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Progressive histopathological changes and β-cell loss in the pancreas of a new spontaneous rat model of type 2 diabetes

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ABSTRACT

The eSMT rat is a new spontaneous model of type 2 diabetes that develops a progressive diabetic syndrome with a stronger incidence in males than in females. We decide to investigate the progression of the pancreatic histopathological changes during the lifespan of the eSMT rat, especially those associated with islet cell populations. Besides that, some plasmatic parameters were evaluated in order to correlate them with the morphological findings. Male eSMT and Sprague-Dawley control rats were used.

The results showed a dramatic decrease of the volume density (VD) of endocrine tissue in the eSMT rats without evidence of insulitis. Islets became fragmented structures with strong presence of interstitial fibrosis. Consequently, plasma insulin levels showed a significant decrease, while plasma glucose, cholesterol and triglyceride levels were increased. Normal rats showed no significant changes in the VD of endocrine tissue, except for the older animals, where the VD of β-cell population was increased.

Early derangements observed in islets, together with the progressive decrease of endocrine tissue and the metabolic disorders described, would be responsible for an irreversible pathologic condition which avoids the animal survival beyond about 18 months of age.

However, there is still a need to investigate the causes of endocrine tissue decrease and its possible association with an inflammatory process that it could be associated with the development and progression of fibrosis.

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1. Introduction

Pancreatic β-cell mass is determined by the balance of cell renewal and cell loss. This turnover should be dynamically adjusted by external demand during adult life (Finegood et al., 1995; Bernard et al., 1999; Bernard and Ktorza, 2001; Bonner-Weir, 2001). In type 2 diabetes, β-cells damage and apoptosis are thought to be significantly accelerated by several causes. Gluco- and lipotoxicity (Robertson et al., 2003, 2004), low-grade chronic inflammation (Donath et al., 2003; Homo-Delarche et al., 2006), amylin deposition with fibrotic islet destruction (Clark and Nilsson, 2004; Homo-Delarche et al., 2006; Haataja et al., 2008; Porte, 1991), and oxidative stress (Robertson et al., 2004) are the leading candidates for the development of type 2 diabetes. The pathologic manifestations of the pancreatic islets in type 2 diabetes, include since slight alterations until a markedly reduced β-cells mass, hyaline material deposition, and also an eventual fibrotic destruction of islet. These pathological changes have been observed in humans as well as in several spontaneous animal models of type 2 diabetes (Shafrir, 1992; Shafrir et al., 1999).

While it is clear that hyperglycemia is associated with both insulin resistance and β-cell dysfunction, there has been much debate over the past few decades regarding the relative importance of these two abnormalities (DeFronzo and Ferrannini, 1991; Gerich, 1998, 2003; Kahn, 2001, 2003; Pimenta et al., 1995).

It was also well established that obesity is a high risk factor in the development of type 2 diabetes. In fact, increases in levels of plasma lipids are usually associated with insulin-resistant states (Boden and Shulman, 2002; Hardy et al., 2002; Unger et al., 1999). Furthermore, abnormalities in the metabolism of carbohydrates and fat have been also associated with the decline and damage in β-cell population (Bergman and Ader, 2000; Poitout and Robertson, 2008; Unger and Zhou, 2001; Unger and Orci, 2001).

The eSMT strain (IIMe/Fm eSMT) was derived from a cross between eSS and β rats, both parent strains belonging to the IIM stock. β is a line of moderately obese rats (Calderari et al., 1995) and

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eSS rats are known to develop a mild diabetic syndrome unrelated with obesity and represent a model of type 2 diabetes (Martínez et al., 1993). The eSMT rats develop a diabetic syndrome related to early corpulence, fasting hyperglycemia and impaired glucose tolerance that worsens with age and is more severe in males. Furthermore, it is also known that eSMT males have an excess of circulating insulin compared with age-matched eSS animals (Picena et al., 2007).

In our preliminary studies, islets of 5-month-old eSMT rats showed a disrupted structure, including insular polymorphism with images of cells or groups of cells separated by severe fibrosis ("starfish-like islets") and intermingled with exocrine acini and adenomatous ductal hyperplasia.

Based on these observations, we decide to investigate the progression of the pancreatic histopathological changes during the lifespan of the eSMT strain, especially those associated with islet cell populations. Moreover, in order to establish some kind of correlation with the morphological findings, we measured the plasma levels of insulin, glucose and lipids in the different ages studied.

In short, our goal is to advance into the characterization of the eSMT strain, to provide a spontaneous new model for studying the pathogenesis of human type 2 diabetes that it would be very useful to investigate and test new therapies for this disease.

2. Materials and methods

2.1. Animals and specimen collection

Male eSMT rats of different ages (2, 5, 10, 14 and 18 months) and age matched Wistar control rats were used. For each age group 5 animals were used. The eSMT line was obtained from School of Medical Sciences and Research Council (National University of Rosario, Santa Fe, Argentina). Five animals for each group were housed in a temperature-controlled room ($22 \pm 2\,^{\circ}C$) on a 12:12-h light–dark cycle. Food (Cargill®, Buenos Aires) and water were available *ad libitum*.

Maintenance and treatment of animals were in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

2.2. Tissue preparation

Rats were sacrificed by rapid decapitation, and samples of the splenic portion (tail) of the whole pancreas were rapidly removed and cleared of fat.

Pancreas were fixed in Bouin's solution, rinsed in phosphate-buffered saline (PBS), dehydrated in ascending ethanols and embedded in paraffin. Serial sections (4 μm) of two different levels were mounted and kept overnight at 37 °C.

2.3. Histological stainings

For routine histological study tissue sections were dewaxed with xylene, rehydrated through graded ethanol, rinsed in distilled water and stained in hematoxylin for 1 min. After rinsed in tap water for 10 min sections were stained with eosin for 5 min and subsequently dehydrated through an ascending grade of ethanol and mounted. To visualize connective tissue Gomori trichrome staining was used.

