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Obese mothers supplemented with melatonin during gestation and lactation ameliorate the male offspring's pancreatic islet cellular composition and beta-cell function

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Original Article

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Abstract

Melatonin supplementation to obese mothers during gestation and lactation might benefit the pancreatic islet cellular composition and beta-cell function in male offspring adulthood. C57BL/6 females (mothers) were assigned to two groups ($n = 20$/each) based on their consumption in control (C 17% kJ as fat) or high-fat diet (HF 49% kJ as fat). Mothers were supplemented with melatonin (Mel) (10 mg/kg daily) during gestation and lactation, or vehicle, forming the groups ($n = 10$/each): C, CMel, HF, and HFMel. The male offspring were studied, considering they only received the C diet after weaning until three months old. The HF mothers and their offspring showed higher body weight, glucose intolerance, insulin resistance, and low insulin sensitivity than the C ones. However, HFMel mothers and their offspring showed improved glucose metabolism and weight loss than the HF ones. Also, the offspring's higher expressions of pro-inflammatory markers and endoplasmic reticulum (ER) stress were observed in HF but reduced in HFMel. Contrarily, antioxidant enzymes were less expressed in HF but improved in HFMel. In addition, HF showed increased beta-cell mass and hyperinsulinemia but diminished in HFMel. Besides, the beta-cell maturity and identity gene expressions diminished in HF but enhanced in HFMel. In conclusion, obese mothers supplemented with melatonin benefit their offspring's islet cell remodeling and function. In addition, improving pro-inflammatory markers, oxidative stress, and ER stress resulted in better glucose and insulin levels control. Consequently, pancreatic islets and functioning beta cells were preserved in the offspring of obese mothers supplemented with melatonin.

Introduction

The overweight/obesity status continues its global rise,[1] compromising women of reproductive age since the risk of developing gestational diabetes mellitus is approximately two, four, and eight times higher in overweight, obese, and severely obese women.[2]

The Developmental Origin of Health and Disease (DOHaD) hypothesis associates environmental conditions in early life, such as maternal nutritional status, with the offspring's metabolic health in the long term.[3,4] In addition, maternal obesity contributes to a raised risk of obesity and insulin resistance in the offspring in childhood, adolescence, and adult life.[5] Indeed, maternal obesity during pregnancy and lactation in mice increased beta-cell mass due to beta-cell proliferation,[6] inducing adverse pancreatic changes in the progeny.[7]

Melatonin (N-acetyl-5-methoxy tryptamine), a pleiotropic hormone, is implicated in circadian rhythm and is involved in glucose homeostasis.[8] Melatonin is an endogenous indoleamine secreted by the pineal gland and shows bioactive anti-inflammatory properties in epigenetic regulation and fetal development.[9,10] Its short-term use does not cause adverse effects, even at extreme doses.[11] In addition, melatonin has been studied as a reprogramming factor for diseases related to maternal metabolic programming.[12,13]

Melatonin might protect against oxidative stress,[14] facilitating electron transfer antioxidant processes in the mitochondrial membrane.[15] Also, melatonin induces the endogenous synthesis of superoxide dismutase (*Sod*), glutathione peroxidase (*Gpx*), and glutathione reductase, enzymes with antioxidant activities or stimulates enzymes that metabolize reactive species.[16]

The role of melatonin in pregnancy is emerging, but maternal melatonin supplementation's long-term metabolic effects are not well-known.[13] Also, melatonin signaling influences the placenta directly[17] and regulates the proliferation, apoptosis, and invasion of trophoblasts in preeclampsia by inhibiting endoplasmic reticulum (ER) stress.[18] In addition, we have demonstrated recently that melatonin supplementation in obese mothers can alleviate the

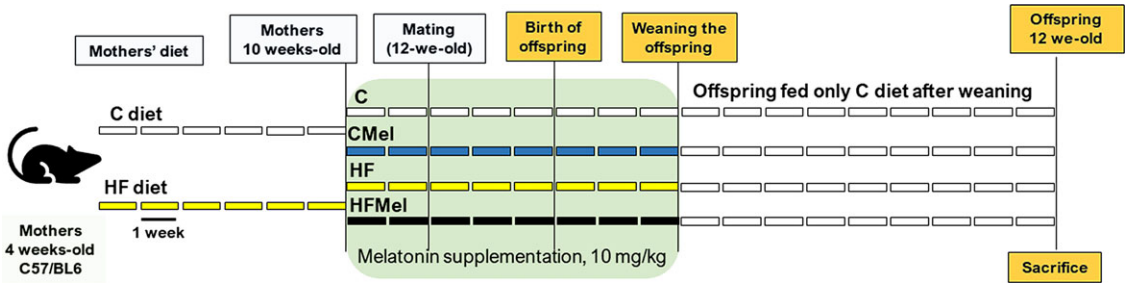

Figure 1. Experimentation timeline. 4th-week-old female C57BL/6 (future mothers) were grouped according to the allocated diet control (C) or high fat (HF). Melatonin supplementation (Mel) started in the 10th week, and females were regrouped as C, CMel, HF, and HFMel (Mel extended for the preconception, pregnancy, and lactation periods). The animals were mating with nonconsanguineous males of the same age in the 12th week. Male offspring were randomly grouped at weaning, fed the C diet, and sacrificed at the 12th-week-old.

development of nonalcoholic liver disease in their male offspring by decreasing lipogenesis and increasing beta-oxidation in the liver tissue.[19]

These findings allow us to hypothesize that maternal melatonin supplementation during gestation and lactation in a known model of diet-induced obesity (DIO) in mice[20,21] would mitigate the development of altered glucose metabolism and insulin resistance, inflammation, ER stress, oxidative stress, and islet remodeling and beta-cell dysfunction in adult male offspring.

Material and methods

Animals and procedures

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals (National Academies Press, 8th Edition, Washington, DC, 2011) and ARRIVE guidelines.[22] The local ethics committee approved animal experimentation (CEUA protocol n° 003/2021). Male and female mice of the C57BL/6 lineage at one month of age were maintained in ventilated cages under a controlled and enriched environment (NexGen system, Allentown Inc., PA, USA, 21 ± 2°C, 12 h/12 h dark/light cycle) with free access to water and food.

Females (mothers) were randomly assigned to one of two experimental diets for eight weeks before the mating: control diet (17 % kJ as fat, defined as C group, $n = 20$) or high-fat diet (49 % kJ as fat, defined as HF group, $n = 20$) to allow for the development of obesity. Males (fathers) received just the C diet. The Pragsoluções (Jau, SP, Brazil) manufactured the diets based on rodents' AIN-93G recommendations[23,24] (Supplementary Table S1). Then, two weeks before mating, the C and HF female mice were again randomly allocated to one of two supplemented groups, Melatonin (Mel) or vehicle, forming four groups: C; CMel, HF; HFMel (Fig. 1).

Melatonin (M5250 Sigma-Aldrich Co., St Louis, MO, USA) was daily subcutaneously administrated at 10 mg/Kg one hour after starting the dark vivarium cycle.[25] The melatonin supplementation lasted eight weeks during the gestation and lactation until the offspring weaning. The mothers assigned to the groups without melatonin supplementation received an equal dose of an alcoholic-saline solution as a vehicle. In addition, the mothers' body weight (BW) and food intake (FI) were measured daily.

At three months old, one female from each group was crossed for mating. After confirming a vaginal plug formation (day 1 of pregnancy), the mother mice were individually housed and continued their diet. At birth, offspring sex was assessed based on the anogenital distance,[26] weighed, and continued with their mother until weaning (Fig. 1).

At weaning, one male offspring was randomly taken off each litter to form the experimental groups, and they were fed with the C diet. The male offspring groups were named considering the mother's diet and supplementation, thus: C, CMel, HF, and HFMel ($n = 10$/each). In addition, the offspring's BW was measured at birth and then weekly, and FI was measured daily.

Carbohydrate metabolism

The mother's oral glucose tolerance test (OGTT) was performed two days before mating and one day after weaning. Offspring OGTT was analyzed at 12 weeks old. First, the animals fasted for six hours and took a 2 g/kg glucose load by orogastric gavage. Then, blood was collected from the tail vein after zero, 15, 30, 60, and 120 min, and glucose was measured (glucometer Accu-Chek, Roche, SP, Brazil), allowing the "area under the curve" calculation (GraphPad Prism, v. 9.5.1 for Windows, La Jolla CA, USA).

Furthermore, the fasting insulin resistance index (FIRi) and quantitative insulin sensitivity check index (QUICKi) were performed on mothers and on offspring to measure insulin resistance and insulin sensitivity, respectively: FIRi = (fasting glucose × fasting insulin)/25)[27] and QUICKI = 1/[log(fasting insulin (μU/mL) + log(fasting glucose (mg/dL)].[28]

Sacrifice and tissue extraction

We sacrificed the mothers two days after weaning and the adult offspring at 12 weeks old. The animals fasted for six hours and were heparinized (Dalteparin Sodium, Fragmin, Pfizer, SP, Brazil, 200 mg/kg) and anesthetized (intraperitoneal Ketamine 240 mg/kg and Xylazine 30 mg/kg). Blood was collected through the cervical vessels section, and plasma was separated from the blood by centrifugation (712 xg for 15 min).

