Melatonin can improve insulin resistance and aging-induced pancreas alterations in senescence-accelerated prone male mice (SAMP8)

Sara Cuesta · Roman Kireev · Cruz García ·
Lisa Rancan · Elena Vara · Jesús A. F. Tresguerres

Received: 30 November 2011 / Accepted: 20 February 2012 / Published online: 13 March 2012
© American Aging Association 2012

Abstract The aim of the present study was to investigate the effect of aging on several parameters related to glucose homeostasis and insulin resistance in pancreas and how melatonin administration could affect these parameters. Pancreas samples were obtained from two types of male mice models: senescence-accelerated prone (SAMP8) and senescence-accelerated-resistant mice (SAMR1). Insulin levels in plasma were increased with aging in both SAMP8 and SAMR1 mice, whereas insulin content in pancreas was decreased with aging in SAMP8 and increased in SAMR1 mice. Expressions of glucagon and GLUT2 messenger RNAs (mRNAs) were increased with aging in SAMP8 mice, and no differences were observed in somatostatin and insulin mRNA expressions. Furthermore, aging decreased also the expressions of Pdx-1, FoxO 1, FoxO 3A and Sirt1 in

pancreatic SAMP8 samples. Pdx-1 was decreased in SAMR1 mice, but no differences were observed in the rest of parameters on these mice strains. Treatment with melatonin was able to decrease plasma insulin levels and to increase its pancreatic content in SAMP8 mice. In SAMR1, insulin pancreatic content and plasma levels were decreased. HOMA-IR was decreased with melatonin treatment in both strains of animals. On the other hand, in SAMP8 mice, treatment decreased the expression of glucagon, GLUT2, somatostatin and insulin mRNA. Furthermore, it was also able to increase the expression of Sirt1, Pdx-1 and FoxO 3A. According to these results, aging is associated with significant alterations in the relative expression of pancreatic genes associated to glucose metabolism. This has been especially observed in SAMP8 mice. Melatonin administration was able to improve pancreatic function in old SAMP8 mice and to reduce HOMA-IR improving their insulin physiology and glucose metabolism.

S. Cuesta · R. Kireev · J. A. F. Tresguerres
Department of Physiology, Medical School,
University Complutense of Madrid,
Madrid, Spain

C. García · L. Rancan · E. Vara
Department of Biochemistry and Molecular Biology,
Medical School, University Complutense of Madrid,
Madrid, Spain

J. A. F. Tresguerres (✉)
Laboratory of Experimental Endocrinology,
Department of Physiology, School of Medicine,
Complutense University,
Avda, Complutense s/n,
28040 Madrid, Spain
e-mail: guerres@med.ucm.es

Keywords Pancreas · Aging · Melatonin · Senescence-accelerated mouse

Introduction

The prevalence of obesity, insulin resistance and type 2 diabetes is reaching epidemic proportions worldwide. Type 2 diabetes is a disease with a slow but progressive pathogenesis. Both genes and the environment play a critical role in its development. Insulin

resistance is the earliest detectable defect that may occur 15–25 years or more before the clinical onset of disease. Insulin-resistant states are characterized by the inability of insulin to promote glucose uptake in the tissues (Duplain et al. 2001; Vollenweider et al. 1994). Thus, insulin resistance constitutes what epidemiologists call an ‘intervening phenotype’, as well as a marker for the disease. Initially, there is an attempt to compensate for this resistance with increased insulin secretion, but eventually hormone secretion fails, and type 2 diabetes develops. The reasons for the failure to maintain sufficient insulin secretion would be a decrease in β cell mass combined with defective insulin secretion. The exact molecular site for the insulin resistance is unknown. Environmental factors, particularly those leading to obesity, further enhance this diabetogenic tendency by accentuating insulin resistance. Increasing evidence suggests that an inflammatory process promotes islet dysfunction in type 2 diabetes (Donath et al. 2008). Indeed, pancreas of patients with type 2 diabetes is characterized by the presence of cytokines, NF- κ B activation, immune cells, beta cell apoptosis, amyloid deposits and fibrosis. Elevated free fatty acid levels establish the link between obesity and insulin resistance. Patients with insulin resistance or type 2 diabetes mellitus usually exhibit obesity and elevated free fatty acid levels. However, insulin resistance can also originate in the adipose tissue, where it leads to an increase in lipolysis, with a subsequent release of glycerol and FFA into the circulation. It is widely accepted that increased availability and utilization of FFA contribute to the development of insulin resistance in skeletal muscle, as well as to increased hepatic glucose production. In fact, the progression of this situation in rats on a high-fat diet is closely related to plasma FFA levels. The clinical consequences of insulin resistance and compensatory hyperinsulinemia have become a major public health problem.

