Research Article

# A combination of melatonin and moderate-intensity aerobic exercise improves pancreatic beta-cell function and glycemic homeostasis in type 2 diabetic model of animals

**Eduardo de Almeida Leite[1a], Patricia Rodrigues Lourenço Gomes[1a], Eloisa Aparecida Vilas-Boas[1], Ana Cláudia Munhoz[1], Lívia Clemente Motta-Teixeira[1], José Sinésio da Silva Junior[1], Angelo Rafael Carpinelli[1] and José Cipolla Neto[1*]**

[1]Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil
[a]These authors contributed equally
*Correspondence: cipolla@icb.usp.br, Tel: +551130917466 or +5511971695000

**Running title:** Melatonin and aerobic exercise improves pancreatic beta-cell function and glycemic homeostasis

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## ABSTRACT

Nocturnal melatonin secretion is important for preservation of ß-cell mass and function. Knowing that type 2 *diabetes mellitus* (T2DM) is a chronic metabolic disorder characterized by hyperglycemia caused by the elevated resistance of peripheral tissues to insulin, reduction in pineal melatonin and disturbances of insulin secretion by pancreatic ß-cells. In this context, exercise is considered one of the most valuable non-pharmacological approaches for treatment of T2DM. Considering the beneficial role of melatonin on glycemic metabolism in physical exercise, we investigated the effects of moderate-intensity aerobic exercise plus melatonin on glycemic homeostasis, the morphology and architecture of pancreas in spontaneous T2DM animals [Goto-Kakizaki (GK) rats]. The results confirmed that melatonin alone reduced the mass of epididymal white adipose tissue (WAT); however, only the combination of melatonin and physical exercise significantly reduced caloric intake, body weight, WAT and improved glucose tolerance and insulin sensitivity in T2DM rats. This combination also reduced apoptosis of cells in pancreatic islets. We observed either melatonin or the combination was able to reduce insulinemia. However, only the combination improved the morphology of the pancreatic islets. Thus, we conclude that in GK rats, melatonin plays a crucial role in the functionality of the pancreas to improve insulin sensitivity of peripheral tissues and, consequently, to maintain the glucose homeostasis. In addition, the combination is more efficiency to improve glucose tolerance and integrity of pancreatic islets in GK rats than melatonin alone.

**Key words:** aerobic exercise training, melatonin, pancreatic islets, type 2 *diabetes mellitus*.

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## 1. INTRODUCTION

Melatonin and insulin have strong association in vertebrates. Insulin potentiates pineal melatonin production (1, 2) while melatonin inhibits insulin production and release (3). Additionally, nocturnal melatonin secretion is responsible for preservation of ß-cell mass and

function in humans (4-6). Type 2 *diabetes mellitus* (T2DM) is a chronic metabolic disorder characterized by hyperglycemia, the resistance of peripheral tissues to insulin and disturbances of insulin secretion (7). An association between the pathology of T2DM and decreased melatonin secretion in diabetic patients and Goto-Kakizaki rats (GK) has been observed (8-10). GK rats is a non-obese and spontaneous (genetic) T2DM model and has been widely used to investigate the development of T2DM and its complications (11-17). In uterus, GK fetus shows reduced pancreatic ß-cell mass, shortly after birth, these rats present normal blood glucose. However, when GK rats reach 28 days of age, they have basal hyperglycemia, impaired insulin secretion and increased hepatic glucose production (18-20) and after 56 days they develop peripheral insulin resistance(21).

Exercise is considered one of the most valuable non-pharmacological approaches in T2DM treatment (22). It improves glucose tolerance(23) and glucose-dependent insulin release. In addition, it also preserves islet architecture, ß-cell viability and insulin content (24, 25). On other hand, melatonin supplementation restores the metabolic adaptations evoked by physical exercise in pinealectomized animals (26) and in elderly who have decreased melatonin synthesis and secretion (27). Therefore, considering the beneficial effects of melatonin on glycemic metabolism in physical training process, we investigated the effects of the combination of moderate-intensity aerobic exercise and melatonin supplementation in glycemic homeostasis, morphology and architecture linked to the function of the endocrine pancreas in GK rats.

## 2. METHODS

### 2.1. Animals.

Male Goto Kakizaki (GK) rats, 12 weeks old, obtained from Charles River Laboratories International, Inc. (Wilmington, MA, USA). The animals were kept in our facility at 22 ± 2ºC on a 12L:12D light-dark cycle. Standard chow (Nuvilab1, Curitiba, Brazil) and water were available *ad libitum*. Three-month-old rats were randomly divided into 4 experimental groups, so that the mean body weight between groups was approximately 300 grams. GK (sedentary control), GM (sedentary GK treated with melatonin), TG (trained GK) and TGM (trained GK treated with melatonin). Then, the exercised groups underwent adaptation to the treadmill. Finally, both the melatonin supplementation protocol and the exercise protocol were started. All experimental procedures were approved by the Animal Ethical Committee (protocol number 86/2016).

### 2.2. Production of spontaneous type 2 diabetic animals (GK strain).

The GK strain was generated under the hypothesis that spontaneous diabetic rats would be produced from normal rats by repeated selective breeding, promoting high blood glucose levels in the offspring and a spontaneous DM2 state (28). Briefly, 211 rats of the Wistar strain were submitted to oral GTT. Of these, 9 males and 9 females were selected as a breeding stock (P). By the breeding, 162 offspring were obtained and GTT was repeated in these offspring. According to the test result, 12 male and 13 female rats were finally selected as a breeding stock (F1). 204 offspring (F2) were obtained. The same procedure was repeated in this generation. For the selection of breeding rats, the second GTT was repeated within two weeks in rats selected by the first test. Animals with two impaired tests were chosen. Consanguinity, especially sibling reproduction, was avoided. Goto *et al* (28), demonstrated that impaired glucose tolerance was observed after crossing selected animals for 16 generations.

### 2.3. Melatonin treatment.

The melatonin groups received 3mg/kg of melatonin (Sigma Chemical Company, St Louis, MO, USA) in drinking water during the entire study (three months). Melatonin water was offered only during the 12 hours of the dark phase. At light phase melatonin-free water was provided. Melatonin solution was prepared daily based on the water consumption and body weight of each animal.

### 2.4. Exercise training protocol.

The physical training protocol lasted 12 weeks, 5 times/week, and was performed on a programmable treadmill, adapted to train 12 rats simultaneously. The protocol used was adapted from Mendes *et al*. 2013 (27). Before starting training, the rats of the TG and TGM groups were adapted to the treadmill for one week, 10 minutes/day, at low speed (0.5 km / h). The training was performed by gradual increases in exercise intensity, defined by speed and time, as shown in Table 1. The protocol did not include changes in the inclination of the treadmill. The protocol maintained the training intensity between 50-80% of the $VO_2$ max.

**Table 1. Exercise training protocol.**

| WEEK | ADAP. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SPEED (KM/H)** | 0.5 | 0.5 | 0.6 | 0.6 | 0.7 | 0.8 | 0.9 | 1 | 1 | 1.1 | 1.2 | 1.3 | 1.3 |
| **TIME (MIN)** | 10 | 10 | 15 | 20 | 20 | 30 | 30 | 35 | 35 | 40 | 40 | 40 | 40 |

The animals were trained in the dark phase of the light-dark cycle, in accordance to their circadian phase of activity. In order to avoid any putative synchronizing effect of the training activity, animals were trained at random times within the 12 hours of the dark phase (29).