Immediately the pancreas was dissected, weighed, and fixed ($n = 5$, formaldehyde at 4 % w/v, phosphate buffer 0.1 M pH 7.2), then embedded in Paraplast plus (Sigma-Aldrich Co., St Louis, MO, USA) or inflated through the pancreatic duct with Hank's solution ($n = 5$, supplemented with bovine serum albumin, BSA, 1.0 mg/mL). Next, the islets were isolated after collagenase digestion (type V 0.8 mg/mL, Sigma-Aldrich Co., St Louis, MO, USA), the exocrine portion was discarded, and the islets were manually collected in a Petri dish and used to analyze static insulin secretion in vitro ($n = 15$ islet/group) or frozen at −80°C for molecular analysis.

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Plasma

In mothers, we measured adiponectin (Mouse adiponectin ELISA kit #EZMADP-60K, Millipore, Missouri, USA) and insulin (Rat/mouse Insulin ELISA Kit #EZRMI-13K, Millipore, Missouri, USA).

In offspring, we measured the C-Peptide, Glucose-dependent Insulinotropic Peptide (GIP), Glucagon, Interleukin (IL)-6, Insulin, Leptin, Peptide YY (PYY), and Tumor Necrosis Factor-alpha (TNFa) by Multiplex Biomarker Immunoassays for Luminex xMAP technology (Millipore, Billerica, MA, USA, cat. #MMHMAG-44K). Furthermore, to measure adiponectin, we used an enzyme-linked immunosorbent assay (Mouse adiponectin ELISA kit #EZMADP-60K, Millipore, Missouri, USA).

Pancreas

The pancreas prepared for light microscopy was entirely sectioned at 5 µm thickness, and sections were stained with hematoxylin and eosin or incubated with anti-insulin antibodies for immunohistochemistry analysis. The observations and digital photomicrographs were obtained in a Nikon microscope (model 80i and DS-Ri1 digital camera, Nikon Instruments, Inc., New York, USA).

We analyzed 15 nonconsecutive random sections in each animal. First, the islet volume density (Vv [islet, pancreas]) was estimated by point-counting, and islet mass (M [islet, pancreas]) was estimated as the product of Vv [islet, pancreas], and pancreas mass. Second, the numerical density per area of the islets (Q_A [islet, pancreas]) was determined by taking into consideration the edge effect in the counts).[29] Then, the islet cross-sectional area was determined as A [islet, pancreas] = Vv [islet, pancreas]/2*Q_A [islet, pancreas].[30,31]

Furthermore, we used image analysis in sections incubated with anti-glucagon (CSB-PA002654, Cusabio, 1:100) and anti-insulin (sc-9168, Santa Cruz Biotech, CA, USA; 1:100) to estimate the volume density of alpha and beta cells. Briefly, the sections were incubated with biotinylated secondary antibodies and streptavidin-peroxidase conjugates, washed in PBS, revealed with liquid diaminobenzidine (DAB, Histostain Plus Kit, Invitrogen, CA, USA), and counterstained with hematoxylin. Then, using the ImagePro Plus 7.1 for Windows (Media Cybernetics Corp., Rockville, MD, USA), islets were outlined, and the deconvoluted color was the DAB image measured in intensity units, then converted to the optical density.[32] Finally, alpha and beta-cell mass was estimated as the product of [Vv [alpha-cell] (or Vv [beta-cell]), and M [islet].[33,34]

Glucose-stimulated insulin secretion in vitro

Fifteen isolated islets per group were incubated for 30 min at 37°C in a Krebs–Ringer bicarbonate buffer (KRB) growth medium containing 115 mM NaCl, 5 mM KCl, 10 mM $NaHCO_3$, 2.56 mM $CaCl_2$, 1 mM $MgCl_2$, and 15 mM HEPES. This solution was supplemented with glucose 5.6 mM and BSA 0.3% (pH 7.4, Sigma-Aldrich Co., St Louis, MO, USA) and continuously gassed with 95% O_2/5% CO_2. Next, the culture medium was replaced with fresh buffer, and five islets per group were incubated for another hour at three different concentrations of glucose (2.8, 11.1, or 22.2 mM.). Finally, the insulin level was measured (rat/mouse Insulin ELISA Kit #EZRMI-13K, Millipore, Missouri, USA).

Quantitative real-time polymerase chain reaction (RT-qPCR)

Briefly, the isolated pancreatic islets had the total RNA extracted using Trizol (Invitrogen, CA, USA), and 1 µg of mRNA was treated with DNAse I (Invitrogen) and evaluated with Nanovue (GE Healthcare Life Sciences, Piscataway, NJ, USA). cDNA was synthesized (Oligo dT) from the mRNA of the samples, and cDNA was mixed with the gene of interest primer and SYBR Green Mix (Invitrogen). First, the expression of the TATA- box binding protein (*Tbp*) gene was performed and used as the reference gene for mRNA standardization. Next, negative controls were performed in wells in which the cDNA was replaced by deionized water. Then, the qPCR was evaluated with the Step One Plus real-time PCR cycler system (Applied Biosystems by Life Technologies, Waltham, Massachusetts, USA) and SYBR Green mix (Invitrogen). In addition, signal amplification was measured using the $2^{-\Delta\Delta Ct}$ method to estimate the difference between target gene cycles and endogenous control.[35] Primer sequences were designed by Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/).

Statistical analysis

Data were tested for normality (for small samples, Shapiro–Wilk test) and homogeneity of variances (Bartlett test) and then shown as mean and standard deviation. The differences between groups were tested using Student's *t*-test with Welch's correction (mothers, two groups) or two-way ANOVA followed by Tukey's multiple comparison test (GraphPad Prism v.9.5.1 for Windows, GraphPad Software, San Diego, CA, USA). We accepted the *P*-value <0.05 statistical significance.

Results

Mother data

Before mating, after eight weeks of diet, the HF mother was heavier than the C mother, and melatonin supplementation did not alter BW. However, the OGTT's area under the curve (AUC) was 20% higher in HF mothers than in C mothers and 12% lower in HFMel mothers than in HF mothers. After weaning, BW increased by +14% in HF mothers than in C mothers and decreased by −12% in HFMel mothers than in HF mothers. Food intake did not show a difference among the groups. However, the energy intake was higher in HF mothers than in C mothers (Table 1).

The HF mothers showed hyperinsulinemia (+130%), insulin resistance (FIRi +236 %), and diminished insulin sensitivity (QUICKi −21%) compared to the C mothers, which were restored in the HFMel vs. HF. In addition, lower plasmatic adiponectin levels (−34%) were observed in the HF mothers than in the C mothers, but higher levels of adiponectin (+58%) and an improvement of glucose metabolism was seen in the HFMel mothers in comparison to the HF mothers (Table 1).

Offspring data

At birth, the HF offspring was heavier by +16%, and the CMel by +8% than the C offspring (Fig. 2B). The initial difference observed in CMel vs. C was not maintained in the following weeks, but a heavier HF offspring existed until week 12 compared to the C offspring. However, BW decreased in the HFMel offspring from the fourth week compared to the HF offspring. At twelve weeks, the HF offspring was heavier by +9% than the C offspring, and HFMel lost weight by −7% than HF (Fig. 2A). In addition, FI and EI were increased in the HF offspring compared to the C offspring, but reduced in HFMel than in HF.

There was an "area under the curve" increase (OGTT +15%, Fig. 1B-C), hyperinsulinemia (+30%), insulin resistance (FIRi +46%), and reduced insulin sensitivity (QUICKi −8%) in

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Table 1. Biometry and plasma analyses of mothers

Data	C	CMel	HF	HFMel
BW (g, $n = 20$)	12.7 ± 1.09		13.1 ± 1.60	
Pre-mating				
BW (g, $n = 10$)	19.4 ± 0.71	20.1 ± 0.57	22.0 ± 0.40†	21.3 ± 0.70
OGTT (auc, mmol/L/min, $n = 5$)	1047.1 ± 49.60	1053.0 ± 49.40	1246.2 ± 32.15†	1099.1 ± 90.54‡
After weaning				
BW (g, $n = 10$)	21.1 ± 0.55	20.9 ± 1.10	24.2 ± 0.54†	21.4 ± 1.32‡
FI (g/day/mouse, $n = 10$)	1.5 ± 0.08	1.5 ± 0.06	1.4 ± 0.20	1.4 ± 0.20
EI (g/day/mouse, $n = 10$)	22.5 ± 3.19	22.7 ± 2.75	28.7 ± 3.88†	27.5 ± 3.77
PW (g, $n = 10$)	0.114 ± 0.02	0.108 ± 0.02	0.134 ± 0.01	0.121 ± 0.01
PW/BW (%, $n = 10$)	0.54 ± 0.08	0.49 ± 0.07	0.58 ± 0.05	0.57 ± 0.05
OGTT (auc, mmol/L/min, $n = 5$)	827.1 ± 5.70	789.2 ± 47.53	1150.0 ± 111.62†	844.1 ± 43.75‡
Adiponectin (10^6 pg/mL, $n = 5$)	14.0 ± 1.75	13.4 ± 0.69	9.2 ± 0.95†	14.4 ± 1.80‡
Insulin (pg/mL, $n = 5$)	606.0 ± 90.00	660.0 ± 79.00	1388.0 ± 193.46†	711.0 ± 68.27‡
FIRi ($n = 5$)	3.3 ± 0.40	3.8 ± 0.50	11.1 ± 1.60†	5.0 ± 0.60‡
QUIKi ($n = 5$)	0.5 ± 0.01	0.5 ± 0.01	0.4 ± 0.01†	0.5 ± 0.01‡

AUC, area under the curve; BW, body weight; EI, energy intake; FI, food intake; FIRi, Fasting Insulin Resistance index; OGTT, oral glucose tolerance test; PW, pancreas weight; QUICKi, Quantitative Insulin Sensitivity Check Index. Groups: C, control; HF, high fat; Mel, melatonin. Mean ± SD, $P < 0.05$ when: †≠ C; ‡≠ HF.