Melatonin (*N*-acetyl-5-methoxytryptamine), a tryptophan derived small indolic molecule, is mainly produced by the pineal gland but is also produced in other tissues (Erren and Reiter 2008; Hardeland 2008; Reiter et al. 2009). Melatonin is a highly phylogenetically conserved molecule that exerts many regulatory functions, modulating cellular metabolism through its binding to specific membrane and nuclear receptors (Zanquetta et al. 2003; Lima et al. 1998). Data obtained from experimental animal models in vivo and studies in

vitro suggest that melatonin might regulate glucose homeostasis (Zanquetta et al. 2003; Lima et al. 1998; Ha et al. 2006). In normal rats, pinealectomy induces insulin resistance and glucose intolerance (Zanquetta et al. 2003; Lima et al. 1998). In obese insulin-resistant rats, parenteral melatonin administration was able to decrease body weight and to lower plasma glucose (Prunet-Marcassus et al. 2003) and insulin (Hussein et al. 2007) levels, but the underlying mechanisms are not known. Melatonin was also shown to reduce serum cholesterol levels in mammalian species (Hoyos et al. 2000; Mori et al. 1989; Aoyama et al. 1988; Montilla et al. 1998) and to prevent oxidative stress in diabetic subjects (Montilla et al. 1998; Nishida et al. 2002). It has also been shown to reduce hyperglycemia, hyperinsulinemia and hyperleptinemia, and to restore hepatic Δ -5 desaturase (an insulin-permissive enzyme) activity in type 2 diabetic rats. These animals also exhibited a restoration in their altered polyunsaturated fatty acids (PUFA) levels to near normal values (Nishida et al. 2002). Thus, melatonin may prevent changes in membrane fluidity during lipid peroxidation induced by oxidants (Garcia et al. 1997) and diabetes (Nishida et al. 2002).

The decrease in the production of melatonin, the increase in insulin secretion and the decrease in sensitivity of peripheral tissues and of pancreatic islet β cells to insulin have been described as typical for aging in humans (Barbieri et al. 2002; Bellino and Wise 2003; Ferrari et al. 1995, 1996; Touitou and Haus 2000).

Establishing pertinent animal models which have characteristics closely similar to humans is essential to elucidate the fundamental mechanisms of age-related changes and to develop effective drugs for the prevention of age-related diseases. SAM (senescence-accelerated mouse) has been already established as a murine model of accelerated aging. It is composed actually by a group of related inbred mice including nine strains showing accelerated, senescence-prone, short-lived animals (SAMP) and three resistant to accelerated senescence, long-lived mice (SAMR) (Kitado et al. 1994; Takeda et al. 1997). SAMP animals show relatively specific age-associated phenotypic pathologies such as a shortened life span and early manifestation of senescence. Previous studies from our group have investigated age-associated changes in SAMP8 and SAMR1 mice in heart (Forma et al. 2010; Cuesta et al. 2011b). In this study biochemical parameters associated with glucose

homeostasis have been investigated in SAMP8 mice as compared with SAMR1 mice which serve as the controls.

Due to all the aforementioned facts, in this paper the effect of aging on glucose metabolism, cell proliferation and differentiation in pancreas of SAMP8 mice has been investigated as compared with SAMR1 mice. The effect of a chronic treatment with melatonin at two different doses has been also tested.

Materials and methods

Animals and treatment

Melatonin was obtained from Actafarma (Madrid, Spain). Other reagents were of the highest quality available and obtained from different commercial companies.

Male senescence-accelerated mice (SAMP8/SAMR1) of 2 (young) and 10 months (old) of age were used ($n=64$). Animals were divided into eight experimental groups ($n=8$ animals per group): (1) young SAMP8 untreated (2-month age), (2) old SAMP8 untreated (10-month age), (3) old SAMP8 (10-month age) treated with melatonin 1 mg, (4) old SAMP8 (10-month age) treated with melatonin 10 mg, (5) young SAMR1 untreated (2-month age), (6) old SAMR1 untreated (10-month age), (7) old SAMR1 (10-month age) treated with melatonin 1 mg and (8) old SAMR1 (10-month age) treated with melatonin 10 mg. The animals remained during all the time including the treatment period in conditions of controlled light (12-h light/dark cycle) and temperature (20–24°C) and received a standard diet and water ad libitum. Mice were treated with melatonin during 4 weeks between 9 and 10 months of age. Melatonin was dissolved in absolute ethanol and added to the drinking water in a final ethanol concentration of 0.066%. Water bottles were covered with aluminium foil to be protected from light, and the drinking fluid was changed three times a week, depending on the water consumption and the weight of the animals. Untreated animals received 0.1% alcohol in tap water. After 30 days of treatment, animals were killed by cervical dislocation followed by decapitation, and pancreas samples were collected in RNA later (for PCR determinations). All animals received humane care according to the Guidelines for Ethical Care of Experimental Animals of the European Union.

RNA isolation and PCR quantification

RNA was isolated from pancreas samples of male mice using the kit RNeasy total rna kit ref.50974104 (Qiagen) and following the manufacturer's protocol. The purity of the RNA was estimated by 1.5% agarose gel electrophoresis, and RNA concentration was determined by spectrophotometry. Reverse transcription of 2 µg RNA for complementary DNA (cDNA) synthesis was performed using the Reverse Transcription System (Promega, Madison, WI, USA) and a pd (N) 6 random hexamer. RT-PCR was performed in an Applied Biosystems 7300 apparatus using the SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK) and 300-nM concentrations of specific primers (Table 1). The thermocycling profile conditions used were 50°C for 2 min, 95°C for 10 min (followed by 40 cycles), 95°C for 15 s, 60°C for 1 min, 95°C for 15 s, 60°C for 30 s and 95°C for 15 s. For the normalization of cDNA loading in the PCR reaction, the amplification of 18S ribosomal RNA (rRNA) for every sample was used. The primers used were for the estimation of glucagon, GLUT2, insulin, somatostatin, FoxO 1, FoxO 3A, Sirtuin 1, Sei1 and Pdx-1 (Table 1). Relative changes in gene expression were calculated using the 2-DDCT method (Livak 2001).