Both at the beginning and at the end of the training protocol, the incremental load test was performed in order to confirm the training efficiency. Therefore, the trained animals were submitted to exercise on a treadmill with a gradual increase in speed (0.5 km/h every 3 min) until their exhaustion (30) .

### 2.5. Body weight, food intake and blood glucose levels.

The weekly body weight and daily food intake were measured during the experimental period. In addition, food intake was calculated in Kcal by converting the sum of the weekly consumption during the experimental protocol from the nutritional table provided by the manufacturer (Nuvilab1, Curitiba, Brazil).

For blood glucose analysis, blood samples from the animals' tails were measured on test strips and glucometer (Optium Blood Glucose Test Strips® and Optium Xceed®, Medisense®, Oxford, UK) at the beginning and at the 10$^{th}$ experimental week, in the light period (ZT 14) after a 10-hour fast.

### 2.6. Intraperitoneal glucose tolerance test (GTT).

The animals were submitted to an intraperitoneal (i.p.) injection with 2g/kg of 20% glucose, at ZT 10, after fasting for 8 hours. Tail blood samples were collected at times 0, 5, 10, 15, 30, 60 and 120 min for menstruation blood glucose. To estimate glucose tolerance,

the area under the curve (AUC) was calculated from the basal blood glucose using Prism software, version 8.0 (GraphPad Software, LLC).

**2.7. Intraperitoneal insulin tolerance test (ITT).**

The animals were submitted to an i.p. injection with 0.5 IU/kg of insulin (Humulin R; Eli Lilly), at ZT 10, after 5 hours of food deprivation. Tail blood samples were collected at times 0, 5, 10, 15, 20, 25 and 30 min for menstruation blood glucose. The constant rate for glucose disappearance (Kitt) was calculated using the formula 0.693/half-life using Prism software, version 8.0 (GraphPad Software, LLC)(31).

**2.8. Tissue collection - Pancreatic islet isolation**.

24 hours after the last exercise training session (ZT 14), animals were deeply anesthetized with an i.p. injection of ketamine and xylazine (7.5:1/kg) and pancreas was collected and immediately processed. Pancreatic islets were isolated as described previously(32). Briefly, after distension via pancreatic duct injection with collagenase (0.68 mg/mL), pancreas was removed and digested in a shaking water bath at 37 $^{o}$C. Pancreatic islets were isolated from GK, GM, TG and TGM groups.

**2.9. Cell viability, apoptosis and death.**

Cell viability, apoptosis and cell death were identified by flow cytometry using the Guava ViaCount Assay (Millipore, Billerica, MA)(32). Briefly, groups of 30 islets were disrupted with trypsin and resuspended in 200 µL of RPMI-1640 medium. Then, 175 µL of the cell suspension was added to 25 µL of ViaCount reagent and incubated for five minutes at room temperature. Data acquisition was performed in the flow cytometer Guava EasyCyte (Millipore) and data analysis was performed using the ViaCount software module.

**2.10. Pancreatic histological assessment.**

Tails of the pancreas were immersed in 4% (wt/vol) paraformaldehyde fixative solution for 24 h and submerged in a solution for cryoprotection (sucrose 20%) for 48h. The samples were dehydrated by serial increasing concentrations of ethanol, paraffin embedded. Serial sections (5 µm tick and 200 µm apart from each other) were mounted using glass slides and stained with hematoxylin and eosin (HE). All slides were observed under a microscope (Leica Imaging System, Germany). The images were calibrated and analyzed with the Aperio ImageScope software (Leica Imaging System, Germany), then, (i) islets density (islets/cm$^2$), (ii) pancreatic islets area and (iii) islet size were measured according to Weibel (33).

To calculate circularity, pancreatic islets were delimited, and both their area and circularity were automatically calculated using ImageJ 1.50 software (National Institutes of Health, Bethesda, MD). A circularity value closer to 1 indicates a circular shape and closer to 0 indicates more irregular shape(34).

**2.11. Measurement of insulinemia by enzyme-linked immunosorbent (ELISA) assay.**

20 µL of venous blood were collected from the animal's tail at the beginning of the glucose tolerance assay. Thus, blood was collected at times 0, 5, 10, 15 and 120 minutes after intraperitoneal injection with a 20% glucose solution, using a dose of 2 g/Kg at ZT 10. Then, the serum was extracted to measure insulinemia by Rat/Mouse Insulin enzyme-linked

immunosorbent assay (Merck-Millipore, Massachusetts, EUA). Data were normalized and transformed into ng/mL using a dose-response curve with sigmoidal equation.

### 2.12. Statistical analyses.

For incremental load test analysis was used paired Student's t test (supplementary data). For the other analyses, Two-way ANOVA was used, followed by the Bonferroni's multiple comparison test. Both performed using Prism version 8.0 (GraphPad), considering $P \leq 0.05$.

## 3. RESULTS

### 3.1. Effects of melatonin and/or exercise on WAT, caloric intake, body weight.

Body weight was assessed every week throughout the experimental protocol. At the beginning of the experiment, animals in the four groups had similar body weight (Figure 1a), but at the end of 12 weeks, the exercise plus melatonin group (TGM) showed decrease in body weight when compared to the sedentary group (GK) and the melatonin alone group (GM) (Figure 1b). Moreover, after the sixth week of exercise, TGM maintained the lower body weight compared to the GK until the end of the study (Figure 1c). Besides, the weight variation, calculated by the difference between the final and initial values (delta of body weight) (Figure 1d), showed that both TG and TGM presented less body weight variation, indicating that the body weight gain was lower in these groups compared to GK and GM.