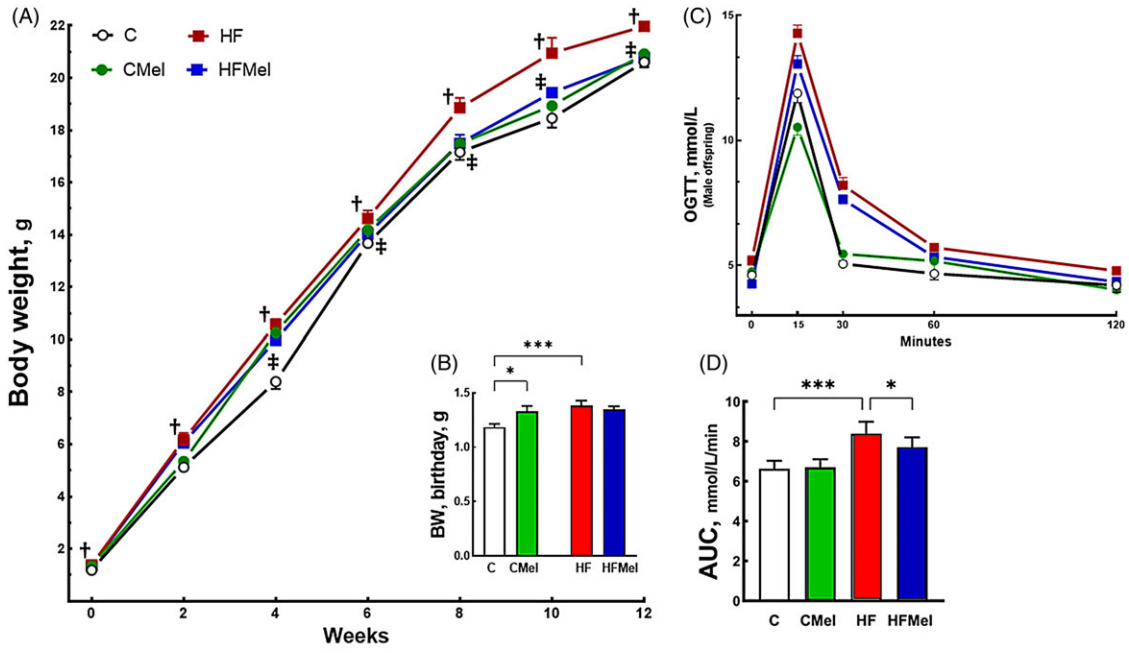

Figure 2. Offspring body weight evolution and oral glucose tolerance test. A. Body weight evolution; B. Birthday body weight. C. Oral glucose tolerance test curves; D. Area under the curve. Data are mean ± SD, $n = 10$/group, Groups: C (control), CMel (control melatonin), HF (high fat), and HFMel (high-fat melatonin) $P < 0.05$ when †≠ C e ‡≠ HF.

HF than in C (Table 2). However, HFMel vs. HF showed lessened OGTT (−8%, Fig. 2C-D), plasma insulin (−40%), insulin resistance (FIRi −50%), and increased insulin sensitivity (QUICKi +18%) (Table 2).

Plasma

The HF offspring, compared to the C one, showed lower adiponectin (−25%), GIP (−60%), and PYY (−45%), and higher IL6 (+88%), TNFalpha (+18%), C-Peptide (+45%), and leptin (+27%). Also, HFMel vs. HF showed higher adiponectin (+18%) and diminished IL6 and TNF alpha (−30%). In addition, the groups did not differ in glucagon (Table 2).

Pancreatic islets

Typical rodent Islet alpha and beta-cell distributions were observed in C and CMel and showed in the first two rows of

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Table 2. Biometry and plasma analyses of offspring

Data	C	CMel	HF	HFMel
FI (g/day/mouse, $n = 10$)	1.5 ± 0.1	1.5 ± 0.04	1.8 ± 0.02†	1.5 ± 0.06‡
EI (g/day/mouse, $n = 10$)	24.3 ± 2.15	25.0 ± 0.70	29.0 ± 0.30†	25.3 ± 0.90‡
PW (g, $n = 10$)	0.10 ± 0.02	0.10 ± 0.02	0.11 ± 0.01	0.11 ± 0.01
PW/BW (%, $n = 10$)	0.51 ± 0.05	0.53 ± 0.03	0.51 ± 0.07	0.53 ± 0.08
Plasmatic levels ($n = 5$)				
Insulin (pg/mL)	619.0 ± 93.00	565.0 ± 87.20	804.3 ± 106.37†	488.0 ± 54.66‡
FIRi	2.8 ± 0.44	2.7 ± 0.45	4.1 ± 0.46†	2.1 ± 0.43‡
QUIKi	0.6 ± 0.0	0.6 ± 0.02	0.5 ± 0.01†	0.6 ± 0.03‡
Adiponectin (10^6 pg/mL)	8.5 ± 0.40	9.8 ± 0.71†	6.4 ± 0.45†	7.6 ± 0.16 ‡ #
C-Peptide (pg/mL)	282.9 ± 38.68	256.5 ± 26.94	413.0 ± 25.90†	215.1 ± 14.30‡
GIP (pg/mL)	590.2 ± 33.73	578.8 ± 37.48	236.4 ± 35.45†	412.8 ± 35.53‡#
Glucagon (pg/mL)	51.2 ± 5.52	40.7 ± 12.61	48.6 ± 6.62	40.1 ± 15.90
IL-6 (pg/mL)	6.5 ± 0.47	7.7 ± 0.49	12.1 ± 0.73†	8.4 ± 1.40‡
Leptin (pg/mL)	417.4 ± 52.99	391.4 ± 58.38	529.7 ± 32.28†	324.1 ± 20.37‡
PYY (pg/mL)	323.3 ± 51.20	348.5 ± 28.87	177.8 ± 29.82†	243.2 ± 32.89 #
TNFa (pg/mL)	6.1 ± 0.32	5.5 ± 0.30	7.2 ± 0.50†	4.8 ± 0.57‡

BW, body weight; EI, energy intake; FI, food intake; FIRi, Fasting Insulin Resistance index; GIP, gastric inhibitory polypeptide; IL, Interleukin; PW, pancreas weight; PYY, intestinal polypeptide; QUICKi, Quantitative Insulin Sensitivity Check Index; TNFa, tumoral necrosis factor-alpha. Groups: C, control; HF, high fat; Mel, melatonin; Mean ± SD, $P < 0.05$ when: †≠ C; ‡≠ HF; #≠CMel.

photomicrographs of Fig. 3. However, islets were hypertrophied in the HF group, but restored in the HFMel group (Fig. 3A). Consequently, alpha and beta cells were hypertrophied in the HF group than in the C group but reduced in the HFMel vs. HF (Fig. 3B-C).

Glucose-stimulated insulin secretion in vitro

Islet insulin secretion was higher in the HF offspring than in the C one (+530 % at 2.8 mM) but lower in the HFMel offspring than in the HF one (−85 %) (Fig. 4A) as well as at 11.1 and 22.2 mM of glucose (higher secretion in HF than in C, and lower secretion in HFMel than in HF) (Fig. 4B-C). In addition, at 22.2 mM, insulin secretion was lower in CMel than in C (Fig. 4C).

Pro-inflammatory cytokines

Il6, *Il1b*, and *Tnfa* were augmented, but *Sirt1* was diminished in the HF offspring than in the C offspring. These cytokines were mitigated, and *Sirt1* was increased in the HFMel offspring compared to the HF one. Also, CMel offspring showed diminished *Il1b* and augmented *Sirt1* expressions compared to the HFMel offspring (Fig. 5A-D).

Oxidative stress and ER stress

Sod, *Catalase,* and *Gpx* were reduced, and *Chop, Gadd45,* and activating transcription factor 4 (*Atf4)* were improved in HF than in C. However, HFMel showed higher *Sod*, *Catalase,* and *Gpx* and lower *Chop, Gadd45,* and *Atf4* than HF. In addition, *Chop* and *Atf4* genes were downregulated in the CMel vs. HFMel (Fig. 6A-F).

Transcriptional factors and beta-cell identity markers

There were decreased expressions of *Pdx1* (−36%, Fig. 7A), *Mafa* (−60%, Fig. 7B), *Neurod1* (−65%, Fig. 7C), *Pax6* (−31%, Fig. 7F), and *Pparg* (−75%, Fig. 7H) in HF than in C. However, the expressions of these genes were regulated in HFMel than in HF, except *Pax6*. In addition, although it has not been decreased in the HF group, aristaless-related homeobox (*Arx)* (+70%, Fig. 7D) and *Ppara* (+400%, Fig. 7G) were enhanced in HFMel vs. HF. Conversely, *Pax4* was augmented in HF than in C (+260%) but diminished in HFMel vs. HF (−25%, Fig. 7E). Furthermore, *Neurod1* and *Pax6* were enhanced in CMel vs. HFMel (Fig. 7C and 7F).