Insulin and glucose

Insulin was measured in plasma by an RIA kit according to the manufacturer's instructions (DRG Instruments, GmbH, Germany).

Glucose was measured in plasma by an assay kit according to the manufacturer's instructions (GAG 020-1kt, SIGMA).

Plasma glucose and insulin values were used to calculate a homeostasis model assessment of insulin resistance (HOMA-IR) as follows: glucose (nanomole per liter) $\times$ insulin (milli insulin units per liter)/22.5 (Matthews et al. 1985).

Free fatty acids

Free fatty acids were measured in plasma by an assay kit according to the manufacturer's instructions (NEFA ACS-ACOD Method, Wako Chemicals GmbH).

Table 1 Primers used in the study

Primers	Sequence (5'-3')
18 s	
Forward	GGTGCATGGCCGTCTTA
Reverse	TCGTTCTTATCGGAATTAACC
Glucagon	
Forward	CACGCCCTTCAAGACACAG
Reverse	GTCCTCATGCGCTTCTGTC
GLUT2	
Forward	TGTGATCCAGTGAGTCTCAA
Reverse	GGCGCACATCTATAATGCTCT
Insulin	
Forward	AGCAAGCAGCTCATTGTTC
Reverse	TTGCGGGTCTCCACTTC
Somatostatin	
Forward	CTGGAGCCTGAGGATTGTC
Reverse	CTGCAGCCTGAGCCTCAT
FoxO 1	
Forward	CTTCAAGGATAAGGGCGACA
Reverse	GACAGATTGTGGCGAATTGA
FoxO 3A	
Forward	GCTAAGCAGGCCTCATCTCA
Reverse	TTCCGTCAGTTTGAGGGTCT
Sirtuin 1	
Forward	TCGTGGAGACATTTTAATCAGG
Reverse	GCTTCATGATGGCAAGTGG
Sei1	
Forward	CAAGCGGGAGGAGGAGGAGACGAT
Reverse	AGAAGGGGCTGGGGGCTGGAT
Pdx-1	
Forward	GAAATCCACCAAAGCTCACG
Reverse	CGGGTTCGCTGTGTAAG

18 s was used as a housekeeping gene to compare the samples

Statistical analyses

The results were statistically analyzed with the ANOVA method, with a confidence level of 95% ($p < 0.05$) considered significant. Results are expressed as the mean $\pm$ SEM. Mean comparison was done by ANOVA followed by a Fisher test. In each case analysis for PCR was carried out and normalized according to 18S rRNA endogenous gene run in parallel.

Relative changes in gene expression were calculated using the 2-DDCT method.

Results

Plasma insulin levels were increased in both old SAMP8/SAMR1 mice as compared with their respective young controls ($p < 0.001$). Treatments with melatonin were able to restore these levels in a dose-dependent manner ($p < 0.05$).

Pancreatic content of this hormone was decreased with aging ($p < 0.001$) in SAMP8 animals. Treatments with melatonin at 1 and 10 mg/kg/day were able to restore values of insulin to those present in young animals and in a dose-dependent manner ($p < 0.05$). In SAMR1 mice, pancreatic content of this hormone was increased ($p < 0.001$), and treatments with melatonin 1 and 10 mg were able to decrease its values ($p < 0.05$) (Table 2).

Glucose was measured as a metabolic index for insulin action, and no differences were found with aging in SAMP8 animals. Melatonin treatments at 1 and 10 mg were able to decrease plasma glucose levels of these mice ($p < 0.05$). In SAMR1 mice, no differences were observed either with aging or with melatonin treatment (Table 2).

HOMA-IR index showed significant increases in old SAMP8 and SAMR1 male mice as compared to young animals ($p < 0.001$). The index was also significantly higher in old SAMP8 mice as compared with old SAMR1 mice ($p < 0.01$). Melatonin administration was able to reduce the HOMA-IR index in both old SAMP8 and SAMR1 mice in a dose-dependent manner ($p < 0.05$) (Table 2).

Whereas gene expressions of glucagon ($p < 0.05$) and GLUT2 ($p < 0.05$) were significantly increased with aging in pancreas of SAMP8 mice, no differences were observed in its expression on SAMR1 animals (Fig. 1). Expression of GLUT2 was lower ($p < 0.01$) in young SAMP8 mice as compared with young SAMR1 animals (Fig. 1b), but old SAMP8 mice showed higher values than old SAMR1 mice.

Expression of insulin and somatostatin did not change during aging in pancreas of male SAMP8 mice, and also no significant differences were observed between old and young SAMR1 animals (Fig. 2). Expressions of insulin ($p < 0.05$) and somatostatin ($p < 0.001$) mRNA were increased in pancreas of young and old SAMP8 animals as compared with young and old SAMR1 mice (Fig. 2).