2.4. Immunohistochemistry

Tissue sections were also processed for immunohistochemical identification of insulin-, glucagon-, somatostatin-, pancreatic polypeptide-secreting cells: B, A, D and PP cells respectively, and proliferating cell nuclear antigen (PCNA). It is important to point out that each of the tissue samples was especially oriented during inclusion, in such a way as to obtain the widest possible area of tissue to be evaluated. For immunodetection, sections were incubated at room temperature for 1 h with ready-to-use primary antibodies against either rat insulin, glucagon, somatostatin and pancreatic polypeptide (Biogenex Laboratories, San Ramón, CA, USA). After washing, the sections were immunostained by means of a Dako EnVision System and Dako LSAB System. Diaminobenzidine (DAB) and alkaline phosphatase were respectively used as chromogens. The specificity of the primary antiserum was monitored by replacing the first antiserum by normal rabbit serum or PBS. The sections were counterstained with hematoxylin.

2.5. Pancreas morphometry

Measurement of cell parameters was made by means of an image analysis system (ImageJ 1.39f, NIH – Bethesda, MD, USA). Four serial sections were obtained from each level for each of the pancreatic hormones. All the sections were introduced in the PC using an analog Sony video camera (PAL system), after being converted to the RGB (red–green–blue) system necessary for digitizing and processing the sections. The totality of islets found was considered for each section, an image being generated for each islet detected (average = 15 images/section) depending on the size and nearness of the islets. For this purpose, a 25× objective was used. The islets under approximately 40 μm in diameter were not measured since they were considered possible error factors, as it could not be determined whether they were islets superficially cut or small groups of 4 or 5 cells. These measurements were recorded and processed automatically, and the following parameters were afterwards calculated: volume density (VD = $\sum$ cell area/RA) and cell density (CD = number of cells/RA). Both of them were referred to different reference areas (RA): endocrine reference area (RA_e) and total pancreas reference area (RA_t). RA_e represents the total endocrine area scanned, in which islet populations were scored. Then, with the sum (Σ) of the areas (A) of each endocrine cell type, referred to as RA_e, we obtained the respective VD, which indicates cell mass, according to a usually accepted concept.

The number of cells was calculated dividing the immunostained area of each cell population by the mean individual cell area of each cell type. For this parameter, 100 cells of each type were recorded. The individual islet areas (μm^2) and the number of islet profiles were also measured. The latter parameter was referred to as total pancreas (mm^2). The islet profiles are representative of the number of islets, since the sections between two levels were sufficiently separated as not to count twice the same islet. All of these data were calculated from the totality of islets finding in each slide of each experimental group. Finally, the VD of the pancreatic adipose tissue was also calculated, taking the total pancreas as a RA. For this parameter, the volume density logarithm was calculated.

2.6. Plasma measurements

After rapid decapitation, trunk blood was collected and centrifuged at 2500 rpm, and plasma was frozen at −20 °C until assay. The glucose, plasma cholesterol and triglyceride level determination were carried out in the same day from the extraction to avoid enzymatic degradation and possible errors in the measurements.

The blood glucose was determined using a commercial kit (Wiener Lab) based on the method of enzyme glucose oxidase (GOD).

The triglyceride level was determined using an enzymatic-colorimetric commercial kit GPO/PAPAA (Wiener Lab).

Plasma insulin levels were determined by radioimmunoassay using specific commercial antibodies (Lincos Research, USA).

TBARS levels were measured through the method of thiobarbituric acid (Ohkawa et al., 1979). After heating tubes with samples

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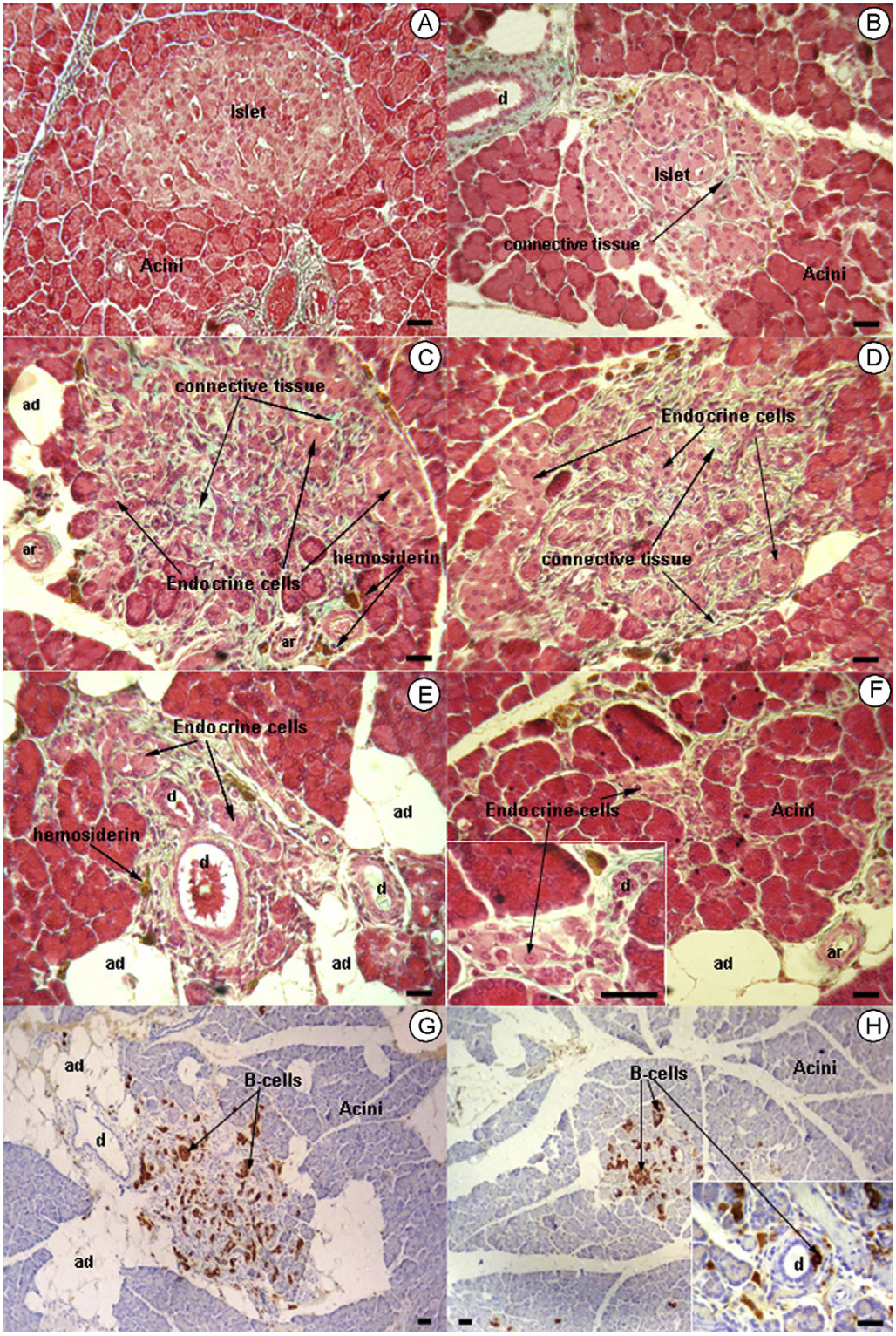