Discussion

It is known that obesity in the mother might implicate the offspring's adverse pancreatic islet cell remodeling and altered metabolism.[36-38] These alterations are associated with changes in islet pro-inflammatory regulators, oxidative stress, ER stress, and beta-cell integrity in adult offspring, consequently deteriorating insulin production and glycemic control.[39,40] However, these alterations were mitigated in the offspring when the obese mother received melatonin supplements during pregnancy and lactation, which agreed with our initial hypothesis that melatonin treatment improves insulin sensitivity and glucose tolerance in mice.[41] Fig. 8 summarizes our findings.

Fetal programming affects male and female offspring differently.[42-44] Therefore, sexual dimorphism merits a more detailed study to investigate the link with maternal melatonin supplementation. Usually, studying male offspring is suitable when we are not interested in sexual dimorphism because males suffer less influence from the variety of sexual hormones. On the contrary, mature female cycle periodically (they go through the four phases of the estrous cycle at each period), which would be another variable to consider in the study, including the moment of sacrifice. Hence, to avoid the inherent aspect of females and focus on the study's central theme, only males were sampled.

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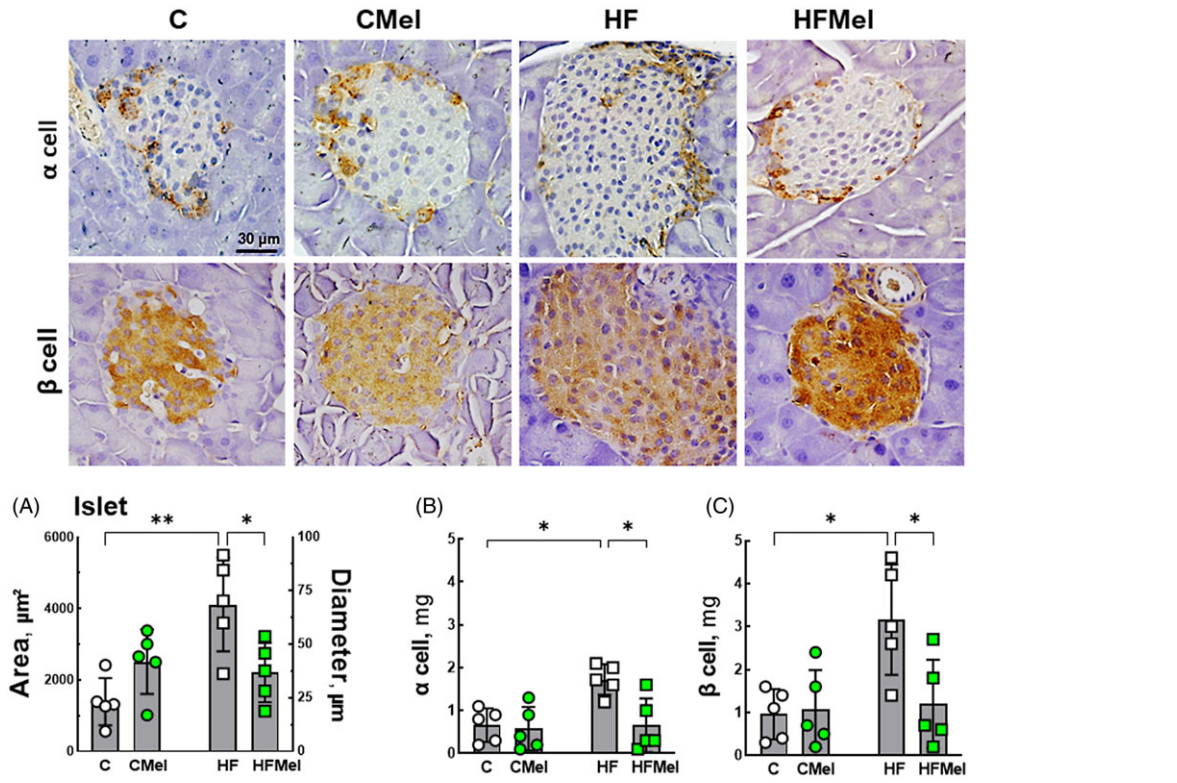

Figure 3. Pancreatic islets in adult offspring. The two rows show islets immunolabeled by anti-glucagon (alpha cells) and anti-insulin (beta cells) (same magnification in all images). A. Islet cross-sectional area, *B. alpha*-cell mass, *C. beta*-cell mass. Data are mean ± SD, $n = 5$/group, $^{*}P < 0.05$, $^{**}P < 0.01$. Groups: C (control), CMel (control melatonin), HF (high fat), HFMel (high-fat melatonin).

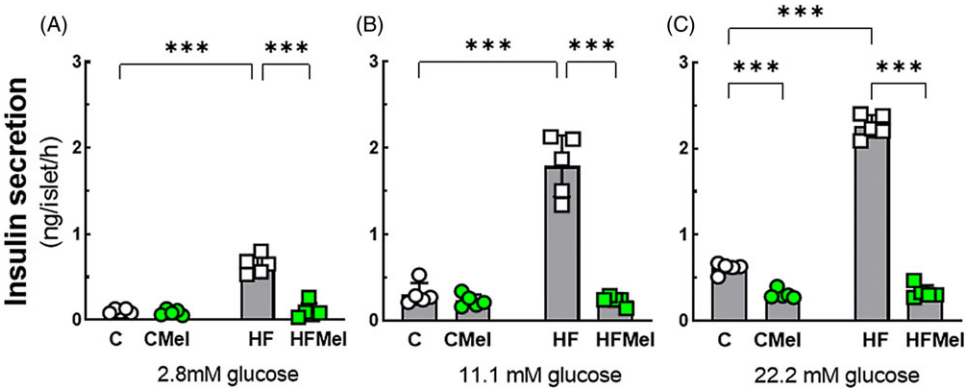

Figure 4. Insulin secretion in isolated islets in adult offspring. Data are mean ± SD, $n = 5$/group, $^{***}P < 0.001$. Groups: Groups: C (control), CMel (control melatonin), HF (high fat), HFMel (high-fat melatonin).

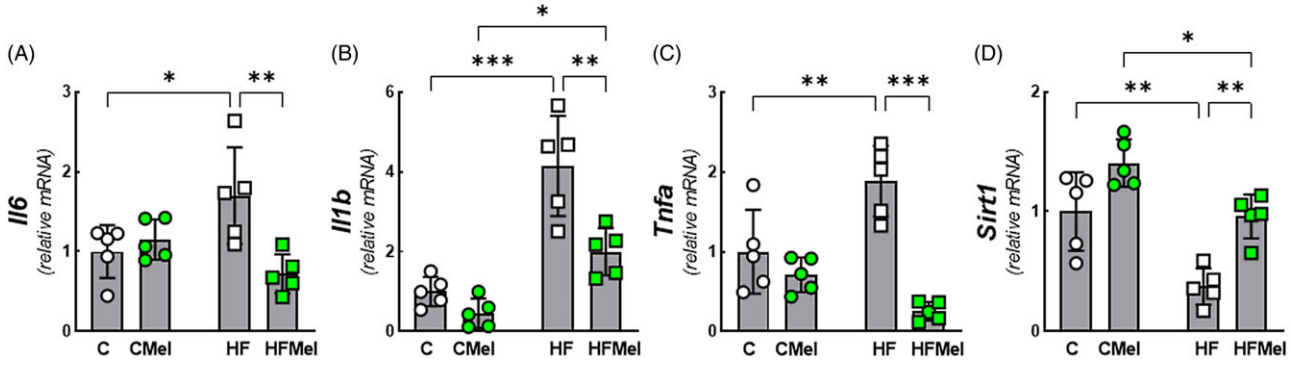

Figure 5. Pro-inflammatory markers in the pancreatic islet of adult offspring. A. *Il6,* interleukin6; B. *Il1b,* interleukin1 beta; C. *Tnfa*, tumor necrosis factor-alpha; D. *Sirt1*, Sirtuin 1. Data are mean ± SD, $n = 5$/group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Groups: Groups: C (control), CMel (control melatonin), HF (high fat), HFMel (high-fat melatonin).

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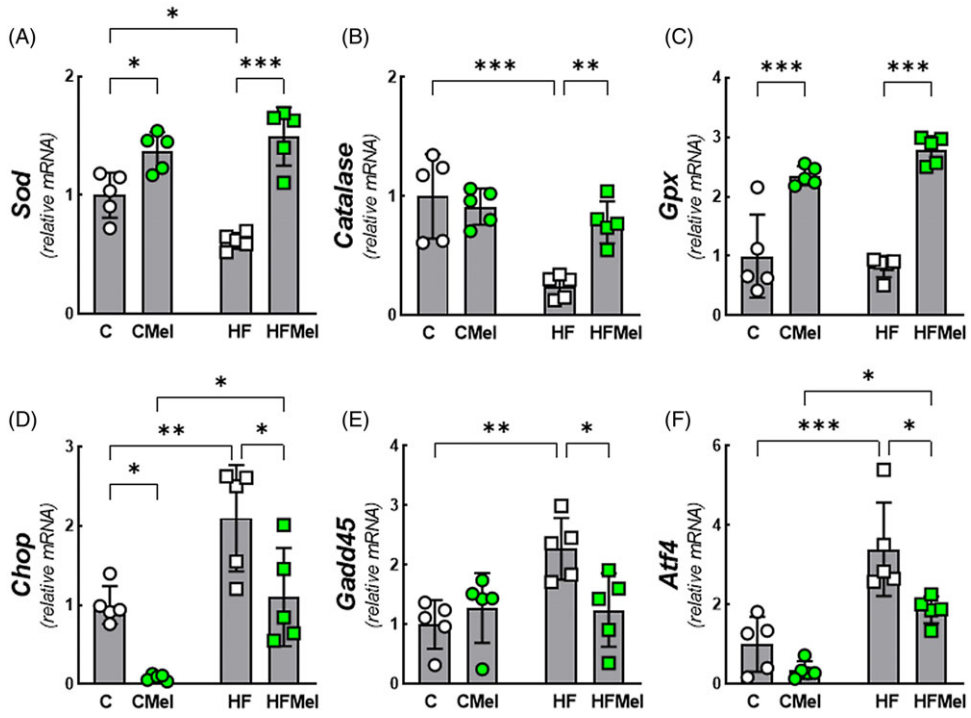

Figure 6. Obese mother melatonin supplementation restored biomarkers of oxidative and endoplasmatic reticulum stress in pancreatic islets of adult offspring. A. *Sod*, superoxide dismutase; B. *Catalase*; C. *Gpx*, glutathione peroxidase. D. *Chop*, DNA-damage-inducible transcript 3; E. *Gadd45*, growth arrest and DNA-damage-inducible 45; F. *Atf4*, activating transcription factor 4. Data are mean ± SD, $n = 5$/group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Groups: C (control), CMel (control melatonin), HF (high fat), and HFMel (high-fat melatonin).