Treatment with melatonin at 1 and 10 mg/kg/day was able to reduce expression of glucagon ($p < 0.05$),

Table 2 Levels of insulin and glucose on plasma of SAMP8 and SAMR1 mice and HOMA-IR

	SAMP8				SAMR1			
	Young	Old	Old+Mel 1 mg	Old+Mel 10 mg	Young	Old	Old+Mel 1 mg	Old+Mel 10 mg
Glucose (mmol/L)	5.43±0.31	5.80±0.32 ^b	1.97±0.41	1.25±0.24	4.48±0.69	5.42±0.35	4.91±0.41	3.24±0.76
Insulin plasma (mUi/L)	4.72±0.95	14.96±2.57 ^{ab}	4.62±1.11	4.66±0.64	4.72±0.95	7.47±0.69 ^{ab}	3.65±0.69	1.12±0.16
Insulin pancreas (ng/15 mg tissue)	0.28±0.07	0.12±0.02 ^{ab}	0.27±0.04	0.37±0.13	0.12±0.01	0.17±0.0005 ^{ab}	0.14±0.01	0.12±0.01
HOMA (I/R)	1±0.17	3.16±0.58 ^{abc}	1.22±0.31	0.77±0.39	1.25±0.17	1.71±0.13 ^{ab}	1.22±0.31	0.42±0.19

Insulin was measured in pancreas too

Mel melatonin

^a Old mice compared with young animals ($p<0.001$)

^b Old mice compared with melatonin treatments ($p<0.05$)

^c Old SAMP8 as compared with old SAMR1 ($p<0.01$)

GLUT2 ($p<0.01$), somatostatin ($p<0.001$) and insulin ($p<0.01$) in old SAMP8 male mice as compared with untreated animals (Figs. 1 and 2).

mRNA expressions of FoxO 1 ($p<0.05$) and FoxO 3A ($p<0.001$) and Sirtuin ($p<0.05$) were decreased with aging in old SAMP8 mice (Fig. 3). No significant differences in these parameters were observed between old and young SAMR1 mice. The comparative analysis of the gene expression of FoxO 1 ($p<0.05$) between old SAMP8 and old SAMR1 mice demonstrated significantly lower values in SAMR1 mice (Fig. 3).

Treatment with melatonin (1 and 10 mg/kg/day) significantly increased mRNA expression of FoxO 3A ($p<0.001$), Sirtuin ($p<0.05$) and FoxO 1 ($p<0.05$) in old male SAMP8 mice as compared with untreated animals (Fig. 3). We observed a dose-dependent increase in mRNA expression of FoxO 3A and Sirtuin.

No significant differences were observed with aging in the pancreatic expression of proliferation gene (Sei1) between SAMP8 and SAMR1 mice. Melatonin administration did not influence these parameters in old SAMP8 mice. No differences were observed also in SAMR1 mice.

Pdx-1 expression was decreased in both old SAMP8 and SAMR1 mice ($p<0.001$), and only melatonin 10 mg/kg/day treatment increased this expression in SAMP8 mice ($p<0.001$) (Fig. 4).

No significant differences were observed between young and old SAMP8 mice in plasma free fatty acids levels. Treatments with melatonin did not change

these levels of FFA. No differences were observed either between young and old SAMR1 or between young and old SAMP8 mice (Fig. 5).

Discussion

One of the greatest risk factors for the development of type 2 diabetes in humans is age (Wilson et al. 1986). Type 2 diabetes is characterized by a combination of defective insulin secretion that results from a progressive age-associated decline in pancreatic function and appearance of insulin resistance (Iozzo et al. 1999; Basu et al. 2003; Moller et al. 2003). In our data a decrease in pancreatic insulin content of old SAMP8 mice as compared with young mice has been observed. However, mRNA expression of insulin did not change with aging, either in SAMP8 or in SAMR1 mice. On the other hand, enhanced plasma levels of this hormone were also observed but without changes in glycemia (Table 2). So according to our results, the decrease in pancreatic insulin content observed in old SAMP8 mice was surely still preceding the appearance of hyperglycemia. In addition, levels of plasma insulin were elevated in both old SAMP8 and SAMR1 mice. Furthermore, HOMA-IR index was calculated and in old SAMP8 and SAMR1 mice was found to be elevated as compared with young animals. So we considered that animals were in an initial insulin resistance state. Two different approaches have been considered in order to solve the insulin resistance problem. In SAMR1 mice, when tissues become less

Fig. 1 Effect of aging and melatonin administration on mRNA expression of **a** glucagon and **b** GLUT2 in pancreas from male SAMP8 and SAMR1 mice. $n=8$ animals per group

sensitive and more resistant to insulin, pancreas did increase insulin secretion (and therefore its level) to maintain normal glucose levels, but HOMA-IR index did not change in these animals. In SAMP8 mice the insulin content of pancreas decreased with aging, due to the fact that pancreas in these animals could not secrete enough insulin, since beta cell in these animals were already damaged due to the aging process. Furthermore, an increase in plasma insulin levels with aging was observed in these animals. Glucose levels did not change between young and old animals, due to the increased plasma levels of insulin and also to the increase in GLUT2 expression in these animals (Fig. 6). People suffering from type 2 diabetes mellitus normally experience normoglycemic hyperinsulinism for many years before hyperglycemia and clinical characteristics occur (di Giulio et al. 2009; Weinberg

et al. 2009). However, if insulin resistance worsens, plasma insulin levels are finally reduced, and glucose levels should begin to rise. These are then two of the major symptoms of an established type 2 diabetes mellitus.

