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An Investigation into the Protective Effects of Melatonin on Pancreatic B-Cells under Chronic Lipid Loading during the Aging Process

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Abstract: ***Our research aims to investigate the potential protective influence of melatonin on pancreatic β-cells during chronic lipid loading, a condition that often precipitates the onset of diabetes mellitus in aging populations. The study was conducted on a sample of 140 white male rats, divided into two age groups: adult and late-senescence. For the study, rats from Groups I and II were subjected to a high-lipid diet over a three-month period. In addition to the diet, Group II rats received daily melatonin doses (0.15 mg/kg) throughout the same timeframe. All animals were subsequently euthanized under ether anesthesia after the lipid-loading phase, and again one and two months post-lipid loading, with each phase involving ten rats. Group III, the control group, consisted of undisturbed rats. Tissue samples from the pancreas were processed for electron microscopy, with the ultrastructural study conducted using electronograms. In each experimental stage, we examined 100 pancreatic β-cells. We analyzed the resulting data using the SPSS-12-ANOVA statistical software. Our findings revealed that chronic lipid loading incites functional activation in pancreatic β-cells, subsequently leading to irreversible damages in the β-cells of older organisms. This outcome suggests that chronic lipid loading can significantly contribute to the development of diabetes, particularly in the later stages of life. However, our study also demonstrated that melatonin could potentially alleviate the severity of β-cell damage under chronic lipid loading. Given melatonin's high β-cell protective activity, we advocate for its co-administration with anti-diabetic drugs, particularly in older individuals.***

Keywords: Melatonin, Pancreatic β-cells, Chronic lipid loading, Diabetes mellitus, Aging populations, High-lipid diet, Ultrastructural study on pancreas tissue

1. Introduction

The main lipids present in the blood plasma are fatty acids, triglycerides, cholesterol and phospholipids [8, 9]. Elevated plasma concentrations of lipids, particularly cholesterol, are related to the pathogenesis of atherosclerosis, the process responsible for the majority of cardiovascular disease (coronary, cerebrovascular and peripheral vascular disease) [12].

Distarbances of fat metabolism are fundamental to the diabetic process. Diabetes constitutes a major cardiovascular risk. The causes of insulin deficiency and deranged insulin secretion (synthesis/extrusive) are not completely clear [3, 2, 12]. Type 2 diabetes mellitus is the result of multiple genetic defects, the obvious risk of which is increased by environmental factors and first of all – by modern life-style characterized by psycho-emotional load, stressors-homotoxins, overfeeding by high-caloric meals, low physical activity, obesity and etc. [10, 12].

Our attention was especially paid to diets overloaded with high caloric meals, particularly fats [6, 7]. Intracellular triglycerides and products of fatty acid metabolism are potent inhibitors of insulin signaling and result in an insulin resistance state [12]. It is important that chronic conditions of lipid loading cause the mobilization of pancreatic β-cells reserves and, correspondingly, promote the occurrence of secretory-extrusive functions intensity in mentioned cells, followed in certain β-cells (especially in old organisms) by irreversible changes[6, 7, 8].

Besides, the abnormal night-sleep is overlooked as a risk factor; sleep-deprivation has been associated with both obesity and type 2 diabetes mellitus [4, 9]. There's also evidence that disturbances to "sleep hormone" melatonin production may affect the body's insulin level [13]. Melatonin increases the osteocalcin gene expression; osteocalcin is secreted by osteoblasts and thought to play a role in the body's metabolic regulation: osteocalcin acts as a hormone in the body, causing pancreatic β-cells to release more insulin and the same time directing fat cells to release the hormone adiponectin, which increases sensitivity to insulin [5]. All mentioned suggest that that melatonin has a main role in glucose metabolism. Melatonin (main hormone of pineal gland) has a great role in animal organisms. It is considered as a strong antioxidant; it also has immunomodulate, genoprotective, cytostatic activity; it is important that receptors are located on pancreatic β-cells [11].

According to the above mentioned, the search for every potential factor to prevent pancreatic β-cells' damage is of high interest.

2. Material and Methods

The aim of our research was to study the influence of melatonin on pancreatic β-cells under the condition of chronic lipid loading – especially in old age, when the diabetes mellitus frequently takes place.

Experiment was performed on 140 male white rats. Two age groups were selected: adult period of life and late senescent period of life. This number of rats was divided into three groups: I – experimental group (30 adult and 30 old animals), II- experimental group (30 adult and 30 old animals), III - experimental group (10 adult and 10 old animals). The animals of I and II experimental groups were on a lipid-rich diet during three months. The animals of II experimental group were also given melatonin (0,15 mg/kg) daily during three months. Under the ether narcosis the animals were sacrificed after lipid loading and 1 and 2 month later from finishing lipid loading (in each experimental case 10 rats have been used). In III – control group the intact animals have been used. The tissue taken from pancreas has been processed for electron microscopy. Ultrastructural study was performed using electronograms. In each experimental case 100 pancreatic β-cells have been studied. Results of research were studied statistically using computer program SPSS-12-ANOVA.

