

# A comparative study of the ultrastructure of submandibular, parotid and exocrine pancreas in diabetes and fasting

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## ABSTRACT

**Objective:** To comparatively analyze the ultrastructural changes in the submandibular and parotid glands and in the exocrine pancreas following diabetes induced by Streptozotocin exposure and the effects of fasting and insulin treatment on these alterations.

**Methods:** For experimental procedure, we included 48 Sprague-Dawley type rats in July 2001-March 2002 at Gazi University, Turkey. We divided the rats into 8 groups following the infusion of Streptozotocin.

**Results:** While the degeneration manifested itself as accumulation of secretions within the mucous cells in the submandibular gland, lipid droplets were absent, being replaced by vacuolar structures. The parotid gland and exocrine pancreas, having similar properties, were affected similarly. Diabetes-induced loss of granules was observed in the serous cells in both glands. There was diffuse lipid accumulation within these cells. Regarding granule content, we observed the most prominent degenerative changes in the parotid gland. While cellular loss was observed in neither the submandibular, nor the parotid gland, we noted presence of apoptotic cells was noted in the pancreas. State of fasting was found to cause alterations within the glands indicating increased activity. While insulin treatment was seen to restore the structure to normal in general in both of the 3 glands.

**Conclusion:** This study demonstrated that both of the 3 glands are affected by diabetes and concomitant fasting, and this effect manifests itself via the granule content.

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Diabetes mellitus is a chronic metabolic disease. It is caused by lack of either free or conjugated insulin. Several side effects of chronic diabetes are reported, including microangiopathies, neuropathy, and accelerated atherosclerosis.<sup>1</sup>

Beside the pathologic consequences of diabetes, several functional alterations on various organs that are not considered as side effects have also been reported in studies based on animal models of diabetes. Profound metabolic and functional alterations in salivary glands have been demonstrated in studies of Streptozotocin (STZ)-induced diabetes models in rats. In diabetes models, alterations in carbohydrate metabolism<sup>2,3</sup> and changes in salivary secretions and their compositions have been reported.<sup>4,5</sup>

As a result, though various studies performed to date regarding the effects of diabetes and subsequent insulin treatment on salivary glands and pancreas, none of the studies were comparative and sufficient information on the effects of diabetes accompanied by fasting on these glands has not been gained. With this purpose, this study focuses on a comparative analysis of ultrastructural changes using electron microscopy in the submandibular and parotid glands, which secrete approximately 95% of the total salivary volume, and those in the exocrine glands of pancreas, which have structural and functional similarity to parotid glands, following a 2-month diabetes induced by low-dose STZ exposure and the effects of insulin treatment and fasting on these alterations.

**Methods.** In this study, 48 Sprague-Dawley type rats were used. The experimental protocol was approved by the local Ethical Committee for animal studies and conducted at Gazi University Faculty of Medicine, Ankara, Turkey in July 2001 to March 2002. Every rat was given a single dose of intravenous 45 mg/kg STZ (Sigma Inc.) in citrate buffer through the tail vein.<sup>6</sup> The rats in the control group were given only a single dose of sodium citrate buffer each. The rats were divided into 8 groups following the infusion: Group 1: control group (n=6). Group 2: control + 24

hours of fasting group (n=6). Group 3: control + 48 hours of fasting group (n=6). Group 4: 2 months of diabetes group (n=6). Group 5: 2 months of diabetes + 24 hours of fasting group (n=6). Group 6: 2 months of diabetes + 48 hours of fasting group (n=6). Group 7: Short-term (3 days) insulin treatment group (n=6). Group 8: Long-term (7 days) insulin treatment group (n=6). The rats in each group were kept in media with 12 hours of daylight and 12 hours of darkness periods, and were fed with chow and water. The experimental protocol was approved by the Local Ethical Committee for Animal Studies.