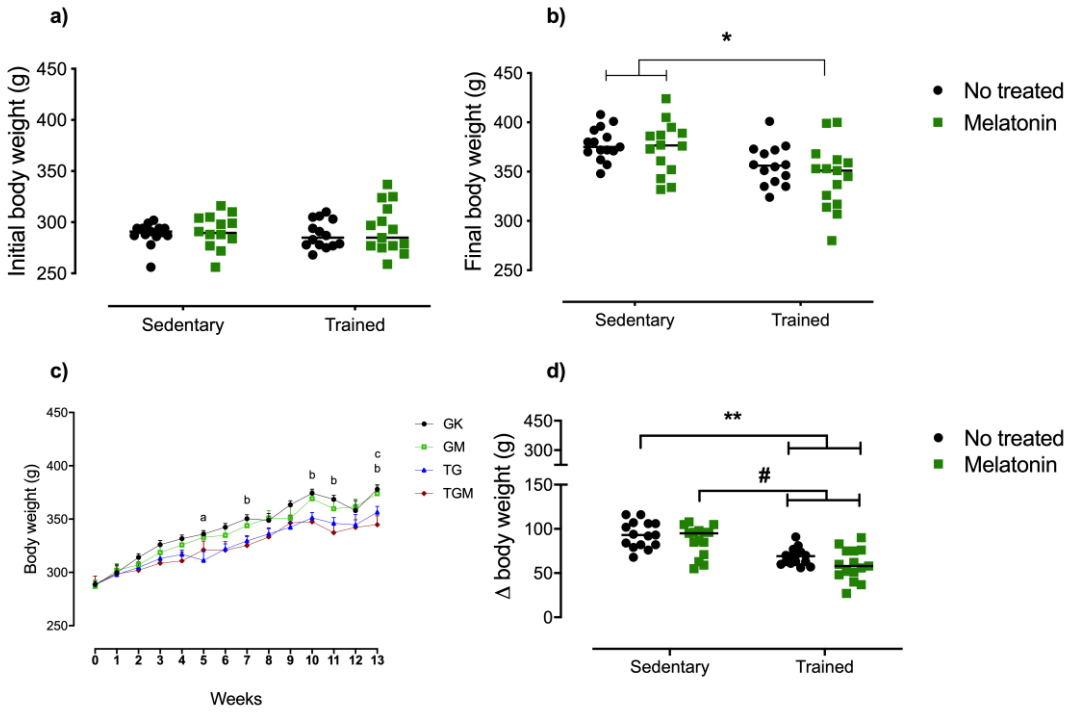


**Fig. 1. Changes of the body weight.**

*a) At the beginning of the study; b) at the end of the study; c) changes of body weight over the 12 weeks of study and d) difference between initial and final body weight. Data are expressed as mean ± SEM (n=14-15 rats/group). * TGM vs. GK and GM; ** GK vs TG and TGM, # GM vs TG and TGM. [a] GK vs TG; [b] GK vs TGM; [c] GM vs TGM, respectively. All symbols represent P < 0.05.*

Food intake was monitored weekly (Figure 2a). The average intake over the period (Figure 2b) and the caloric intake in Kcal were calculated (Figure 2c), respectively. There were no differences among groups in the average food intake (Figure 2b). However, TGM consumed less calories compared to GK and GM (Figure 2c). The combination of exercise and melatonin supplementation was more efficient to maintain lower body weight at the end of the study than other groups (Figure. 2c).

There were no significant differences as to the body size, measured by the length of the tibia (Figure 2d), muscle hypertrophy of the soleus (Figure. 2e) and EDL muscles (Figure. 2f) and the inguinal WAT (Figure. 2g) among groups. However, melatonin supplementation was able to significantly decrease the amount of epididymal WAT (Figure. 2h), and the combination of exercise and melatonin significantly reduced the amount of retroperitoneal WAT (Figure. 2i). Thus, the TGM group showed significant reduction of epididymal and retroperitoneal WAT mass compared to the GK and GM groups.

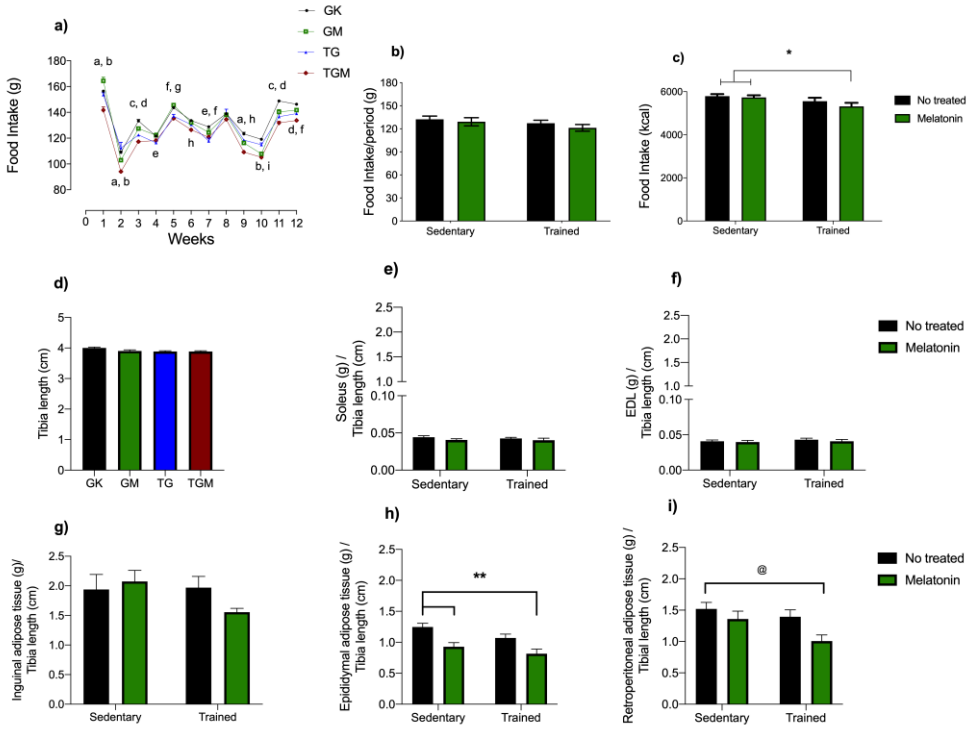


**Fig. 2. Food intake of the different groups.**

*a) During the period of the entire study.; b) average food intake and c) Kcal consumption; d) Tibial length, (e – f) weight of muscles and (g – i) weight of adipose tissues. Data are expressed as mean ± SEM (n=14-15 rats/group). [a] TGM vs GK, GM and TG, [b] GM vs GK and TG, [c] GK vs GM, TG and TGM, [d] TGM vs GM, [e] GM vs TG, [f] GK vs TG and TGM, [g] GM vs TG and TGM, [h] GK vs GM, [i]TGM vs GK and TG, *vs GK and GM, **vs GM and TGM, [@]GK vs TGM; respectively All symbols represent P < 0.05.*

### 3.2. Exercise and melatonin supplementation improve glucose tolerance and insulin sensitivity.

There were no significant differences as to the fasting glucose levels among groups, comparing the 1st and the last (10th) week of the study periods (Figure 3e). In the beginning of the study, no significant differences in glucose tolerance were observed among groups (Figure 3a). However, glucose tolerance was significantly improved in TGM group compared to other groups after 10 weeks of treatment (Figure 3b). Regarding the insulin sensitivity, there were no obvious differences among groups in the 1st week of the study (Figure 2c); but

in the 10th week of the study, both GM and TGM groups had significantly improved their insulin sensitivity compared to the rest of the groups and the TGM had better effect even than that of GM group (Figure 3d).