Fig. 1. Changes in the histoarchitecture of the eSMT rat pancreas along the different ages studied. Note the progression of fibrotic tissue within the structure of the islets (starfish-like islets). (A–F) Gomori trichrome (bar = 50 μm). (A) Control Sprague-Dawley rat, (B–F) eSMT strain, (B) 2 months, (C) 5 months, (D) 10 months, (E) 14 months, (F) 18 months. (G and H) Insulin-positive cells showing the fragmented and star-like islets in 5-month-old rat (G) and 10-month-old rat (H). References: ad, adipocytes; ar, arteriole, d, duct.

of plasma, white (H_2Od) and standard (malondialdehyde) in the presence of thiobarbituric acid and acetic acid, at 95 °C for 60 min in a boiling water bath, mixtures were centrifuged at 2500 rpm and the supernatant was available to be read in its optical density at 532 nm.

2.7. Statistical analysis

Statistical analysis was performed using one-way analysis of variance, followed by Tukey's multiple-range test. Data were expressed as mean ± SEM.

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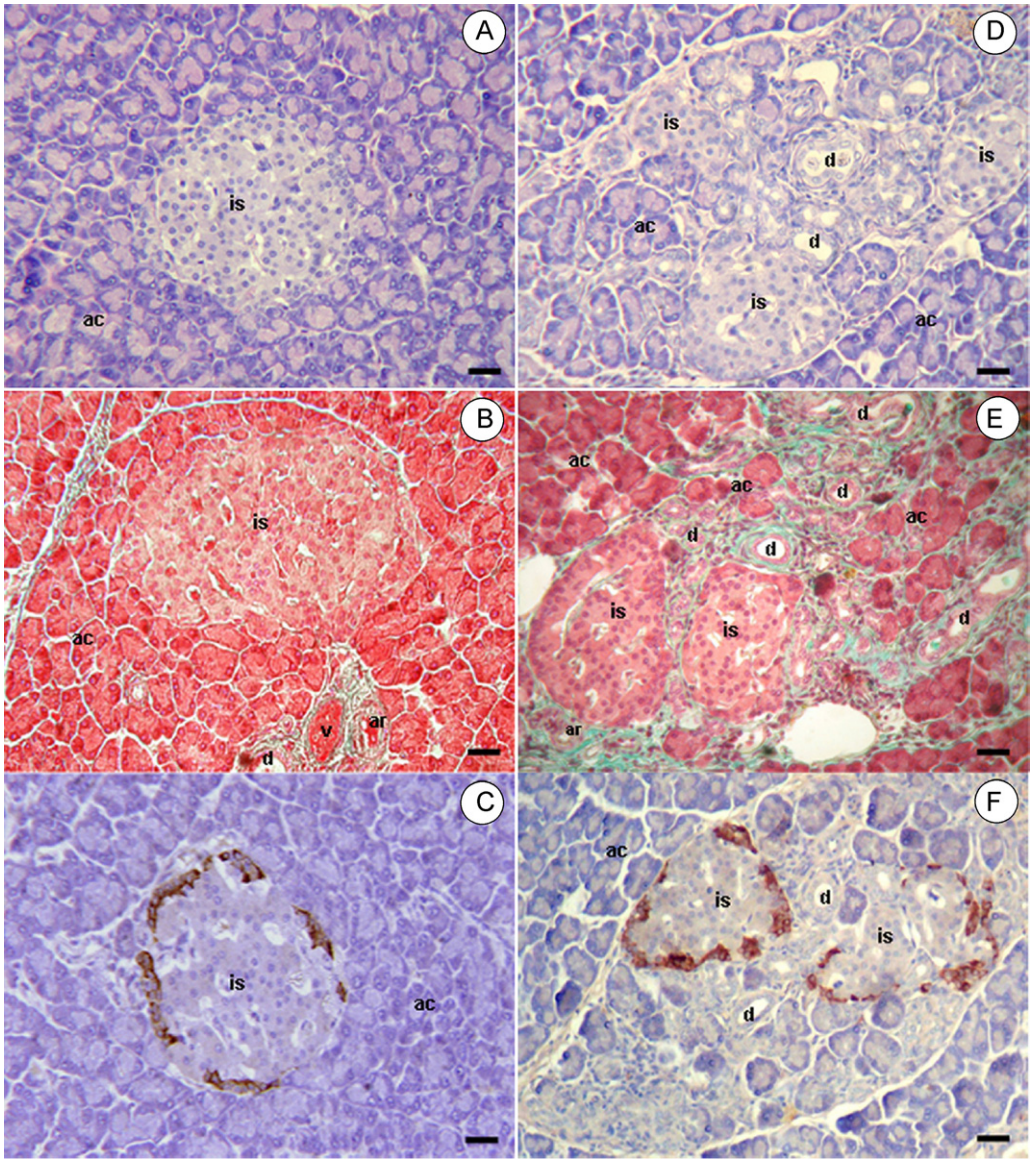

Fig. 2. Hematoxylin–eosin staining (A and D), Gomori trichrome staining (B and E) and glucagon immunostaining (C and F) of pancreatic parenchyma of young (A–C) and old (D–F) control rats. The older rats showed the presence of connective septa between exocrine and endocrine tissue. Despite the presence of interstitial connective septa, in these rats, the islets keep their shape and structural integrity, unlike what is observed in the diabetic strain (bar = 50 μm). References: ac, acini; ad, adipocytes; ar, arteriole; d, duct; is, islet; v, venule.