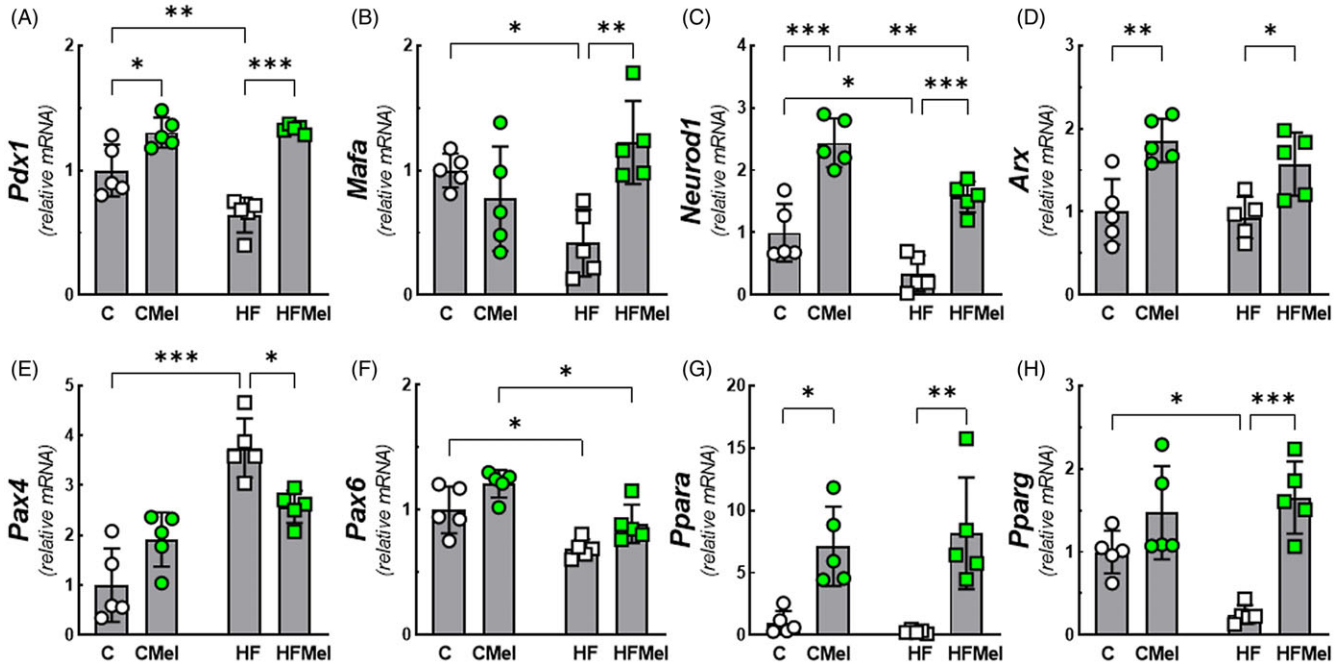

Figure 7. Melatonin supplementation of obese mothers improves transcription factors and beta-cell identity markers in adult offspring. A. *Pdx 1*, pancreatic duodenal homeobox 1; *Mafa,* v-maf musculoaponeurotic fibrosarcoma oncogene family; C. *Neurod1,* neurogenic differentiation 1; D. *Arx,* transcription factor aristaless-related homeobox gene; E. *Pax4*, paired box 4; F. *Pax6*, paired box 6; G. *Ppara*, peroxisome proliferator-activated receptor alpha; H. *Pparg*, peroxisome proliferator-activated receptor gamma. Data are mean ± SD, $n = 5$/group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Groups: C (control), CMel (control melatonin), HF (high fat), HFMel (high-fat melatonin).

Maternal obesity induces inflammation in offspring,[45,46] leading to impaired oxidative stress,[47,48] and ER stress.[49] Melatonin might restore regular physiological function during pregnancy by alleviating oxidative damage in the placenta, favoring nutrient transfer, and improving the placenta vascular dynamics at the uterine-placental interface.[50-52] Additionally, maternal

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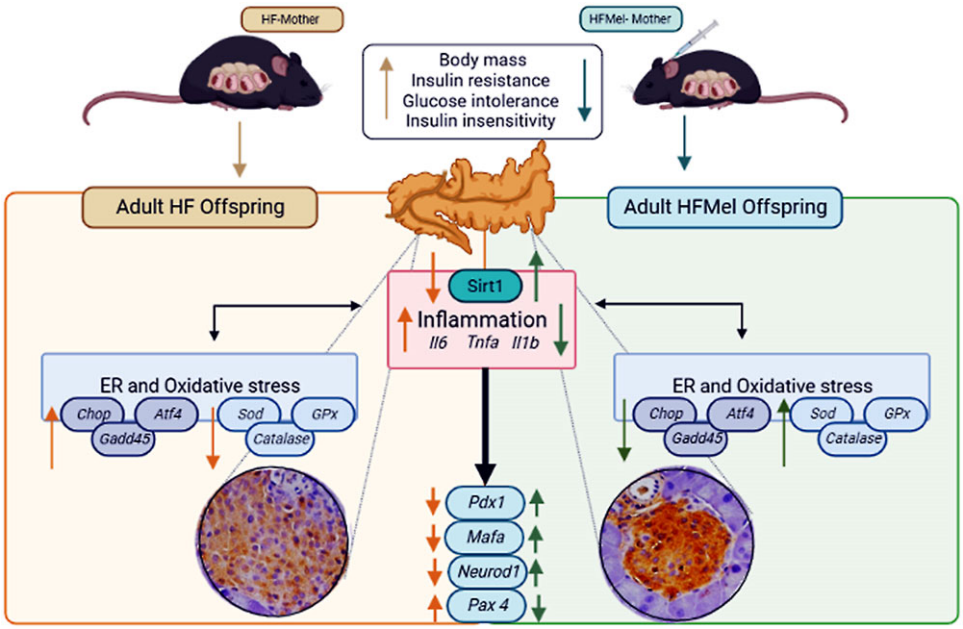

Figure 8. Obese mothers supplemented with melatonin have beneficial effects on their adult offspring (reduced body weight and insulin resistance). In addition, pro-inflammatory cytokines and endoplasmic reticulum stress markers were mitigated, and enzymes related to oxidative stress increased. The hypertrophied islets due to obese mothers were mitigated by melatonin supplementation to mothers. Arrow-up indicates an increase, and arrow-down indicates a decrease. *Atf4*, activating transcription factor 4; *Catalase*; *Chop*, DNA-damage inducible transcript; *Gadd45*, growth arrest and DNA-damage-inducible 45; *Gpx*, glutathione peroxidase.; *Il1b,* interleukin1 beta; *Il6,* interleukin6; *Mafa,* v-maf musculoaponeurotic fibrosarcoma oncogene family; *Neurod1,* neurogenic differentiation 1*; Pax4*, paired box 4*; Pdx 1*, pancreatic duodenal homeobox 1; *Sirt1*, Sirtuin 1; *Sod*, superoxide dismutase; *Tnfa*, tumor necrosis factor-alpha.

melatonin freely crosses the placenta and can influence offspring development, programming several functions related to neural and brain development, energy, and glucose metabolism.[53]

Melatonin plays an anti-oxidative effect and protects against obesity-related insulin resistance[54] and prevents the generation of reactive oxygen species, regulating the redox state in the beta cell.[55] Also, melatonin is a potent antioxidant molecule and has been reported to increase the antioxidant enzyme expression and activity,[56] and melatonin reduces SODK68 acetylation in oocytes in culture.[57] In agreement, our findings demonstrated enhanced *Sod, catalase*, and *Gpx*, decreasing pro-inflammatory cytokines in offspring, emphasizing the interplay between inflammation and oxidative stress.[58]

Melatonin regulates GLUT4 expression and triggering via its G-protein-coupled membrane receptors, the phosphorylation of the insulin receptor, and intracellular substrates mobilizing the insulin-signaling pathway.[59] In the current study, melatonin supplementation was linked with weight loss in obese mothers and their offspring, possibly because melatonin determined an adequate energy balance mainly by regulating energy flow to and from the stores.[59] Furthermore, maternal melatonin supplementation diminished glucose intolerance and insulin resistance in mothers and their offspring, improving *Pparg* expression, an insulin sensitizer.