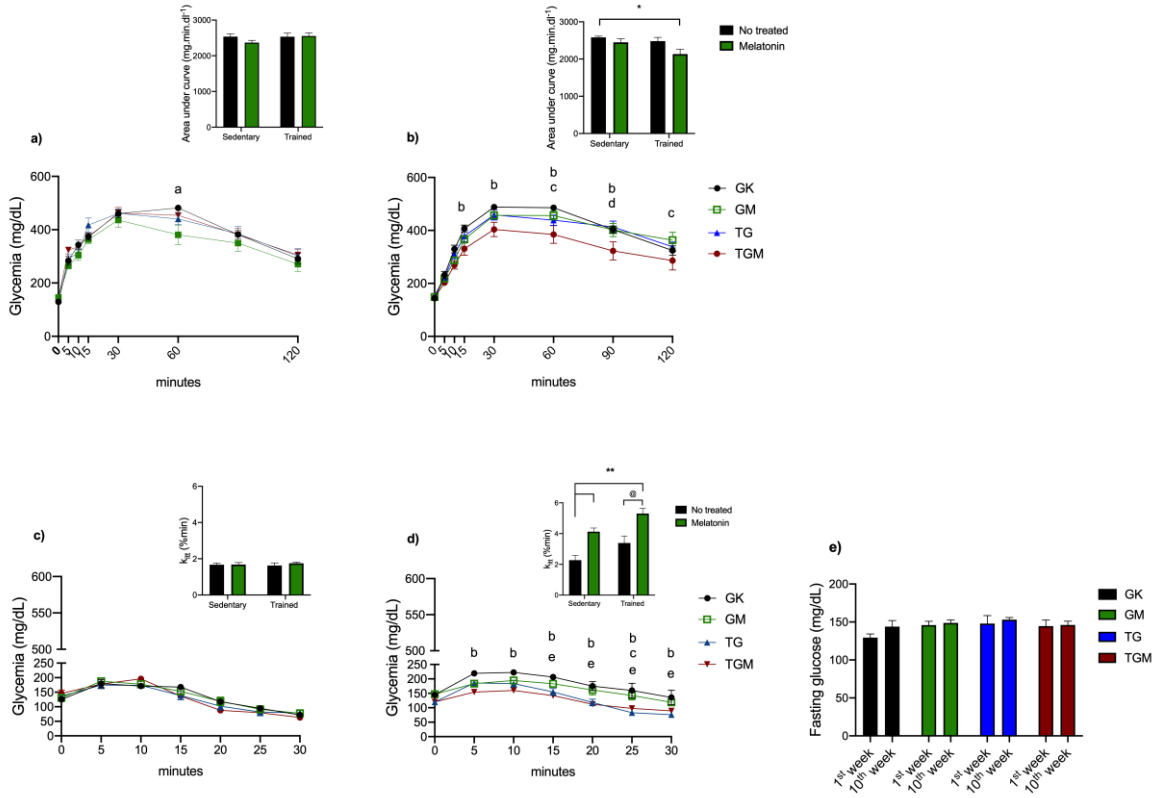


**Fig. 3. Effects of melatonin and/or exercise on glucose and insulin tolerances in GK rats.**

*(a and c) at the 1st week and (b and d) at the 10th week of the study. Timeline changes in glucose levels vs time and the AUC or Kitt are shown in each panel. e) Fasting glucose at the 1st and 10th week of the studyl. Data are expressed as mean ± SEM (n=6-7 rats/group).. [a] GK vs GM, [b] GK vs TGM, [c] TGM vs GK and GM, [d] TGM vs GM and TG, [e] GK vs. TG, *GK vs TGM, **GK vs GM and TGM, [@]TG vs TGM, respectively. All symbols represent P<0.05.*

### 3.3. Effects of melatonin and/or exercise on endocrine and apoptotic cells of islets.

A significant decrease in pancreatic endocrine area was observed in both GM and TGM groups compared to the GK group (Figure 4b). The same pattern was observed as to the islet density (size of the islets per field) (Figure 4d). There were no significant differences in size of the islets (Figure 4c), cell viability (Figure 5a), cell death (Figure 5c) and the pancreatic islets among all groups. Interestingly, the percentage of apoptotic cells was reduced in TGM group compared to groups that did not receive melatonin supplementation (Figure 5b).

### 3.4. Effects of melatonin and/or exercise on insulinemia.

Animals treated with melatonin and/or exercise, had significant low insulinemia at 10 weeks of study compared to the first week (Figure 6a and c). For example, at the first phase of insulin secretion, 15 minutes after glucose administration, insulinemia remained low compared to the end of the test (Figure 6d); however, this pattern was not observed in the first week of the study (Figure 6b). Regarding the function of ß-cells, which is commonly related to the shape and size of the islets, we observed that the TGM group had increased

circularity compared to the other groups (Figure 5d). No difference in the size of the islets was observed among groups (Figure 5e and 5f).

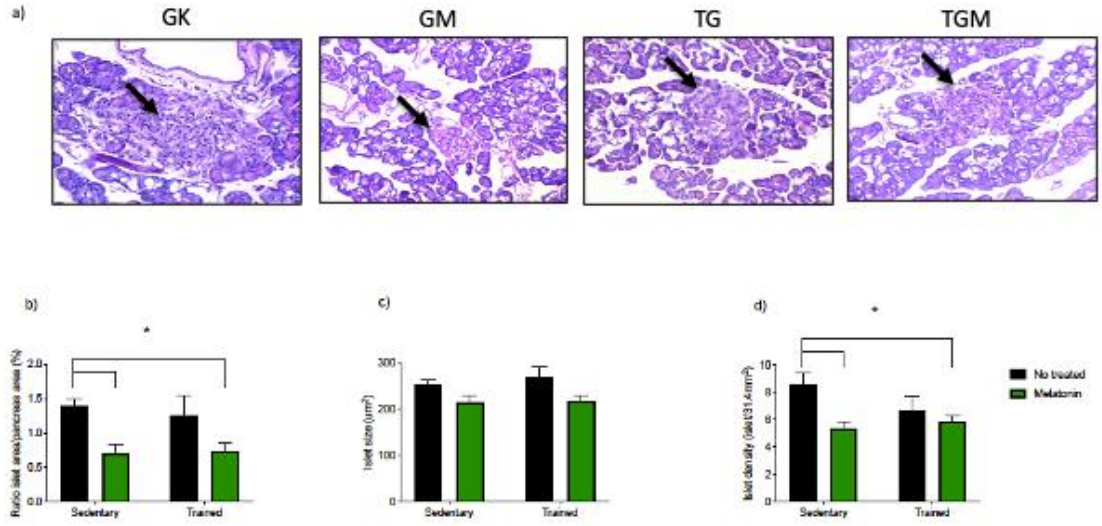


**Fig. 4. Effects of melatonin and/or exercise on morphology of pancreatic islets**.

*a) micrography of a segment of pancreas from GK, GM, TG and TGM groups at the 12th week (HE, 100x). Black arrows indicate the pancreatic islet (endocrine pancreas) in relation to the exocrine pancreas. (b) pancreatic islets area, (c) islet size and (d) islet density. Data are expressed as mean ± SEM (n=6-8 rats/group). *GK vs GM and TGM, P <0.05.*