Islets of Langerhans are heterogeneous cell aggregates containing β , α , δ and PP cells, which secrete insulin, glucagon, somatostatin (SST) and pancreatic polypeptide, respectively. The different cell types within the islet are affected by changes in extracellular glucose concentrations. Thus, elevations in circulating glucose should stimulate insulin secretion from β cells and somatostatin secretion from δ cells (Vieira and Salehi 2007). Our results showed that insulin and somatostatin mRNA expressions seem to behave in the same way, and no differences were observed between young and old animals. These results showed

Fig. 2 Effect of aging and melatonin administration on mRNA expression of **a** insulin and **b** somatostatin in pancreas from male SAMP8 and SAMR1 mice. $n=8$ animals per group

correlation with plasma glucose levels. Other pancreatic hormones were also analyzed in these mice. The mRNA expression of glucagon was increased in old SAMP8 and SAMR1 mice ($p < 0.05$). Other studies have found that type 2 diabetes is characterised not only by problems with β cell function and mass but also by a relative increase of α cells, resulting in a relative hyperglucagonemia due to a certain type of α cell dysfunction (Deng et al. 2004; Unger and Orci 1975; Yoon et al. 2003) that these mice could also display.

Cuesta et al. (2011b) showed that the proliferative genes PCNA and Se1 did not change with aging in SAMP8 old mice, but Pdx-1 expression was lower in aged mice. Pancreatic and duodenal homebox-1 (Pdx-1) is a transcription factor necessary for pancreatic development and β cell maturation. In our results, mRNA expression of Pdx-1 was lowered in old SAMP8 mice. The reduced Pdx-1 expression has been shown to accompany the development of full blown diabetes, in complex genetic or

environmentally related animal models of the disease, showing a correlation between low Pdx-1 levels and β cell failure (Seufert et al. 1998; Ahn et al. 2007; Bonner-Weir et al. 2000).

FOXO factors are associated with a wide range of biological processes, including cell cycle arrest, apoptosis, DNA repair, glucose metabolism, anti-oxidative stress and longevity (Accili and Arden 2004; Barthel et al. 2005). mRNA expressions of FoxO 1 and FoxO 3A were measured showing a decrease with aging in SAMP8 mice.

SIRT1 is a known regulator of hepatic gluconeogenesis; it deacetylates peroxisome proliferator-activated receptor γ (PPAR γ)-coactivator1 α (PGC1- α), thereby increasing liver gluconeogenic gene transcription and repressing glycolytic gene expression. Furthermore, SIRT1 has been shown to bind and to deacetylate FOXO proteins (Daitoku et al. 2004; Kobayashi et al. 2005; Brunet et al. 2004), and in our results mRNA expression of SIRT1 was also decreased with aging in SAMP8 mice. SIRT1 effects on

Fig. 3 Effect of aging and melatonin administration on mRNA expression of **a** FoxO 1, **b** FoxO 3A and **c** Sirtuin 1 in pancreas from male SAMP8 and SAMR1 mice. $n=8$ animals per group

FOXO activities vary depending on FOXO target genes. The consensus that emerges from these studies is that SIRT1 may play a crucial role in tilting the balance of FOXO functions away from cell death and more towards resistance to stress (Wang and Tissenbaum 2006).

Pineal melatonin biosynthesis declines with aging. Nocturnal plasma melatonin levels are significantly decreased already by middle age, and this reduction

has been hypothesized to be associated with a variety of age-related physiological changes (Srinivasan et al. 2005). Melatonin levels tend to decrease significantly by middle age and then continue to decline throughout old age (Reiter 1992; Pang et al. 1990); intraabdominal adiposity, plasma insulin and plasma leptin levels tend to increase at the same time (Bjorntorp 1995; Rasmussen et al. 1999) due to the development of insulin resistance, leading to diabetes, dyslipidemia

Fig. 4 Effect of aging and melatonin administration on mRNA expression of **a** *Sei1* and **b** *Pdx-1* in pancreas from male SAMP8 and SAMR1 mice. $n=8$ animals per group

and cardiovascular diseases (Bjorntorp 1995). Reports indicate that factors that increase oxidative stress like hyperglycemia, increased free-fatty acids and adipokines contribute to insulin resistance (Evans et al. 2002; Evans et al. 2003). No significant differences

with age were observed in these mice in plasma free fatty acids levels.