The upregulation of the oxidative stress enzymes may explain the relief of ER stress observed in the offspring, possibly associated with the increased expression of *Sirt1*, which alleviates oxidative stress and ER stress[14] in HFMel offspring. In addition, melatonin strongly inhibits oxidative stress and partially inhibits ER stress in pancreatic beta cell *in vitro*.[60]

Maternal obesity impairs offspring's beta-cell function in rodents[61] and humans.[62] Here, maternal obesity negatively affects the genetic regulation of beta-cell differentiation, maturation, and glucose metabolism in the offspring, such as pancreatic duodenal homeobox 1 (*Pdx1),* v-maf musculoaponeurotic fibrosarcoma oncogene family (*Mafa);* neurogenic differentiation 1 *(Neurod1*); paired box 4 *(Pax4) and* paired box 6 *(Pax6)*. Moreover, some transcription factors are required at specific stages of the islet cell formation stimulating a network of transcription factors regulating cell differentiation genes in the embryonic stages.[63]

Pdx1 is a crucial transcription factor in different stages of pancreatic development, and the process of differentiation of beta cells starts early in the embryonic period.[64] The expression of *Pdx1* assures beta-cell function but decreases during insulin resistance and type 2 diabetes.[65,66]

Mafa is found exclusively in developing and adult insulin cells, linked to insulin cell production.[67] Moreover, induction of *Mafa* expression is essential for regenerative approaches to regenerate functional and mature beta cells from pluripotent stem cells.[68]

An insulin resistance environment includes hyperglycemia, hyperinsulinemia, increased FIRi, and diminished QUICKi. Insulin seems not to perform its role in reducing blood glucose in our HF animals, even with the pancreatic islet hypersecreting insulin at different glucose concentrations. Besides, *Pdx1* deficiency increases beta-cell susceptibility to ER stress, as *Pdx1* regulates a wide range of genes involved in diverse ER functions, including the proper formation of disulfide bonds and protein folding, and the unfolded protein response. Therefore, the reduced expression of the *Pdx1* gene in HF offspring may favor the beta-cell failure to compensate for insulin resistance related to impaired critical ER functions.[69] However, it might indicate an early beta-cell failure in this group, programmed by maternal obesity.

Neurod1 is detected in the developing embryonic pancreas[70] and, in elderly life, plays a predominant role in the maintenance of functional beta cells.[71] *Pax4* is transiently expressed in all endocrine progenitors during pancreatic development and

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downregulated shortly after birth.[66] *Pax4* appears essential for the appropriate initiation of beta-cell differentiation.[72,73] Remarkably, upregulated gene expressions of *Pdx1, Mafa,* and *Neurod1* were observed in the offspring of obese mothers supplemented with melatonin.

Pax6 is detected at the end of the embryonic period as a critical transcriptional regulator of adult beta-cell identity and function.[74] Therefore, diminished *Pax6* expression might be associated with beta-cell failure in diabetes.[75] Here, we determined that maternal obesity reduced *Pax6* expression, which was not altered by maternal melatonin supplementation.

Arx expression begins during mouse pancreatic development and persists into mature alpha cells.[76] Furthermore, maternal melatonin supplementation increased *Arx* in offspring. Although *Arx* is required for early specification and maintenance of alpha-cell mass,[77] is not directly involved in glucagon expression.[78] Therefore, the effect of melatonin on glucagon secretion is controversial.[79,80] Here, the impact of melatonin on the offspring was indirect (administered to the mothers), which might explain the absence of changes in glucagon secretion in programming offspring.

The *Pax4* and *Arx* balance is crucial for cell fate determination of islet alpha and beta cells.[72] In the current study, we determined a decrease in the *Pax4* gene related to an immature beta cell in the offspring of obese mothers supplemented with melatonin.

Our findings demonstrated that obese mothers supplemented with melatonin favored cell remodeling in the adult offspring's pancreatic islet and preserved glucose-stimulated insulin secretion *in vitro*. These findings agree with a report in pinealectomized pregnant rats and impaired glucose metabolism, insulin secretion dysregulation, and failure in the glucose-stimulated insulin secretion.[81]

Beta-cell dedifferentiation is characterized by lessened gene expressions related to mature beta-cell function and enhanced endocrine precursor cells, an adaptive response to avoid apoptosis.[82] Our obese mother's offspring showed alterations indicating more susceptibility to beta-cell failure with advancing age. In addition, maternal melatonin supplementation seems to favor adult offspring beta-cell function.

In conclusion, obese mothers supplemented with melatonin benefit their offspring's islet cell remodeling and function. In addition, improving pro-inflammatory markers, oxidative stress, and ER stress resulted in better glucose and insulin level control. Consequently, pancreatic islets and functioning beta cells were preserved in the offspring of obese mothers supplemented with melatonin.

Supplementary materials. The supplementary material for this article can be found at https://doi.org/10.1017/S2040174423000168.

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Authors' contribution. All authors discussed the results, commented on the manuscript, and approved the article's final version. BAN and MA performed the measurements, processed the experimental data, performed the analysis, and drafted the manuscript; FO and MBA aided in interpreting the results and worked on the manuscript; MBA and CAM-L conceived the research, planned and supervised the work, supported the research, designed the figures, and edited the manuscript's final version.

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Competing interests. None.

Ethical standard. The animals came from the University of the State of Rio de Janeiro vivarium, cared for in the laboratory during the experimentation until sacrifice. Animal care followed the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Pu. No. 85-23, revised in 1996 and National Academies Press, 8th Edition, Washington, DC, 2011) and ARRIVE guidelines. [22] The institutional committee approved the study (CEUA protocol n° 003/2021).

Significance of study and contribution to science. Maternal melatonin supplementation might act as an epigenetic regulator of fetal development, helping prevent disease in adult progeny like that induced by maternal obesity. However, maternal melatonin supplementation's long-term metabolic effects are not well-known. Therefore, we hypothesized that maternal melatonin supplementation during gestation and lactation in a known model of diet-induced obesity (DIO) in mice would mitigate the development of altered glucose metabolism and insulin resistance, inflammation, ER stress, oxidative stress, and islet remodeling and beta-cell dysfunction in adult male offspring. In the current study, we observed the benefits of melatonin supplementation to obese mothers on their offspring's islet cell remodeling and function. In addition, there were benefits in pro-inflammatory markers, oxidative stress, and ER stress resulting in glucose and insulin improvement. Consequently, pancreatic islets and functioning beta cells were preserved in the offspring of obese mothers supplemented with melatonin.

References

1. Afshin A, Forouzanfar MH, Reitsma MB, *et al.* Health effects of overweight and obesity in 195 Countries over 25 years. *N Engl J Med*. 2017; 377(1), 13–27. DOI: 10.1056/NEJMoa1614362.
2. Chu SY, Callaghan WM, Kim SY, *et al.* Maternal obesity and risk of gestational diabetes mellitus. *Diabetes Care*. 2007; 30(8), 2070–2076. DOI: 10.2337/dc06-2559a.
3. Barker DJ. Developmental origins of adult health and disease. *J Epidemiol Commun Health*. 2004; 58(2), 114–115. DOI: 10.1136/jech.58.2.114.
4. Ong TP, Ozanne SE. Developmental programming of type 2 diabetes: early nutrition and epigenetic mechanisms. *Curr Opin Clin Nutr Metab Care*. 2015; 18(4), 354–360. DOI: 10.1097/MCO.0000000000000177.
5. Nicholas LM, Morrison JL, Rattanatray L, Zhang S, Ozanne SE, McMillen IC. The early origins of obesity and insulin resistance: timing, programming and mechanisms. *Int J Obes (Lond)*. 2016; 40(2), 229–238. DOI: 10.1038/ijo.2015.178.
6. Gniuli D, Calcagno A, Caristo ME, *et al.* Effects of high-fat diet exposure during fetal life on type 2 diabetes development in the progeny. *J Lipid Res*. 2008; 49(9), 1936–1945. DOI: 10.1194/jlr.M800033-JLR200.
7. Bringhenti I, Moraes-Teixeira JA, Cunha MR, Ornellas F, Mandarim-de-Lacerda CA, Aguila MB. Maternal obesity during the preconception and early life periods alters pancreatic development in early and adult life in male mouse offspring. *PLoS One*. 2013; 8(1), e55711. DOI: 10.1371/journal.pone.0055711.
8. Karamitri A, Jockers R. Melatonin in type 2 diabetes mellitus and obesity. *Nat Rev Endocrinol*. 2019; 15(2), 105–125. DOI: 10.1038/s41574-018-0130-1.
9. Voiculescu SE, Zygouropoulos N, Zahiu CD, Zagrean AM. Role of melatonin in embryo fetal development. *J Med Life*. 2014; 7(4), 488–492.
10. Tain YL, Huang LT, Chan JY. Transcriptional regulation of programmed hypertension by melatonin: an epigenetic perspective. *Int J Mol Sci*. 2014; 15(10), 18484–18495. DOI: 10.3390/ijms151018484.
11. Andersen LPH, Gögenur I, Rosenberg J, Reiter RJ. The safety of melatonin in humans. *Clin Drug Invest*. 2016; 36(3), 169–175. DOI: 10.1007/s40261-015-0368-5.