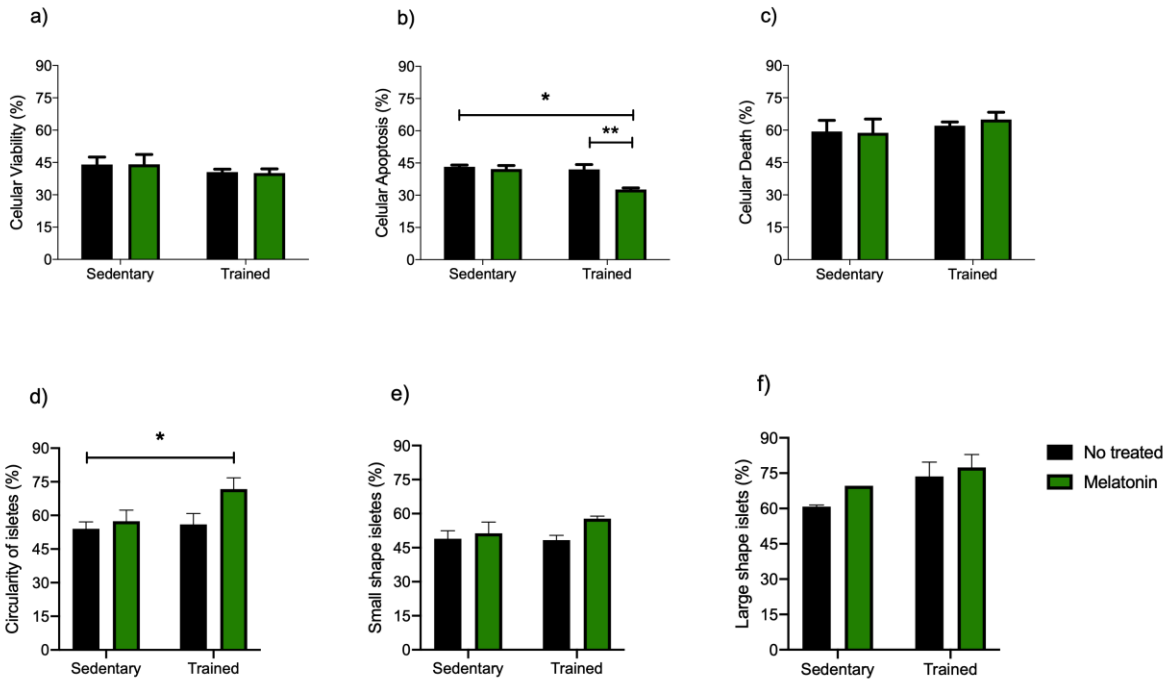


**Fig. 5. Effects of melatonin and/or exercise on viability, apoptosis and death of cells in pancreatic islets.**

*a) Cell viability, b) apoptotic cells, c) cells death and d) percentage of circular-shaped islets; e) relative % of small islets with a circular shape and f) relative % of large islets with a circular shape. Data are expressed as mean ± SEM (n=6-8 rats/group). *GK vs TGM; **TG vs TGM, respectively. All symbols represent P <0.05.*

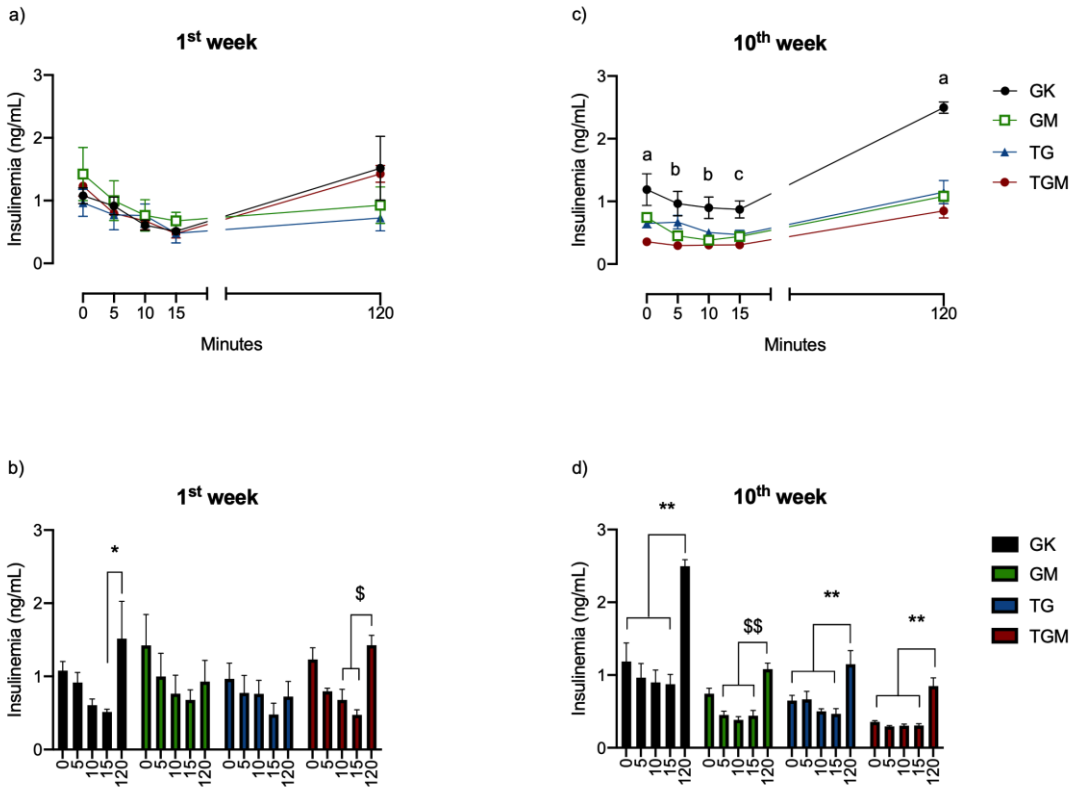


**Figure 6. Effects of melatonin and/or exercise on the basal insulinemia.**

*The data were recorded at 5, 10, 15 and 120 minutes after glucose stimulation in the 1st (a, b) and in the 10th experimental week (c, d). Data are expressed as mean ± SEM (n=4 rats/group). [a]GK vs GM; TG and TGM; [b]GK vs GM and TGM; [c]GK vs TGM; *120 min vs 15 min in GK; **120 min vs 0, 5, 10 and 15 min in GK, TG and TGM; [$]120 vs 10 and 15 min in TGM; and [$$]120 vs. 5, 10 and 15 min in GM, respectively. All symbols represent P <0.05.*

## 4. DISCUSSION

Following the progress of T2DM, the impairment of glucose homeostasis is attributed to the insulin resistance of the peripheral tissues and the reduced insulin production/secretion of the pancreatic ß-cells. T2DM is usually associated with increases in obesity and physical inactivity, thus, exercise appears to be a key strategy for reversing this trend. Physical activity could reduce the risk of T2DM by 47 to 58% in high-risk populations (35-37), and this reduction could persist for 10 years following the initial intervention (38). In this context, we investigated the potential effects of melatonin and/or aerobic physical exercise on the T2DM animal model. In order to analyze the possible benefits of these interventions on glucose metabolism, the focus was given to the ß-cell function and viability.

Melatonin is an important player in the regulation of energy metabolism, including body weight, insulin sensitivity, and glucose tolerance (39). In our study, we observed that melatonin is able to decrease body weight when combined with physical exercise for five weeks in the GK rats. From the seventh week onwards, the TGM group maintained a significant weight reduction compared to control and melatonin alone groups. Usually, T2DM patients are suggested to perform the aerobic exercise (40). Considering weight loss at the end of the study, it is evident that after 12 weeks of interventions, the TGM and the TG groups achieved the similar result. However, the caloric intake over 12 weeks in the TGM group is significantly low compared to the other groups.

One of the main actions of melatonin is to participate in the circadian organization of metabolic functions and their association to the circadian behavioral cycles which regulates food intake and energy expenditure (39). However, Frese *et al*. (41) demonstrated that the pineal glands of GK animals exhibited different pattern in expression of melatonin synthetic enzymes compared to the wild type animals. For example, the precursors for melatonin synthesis in their pineal glands were reduced as well as the reduced noradrenaline level. These alterations indicate the reduced nocturnal synthesis of melatonin in the *in vivo* and *in vitro* conditions And, Buonfiglio *et al*. (42) reported that melatonin replacement promoted reduction in food intake, body weight, adiposity and hyperphagia after a 24-hour fasting. In this context, our findings showed that GK animals did not have the high caloric consumption compared to TG animals, thus the caloric expenditure by exercise can explain the decrease in body weight of the TG group with no apparent change in body composition. On other hand, the combination of aerobic exercise and melatonin supplementation promoted a reduction in caloric intake, loss of body weight and less adiposity.

It was reported that even having an intact pineal gland with normal melatonin production in young rats, melatonin supplementation was still able to achieve a long-term body weight loss (roughly by 25%) and the reduced size of the visceral fat deposits (by 50%) (43). The same anti-obesogenic and weight-gain effects of melatonin were seen in animals of diet-induced obesity (44, 45) and also in older animals. Our results indicate that the mechanisms that involve weight loss via melatonin and physical exercise are present in the TGM group.