On the 3rd day following the application, blood was sampled from the tail veins of each rat, and blood glucose was assessed with Glucotrend®. Blood glucose levels of the rats in diabetic group were found to be 310 – 399 in average. In the control group, blood sugar values ranged from 80 to 90 in average. The rats were grown for 2 months. In this period, their environments were cleaned daily and chow and water were supplied continuously. Their blood sugar was assessed twice weekly. At the end of the second month, the first groups of rats (diabetes and control groups) were operated under anesthesia with 50 mg/kg pentothal sodium, and their parotid and submandibular glands and pancreas were removed. At the same day, fasting groups were formed. For this purpose, chow was withdrawn, but the rats were given water. In the following 24th and 48th hours respectively, the rats were anesthetized in the way described above, and the same tissues were removed.

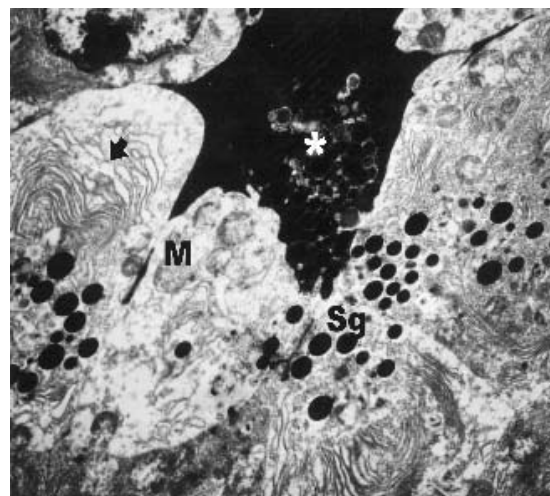
The blood glucose levels of the remaining rats were assessed individually and 8–15 U of NPH insulin was injected subcutaneously, dose depending on the assessed glucose level. Beginning on the day of the first insulin injection, blood glucose was assessed daily at the same hour, and insulin was injected at the same hour, at doses depending on the outcome of the assessment. On the third day, a group of rats were operated for removal of their tissues with the purpose of examining the influences of short-term insulin treatment. The last group was the one with insulin treatment for 7 days, and the rats in this group were operated for removal of their tissues at the end of the treatment for a week.

For electron microscopic examination, all tissues were fixed in 0.1M phosphate-buffer containing 2.5% glutaraldehyde for 2-3 hours; then they were postfixed in 1% osmium tetroxide ( $\text{OsO}_4$ ) and dehydrated in a series of graded alcohols (50, 60, 70, 80, 90, 96 and 100% ethanol). After passing through propylene oxide, the specimens were embedded in Araldite CY 212, 2-dodecenyl succinic anhydride, benzyltrimethyl amine and dibutylphthalate. Semi-thin sections were cut and stained with toluidine blue and examined with a BH<sub>2</sub> Olympus light microscope. Ultra-thin sections

were stained with uranyl acetate and lead citrate and examined with a Carl Zeiss EM 900 transmission electron microscope.

**Results.** In the control group of electron microscopy photographs, nuclei of the cells constituting the mucous acinars were found to be heterochromatic with single dense nucleoli. The cytoplasm was filled with electron lucent granules and the granules were characteristically forming large granular structures in general. Rough endoplasmic reticulum (RER) was observed as tubules with parallel arrangement at the basal regions of the cells. Few smooth endoplasmic reticulum (SER) were observed in groups in the basal cytoplasm (not shown in figures). During the electron microscopic examinations of the control group of parotid, the lumen was seen to be filled with a dense secretion. The apical cytoplasm was filled with considerably electron dense, round or oval secretory granules. The tubules of RER tubules were narrow. The mitochondria were oval or rounded in their normal structure (not shown in figures). In the control group of exocrine pancreas, the lumen was found to be filled with a considerably dense secretion and a prominent excretion was noted. Considerably electron dense round or oval secretory granules were distinguished in the apical cytoplasm. Among the secretory granules, tubules of RER were noticed as vesicles of small and large size (not shown in figures).