12. Tain YL, Huang LT, Hsu CN. Developmental programming of adult disease: reprogramming by melatonin? *Int J Mol Sci*. 2017; 18(2), 426. DOI: 10.3390/ijms18020426.
13. Feng X, Zhang Y, Li N, *et al.* Melatonin in reproductive medicine: a promising therapeutic target? *Curr Med Chem*. 2022; 30, 3090. DOI: 10.2174/0929867329666221005101031.
14. Xu D, Liu L, Zhao Y, *et al.* Melatonin protects mouse testes from palmitic acid-induced lipotoxicity by attenuating oxidative stress and DNA damage in a SIRT1-dependent manner. *J Pineal Res*. 2020; 69(4), e12690. DOI: 10.1111/jpi.12690.
15. Tan DX, Manchester LC, Qin L, Reiter RJ. Melatonin: a mitochondrial targeting molecule involving mitochondrial protection and dynamics. *Int J Mol Sci*. 2016; 17(12), 2124. DOI: 10.3390/ijms17122124.
16. Tamura H, Nakamura Y, Korkmaz A, *et al.* Melatonin and the ovary: physiological and pathophysiological implications. *Fertil Steril*. 2009; 92(1), 328–343. DOI: 10.1016/j.fertnstert.2008.05.016.
17. Berbets AM, Davydenko IS, Barbe AM, Konkov DH, Albota OM, Yuzko OM. Melatonin 1A and 1B receptors' expression decreases in the placenta of women with fetal growth restriction. *Reprod Sci*. 2021; 28(1), 197–206. DOI: 10.1007/s43032-020-00285-5.
18. Zhou C, Ding Y, Yu L, Nie Y, Yang M. Melatonin regulates proliferation, apoptosis and invasion of trophoblasts in preeclampsia by inhibiting endoplasmic reticulum stress. *Am J Reprod Immunol*. 2022; 88(2), e13585. DOI: 10.1111/aji.13585.
19. Ajackson M, Nagagata BA, Marcondes-de-Castro IA, Mandarim-de-Lacerda CA, Aguila MB. Adult mice offspring of obese mothers supplemented with melatonin show lessened liver steatosis, inflammation, oxidative stress, and endoplasmic reticulum stress. *Life Sci*. 2023; 312, 121253. DOI: 10.1016/j.lfs.2022.121253.
20. Fraulob JC, Ogg-Diamantino R, Santos CF, Aguila MB, Mandarim-de-Lacerda CA. A mouse model of metabolic syndrome: insulin resistance, fatty liver and non-alcoholic fatty pancreas disease (NAFPD) in C57BL/6 mice fed a high fat diet. *J Clin Biochem Nutr*. 2010; 46(3), 212–223. DOI: 10.3164/jcbn.09-83.
21. Mandarim-de-Lacerda CA, Del Sol M, Vazquez B, Aguila MB. Mice as an animal model for the study of adipose tissue and obesity. *Int J Morphol*. 2021; 39(6), 1521–1528. DOI: 10.4067/S0717-95022021000601521.
22. du Sert NPercie, Hurst V, Ahluwalia A, *et al.* The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *Exp Physiol*. 2020; 105(9), 1459–1466. DOI: 10.1113/EP088870.
23. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr*. 1993; 123(11), 1939–1951. DOI: 10.1093/jn/123.11.1939.
24. Aguila MB, Ornellas F, Mandarim-de-Lacerda CA. Nutritional research and fetal programming: parental nutrition influences the structure and function of the organs. *Int J Morphol*. 2021; 39(1), 327–334. DOI: 10.4067/s0717-95022021000100327.
25. Baydas G, Nedzvetsky VS, Nerush PA, Kirichenko SV, Demchenko HM, Reiter RJ. A novel role for melatonin: regulation of the expression of cell adhesion molecules in the rat hippocampus and cortex. *Neurosci Lett*. 2002; 326(2), 109–112. DOI: 10.1016/s0304-3940(02)00328-2.
26. Wolterink-Donselaar IG, Meerding JM, Fernandes C. A method for gender determination in newborn dark pigmented mice. *Lab Anim (NY)*. 2009; 38(1), 35–38. DOI: 10.1038/laban0109-35.
27. Pang J, Xi C, Huang X, Cui J, Gong H, Zhang T. Effects of excess energy intake on glucose and lipid metabolism in C57BL/6 mice. *PLoS One*. 2016; 11(1), e0146675. DOI: 10.1371/journal.pone.0146675.
28. Katz A, Nambi SS, Mather K, *et al.* Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab*. 2000; 85(7), 2402–2410. DOI: 10.1210/jcem.85.7.6661.
29. Gundersen HJG. Notes on the estimation of the numerical density of arbitrary profiles: the edge effect. *J Microsc*. 1977; 111, 219–223. DOI: 10.1111/j.1365-2818.1977.tb00062.x.
30. Mandarim-de-Lacerda CA, Del-Sol M. Tips for studies with quantitative morphology (morphometry and stereology). *Int J Morphol*. 2017; 35(4), 1482–1494. DOI: 10.4067/s0717-95022017000401482.
31. Mandarim-de-Lacerda CA. Stereological tools in biomedical research. *An Acad Bras Cienc*. 2003; 75(4), 469–486. DOI: 10.1590/S0001-37652003000400006.
32. Mandarim-de-Lacerda CA, Santos C, Aguila MB. Image analysis and quantitative morphology. *Methods Mol Biol*. 2010; 611, 211–225. DOI: 10.1007/978-1-60327-345-9_17.
33. Marinho TS, Aguila MB, Mandarim-de-Lacerda CA. Pancreatic islet stereology: estimation of beta cells mass. *Int J Morphol*. 2019; 37(4), 1331–1334. DOI: 10.4067/s0717-95022019000401331.
34. Marinho TS, Martins FF, Cardoso LEM, Aguila MB, Mandarim-de-Lacerda CA. Pancreatic islet cells disarray, apoptosis, and proliferation in obese mice. the role of Semaglutide treatment. *Biochimie*. 2022; 193, 126–136. DOI: 10.1016/j.biochi.2021.10.017.
35. Rao X, Lai D, Huang X. A new method for quantitative real-time polymerase chain reaction data analysis. *J Comput Biol*. 2013; 20(9), 703–711. DOI: 10.1089/cmb.2012.0279.
36. Mandarim-de-Lacerda CA. L'îlot pancréatique: ce que nous savons 150 ans après Langerhans. *Bull l'Acad Natl Méd*. 2019; 203(8-9), 670–682. DOI: 10.1016/j.banm.2019.06.015.
37. Mandarim-de-Lacerda CA. Pancreatic islet (of Langerhans) revisited. *Histol Histopathol*. 2019; 34(9), 985–993. DOI: 10.14670/HH-18-118.
38. Ornellas F, Karise I, Aguila MB, Mandarim-de-Lacerda CA. Pancreatic islets of langerhans: adapting cell and molecular biology to changes of metabolism. In *Obesity and Diabetes* (eds. Faintuch J, Faintuch S), 2020; pp. 175–190. Springer International Publishing, Cham. DOI: 10.1007/978-3-030-53370-0_13.
39. Cerf ME. Parental high-fat programming of offspring development, health and beta-cells. *Islets*. 2011; 3(3), 118–120. DOI: 10.4161/isl.3.3.15420.
40. Cerf ME. High fat programming of beta cell compensation, exhaustion, death and dysfunction. *Pediatr Diabetes*. 2015; 16(2), 71–78. DOI: 10.1111/pedi.12137.
41. Sartori C, Dessen P, Mathieu C, *et al.* Melatonin improves glucose homeostasis and endothelial vascular function in high-fat diet-fed insulin-resistant mice. *Endocrinology*. 2009; 150(12), 5311–5317. DOI: 10.1210/en.2009-0425.
42. Ornellas F, Mello VS, Mandarim-de-Lacerda CA, Aguila MB. Sexual dimorphism in fat distribution and metabolic profile in mice offspring from diet-induced obese mothers. *Life Sci*. 2013; 93(12-14), 454–463. DOI: 10.1016/j.lfs.2013.08.005.
43. Palou M, Priego T, Sanchez J, Palou A, Pico C. Sexual dimorphism in the lasting effects of moderate caloric restriction during gestation on energy homeostasis in rats is related with fetal programming of insulin and leptin resistance. *Nutr Metab (Lond)*. 2010; 7, 69. DOI: 10.1186/1743-7075-7-69.
44. Thompson LP, Song H, Polster BM. Fetal programming and sexual dimorphism of mitochondrial protein expression and activity of hearts of prenatally hypoxic guinea pig offspring. *Oxid Med Cell Longev*. 2019; 2019; 2019, 7210249. DOI: 10.1155/2019/7210249.
45. Kretschmer T, Schulze-Edinghausen M, Turnwald EM, *et al.* Effect of maternal obesity in mice on IL-6 levels and placental endothelial cell homeostasis. *Nutrients*. 2020; 12(2), 296. DOI: 10.3390/nu12020296.
46. Enstad S, Cheema S, Thomas R, *et al.* The impact of maternal obesity and breast milk inflammation on developmental programming of infant growth. *Eur J Clin Nutr*. 2021; 75(1), 180–188. DOI: 10.1038/s41430-020-00720-5.
47. Igosheva N, Abramov AY, Poston L, *et al.* Maternal diet-induced obesity alters mitochondrial activity and redox status in mouse oocytes and zygotes. *PLoS One*. 2010; 5(4), e10074. DOI: 10.1371/journal.pone.0010074.
48. Napso T, Lean SC, Lu M, *et al.* Diet-induced maternal obesity impacts feto-placental growth and induces sex-specific alterations in placental morphology, mitochondrial bioenergetics, dynamics, lipid metabolism and oxidative stress in mice. *Acta Physiol (Oxf)*. 2022; 234(4), e13795. DOI: 10.1111/apha.13795.
49. Park S, Jang A, Bouret SG. Maternal obesity-induced endoplasmic reticulum stress causes metabolic alterations and abnormal hypothalamic development in the offspring. *PLoS Biol*. 2020; 18(3), e3000296. DOI: 10.1371/journal.pbio.3000296.