In addition to the aforementioned findings, in the 10$^{th}$ week of study, the TGM animals showed improvement in glucose tolerance. This was reflected by the better glycemic control during the process of GTT of this group of rats compared to the GK animals. However, despite the improved glucose tolerance in TGM group at the end of the test (minute 120) their glycemia had not completely returned to baseline level. On the other hand, both GM and TGM groups showed improved insulin sensitivity with better result in TGM group. . It is well known that regular physical activity improves glucose tolerance (36, 46). However, our study shows that only the combination of moderate-intensity aerobic exercise and melatonin supplementation could improve glucose tolerance in GK rats.

Melatonin plays a fundamental role for metabolic adaptations associated to physical activity in both adipose and muscle tissues (26, 47). Previously our group have reported that in older animals, exercise plus melatonin improve glucose tolerance, physical capacity, citrate synthase activity, liver and muscle glycogen content, body weight, PI3K protein expression, MAPK and AMPK in the liver and upregulate the expression of GLUT4 and AMPK proteins in muscle. This indicates that in hypomelatoninemic condition (as diabetes, aging and pinealectomy) melatonin supplementation is crucial for the metabolic adaptations under the aerobic exercise (27).

Goto and Kakizaki (28) reported that GK rats have already developed insulin resistance and glucose intolerance in the absence of an obese phenotype at 10 weeks of age. The mechanisms remain unclear (12). However, we show that supplementation of melatonin with 3 mg of /kg/day for 10 weeks improved insulin sensitivity, regardless of physical exercise. An association of glucose homeostasis and circadian rhythm is supported by studies of circadian misalignment which show profound perturbations of plasma glucose and insulin levels in humans (3, 48) as well as in rats (49). Considering that T2DM is a chronic metabolic disorder characterized by hyperglycemia, as a consequence of both the resistance of peripheral tissues to insulin and disturbances of insulin secretion by the pancreatic ß-cell(7), therefore, alterations of the pancreatic function is the key feature of this disease.

It is known that GK animals present complications in the synthesis and secretion of insulin, and less mass of pancreatic ß-cells is already in prenatal periods (11, 13). Another

important aspect is that peripheral hyperglycemia reduces the synthesis and secretion of pineal melatonin in these animals (41). Our findings show that supplementation with melatonin, regardless of physical exercise, decreased the area of the endocrine pancreas, as well as its density. Interestingly, only the combination of melatonin and physical exercise decreased the apoptotic cells, in addition to improving the circularity of islets. In the study, we observed that there was an elevated insulinemia in the GK group at the age of 10 weeks, which was not surprised since the aging worsens the T2DM in these animals. However, all other groups (GM, TG and TGM) showed a improve insulinemia, evidencing a possible protection against the worsening of the diabetic condition.

Maintenance of healthy ß-cell mass involves a dynamic balance between cell replication, neogenesis and apoptosis. Prior to the development of T2DM, ß-cell mass reflects whole body adiposity and insulin resistance. Therefore, as ß-cell mass increases to compensate for greater whole-body insulin demand, the feedback system is maintained by increases in ß-cell turnover through controlled mechanisms of proliferation/neogenesis and ß-cell death (50). In humans with T2DM, a shift is toward an increase in apoptosis that outweighs cell neogenesis in ß-cells (51).

Individuals with T2DM are thought to have improper ß-cell adaptations that hinder ß-cell growth and regeneration and promote premature ß-cell death, resulting in impaired glucose tolerance and reduced fasting glucose clearance (52, 53). Interestingly, both intermittent stress and regular exercise stimulate ß-cell neogenesis through various actions including signaling through insulin receptor substrate (IRS-2), thereby allowing for normal ß-cell function (54). In current study, we did not observe any increase in the ß-cell neogenesis in the exercise or exercise plus melatonin animals. Notably, the melatonin treatment alone reduced the area and density of the endocrine tissue. In this case, we must consider the genetic characteristic of the animal to develop T2DM without an obese phenotype, different from the more prevalent T2DM caused by the association between genetic predisposition and environmental factors. On the other hand, there was a decrease in insulinemia in all groups with interventions.

The results are in accordance with previous reports related to the insulin sensitivity and glucose tolerance. Thus, these results demonstrate that the combination of aerobic exercise and melatonin supplementation plays a protective role on pancreatic function and peripheral metabolic response. Thereby, we observed that, despite the decrease in the mass of pancreatic islets, the improved circularity and the decrease in apoptosis were able to improve glucose homeostasis (both in glucose tolerance and insulin sensitivity). The lower demand for circulating insulin observed also reflects in a lower ß-cell overload, which may explain the decrease of apoptosis. Although insulinemia was also decreased in the GM and TG groups, only the TGM group had its glucose tolerance be improved, while only the groups supplemented with melatonin had an improvement in insulin sensitivity.

The short-term exercise improves insulin sensitivity in humans (55), and regular daily exercise decreases the risk of developing T2DM in rodents (56-59) and in humans (37, 60, 61). Indeed, exercise is an important regulator of normal glucose tolerance as it increases GLUT4 translocation within stimulated skeletal muscle, which is protective against T2DM development (62). Moreover, long-term daily exercise improves insulin action, as well as GLUT4 translocation in skeletal muscle, in both healthy and insulin-resistant individuals (63). Regular exercise improves insulin action on peripheral skeletal muscle while decreasing insulin requirements. These changes are hypothesized to be direct improvements in ß-cell adaptation to chronic exercise stimulus that helps to prevent ß-cell exhaustion by preserving mass (64).

In addition to physical exercise, melatonin also regulates energy metabolism (5). In T2DM, nocturnal melatonin was shown to be necessary for the preservation and survival of

ß-cells (65). Through MT1 receptors linked to Gi protein, in addition to decreasing cAMP, melatonin activates additional signaling pathways that act in the phosphorylation of IGF-R, IR, IRS-1, AKT, MAPK, ERK1/2 and STAT3 in pancreatic islets (6). Phosphorylation of these proteins triggers signaling pathways that regulate processes essential to cell survival and proliferation in mammals, including pancreatic islet cells. Furthermore, there is consistent scientific evidence supporting that melatonin inhibits insulin secretion in islets (66) and clonal ß-cell lines (67-71). As indicated by previous studies, melatonin in pharmacological, as well as physiological, concentrations distinctively reduces receptor-mediated insulin release from isolated rat pancreatic islets (72, 73) and from rat insulinoma ß-cells (INS-1) in a dose-dependent manner (71).

Thus, we conclude that melatonin plays a crucial role in the functionality of the endocrine pancreas, as well as in the mechanisms involved in insulin sensitivity by insulin-dependent tissues and, consequently, in glucose homeostasis. In addition, although the mechanisms are not fully understood, it appears that only the combination of melatonin and moderate-intensity aerobic exercise is able to improve glucose tolerance, playing a protective role in pancreatic islets. Considering the worldwide prevalence of T2DM, the rates of physical inactivity in the population and, sometimes, shift work and even the use of night lights for leisure activities, our findings are relevant to open paths to be explored in an attempt to minimize health damage related to T2DM, physical inactivity and use of melatonin for therapeutic purposes.