Electron microscopic investigation of control with 24 (not shown in figures) and 48 hours fasting groups showed similar ultrastructural appearance in all groups but degenerative alternations were more prominent in 48 hours fasting groups. In the control group of submandibular gland with 48 hours of fasting, electron



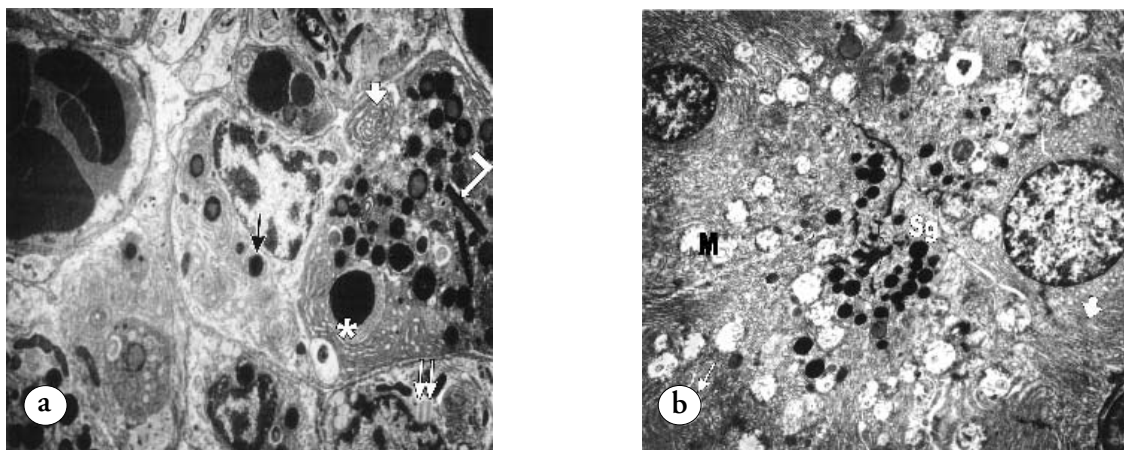
**Figure 1** - Exocrine pancreas from diabetic group: \*apoptotic cell, Sg - secretory granules, → - rough endoplasmic reticulum, M - mitochondria (uranyl acetate, lead citrate x9000).

microscopic examination of the submandibular gland revealed large, electron lucent secretory granules in the mucous cells, occasionally forming accumulation. The regions of these granules opening to the lumen were seen. In the basal cytoplasm, mitochondria and RER were observed. The RER tubules were seen to enlarge in some regions (not shown in figures). In the parotid gland of the same group, electron microscopic examinations have shown that the majority of the cells were filled with secretory granules. Some cells were devoid of granular content. Such cells were distinguished with their electron lucent cytoplasm (not shown in figures). In the control group with 48 hours of fasting electron microscopic photographs of exocrine pancreas revealed cells in both accumulated secretion and production phases of secretion in the serous cells. The mitochondria were considerably large. The RER tubules were regionally enlarged, filled with electron lucent secretory material, and arranged paralleling each other. The structure of centroacinar cells was seen in their normal structure, while some mitochondria were found to be swollen with loss of cristae, the mitochondrion structure was normal in general (not shown in figures).

The electron microscopic examinations of submandibular gland in the diabetic group have shown electron lucent granules of mucous cells occasionally forming accumulation. Vacuoles were distinguished in the cytoplasm. The RER tubules were circularly localized and paralleling each other. Groups of SER were prominent. Myelin figures were noted in between the secretory granules in the cytoplasm. A group of the secretory granules was observed to enlarge excessively

and form autophagic vacuoles (not shown in figures). In the electron microscopic examinations of parotid gland of the diabetic group, electron dense secretory granules were distinguished in the apical cytoplasm. In various regions of the serous cells, RER tubules was observed as dilated or paralleled each other. Lipid droplets were prominent at the basal regions of the cells. The mitochondria were found to be large, and swollen and with occasionally absence of cristae (not shown in figures). In the same group of exocrine pancreas, cells undergoing apoptosis were found in the serous acini. In these cells, the nuclei were excessively heterochromatic. Their cytoplasm were also densely stained, disallowing observation of integrity. Few electron dense and electron lucent granules were noticed to aggregate in the apical cytoplasm. In the basal part of the acinar cells, RER were found to enlarge and be arranged paralleling each other in general. The mitochondria were considerably large, and degenerative changes were noticed within some of them (Figure 1).