3. Result and Discussion

The results of study shown, that after stopping of lipid loading in the pancreatic β-cells of all animas (adult/old rats) from I experimental group the number and size of mitochondria are significantly increased. The number of GER membranes are markedly increased as well together with elevation of number and sizes of membrane-attached and free ribosomes and polisomes, the Golji apparatus is well defined, the number of secretoy granules is significantly decreased.

After 1 month from stopping of lipid loading the ultrastructural patern in the pancreatic β-cells of all adult rats is the same, as it has been in the previous term of experiment; whereas in the pancreatic β-cells of all old rats the number of mitochondria in comparison with the previous term of observation is decreased, though, their sizes are not impaired, on the contrary, frequently are revealed the ball-like, fish-like, cigar-like and other bizarre-form mitochondria (Fig. 1), the number and sizes of GER membrane-attached ribosomes and polisomes are decreased, Golji apparatus is ill defined, the number of secretory granules is still decreased.

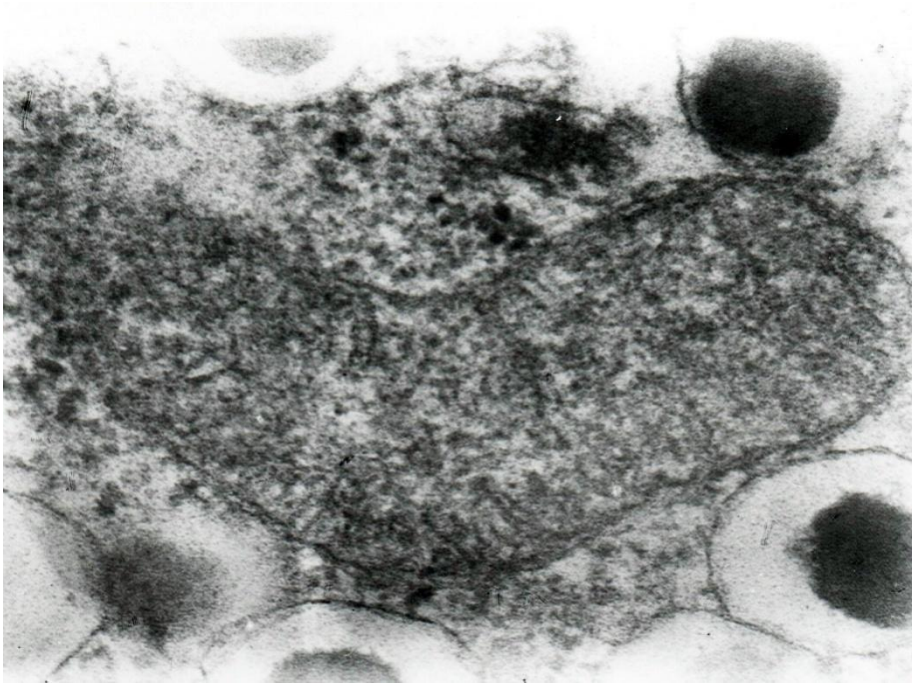

Figure 1: Bizarre-form mitochondrion in the pancreatic β-cell. Electron microscopy: x 160 000, JEM-1200 ex. After 1 months from stopping of the lipid loading. Old white rat. I experimental group

After 2 months from stopping of lipid loading the ultrastructural patern in the pancreatic β-cells of all adult rats is identical to norm; whereas the number of mitochondria in the β-cells of all old rats is markedly decreased, while there is plenty of giant mitochondria and part of them is disorganized, the number of GER membranes is markedly decreased, the membranes are partly fragmented and disorganized, the number of GER membrane-attached and free ribosomes and polisomes is significantly decreased, the Golji apparatus is reduced, the stagnation of secretory granules is revealed (Fig. 2).