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## AUTHORSHIP

Almeida-Leite E and Cipolla-Neto J designed the study. Almeida-Leite E, Gomes PRL, Vilas-Boas EA, Munhoz AC, Motta-Teixeira LC, Silva-Junior JS developed the experimental techniques and data collection. Almeida-Leite E, Gomes PRL, Carpinelli AR and Cipolla-Neto J contributed to the data analysis and writing of the manuscript. Cipolla-Neto J provided funding and support. All authors read and approved the final format of the manuscript

## CONFLICT INTEREST

There is no commercial or financial relationship in this study. There is no conflict of interest.

## DATA AVAILABILITY

For additional information on data from this study please contact the corresponding author.

## REFERENCES

1. Garcia RA *et al.* (2008) Insulin modulates norepinephrine-mediated melatonin synthesis in cultured rat pineal gland. *Life Sci.* **82**: 108-114.

2. Peliciari-Garcia RA *et al.* (2010) Insulin temporal sensitivity and its signaling pathway in the rat pineal gland. *Life Sci.* **87**: 169-174.
3. Picinato MC *et al.* (2002) Melatonin inhibits insulin secretion and decreases PKA levels without interfering with glucose metabolism in rat pancreatic islets. *J Pineal Res.* **33**: 156-160.
4. Costes S, Boss M, Thomas AP, Matveyenko AV (2015) Activation of melatonin signaling promotes beta-cell survival and function. *Mol. endocrinol.* **29**: 682-692.
5. Cipolla-Neto J, Amaral FGD. (2018) Melatonin as a Hormone: New Physiological and Clinical Insights. *Endocr. Rev.* **39**: 990-1028.
6. Picinato MC *et al.* (2008) Activation of insulin and IGF-1 signaling pathways by melatonin through MT1 receptor in isolated rat pancreatic islets. *J Pineal Res.* **44**, 88-94.
7. American Diabetes Association (2017) Classification and Diagnosis of Diabetes. *Diabetes care* **40** (Supplement 1): S11-S24.
8. Mellado C, *Rodríguez V, Diego JG, Alvarez E, Blázquez E (1989)* Effect of pinealectomy and of diabetes on liver insulin and glucagon receptor concentrations in the rat. *J Pineal Res.* **6**: 295-306.
9. Mantele S *et al.* (2012) Daily rhythms of plasma melatonin, but not plasma leptin or leptin mRNA, vary between lean, obese and type 2 diabetic men. *PloS one* **7**: e37123.
10. Peschke E *et al. (2006)* Diabetic Goto Kakizaki rats as well as type 2 diabetic patients show a decreased diurnal serum melatonin level and an increased pancreatic melatonin-receptor status. *J. Pineal Res.* **40**: 135-143.
11. Movassat J *et al.* (2007) Type 2 diabetes - a matter of failing beta-cell neogenesis? Clues from the GK rat model. *Diabetes Obes Metab.* **9 (**Suppl 2): 187-195.
12. Ehses JA *et al. (2007)* Increased number of islet-associated macrophages in type 2 diabetes. *Diabetes* **56**: 2356-2370.
13. Portha B *et al.* (2009) The GK rat beta-cell: a prototype for the diseased human beta-cell in type 2 diabetes? *Mol. Cell Endocrinol.* **297**: 73-85.
14. Giroix MH *et al.* (2011) Hypercholesterolaemia, signs of islet microangiopathy and altered angiogenesis precede onset of type 2 diabetes in the Goto-Kakizaki (GK) rat. *Diabetologia* **54**: 2451-2462.
15. Portha B *et al.* (2007) Defective functional β-cell mass and Type 2 diabetes in the Goto-Kakizaki rat model. *Expert Rev. Endocrinol. Metab.* **2**: 785-795.
16. Portha B (2005) Programmed disorders of beta-cell development and function as one cause for type 2 diabetes? The GK rat paradigm. *Diabetes Metab. Res. Rev.* **21**: 495-504.
17. Kuwabara WMT *et al.* (2017) Comparison of Goto-Kakizaki rats and high fat diet-induced obese rats: Are they reliable models to study type 2 *diabetes mellitus*? *PLoS One* **12**: e0189622.
18. Picarel-Blanchot F, Berthelier C, Bailbé D, Portha B (1996) Impaired insulin secretion and excessive hepatic glucose production are both early events in the diabetic GK rat. *Am. J. Physiol.* **271**: E755-762.
19. Movassat J, Saulnier C, Serradas P, Portha B (1997) Impaired development of pancreatic beta-cell mass is a primary event during the progression to diabetes in the GK rat. *Diabetologia* **40:** 916-925.
20. Portha B *et al.* (2001) beta-cell function and viability in the spontaneously diabetic GK rat: information from the GK/Par colony. *Diabetes* **50 (**Suppl 1): S89-93.
21. Portha B, Giroix MH, Tourrel-Cuzin C, Le-Stunff H, Movassat J (2012) The GK rat: a prototype for the study of non-overweight type 2 diabetes. *Methods Mol. Biol.* **933**: 125-159.

22. Raza H, John A , Shafarin J , Howarth FC (2016) Howarth, Exercise-induced alterations in pancreatic oxidative stress and mitochondrial function in type 2 diabetic Goto-Kakizaki rats. *Physiol. Rep.* **4** (8): e12751.
23. Knowler WC *et al.* (2002) Reduction in the incidence of type 2 diabetes with life style intervention or metformin. *N. Engl. J. Med.* **346**: 393–403.
24. Rawal S *et al.* (2013) Effects of exercise on pancreatic islets in zucker diabetic fatty rats. *J Diabetes Metab*. **S10**: 007.
25. Paula FMM *et al.* (2015) Exercise increases pancreatic beta-cell viability in a model of type 1 diabetes through IL-6 signaling. *FASEB J.* **29**: 1805-1816.
26. Borges-Silva CN *et al.* (2005) Pinealectomy impairs adipose tissue adaptability to exercise in rats. *J Pineal Res.* **38**: 278-283.
27. Mendes C *et al.* (2013) Adaptations of the aging animal to exercise: role of daily supplementation with melatonin. *J. Pineal Res.* **55**: 229-239.
28. Goto Y, Kakizaki M, Masaki N (1976) Production of spontaneous diabetic rats by repetition of selective breeding. *Tohoku J. Exp. Med.* **119**: 85-90.
29. Buxton OM *et al.* (1997) Roles of intensity and duration of nocturnal exercise in causing phase delays of human circadian rhythms. *Am. J. Physiol.* **273**: E536-542.
30. Brooks GA, White TP. (1978) Determination of metabolic and heart rate responses of rats to treadmill exercise. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol*. **45**: 1009-1015.
31. Bonora E, Manicardi V, Zavaroni I, Coscelli C, Butturini U (1987) Relationships between insulin secretion, insulin metabolism and insulin resistance in mild glucose intolerance. *Diabete Metab.* **13**: 116-121.
32. Gomes PRL *et al.* (2021) Melatonin regulates maternal pancreatic remodeling and B-cell function during pregnancy and lactation. *J. Pineal Res*. **71** (1): e12717. doi: 10.1111/jpi.12717.
33. Weibel ER (1063) Principles and methods for the morphometric study of the lung and other organs. *Lab. Invest.* **12**: 131-155 .
34. Choi EY *et al.* (2020) Association between clinical biomarkers and optical coherence tomography angiography parameters in type 2 diabetes mellitus. *Invest. Ophthalmol. Vis. Sci.* **61**: 4.
35. Pan XR *et al.* (1997) Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes Care* **20**: 537-544.
36. Tuomilehto *J et al.* (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N. Engl. J. Med.* **344**: 1343-1350.
37. Knowler WC *et al.* (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N. Engl. J. Med.* **346**: 393-403.
38. Knowler WC *et al.* (2009) 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. *Lancet* **374**: 1677-1686.
39. Cipolla-Neto J, Amaral FG, Afeche SC, Tan DX, Reiter RJ (2014) Melatonin, energy metabolism, and obesity: a review. *J. Pineal Res.* **56**: 371-381.
40. Lumb A (2014) Diabetes and exercise. *Clin. Med.* **14**: 673-676.
41. Frese T *et al.* (2009) Pineal melatonin synthesis is decreased in type 2 diabetic Goto-Kakizaki rats. *Life Sci.* **85**: 526-533.
42. Buonfiglio D *et al.* (2018) Melatonin absence leads to long-term leptin resistance and overweight in rats. *Front Endocrinol.* **9**: 122.
43. Tan DX, Manchester LC, Fuentes-Broto L, Paredes SD, Reiter RJ. (2011) Significance and application of melatonin in the regulation of brown adipose tissue metabolism: relation to human obesity. *Obes. Rev.* **12**: 167-188.