https://doi.org/10.1017/S2040174423000168 Published online by Cambridge University Press

50. Saat N, Risvanli A, Dogan H, *et al.* Effect of melatonin on torsion and reperfusion induced pathogenesis of rat uterus. *Biotech Histochem*. 2019; 94(7), 533–539. DOI: 10.1080/10520295.2019.1605456.
51. Tamura H, Takasaki A, Miwa I, *et al.* Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. *J Pineal Res*. 2008; 44(3), 280–287. DOI: 10.1111/j.1600-079X.2007.00524.x.
52. Chuffa LGA, Lupi LA, Cucielo MS, Silveira HS, Reiter RJ, Seiva FRF. Melatonin promotes uterine and placental health: potential molecular mechanisms. *Int J Mol Sci*. 2019; 21(1), 300. DOI: 10.3390/ijms21010300.
53. Gomes PRL, Motta-Teixeira LC, Gallo CC, *et al.* Maternal pineal melatonin in gestation and lactation physiology, and in fetal development and programming. *Gen Comp Endocrinol*. 2021; 300, 113633. DOI: 10.1016/j.ygcen.2020.113633.
54. Xu L, Li D, Li H, *et al.* Suppression of obesity by melatonin through increasing energy expenditure and accelerating lipolysis in mice fed a high-fat diet. *Nutr Diabetes*. 2022; 12(1), 42. DOI: 10.1038/s41387-022-00222-2.
55. Zephy D, Ahmad J. Type 2 diabetes mellitus: role of melatonin and oxidative stress. *Diabetes Metab Syndr*. 2015; 9(2), 127–131. DOI: 10.1016/j.dsx.2014.09.018.
56. Mayo JC, Sainz RM, Antoli I, Herrera F, Martin V, Rodriguez C. Melatonin regulation of antioxidant enzyme gene expression. *Cell Mol Life Sci*. 2002; 59(10), 1706–1713. DOI: 10.1007/pl00012498.
57. Han L, Wang H, Li L, *et al.* Melatonin protects against maternal obesity-associated oxidative stress and meiotic defects in oocytes via the SIRT3-SOD2-dependent pathway. *J Pineal Res*. 2017; 63(3), e12431. DOI: 10.1111/jpi.12431.
58. Dandekar A, Mendez R, Zhang K. Cross talk between ER stress, oxidative stress, and inflammation in health and disease. *Methods Mol Biol*. 2015; 1292, 205–214. DOI: 10.1007/978-1-4939-2522-3_15.
59. Cipolla-Neto J, Amaral FG, Afeche SC, Tan DX, Reiter RJ. Melatonin, energy metabolism, and obesity: a review. *J Pineal Res*. 2014; 56(4), 371–381. DOI: 10.1111/jpi.12137.
60. Park JH, Shim HM, Na AY, *et al.* Melatonin prevents pancreatic β-cell loss due to glucotoxicity: the relationship between oxidative stress and endoplasmic reticulum stress. *J Pineal Res*. 2014; 56(2), 143–153. DOI: 10.1111/jpi.12106.
61. Cerf ME, Williams K, Nkomo XI, *et al.* Islet cell response in the neonatal rat after exposure to a high-fat diet during pregnancy. *Am J Physiol Regul Integr Comp Physiol*. 2005; 288(5), R1122–1128. DOI: 10.1152/ajpregu.00335.2004.
62. Black MH, Watanabe RM, Trigo E, *et al.* High-fat diet is associated with obesity-mediated insulin resistance and β-cell dysfunction in Mexican Americans. *J Nutr*. 2013; 143(4), 479–485. DOI: 10.3945/jn.112.170449.
63. Murtaugh LC. Pancreas and beta-cell development: from the actual to the possible. *Development*. 2007; 134(3), 427–438. DOI: 10.1242/dev.02770.
64. Offield MF, Jetton TL, Labosky PA, *et al.* PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development*. 1996; 122(3), 983–995. DOI: 10.1242/dev.122.3.983.
65. Yang BT, Dayeh TA, Volkov PA, *et al.* Increased DNA methylation and decreased expression of PDX-1 in pancreatic islets from patients with type 2 diabetes. *Mol Endocrinol*. 2012; 26(7), 1203–1212. DOI: 10.1210/me.2012-1004.
66. Lin CL, Vuguin PM. Determinants of pancreatic islet development in mice and men: a focus on the role of transcription factors. *Horm Res Paediatr*. 2012; 77(4), 205–213. DOI: 10.1159/000337219.
67. Matsuoka TA, Artner I, Henderson E, Means A, Sander M, Stein R. The MafA transcription factor appears to be responsible for tissue-specific expression of insulin. *Proc Natl Acad Sci U S A*. 2004; 101(9), 2930–2933. DOI: 10.1073/pnas.0306233101.
68. Rezania A, Bruin JE, Arora P, *et al.* Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nat Biotechnol*. 2014; 32(11), 1121–1133. DOI: 10.1038/nbt.3033.
69. Sachdeva MM, Claiborn KC, Khoo C, *et al.* Pdx1 (MODY4) regulates pancreatic beta cell susceptibility to ER stress. *Proc Natl Acad Sci U S A*. 2009; 106(45), 19090–19095. DOI: 10.1073/pnas.0904849106.
70. Naya FJ, Huang HP, Qiu Y, *et al.* Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/neuroD-deficient mice. *Genes Dev*. 1997; 11(18), 2323–2334. DOI: 10.1101/gad.11.18.2323.
71. Gasa R, Mrejen C, Lynn FC, *et al.* Induction of pancreatic islet cell differentiation by the neurogenin-neuroD cascade. *Differentiation*. 2008; 76(4), 381–391. DOI: 10.1111/j.1432-0436.2007.00228.x.
72. Collombat P, Hecksher-Sorensen J, Broccoli V, *et al.* The simultaneous loss of Arx and Pax4 genes promotes a somatostatin-producing cell fate specification at the expense of the alpha- and beta-cell lineages in the mouse endocrine pancreas. *Development*. 2005; 132(13), 2969–2980. DOI: 10.1242/dev.01870.
73. Sosa-Pineda B. The gene Pax4 is an essential regulator of pancreatic beta-cell development. *Mol Cells*. 2004; 18(3), 289–294. https://pubmed.ncbi.nlm.nih.gov/15650323/.
74. Lorberbaum DS, Docherty FM, Sussel L. Animal models of pancreas development, developmental disorders, and disease. *Adv Exp Med Biol*. 2020; 1236, 65–85. DOI: 10.1007/978-981-15-2389-2_3.
75. Swisa A, Avrahami D, Eden N, *et al.* PAX6 maintains β cell identity by repressing genes of alternative islet cell types. *J Clin Invest*. 2017; 127(1), 230–243. DOI: 10.1172/jci88015.
76. Collombat P, Mansouri A, Hecksher-Sorensen J, *et al.* Opposing actions of Arx and Pax4 in endocrine pancreas development. *Genes Dev*. 2003; 17(20), 2591–2603. DOI: 10.1101/gad.269003.
77. Bramswig NC, Kaestner KH. Transcriptional regulation of α-cell differentiation. *Diabetes Obes Metab*. 2011; 13(Suppl 1), 13–20. DOI: 10.1111/j.1463-1326.2011.01440.x.
78. Gosmain Y, Cheyssac C, Heddad Masson M, Dibner C, Philippe J. Glucagon gene expression in the endocrine pancreas: the role of the transcription factor Pax6 in α-cell differentiation, glucagon biosynthesis and secretion. *Diabetes Obes Metab*. 2011; 13(Suppl 1), 31–38. DOI: 10.1111/j.1463-1326.2011.01445.x.
79. Bähr I, Mühlbauer E, Schucht H, Peschke E. Melatonin stimulates glucagon secretion in vitro and in vivo. *J Pineal Res*. 2011; 50(3), 336–344. DOI: 10.1111/j.1600-079X.2010.00848.x.
80. Peschke E, Schucht H, Mühlbauer E. Long-term enteral administration of melatonin reduces plasma insulin and increases expression of pineal insulin receptors in both Wistar and type 2-diabetic Goto-Kakizaki rats. *J Pineal Res*. 2010; 49(4), 373–381. DOI: 10.1111/j.1600-079X.2010.00804.x.
81. Gomes PRL, Vilas-Boas EA, Leite EA, *et al.* Melatonin regulates maternal pancreatic remodeling and B-cell function during pregnancy and lactation. *J Pineal Res*. 2021; 71(1), e12717. DOI: 10.1111/jpi.12717.
82. Wang W, Zhang C. Targeting β-cell dedifferentiation and transdifferentiation: opportunities and challenges. *Endocr Connect*. 2021; 10(8), R213–r228. DOI: 10.1530/ec-21-0260.

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