Degenerative findings were very clear in the diabetic with 24 (not shown in figures) and 48 hours of fasting groups. These findings were almost the same in the same organelles but more severe in 48 hours fasting groups. During the electron microscopic examinations of the submandibular gland in the diabetic group with 48 hours of fasting, granules of varying size with frequent vacuolar structure were observed. Myelin figures also were observed in the cytoplasm in this group. The mitochondria were rather large as compared with the other groups, and disappearance of cristae and expression of vacuolar structure was noticed in



**Figure 2** - Diabetic group with 48 hours of fasting. a) Parotid gland, \*autophagic vacuoles, ↓ - crystalloid figures, ↑↑ - lipid droplets, → - circularly localized rough endoplasmic reticulum (RER), ↑ - electron dense granules center of RER. b) Exocrine pancreas, Sg - secretory granules, → - RER, M - mitochondria, ↑ - membranous structure in centre of mitochondria (uranyl acetate, lead citrate x9000)

a group of mitochondria (not shown in figures). In the parotid of same group, crystalline structures and autophagic vacuoles of varying size were found. The presence of circularly arranged RER tubules within the cytoplasm and large vacuoles at their median segments were noticed. Also, lipid droplets were distinguished in some cells. Electron lucent core regions were detected at the median segments of mature granules, with electron dense periphery (Figure 2a). In exocrine pancreas, electron dense secretory material was observed filling the lumens. The secretory granules were localized in the apical cytoplasm, considerably electron dense stained, and few in number. Their mitochondria were considerably large with occasionally absent cristae. They usually included membranous structures median segments. The RER tubules were arranged in parallel. These were found to enlarge regionally, and filled with electron lucent secretory material (Figure 2b).

In the diabetic group with insulin treatment for 3 days, electron microscopic examinations of the submandibular gland revealed frequent accumulated formations of the secretory granules. Mitochondria and junctional complexes were seen in their normal structure. The heterochromatic nuclei were noticeable. The RER tubules were prominent in the basal cytoplasm (not shown in figures). In the parotid gland, the structures were found to be restored to normal in general. The tubules of RER were seen to be distributed regularly at the basal regions of the cells. The mitochondria were less degenerated and normal in general. The granules were varying in size and electron dense. Those with electron lucent were noticed in between (not shown in figures).

In the group with 3 days of insulin treatment of exocrine pancreas, acinar and centroacinar cells were identical to those in the control group. However, the mitochondria in serous cells were considerably large with occasional loss of cristae. Membranous structures were noticed in their centers (not shown in figures).

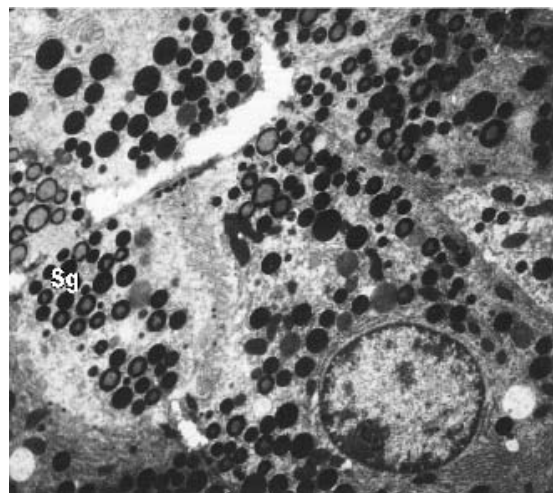
The electron microscopic examinations of the submandibular gland in the diabetic group with insulin treatment for 7 days have shown structures identical to those of the control group. Tubules of RER were paralleling each other in the basal regions of the cells. The mitochondria were normal in structure. Few mitochondria have myelin figures in their median segment (not shown in figures).