3. Results

3.1. Histopathology and architecture of pancreatic islets of eSMT rat (Fig. 1)

In 2-month-old eSMT rats slight enlargement of the islets was observed, and there was an early appearance of interstitial connective tissue. At 5 months of age, the islets of eSMT rats have become fragmented structures, as a result of the progress of interstitial connective tissue, formed by cells or groups of endocrine cells along with hyperplasia foci of ductal cells, leucocytes and hemosiderin deposits in the peri-islet areas and within the islets.

As the eSMT rats were getting older, the islets became more irregular shaped, showing finger-like projections of endocrine tissue into the surrounding exocrine acini. The result was a multinodular islet, in which clusters of endocrine cells are widely separated from each other by traversing bands of connective tissue. The presence of islet cells was significantly decreased with age.

Also, a gradual increase of intraglandular adipose tissue was observed along the different ages of the strain.

In the control rats, although some changes were found in most aged animals, there were no islets found with star-like and irregular boundaries similar to the eSMT line. The changes affected all the entire exocrine parenchyma than specifically the endocrine portion (Fig. 2).

3.2. Quantitative immunohistochemical analysis

Fig. 6A shows a marked decrease of the VD of pancreatic endocrine tissue during the lifespan of the strain. After 5-month-old rats, the VD of endocrine cells was significantly lower, reaching the lowest values in older animals ($p < 0.0001$). When VD of both β and non-β cells was analyzed, it was observed that the two populations decreased by almost parallel (Fig. 7).

This demonstrates that endocrine cells decreased all together and not by higher or lower incidence of some of these island populations.

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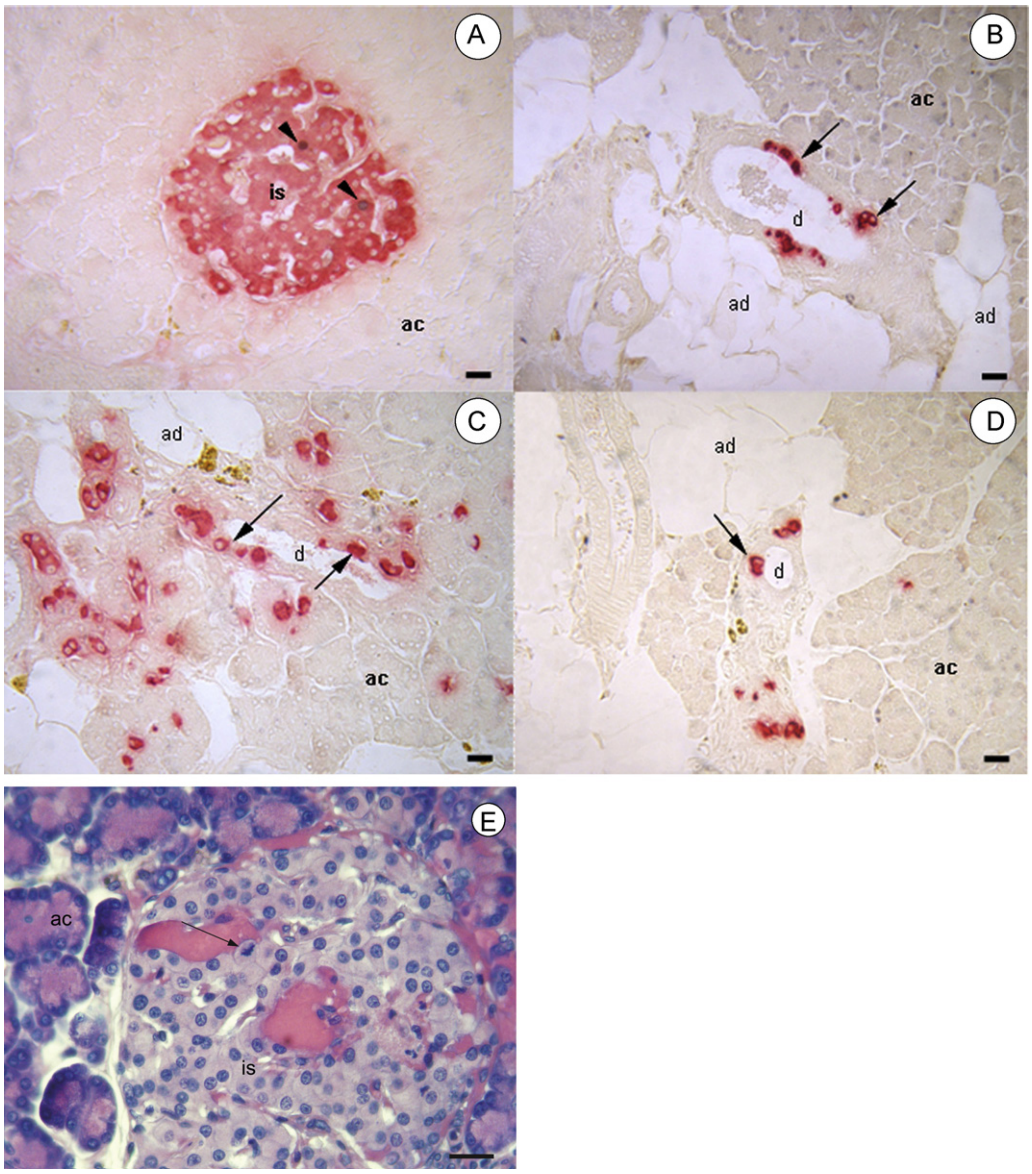

Fig. 3. Insulin immunostaining in eSMT rats. (A) Islet of 2-month-old eSMT rat: insulin (red staining), PCNA-positive nuclei (arrowhead) were detected in some insulin-positive cells. Note the presence of insulin-positive cells (arrows) in pancreatic duct of 10-month-old rat (B), 14-month-old (C) and 18-month-old rat (D). References: d, duct; ad, adipocytes (bar = 50 μm). (E) Mitosis in the islet cells. Arrow indicates a mitotic figure in the islet population. Hematoxylin–eosin. References: is, islet; ac, acini (bar = 50 μm).