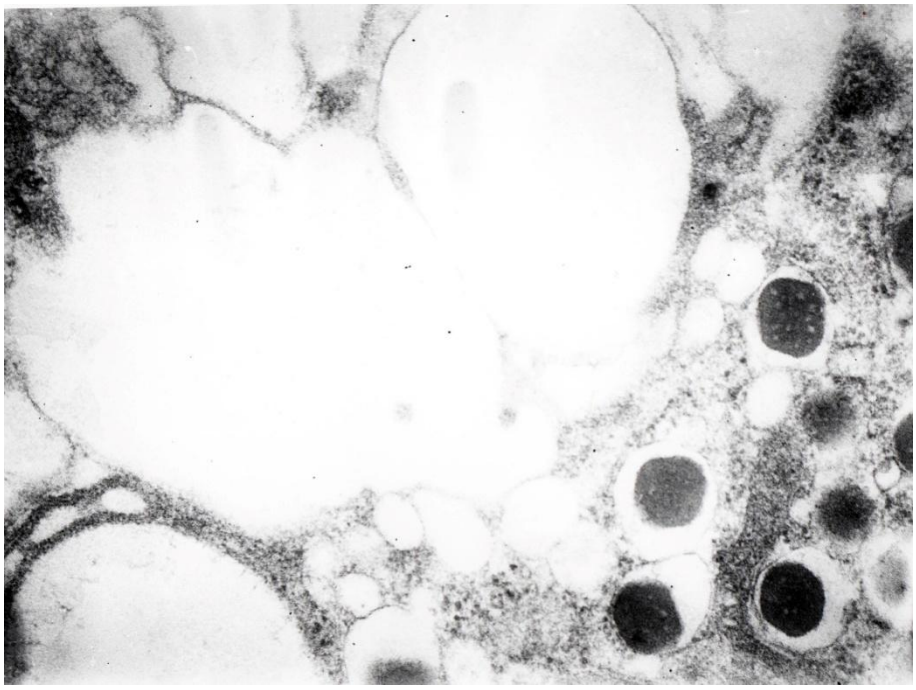

Figure 2: Disorganized mitochondria, fragmented and disorganized membranes of granular endoplasmatic reticulum, stagnated secretory granules in the pancreatic β-cell. Electron microscopy: x 12 000, JEM-1200 ex. After 2 months from stopping of the lipid loading. Old white rat. I experimental group

The results of study of pancreatic β-cells of rats from II experimental group have shown, that after stopping of lipid loading the ultrastructural patern in β-cells of all adult rats is identical to norm (Fig. 3); whereas in the β-cells of 9 old rats (90%; p<0,01) thenumber and size of mitochondria are moderately increased, the number of GER membranes are moderately increased as well together with slight elevation of number and sizes of membrane-attached and free ribosomes and polisomes, the Golji apparatus is well defined, the number of secretory granules is moderately decreased. The ultrastructural patern in the β-cells of 1 old rats (10%; p<0,1) is identical to norm.

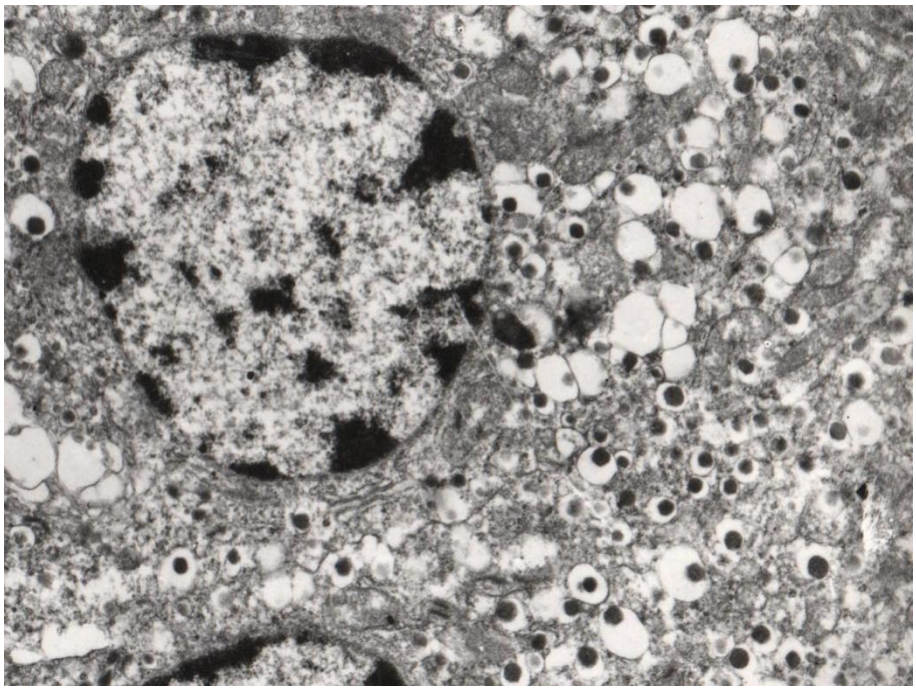

Figure 3: Normal ultrastructural patern in the pancreatic β-cell. Electron microscopy: x 4 000, JEM-1200 ex. After finishing of lipid loading. Adult white rat. II experimental group

After 1 month from stopping of lipid loading the ultrastructural patern in the pancreatic β-cells of all adult rats is the same, as it has been in the previous term of experiment; whereas in 7 old rats (70%; p< 0.05) the number of mitochondria in the β-cells in comparison with the previous term of observation is decreased, the bizarre-form mitochondria are not revealed, the number of GER membranes are decreased as well, fragmentation and disorganization of GER are not seen, the Golji apparatus is not ill defined, the number of secretory granules is slightly

increased. The ultrastructural patern in the β-cells of 3 old rats (30%; p<0, 1) is the same, as it has been in 9 old rats of the previous term of experiment.