In the group with 7 days of insulin treatment of parotid, the structure was similar to that of the controls. However, the granules were noted to include cores with low density and surrounding darker regions due to water-losing properties (Figure 3). In the same group of exocrine pancreas, electron microscopic examinations revealed oval, round or atypically shaped secretory granules of varying size within the serous cells. The lumen

was filled with an exceedingly electron dense secretory material and granule exocytosis was occasionally encountered. While RER appeared normal in general, occasional fragmented tubules were also observed. No degeneration was seen in the mitochondria. However, occasional autophagic vacuoles were present (not shown in figures).

**Discussion.** The salivary glands are directly related with hormones. Several studies have demonstrated the interaction of pancreas and salivary glands.<sup>2,7-10</sup> Also, experimental studies conducted in the last 25 years have shown that diabetes causes metabolic and functional changes in the major salivary glands.<sup>1,11</sup> Polydipsia is one of the classical symptoms of diabetes mellitus. Xerostomia and subsequent thirst are the other symptoms of the illness, and are closely related to polydipsia. Xerostomia in the diabetic subjects primarily is due to reduced salivary flow, which is considered to be related to degenerative changes in the salivary glands.<sup>12</sup> Recent histological studies have demonstrated that the acinar cells of salivary glands are prominently affected by diabetes.<sup>4,11-13</sup>

High et al,<sup>14</sup> in their study published in 1985, have reported that a few small-sized lipid droplets accumulate in the seromucous cells of submandibular gland in diabetes. They detected that formation of autophagic structures in the seromucous cells parallels the duration of diabetes. Cutler et al,<sup>7</sup> have studied the alterations in the submandibular gland in STZ-induced diabetes in rats at the electron microscopic level. The researchers have intravenously injected STZ to male Wistar rats and examined the submandibular glands on the 3rd, 7th and 21st days, and reported observing prominent



**Figure 3** - Parotid gland from diabetic group with insulin treatment for 7 days. SG - secretory granules.

degeneration in every period of diabetes particularly in the acinar cells. They have found that secretions accumulate in the cytoplasm of acinar cells. They proposed that the acinar cells be reduced in number, resulting in a reduction in salivation.<sup>7</sup>

Anderson et al,<sup>15</sup> have reported that replacement of parenchyma by connective tissue in the submandibular gland is incompatible with the physiologic response of the gland, and in their study, in which they induced diabetes in rats using STZ and removed the submandibular glands either 3 weeks, 3 months or 6 months later for examination with light and electron microscopy, they have discovered that the acinar volumes are preserved at a rate of 48% as compared with the controls. In the electron microscopic examinations, they revealed that lipid droplets are formed in the acinar cell cytoplasm, and that autophagic structures, which increase in quantity with increasing duration of diabetes, are built in these cells.<sup>15</sup>

Reuterving et al,<sup>16</sup> in their report in 1987, have observed in the light and electron microscopic examinations of salivary glands in rats with diabetes induced by long-term alloxan exposure that the gland weight is prominently reduced at the end of a month with diabetes, and that this reduction does not change within 12 months. They have stressed that lipid droplets begin to form in the acinar cells in the early stage, and that the morphometric amounts of these droplets are related with the blood glucose levels.<sup>16</sup>

In another study conducted in 1999, Maciejewski et al<sup>3</sup> have investigated the alterations in the lysosomal enzyme activity and ultrastructure of submandibular gland secondary to diabetes with electron microscopy. In their study, they have induced diabetes with alloxan in New Zealand rabbits, and examined the submandibular gland on 21st, 42nd, 90th and 180th days. The researchers have observed degeneration of mitochondria and accumulation of lipid droplets in the cytoplasm of acinar cells in the early stage. On the 90th day of diabetes, they have found vacuoles and swollen lysosomes in the acinar cell cytoplasm and formation of myelin figures in some of the cells.<sup>17</sup> Same researchers reported that lysosomal enzyme levels are related with the blood glucose levels.<sup>3,17</sup>

In our study, results were parallel with literature, but some differences were seen in this study. The electron microscopic examination of the submandibular gland 2 months after the induction of diabetes with STZ has revealed that, as compared with the controls, secretory granules accumulate in the cytoplasm. Marked degenerations were seen in mitochondria and RER. Circular arrangement of the RER tubules shows that these cells go to apoptosis. Also, formation of myelin figures in the cytoplasm, accumulation of the secretory

granules to constitute larger vacuolar structures, and formation of several autophagic vacuoles were noticed.