Mean β-cell area was also analyzed (Fig. 5). This parameter showed a significant increase after 10-month-old eSMT rats (Fig. 5A) with a slight decrease in the last few months of life. In control rats, no significant changes were detected (Fig. 5B).

3.3. Replication and differentiation of endocrine cells

Although some islet cells showed some isolated mitotic figures and PCNA positive nuclei, their number was insufficient to calculate a representative rate of cell replication. It is important to note that the most immunolabeled nuclei were found in β-cells from younger rats and ductal cells of the older groups (not showed), where also was identified insulin-positive cells (Fig. 3A–E).

Besides that, images of endocrine cell differentiation from acinar and/or centroacinar cells in 11 and 14 months-old animals were detected. This phenomenon was extensively studied by Bouwens (1998, 2000) named trans-differentiation or dedifferentiation (Fig. 4).

3.4. Plasma measurements

3.4.1. Glucose and insulin

Fig. 8 shows the changes in plasma glucose and insulin levels during aging of eSMT and control rats.

In eSMT line (Fig. 8A), blood glucose levels showed a significant increase in rats of 14 and 18 months, however, although the tendency was evident from the 5 months-old rats.

On the other hand, insulin levels revealed a marked decline with age. Two and five months-old rats showed similar values without significant differences between them. After 10 months, insulin levels significantly decreased and did not recover until the death of the animals.

Table 1 shows the correlation coefficients between the VD of total endocrine tissue and β-cell respectively, regarding to the values of plasma insulin and glucose, as well as the ratio between the two profiles of variation of both VD. These results demonstrate the correlation between the decline of endocrine tissue and β-cell population with respect to decreased insulin. Moreover, an inverse (−)

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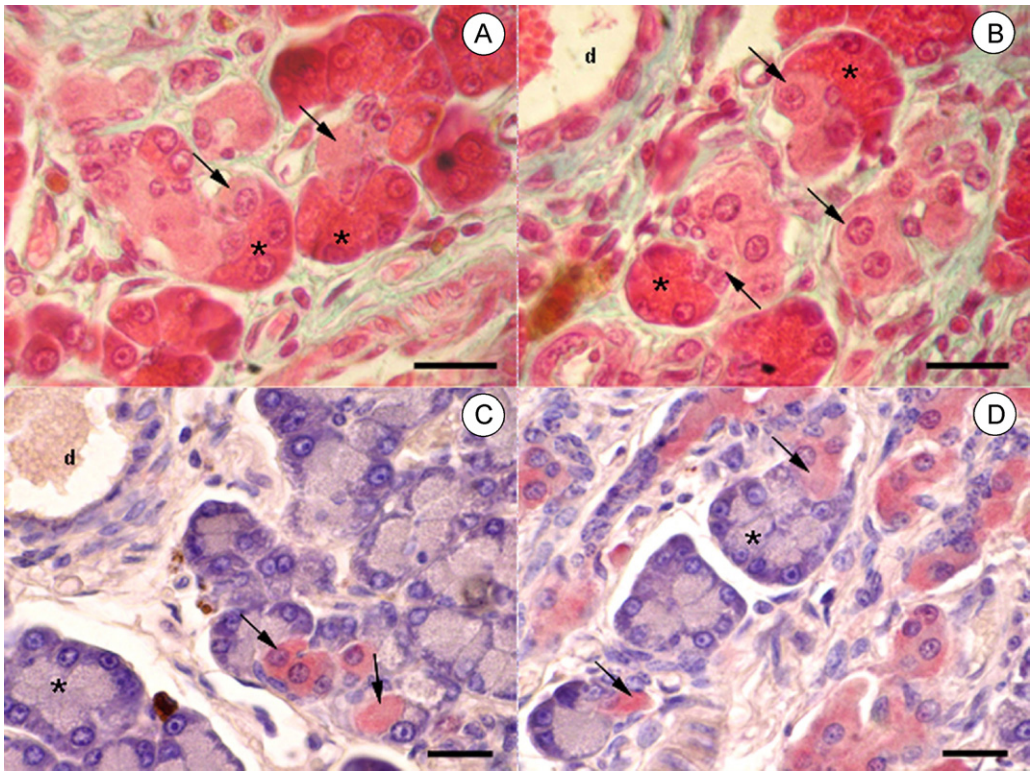

Fig. 4. Differentiation of endocrine cells from exocrine acinar cells in 11-month-old eSMT rat. References: asterisks, acinar cells; arrows, endocrine cells associated with exocrine acini; e, endocrine cells; d, duct; ar, arteriole. (A) and (B): Gomori trichrome. (C) and (D): insulin-positive cells, hematoxylin and eosin counterstain (bar = 50 μm).

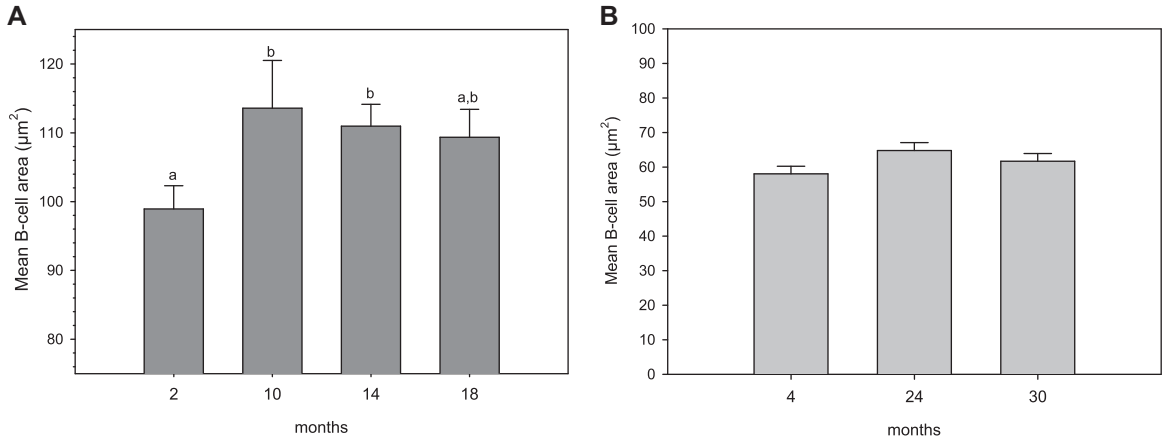

Fig. 5. Mean B-cell area in eSMT (A) and control Sprague-Dawley (B) rats. Note the increased cell area in eSMT strain that could explain a compensatory behavior. In control rats no significant differences were found. Different letters indicate significant differences ($p < 0.05$), $n = 5$.

correlation between these parameters and blood glucose levels was calculated.