Both humans (Alcozer et al. 1956) and rodents seem to have a relationship between circulating melatonin and the regulation of carbohydrate metabolism

Fig. 5 Effect of aging and melatonin administration on levels of free fatty acids in plasma from male SAMP8 and SAMR1 mice. $n=8$ animals per group

Fig. 6 Diagram of two different approaches considered in order to solve the insulin resistance problem in the two different strains of mice

(van Cauter et al. 1991; Peschke and Peschke 1998). Pinealectomy induced diminished glucose tolerance, insulin resistance, decreased hepatic and muscular glycogenesis and an increase in blood pyruvate concentration in rats (Mellado et al. 1986). On the other hand, it has been shown that melatonin was able to reduce insulin secretion under several experimental

conditions (Peschke and Peschke 1998; la Fleur et al. 1999; la Fleur 2001). However, the action of melatonin in the process of glucose-induced insulin secretion has not been well understood. In these mice, treatment with melatonin was able to decrease mRNA expression of insulin. However, insulin content in pancreas was increased, and plasma levels of this hormone were

decreased in old male SAMP8 mice. Glucemic level was decreased with melatonin treatment, and HOMA-IR showed a significant reduction in treated animals, with a clear cut dose–response effect. This seems to indicate that melatonin is able to ameliorate glucose homeostasis.

The absence of a pancreatic insulin increase with age in animals was probably due to a certain damage of its beta cells. Treatment with melatonin induced an increase in pancreatic insulin content, reaching values similar to those found in young mice, and due to this increase, plasma glucose levels were reduced. On the other hand, melatonin treatment was also able to decrease plasma insulin levels since in these animals, melatonin was able to decrease peripheral insulin resistance or increase insulin sensitivity. Furthermore, treatment with melatonin was able to decrease HOMA-IR in a dose–response manner. Similarly, due to the glucemic decrease, glucose sensor GLUT2 expression was also decreased.

Other pancreatic hormones like somatostatin and glucagon showed also a decrease with melatonin treatment. So in these mice, melatonin treatment was able to restore a previous situation of “hyperglucagonemia” and to reduce mRNA expression of somatostatin, decreasing the levels of insulin in plasma and increasing pancreatic content of this hormone.

Pancreas development is the result of an orchestrated series of events. Pancreatic and duodenal homeobox factor-1 (Pdx-1) is a transcription factor that plays an important role in the endocrine pancreas (Kaneto et al. 2008) and is present in all endocrine pancreatic cells during embryonic development. However, its expression is largely restricted to beta cells in the adult pancreas (Prado et al. 2004). In our results mRNA expression of Pdx-1 has been found to be elevated with 10 mg of melatonin. As we mentioned previously in the article, reduced Pdx-1 expression accompanies the development of full-blown diabetes in specific animal models of the disease, showing an evident correlation between low Pdx-1 levels and β cell failure (Loots and Ovcharenko 2004). Melatonin treatment was able to increase the expression of this factor. Pdx-1 is regulated in part by the forkhead transcription factor, FoxO 1, the most abundant forkhead transcription factor in pancreatic beta cells (Kitamura et al. 2002). The maintenance of enough beta cell mass and function is critical for glucose homeostasis. Beta cell mass can be affected by changes in cell size,

proliferation, neogenesis or apoptosis. In previous studies of our group, melatonin was found to be able to decrease the age-dependent oxidative stress, inflammation and apoptosis in pancreas (Cuesta et al. 2011a). In our actual results, an increase in mRNA expression of FoxO factors and SIRT1 on old SAMP8 mice in a dose-dependent manner has been found. FoxO factors could mediate a protective effect by promoting the expression of genes involved in fighting oxidative stress. SIRT1 is a selective activator of FoxO signalling but simultaneously also an inhibitor of the NF- κ B pathway (Yeung et al. 2004; Giannakou and Partridge 2004). This type of regulation can enhance the FoxO-dependent longevity-associated functions, while inhibiting NF- κ B-dependent pro-aging processes. The increase of these genes could help to preserve beta cell function also under conditions of enhanced oxidative stress, such as in insulin-resistant states (Kitamura et al. 2005).

According to our results, aging of the pancreas in SAMP8 mice was associated with alterations in insulin secretion, pancreas differentiation and glucose homeostasis. Melatonin, at two different doses, was able to improve the age-related pancreatic damage, restoring insulin levels, differentiation and glucose metabolism. SAMR1 were not highly affected by aging, and in the same way melatonin effects were not as important.

Acknowledgments This work has been possible through grants from Instituto de Salud Carlos III (RETICEF RD06/0013 RD06/0013/0008), Consejería de Innovación, Ciencia y Empresa, Junta de Andalucía (P07-CTS-03135), PI081644 (ISCIII) and SAF 2007 66878-C02-01. Thanks are given to Daniel and Rocío Campón for their technical support.

References

- Accili D, Arden KC (2004) FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. *Cell* 117:421–426
- Ahn YB, Xu G, Marselli L et al (2007) Changes in gene expression in beta cells after islet isolation and transplantation using laser-capture microdissection. *Diabetologia* 50:334–342
- Alcozer G, Giordano G, Masciocco D (1956) Studi sull' epifisi. Influenza dell' estratto acquoso della pineale su alcuni aspetti del metabolismo glicidico in soggetti sani ed eucrinici. *Arch Pat Clin* 12:1105–1113
- Aoyama H, Nori N, Mori W (1988) Effects of melatonin on genetic hypercholesterolemia in rats. *Atherosclerosis* 69:269–272