It was observed that diabetes with fasting causes broadening of the RER tubules and enlargement of the mitochondria as compared with the control groups with fasting. However, it was noted that secretory granules were small-scaled in general. This discovery was attributed to their being newly synthesized. Also, lipid droplets were found in the cells of this group.

Insulin is a physiologic modifier for the salivary glands. With the purpose of determining the localization of insulin receptors in salivary glands, an immunohistochemical study was conducted on rats via stimulating the receptors with insulin and demonstrating the presence of receptors within salivary glands immunohistochemically using anti-insulin 1 and anti-insulin like growth factor (IGF) 1 receptor antibodies. As a result, they have reported that insulin and IGF-1 receptors exist in salivary glands particularly in the cytoplasm and membrane. Physiologic studies have revealed that insulin causes dose-related phosphorylation at its specific receptor. It was reported that STZ-induced diabetes does not alter the proportion of insulin receptors.<sup>18</sup>

The submandibular glands of rats have insulin-like immunoreactivity (ILI). The ILI activates the autophosphorylation of insulin receptor  $\beta$  subunits and induces lipogenesis within the mucous cells. Therefore, it controls the lipid cycle in the submandibular glands of rats via modifying the formation of insulin-insulin receptor complex. Taouis et al,<sup>19</sup> in a study conducted in 1995, have reported an increase in regions of ILI in the submandibular glands of rats with STZ-induced diabetes. In parallel with the discovery of Taouis et al,<sup>19</sup> another study in which the level of ILI in the rat submandibular gland was assessed has revealed an increase of this activity in rats with STZ-induced diabetes.<sup>20</sup>

Morris et al,<sup>21</sup> in another study in which insulin was used as an anti-diabetic after the induction of diabetes have biochemically and histologically evaluated the alterations in the submandibular gland in rats with insulin treatment for one week which started 7 weeks after inducing diabetes with STZ. They have reported observing maximum accumulation of lipid droplets in the cytoplasm of acinar cells of the rat submandibular gland in the 2nd week following the induction of diabetes, and reduction of these droplets inversely proportional with duration. They have found that, insulin treatment for one week following diabetes of 7 weeks restores the fatty acid profile and lipid droplet contents of the acinar cells in submandibular gland to normal. The researchers have reported that insulin treatment has a rapid effect on reversing lipid metabolism in submandibular gland,

and that disordered lipid metabolism in diabetic rats is secondary to insulin insufficiency.<sup>21</sup>

In our study, electron microscopic examinations of the submandibular gland in the group with insulin treatment following diabetes for 2 months have revealed that insulin restores the structure in general. Disappearance of lipid droplets was noted even in the short-term (3 days) treatment group. The secretory integrity was found to be preserved as compared with the diabetic group without treatment, and the structures of both RER tubules and mitochondria were seen in their normal structure. Insulin treatment for 7 days restored the structure almost to normal, though myelin figures were still apparent.

The salivary glands have a direct relationship with carbohydrate metabolism. This is mediated via the influences of  $\alpha$ -amylase secreted by these glands.<sup>22</sup> In several studies, it has been reported that diabetes reduces the activities of parotid and exocrine pancreatic amylase.<sup>23-26</sup>

In a study conducted in 1988,<sup>22</sup> examination of the parotid gland amylase activities in diabetic rats in fasting and satiety revealed that parotid amylase in the control group was considerably decreased in satiety, and higher than normal in state of fasting. In diabetes, amylase activity was found to be lower in state of fasting as compared with the controls, and even much lower in satiety.<sup>22</sup>

