial food fed to rats.

Component	Amount (g/100 g)
Humidity (Max)	13.0
Crude Protein (Min)	23.0
Ethereal Extract (Min)	4.0
Fibrous Matter (Max)	5.0
Mineral Matter (Max)	10.0
Calcium (Max)	1.3
Phosphorus (Min)	0.85