In control SD rats significant changes in the blood glucose levels were not detected, while insulin content showed a progressive decrease.

Table 1
Correlation coefficients between the endocrine pancreas and insulin values and plasma glucose. VD_{eTotal}, volume density of total endocrine tissue; $VD_{B\text{-}cell}$, density of population B, *r*, correlation coefficient; *p*, significance level of ANOVA.

	Insulin		Glucose		$VD_{B\text{-}cell}$	
	r	*p*	*r*	*p*	*r*	*p*
VD_{eTotal}	0.588	0.005	−0.605	0.004	0.992	0.0001
$VD_{B\text{-}cell}$	0.623	0.003	−0.594	0.005	1	0

3.4.2. Cholesterol and triglycerides (Fig. 9)

Unlike normal rats (Fig. 9B), eSMT strain (Fig. 9A) showed a significant increase ($p < \pm 0.0001$) in plasma triglyceride levels in older animals (14, 18 months-old) compared to younger groups (2–10 months-old). No differences have been detected within the two groups concerned.

3.4.3. TBARS

In the eSMT strain, plasma TBARS levels showed significantly higher values compared with those obtained in normal SD rats, in which they increased progressively. In Fig. 10B are shown the values obtained at different ages, which showed a similarity between the 2, 10 and 14 months (2m = 32.61 ± 4.15 nmol/ml; 10m = 28.71 ± 1.40 nmol/ml; 14m = 37.85 ± 2.40 nmol/ml), with individuals of 18 months reaching the highest plasma levels of

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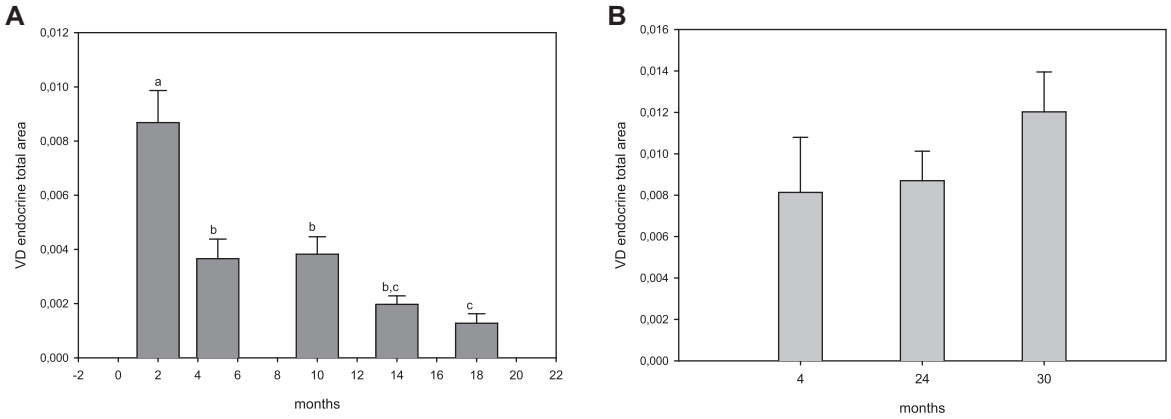

Fig. 6. VD (volume density) of endocrine area in eSMT (A) and control Sprague-Dawley (B) rats. Regard the marked decreased of endocrine tissue in the eSMT rat. In control rats no significant differences were found. Values expressed as mean ± SEM. Different letters indicate significant differences ($p < 0.0001$), $n = 5$.

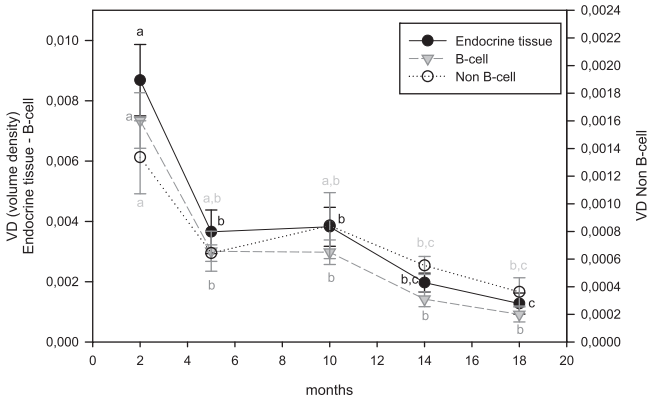

Fig. 7. Comparative VD (volume density) between endocrine tissue, B- and non-B cell population at different ages of eSMT rat. Values expressed as mean ± SEM. Different letters indicate significant differences ($p < 0.05$), $n = 5$.

TBARS (62.60 ± 11.58 nmol/ml). However, this increase was only significant ($p < 0.03$) when compared with animals of 10 months.

4. Discussion

Despite the several factors involved in the development of DM, inadequate β-cell function is an essential component in almost all forms of the disease (Weir et al., 2001). Impaired insulin secretion might be induced by insufficient β-cell mass, by an intrinsic defect in the secretory machinery of the β-cells, or both. In fact, for many years, the contribution of a reduction in β-cell mass to the development of type 2 diabetes was intensely discussed.

It is well established that the β-cell mass exists in a dynamic state determined by the balance of neogenesis, self-replication and apoptosis of the β-cell population. In type 2 diabetes, the increase of the β-cell apoptosis may be due to different causes. Gluco-lipotoxicity (Robertson et al., 2003, 2004), low-grade chronic inflammation (Kolb and Mandrup-Poulsen, 2005), fibrotic islet destruction (Klöppel et al., 2004; Donath et al., 2003; Homo-Delarche et al., 2006), and oxidative stress (Robertson et al., 2004) might be some of the possible pathogenic causes of type 2 diabetes. These pathologic changes are commonly observed in humans and in several animal models of type 2 diabetes.