Hand et al,<sup>1</sup> have reported in 1984 the ultrastructural alterations in the parotid gland in STZ-induced diabetes in rats ranging from 4 hours to 1 year following the induction. The researchers have observed lipid droplets being formed in the basal cytoplasm of acinar cells within the first 24 hours. Within 4.5 months, lipid droplets have risen to maximum in quantity, and transformed into large vacuoles with time. Also, they have reported that Golgi complex developed within the first week following diabetes induction with STZ, and this was prominent on the trans Golgi side of RER. One month later, membrane-enveloped cytoplasmic crystalloids have appeared within the acinar cells, and these structures were found to be higher in quantity in older (10-12 months old) rats. The crystalloids were reported to react with tri-metaphosphatase, which is a chemical marker for lysosomes. The researchers have concluded that parotid gland structural and functional integrity was impaired by insulin deficiency.<sup>1</sup>

In our study, electron microscopic examinations of the parotid gland in diabetes and following state of fasting have revealed multiple large lipid droplets particularly in the basal cytoplasm of acinar cells. The super abundance and large size of the droplets were related with increasing duration of diabetes, which was harmonious with the findings of previous literature.

Broadening of the RER tubules as compared with the controls, and degenerative changes in the mitochondria were also observed. In state of fasting with diabetes, autophagic vacuoles and crystalloid formations that increased in quantity with the duration of fasting were observed in the serous cell cytoplasm. Besides, the RER displayed circular arrangement and were transformed into autophagic vacuoles following 24 hours of fasting, in contrast with the controls. Lipid droplets were also present in the fasting group. While the quantity of granules increased with fasting in the control group, fasting in diabetes was found not to cause an increase in the granule content.

In their study reported in 1998, Szczepanski et al,<sup>27</sup> have intended to determine the effects of diabetes and following insulin treatment on the parotid gland ultrastructure and protein content in rats. Inducing diabetes in rats using STZ, the researchers have examined the parotid glands 30 days later, and treated a group of rats with insulin for 7 days. They have reported the presence of lipid droplets in the acinar cells of diabetic group. Also, they have occasionally observed crystalloid lysosomes. In the diabetic group with insulin treatment for 7 days, it was reported that the structure was restored, as compared with the controls.<sup>27</sup>

In our study, upon examination of the effects of insulin treatment 2 months after the induction of diabetes with STZ on the structure of parotid gland, we observed that the glandular structure was restored to normal in general. Particularly, the lipid droplets have disappeared, even after short-term insulin treatment. This observation was attributed to regulation of lipid metabolism by insulin. It was noted that the properties of RER tubules and the quantity of secretory granules were similar to those in the control group. Insulin treatment for 7 days has restored the localization of RER tubules completely to normal. The secretory granules increased in quantity; however, they displayed paler cores and darker periphery, indicating altered granular content.

Romagnoli et al<sup>28</sup> in a study conducted in 1984 on rats, have reported that following fasting for 12

### Related Topics

Mula-Abed WA. Biochemical analysis of serum pancreatic amylase and lipase enzymes in patients with type 1 and type 2 diabetes mellitus. *Saudi Med J* 2005; 26: 73-77.

Aughsteen AA, Mohammed FI. Insulin enhances amylase and lipase activity in the pancreas of streptozotocin-diabetic rats. An in vivo study. *Saudi Med J* 2002; 23: 838-844.

Al-Shurafa HA, Jawdat MT, Bassas AF, Rogiers XC, Bechstein WO. Innovations in pancreas transplantation. *Saudi Med J* 2002; 23: 265-271.



hours, pancreatic acinar cells are filled with secretory granules, whereas Golgi complexes are inactive. Upon examination of the pancreatic tissue 10 minutes after feeding, they have found that the secretory granules were reduced and Golgi complex cisterns were broadened as compared with the fasting group.

In our study, examination of the pancreatic tissue in fasting group has revealed broadening of the RER tubules with accumulation of electron lucent material. The mitochondria were hypertrophic. Secretory granules varied from cell to cell, and were found to accumulate in some cells while being synthesized in some others. It was noted that the secretory granules coalesced within these cells. In state of fasting, the granular content within serous end segments did not change considerably.

In a study published in 1996,<sup>29</sup> it was reported that exocrine pancreas, which has important functions in carbohydrate metabolism, is also affected by diabetes, and although the secretory granules are not altered in size, their quantity and therefore total volume are decreased.