44. Prunet-Marcassus B *et al.* (2003) Melatonin reduces body weight gain in Sprague Dawley rats with diet-induced obesity. *Endocrinology* **144**: 5347-5352.
45. Sartori C *et al.* (2009) Melatonin improves glucose homeostasis and endothelial vascular function in high-fat diet-fed insulin-resistant mice. *Endocrinology* **150**: 5311-5317.
46. Evans WJ, Cyr-Campbell D (1997) Nutrition, exercise, and healthy aging. *J. Am. Diet Assoc.* **97**: 632-638.
47. Borges-Silva C *et al.* (2007) Pinealectomy reduces hepatic and muscular glycogen content and attenuates aerobic power adaptability in trained rats. *J. Pineal Res.* **43:** 96-103.
48. Scheer FA, Hilton MF, Mantzoros CS, Shea SA (2009) Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc. Natl. Acad. Sci.* **106**: 4453-4458.
49. Picinato MC, Haber EP, Carpinelli AR, Cipolla-Neto J (2002) Daily rhythm of glucose-induced insulin secretion by isolated islets from intact and pinealectomized rat. *J. Pineal Res.* **33**: 172-177.
50. Topp BG, Atkinson LL, Finegood DT (2007) Dynamics of insulin sensitivity, -cell function, and -cell mass during the development of diabetes in fa/fa rats. *Am. J. Physiol. Endocrinol. Metab.* **293**: E1730-1735.
51. Butler AE *et al.* (2003) Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* **52**: 102-110.
52. Porte D (1996) Normal physiology and phenotypic characterization of beta-cell function in subjects at risk for non-insulin-dependent diabetes mellitus. *Diabete Med.* **13**: S25-32.
53. Vinik A (2007) Advancing therapy in type 2 diabetes mellitus with early, comprehensive progression from oral agents to insulin therapy. *Clin. Ther.* **29:** 1236-1253.
54. Park S, Hong SM, Lee JE, Sung SR (2007) Exercise improves glucose homeostasis that has been impaired by a high-fat diet by potentiating pancreatic beta-cell function and mass through IRS2 in diabetic rats. *J. Appl. Physiol.* **103**: 1764-1771.
55. Duclos M, Corcuff JB, Pehourcq F, Tabarin A (2001) Decreased pituitary sensitivity to glucocorticoids in endurance-trained men. *Eur. J. Endocrinol.* **144**: 363-368.
56. Campbell JE, Rakhshani N, Fediuc S, Bruni S, Riddell MC (2009) Voluntary wheel running initially increases adrenal sensitivity to adrenocorticotrophic hormone, which is attenuated with long-term training. *J. Appl. Physiol.* **106**: 66-72.
57. Droste SK, Chandramohan Y, Hill LE, Linthorst ACE, Reul JMHM (2007) Voluntary exercise impacts on the rat hypothalamic-pituitary-adrenocortical axis mainly at the adrenal level. *Neuroendocrinology* **86**: 26-37.
58. Coutinho AE, Campbell JE, Fediuc S, Riddell MC (2006) Effect of voluntary exercise on peripheral tissue glucocorticoid receptor content and the expression and activity of 11beta-HSD1 in the Syrian hamster. *J. Appl. Physiol.* **100**: 1483-1488.
59. Campbell JE *et al.* (2010) Regular exercise prevents the development of hyperglucocorticoidemia via adaptations in the brain and adrenal glands in male Zucker diabetic fatty rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **299**: R168-176.
60. Heath GW *et al.* (1983) Effects of exercise and lack of exercise on glucose tolerance and insulin sensitivity. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **55**: 512-517.
61. Nilsen V, Bakke PS, Gallefoss F ( 2011) Effects of lifestyle intervention in persons at risk for type 2 diabetes mellitus - results from a randomised, controlled trial. *BMC Public Health* **11:** 893.
62. Burr JF, Rowan CP, Jamnik VK, Riddell MC (2010) The role of physical activity in type 2 diabetes prevention: physiological and practical perspectives. *Phys. Sportsmed.* **38**: 72-82.

63. Richter EA, Mikines KJ, Galbo H, Kiens B (1989) Effect of exercise on insulin action in human skeletal muscle. *J. Appl. Physiol.* **66**: 876-885.
64. Beaudry JL, Riddell MC (2012) Effects of glucocorticoids and exercise on pancreatic β-cell function and diabetes development. *Diabetes Metab. Res. Rev.* **28**: 560-573.
65. Costes S, Boss M, Thomas AP, Matveyenko AV (2015) Activation of melatonin signaling promotes β-cell survival and function. *Mol. Endocrinol.* **29**: 682-692.
66. Peschke E *et al.* (2000) Evidence for a melatonin receptor within pancreatic islets of neonate rats: functional, autoradiographic, and molecular investigations. *J. Pineal Res.* **28**: 156-164.
67. Mühlbauer E, Peschke E (2007) Evidence for the expression of both the MT1- and in addition, the MT2-melatonin receptor, in the rat pancreas, islet and beta-cell. *J. Pineal Res.* **42**: 105-106.
68. Stumpf I, Bazwinsky I, Peschke E (2009) Modulation of the cGMP signaling pathway by melatonin in pancreatic beta-cells. *J. Pineal Res.* **46**: 140-147.
69. Stumpf I, Mühlbauer E, Peschke E (2008) Involvement of the cGMP pathway in mediating the insulin-inhibitory effect of melatonin in pancreatic beta-cells. *J. Pineal Res.* **45**: 318-327.
70. Mühlbauer E, Albrecht E, Bazwinsky-Wutschke I, Peschke E (2012) Melatonin influences insulin secretion primarily via MT(1) receptors in rat insulinoma cells (INS-1) and mouse pancreatic islets. *J. Pineal Res.* **52**: 446-459.
71. Peschke E *et al.* (2002) Receptor (MT(1)) mediated influence of melatonin on cAMP concentration and insulin secretion of rat insulinoma cells INS-1. *J. Pineal Res.* **33**: 63-71.
72. Peschke E, D Peschke (1998) Evidence for a circadian rhythm of insulin release from perifused rat pancreatic islets. *Diabetologia* **41**: 1085-1092.
73. Peschke E, D Peschke, T Hammer, V Csernus (1997) Csernus, Influence of melatonin and serotonin on glucose-stimulated insulin release from perifused rat pancreatic islets in vitro. *J. Pineal Res.* **23**: 156-163.



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