- Barbieri M, Rizzo MR, Manzella D et al (2002) Glucose regulation and oxidative stress in healthy centenarians. *Exp Gerontol* 38:137–143
- Barthel A, Schmolli D, Unterman TG (2005) FoxO proteins in insulin action and metabolism. *Trends Endocrinol Metab* 16:183–189
- Basu R, Breda E, Oberg AL et al (2003) Mechanisms of the age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action, and clearance. *Diabetes* 52:1738–1748
- Bellino FL, Wise PM (2003) Nonhuman primate models of menopause workshop. *Biol Reprod* 68:10–18
- Bjorntorp P (1995) Neuroendocrine ageing. *J Intern Med* 238:401–404
- Bonner-Weir S, Taneja M, Weir GC et al (2000) In vitro cultivation of human islets from expanded ductal tissue. *PNAS* 97:7999–8004
- Brunet A, Sweeney LB, Sturgill JF et al (2004) Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* 303:2011–2015
- Cuesta S, Kireev RA, Garcia C et al (2011a) Beneficial effect of melatonin treatment on inflammation, apoptosis and oxidative stress on pancreas of Senescence Accelerated Mice model. *Mech Ageing Dev* Oct 17
- Cuesta S, Kireev R, Forman K et al (2011b) Growth hormone can improve insulin resistance and differentiation in pancreas of senescence accelerated prone male mice (SAMP8). *Growth Horm IGF Res* 21(2):63–68
- Daitoku H, Hatta M, Matsuzaki H et al (2004) Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity. *Proc Natl Acad Sci USA* 101:10042–10047
- Deng S, Vatamaniuk M, Huang X et al (2004) Structural and functional abnormalities in the islets isolated from type 2 diabetic subjects. *Diabetes* 53:624–632
- di Giulio C, Antosiewicz J, Walski M et al (2009) Physiological carotid body denervation during aging. *Adv Exp Med Biol* 648:257–263
- Donath MY, Storling J, Berchtold LA et al (2008) Cytokines and beta-cell biology: from concept to clinical translation. *Endocr Rev* 29(3):334–350
- Duplain H, Burcelin R, Sartori C et al (2001) Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. *Circulation* 104:342–345
- Erren TC, Reiter RJ (2008) A generalized theory of carcinogenesis due to chronodisruption. *Neuro Endocrinol Lett* 29(6):815–821
- Evans JL, Goldfine ID, Maddux BA et al (2002) Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes DOI:dx.doi.org. *Endocr Rev* 23(5):599–622
- Evans JL, Goldfine ID, Maddux BA (2003) Are oxidative stress-activated signaling pathways mediators of insulin resistance and β -cell dysfunction? *Diabetes* 52(1):1–8
- Ferrari E, Magri F, Dori D et al (1995) Neuroendocrine correlates of the aging brain in humans. *Neuroendocrinology* 61:464–470
- Ferrari E, Magri F, Locatelli M et al (1996) Chrono-neuroendocrine markers of the aging brain. *Aging Clin Exp Res* 8:320–327
- Forma K, Vara E, García C et al (2010) Beneficial effects of melatonin on cardiometabolic alterations in a murine model of accelerated aging. *J Pineal Res* 49(3):312–320
- Garcia JJ, Reiter RJ, Guerrero JM et al (1997) Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation. *FEBS* 408:297–300
- Giannakou ME, Partridge L (2004) The interaction between FOXO and SIRT1: tipping the balance towards survival. *Trends Cell Biol* 13:408–412
- Ha E, Yim SV, Chung JH et al (2006) Melatonin stimulates glucose transport via insulin receptor substrate-1/phosphatidylinositol 3-kinase pathway in C2C12 murine skeletal muscle cells. *J Pineal Res* 41:67–72
- Hardeland R (2008) Melatonin, hormone of darkness and more: occurrence, control mechanisms, actions and bioactive metabolites. *Cell Mol Life Sci* 65(13):2001–2018
- Hoyos M, Guerrero JM, Perez-Cano R et al (2000) Serum cholesterol and lipid peroxidation are decreased by melatonin in diet-induced hypercholesterolemic rats. *J Pineal Res* 28:150–155
- Hussein MR, Ahmed OG, Hassan AF et al (2007) Intake of melatonin is associated with amelioration of physiological changes, both metabolic and morphological pathologies associated with obesity: an animal model. *Int J Exp Pathol* 88:19–29
- Iozzo P, Beck-Nielsen H, Laakso M et al (1999) Independent influence of age on basal insulin secretion in nondiabetic humans. European group for the study of insulin resistance. *J Clin Endocrinol Metab* 84:863–868
- Kaneto H, Miyatsuka T, Kawamori D et al (2008) PDX-1 and MafA play a crucial role in pancreatic beta-cell differentiation and maintenance of mature beta-cell function. *Endocr J* 55(2):235–252
- Kitado H, Higuchi K, Takeda T (1994) Molecular genetic characterization of the Senescence-Accelerated Mouse (SAM) strains. *J Gerontol* 49:B247–B254
- Kitamura T, Nakae J, Kitamura Y et al (2002) The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. *J Clin Invest* 110:1839–1847
- Kitamura YI, Kitamura T, Kruse JP et al (2005) FoxO1 protects against pancreatic beta cell failure through NeuroD and MafA induction. *Cell Metab* 2:153–163
- Kobayashi Y, Furukawa-Hibi Y, Chen C et al (2005) SIRT1 is critical regulator of FOXO-mediated transcription in response to oxidative stress. *Int J Mol Med* 16:237–243
- la Fleur SE (2001) The suprachiasmatic nucleus generated rhythm in blood glucose. Ponsen and Looijen, Amsterdam
- la Fleur SE, Kalsbeek A, Wortel J et al (1999) A suprachiasmatic nucleus generated rhythm in basal glucose concentrations. *J Neuroendocrinol* 11:643–652
- Lima FB, Machado UF, Bartol I et al (1998) Pinealectomy causes glucose intolerance and decreases adipose cell responsiveness to insulin in rats. *Am J Physiol* 275:934–941
- Livak KJ (2001) AND SCHMITTGEN TD. Analysis of relative gene expression data using realtime quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods* 25:402–408
- Loots GG, Ovcharenko I (2004) Evolutionary analysis of transcription factor binding sites. *Nucleic Acids Res* 32:W217–W221
- Matthews DR, Hosker JP, Rudenski AS et al (1985) Homeostasis model assessment: insulin resistance and β -cell function