In our strain (eSMT), the decrease of the β-cell mass was one of the outstanding findings, associated with the progression of islet fibrosis through the deposition of extracellular matrix (EM). Also, intraglandular adiposity, pigment deposition (hemosiderin) and hyperplastic ductal cell groups intermingled with the endocrine cells, were found.

While it is difficult to establish a clear relationship between all these aspects, there is strong evidence of similar findings in other murine models, more specifically, those related to the change in the β-cell mass and the presence of islet interstitial fibrosis.

Similar morphological changes have been described in the eSS parent strain, although these changes were less severe and detected

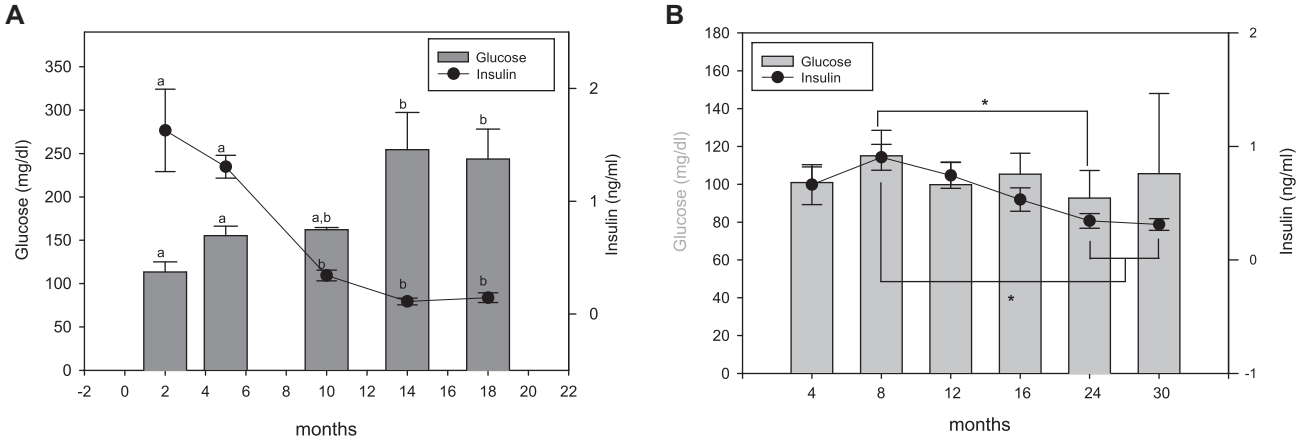

Fig. 8. Plasma insulin and glucose levels in eSMT (A) and control (B) male rats. Values expressed as mean ± SEM. Different letters (eSMT) or asterisk (controls) indicate significant differences, $p < 0.0001$ (ins), $p < 0.005$ (glu), $n = 5$.

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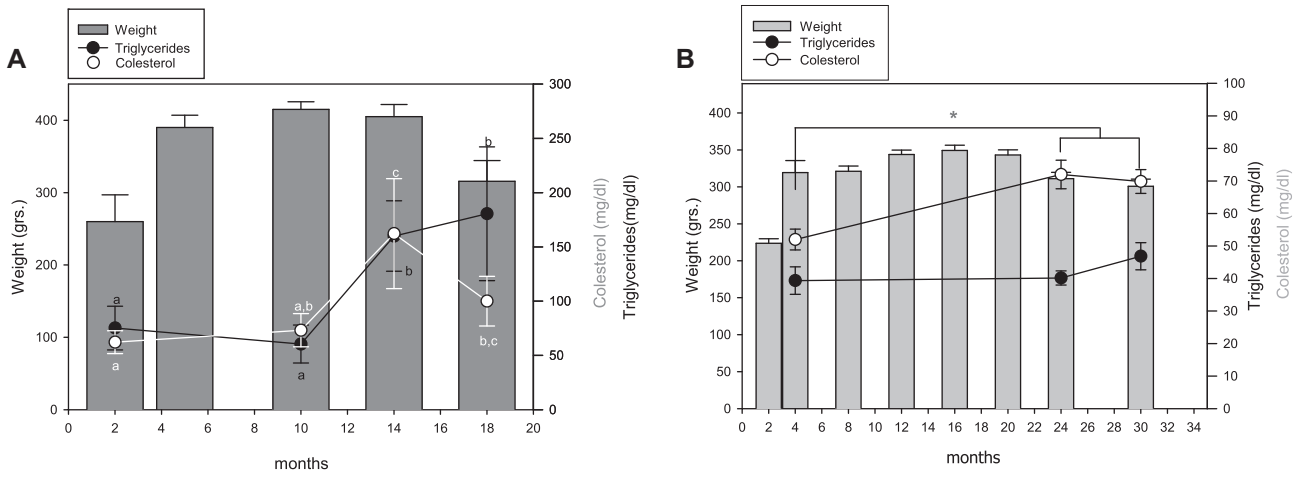

Fig. 9. Body weights compared with plasma triglyceride and cholesterol levels in eSMT (A) and control (B) male rats. Values expressed as mean ± SEM. Different letters indicate significant differences $p < 0.0001$ (triglyc.), $p < 0.0002$ (cholest.), $n = 5$.

only in the older ages (14–18-month-old) compared with the early derangement observed in the eSMT rat. However, islet disruption associated with the presence of fibrosis was also one of the main alterations observed in eSS line (Gómez Dumm et al., 1989; Martínez et al., 1993; Martinez et al., 1994).