After 2 month from stopping of lipid loading the ultrastructural patern in the pancreatic β-cells of all adult and 8 old rats (80%; p<0, 01) is identical to norm (Fig. 4); whereas in the β-cells of 2 old rats (20%; p<0,1) is the same, as it has been in 7 old rats of the previous term of experiment.

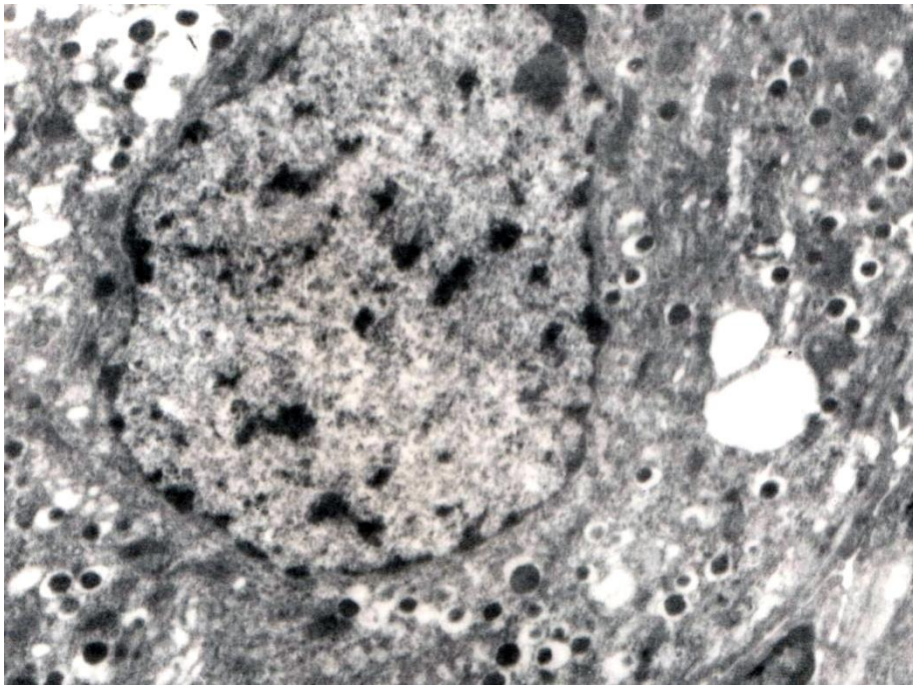

Figure 4: Normal ultrastructural patern in the pancreatic β-cell. Electron microscopy: x 4 000, JEM-1200 ex. After 2 month from stopping of lipid-loading. Old white rat. II experimental group

The results of ultrastructural study of pancreatic cells of rats from III (control) group have shown that cells contain many mitochondria, large part of which have a round form. GER is moderately prominent, on GER membranes the number of ribosomes is moderate. Golji apparatus is composed of smooth membranes, cistern and full number of vesicles. The number of secretory granules is well prominent in cytoplasm.

Our results partly correspond to the data of certain authors [1].

To summarize, the chronic lipid loading causes the activation of the pancreatic β-cells' function, followed in the pancreatic β-cells of old organisms by injury and irreversible changes. Therefore chronic lipid loading seems as a serious condition for development of diabetic process in **s**enescent period of life. Melatonin reduces morphological peculiarities of damage of the pancreatic β-cells at the state of chronic lipid loading. Because of high β-cells-protective activity of melatonin, we recommend that the mentioned hormone be taken with anti-diabetic drugs, especially in old age.

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Informed Consent Statement: Not applicable.

Data Availability: All relevant data are within the paper and its Supporting Information files.

Author Contributions:

A. Tavartkiladze [2,3] (AT) and **D.Kasradze**[1,3] (DK) conceived and designed the experiments.

P.Revazishvili[2,3] and **A.Tavartkiladze**[2,3] (AT) performed the experiments, analyzed the data, and wrote the manuscript.

Alexandre Tavartkiladze[2] (AT), and **D.Kasradze**[1,3](DK) contributed to data collection and manuscript revision.

Alexandre Tavartkiladze[1,2] (AT) provided technical support and assisted in the design of the experiments.
All authors contributed to manuscript revision, read, and approved the submitted version.

Conflict of Interest: The authors declare that they have no conflict of interest.
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