A histological study on this subject was conducted by Yasuda et al,<sup>5</sup> in dogs with STZ-induced diabetes at the levels of both light and electron microscopy. The researchers have examined the exocrine pancreas one year after the administration of STZ, and found that autophagic vacuoles and lipid droplets are formed within the acinar cells, and the structure of RER is fragmented in this cells. Finally, they have stressed that the secretory granules of various sizes decreased in quantity in diabetic dogs, as compared with the controls.

In our study, upon examining the effects of diabetes for 2 months on pancreatic structure we observed atrophic cells particularly in the acinars, which paralleled those reported in the literature. There were occasional broadening and fragmentations of the RER tubules, and degenerative changes in mitochondria. We noted that the quantity of secretory granules was reduced. In the diabetic group with super imposition of fasting, prominent degeneration was observed in the pancreas. The mitochondria were found to enlarge, lacking cristae and including membranous structures, particularly in the serous cells. The autophagic vacuoles appeared within the first 24 hours following diabetes. Occasional broadening and accumulation of material with low density were observed in the RER tubules.

Recent studies have demonstrated that insulin directly regulates the activities of pancreatic serous cells. Endogenous insulin has been found to have a potential role in secretory granule release. Insulin receptors have been found in the serous cells who isolated from rat and mice, and it was reported that insulin stimulates several processes such as carbohydrate transport, protein synthesis and cholecystokinin receptor synthesis within these cells upon binding to its receptor. In conclusion,

the importance of insulin – exocrine pancreas interaction on the regulation of pancreatic functions has been stressed.<sup>30</sup>

Levy et al,<sup>31</sup> in their study published in 1988, have comparatively examined the lipid and fatty acid content in exocrine pancreas of rats with STZ-induced diabetes, diabetic rats receiving insulin treatment and healthy rats in the control group. The researchers have reported a reduction in fatty acid content in diabetes, while cholesterol levels rise 3 to 4 folds. The morphocytochemical studies also support their observations; they have found multiple cholesterol-including lipid droplets within the acinar cells. Insulin treatment has been seen to restore the lipid content to normal. As a result, the researchers have stressed that insulin regulates the lipid profile of the acinar cells in exocrine pancreas.

Insulin treatment following diabetes for 2 months has been found to restore the structure of pancreas to normal in general. The granular content was close to normal. While the degenerative changes were preserved during short-term (3 days) insulin treatment, they were restored to normal after treatment for 7 days. In this period, the RER tubules were noted to be normal in structure, despite the persistence of autophagic vacuoles. Observation of exocytosis within the serous cells has been considered as a sign of cellular activity.

In this study, the purpose of which is to examine the ultrastructural changes caused by diabetes and state of fasting on submandibular and parotid glands and pancreas, and the influences of insulin treatment on these changes, we have observed considerable degeneration caused by diabetes in both glands. While the degeneration manifested itself as accumulation of secretions within the mucous cells in the submandibular gland. In the mucous cells, lipid droplets were absent, being replaced by vacuolar structures. Paralleling the literature, we considered these vacuoles as altered forms of the lipid droplets. Thus, we suggested that the submandibular gland is structurally influenced at the early stages of diabetes. The parotid gland and exocrine pancreas, having similar properties, were found to be affected similarly. Diabetes-induced loss of granules was observed in the serous cells in both glands. This observation was different from that in the mucous cells of submandibular gland. There also was diffuse lipid accumulation within these cells. The presence of this accumulation suggested that both of these 2 glands are affected by diabetes in a later stage than the submandibular gland. Regarding granule content, the most prominent degenerative changes were observed in the parotid gland. While cellular loss was observed in neither the submandibular, nor the parotid gland, presence of apoptotic cells was noted in the pancreas.

State of fasting was found to cause alterations within the glands indicating increased activity, particularly

in the granulated endoplasmic reticulum tubules and the mitochondria. While insulin treatment was seen to restore the structure to normal in general in both of the 3 glands, the degenerative changes were observed to persist partially in the mitochondrial and granular structures.

In conclusion, this study demonstrated that both of the 3 glands are affected by diabetes and concomitant fasting, and this effect manifests itself via the granule content.

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