Furthermore, in the nonobese Goto Kakizaki rat (GK) was described the presence of large irregular islets where endocrine cells were disrupted by the invasion of connective tissue (Koyama et al., 1998; Movassat et al., 1997). In nondiabetic Zucker fatty rats, occasional fibrotic and irregular islets are seen by 12 weeks of age (Pick et al., 1998). At the same age, many Zucker diabetic fatty (ZDF) rat islets are markedly abnormal with fibrosis and irregular projections in the exocrine tissue (Janssen et al., 2001; Pick et al., 1998).The Otsuka Long-Evans Tokushima Fatty (OLETF) strain also shows irregular fibrotic islets, and some abnormalities in their blood supply (Hong et al., 2002; Ko et al., 2004). The Torii rat (SDT), which develops diabetes spontaneously without obesity and hyperglycemia after 5 months of age, shows mainly between 2nd and 3rd month, the earliest alterations of the islet vasculature followed by a process of inflammation and fibrosis (Masuyama et al., 2004).

It is interesting to focus on the analysis of the conclusions about the mechanisms to explain the possible causes of the β-cell mass variations in the different animal models.

While in the ZDF strain the increasing β-cell apoptotic rate could be the main reason in the failed compensatory response (Pick et al., 1998), in the OLEFT rat, the key problem could be associated to a deficiency in the replicative capacity of the β-cells (Zhu et al., 1996; Hong et al., 2002). In the other hand, in the GK rat, the low β-cell mass observed in the adult age, could be a consequence of early development alterations in the insulin secretory cells (Koyama et al., 1998; Movassat et al., 1997; Plachot et al., 2001).

In the case of the eSS rat, the authors suggested that the decline of the β-cell mass in the adult age, may be associated with a probable apoptotic process (Gómez Dumm et al., 1989, 1990; Martínez et al., 1993).

In our strain, as we mentioned before, the alterations in β-cell mass were evident, but it would be necessary to develop new approaches to elucidate the causes of β-cell mass decline and the possible mechanisms involved, since the data of replication and death by apoptosis obtained, do not provide a sufficient basis to address a final conclusion.

However, the presence of mitotic figures on the islet during the first months (2–5 months), the later appearance of insulin in ductal cells and the presence of small groups of islet cells associated with ducts, mainly from 10 months of age, suggest a sustained compensatory effort of β-cell population. In addition, the significant increase in the individual β-cell area after 10 months confirmed this compensatory behavior. However, it is clear that the large decrease in endocrine mass far exceeds any effort of this population to compensate for such decrease. It is likely that the main cause of the decline in endocrine tissue, is a consequence of the induction of apoptosis rather than a deficiency in the proliferation of that population, perhaps associated with the fibrotic process.

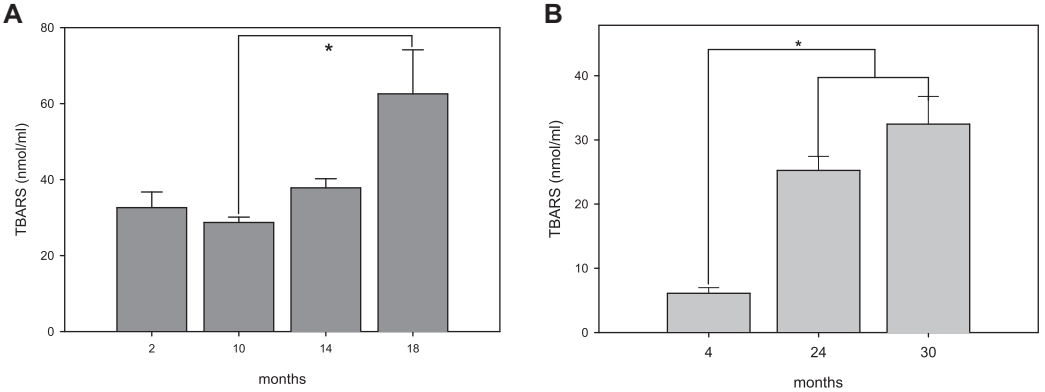

Fig. 10. Plasma TBARS levels showed in eSMT rat significantly higher values compared with those obtained in control SD rats. Values expressed as mean ± SEM. Asterisk indicate significant differences $p < 0.05$ (eSMT), $p < 0.002$ (control), $n = 5$.

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The low rate of apoptotic cell detection could be associated with its brief stay in the tissues, and probably its manifestation has not matched with the established slaughter ages. Therefore, new age of sacrifice should be established in order to confirm the critical involvement of apoptosis in the severe decrease of islet cells.

Regarding the development of fibrosis, although its mechanisms are not yet entirely clear, we suggest that in the rat eSMT the development of fibrosis could be similar to the one described in the GK rat and/or rat OLEFT, based on the close similarity found at the histological level between these lines and our strain.

As is well known, tissue fibrosis is one of the limiting factors for cell proliferation and regeneration in many tissues (Bals, 1997).

Alterations in pancreatic stellate cells (PSC) could be a major cause because these cells are the main source of extracellular matrix (EM) components during development of fibrosis (Apte et al., 1999; Bachem et al., 1998). According to many authors, TGFβ should be a major factor regulating the activity of these cells through the stimulation of collagen synthesis and deposition, and inhibition of specific proteinases (Omary et al., 2007; Shek et al., 2002; Yoshikawa et al., 2002). The connection between fibrosis and inflammation has been strongly studied. However, more recently a systemic subclinic- or low grade inflammation states have been described and associated with the increased fibrosis in the islet (Homo-Delarche et al., 2006; Kolb and Mandrup-Poulsen, 2005).

This subinflammatory condition is considered a nonspecific response to a metabolic/oxidative stress, leading candidate for the accumulation and deposition of extracellular matrix (ME), which would exert its action through the effect of hyperglycemia (glucotoxicity) and associated dyslipidemia (lipotoxicity).

The moderate obese phenotype of the eSMT strain, could explain the progressive fatty infiltration in pancreatic tissue and its contribution to metabolic stress referred previously. Furthermore, the increase in adipose tissue could be responsible for the action of the adipokines, including IL-1, IL-6, TNF-α, resistin, etc., regarded as one of the major mediators in the inflammation process (Gregor and Hotamisligil, 2011; Homo-Delarche et al., 2006; Rocha et al., 2011) leading candidate in fibrotic pathology, as we referred previously.

Further studies are necessary to explain the mechanisms responsible of the deep alterations observed in the eSMT rat.

Finally we thought that this strain represents an interesting new spontaneous model for the study of development and pathogenesis of human type 2 diabetes, as well as to the assay of new therapies for the treatment of this expanding disease.

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