- from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419
- Mellado C, Rodríguez V, Diego JG et al (1986) Effect of pinealectomy and of diabetes on liver and glucagon receptor concentrations in the rat. *J Pineal Res* 6:295–306
- Moller N, Gormsen L, Fuglsang J et al (2003) Effects of ageing on insulin secretion and action. *Horm Res* 60:102–104
- Montilla PL, Vargas JF, Tunez IF et al (1998) Oxidative stress in diabetic rats induced by streptozotocin: protective effects of melatonin. *J Pineal Res* 25:94–100
- Mori N, Aoyama H, Murase T et al (1989) Anti-hypercholesterolemic effect of melatonin in rats. *Acta Pathol Jpn* 39:613–618
- Nishida S, Segawa T, Murai I et al (2002) Long-term melatonin administration reduces hyperinsulinemia and improves the altered fatty-acid compositions in type 2 diabetic rats via the restoration of Delta-5 desaturase activity. *J Pineal Res* 32:26–33
- Pang SF, Tsang CW, Hong GX et al (1990) Fluctuation of blood melatonin concentrations with age: result of changes in pineal melatonin secretion, body growth and aging. *J Pineal Res* 8:179–192
- Peschke E, Peschke D (1998) Evidence for a circadian rhythm of insulin release from perfused rat pancreatic islets. *Diabetologia* 41:1085–1092
- Prado CL, Pugh-Bernard AE, Elghazi L et al (2004) Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. *Proc Natl Acad Sci U S A* 101:2924–2929
- Prunet-Marcassus B, Desbazeille M, Bros A et al (2003) Melatonin reduces body weight gain in Sprague Dawley rats with diet-induced obesity. *Endocrinology* 144:5347–5352
- Rasmussen DD, Boldt BM, Wilkinson CW et al (1999) Daily melatonin administration at middle age suppresses male rat visceral fat, plasma leptin, and plasma insulin to youthful levels. *Endocrinology* 140:1009–1012
- Reiter R (1992) The ageing pineal gland and its physiological consequences. *BioEssays* 14:169–175
- Reiter RJ, Paredes SD, Manchester LC et al (2009) Reducing oxidative/nitrosative stress: a newly-discovered genre for melatonin. *Crit Rev Biochem Mol Biol* 44(4):175–200
- Seufert J, Weir GC, Habener JF (1998) Differential expression of the insulin gene transcriptional repressor CCAAT/enhancer-binding protein beta and transactivator islet duodenum homeobox-1 in rat pancreatic beta cells during the development of diabetes mellitus. *J Clin Invest* 101:2528–2539
- Srinivasan V, Maestroni GJM, Cardinali DP et al (2005) Melatonin, immune function and aging. *Immun Ageing* 2:17
- Takeda T, Matsushita T, Kurozumi M, Takemura K, Higuchi K, Hosokawa M (1997) Pathobiology of the Senescence-Accelerated Mouse (SAM). *Exp Gerontol* 32:117–128
- Toutou Y, Haus E (2000) Alterations with aging of the endocrine and neuroendocrine circadian system in humans. *Chronobiol Int* 17:369–390
- Unger RH, Orci L (1975) The essential role of glucagon in the pathogenesis of diabetes mellitus. *Lancet* 1:14–16
- van Cauter E, Blackman JD, Roland D et al (1991) Modulation of glucose regulation and insulin secretion by circadian rhythmicity and sleep. *J Clin Invest* 88:934–942
- Vieira E, Salehi A, Gylfe E (2007) Glucose inhibits glucagon secretion by a direct effect on mouse pancreatic alpha cells. *Diabetologia* 50:370–379
- Vollenweider P, Randin D, Tappy L et al (1994) Impaired insulin-induced sympathetic neural activation and vasodilation in skeletal muscle in obese humans. *J Clin Invest* 93:2365–2371
- Wang Y, Tissenbaum HA (2006) Overlapping and distinct functions for a *Caenorhabditis elegans* SIR2 and DAF-16/FOXO. *Mech Ageing Dev* 127:48–56
- Weinberg EJ, Schoen FJ, Mofrad MR (2009) A computational model of aging and calcification in the aortic heart valve. *PLoS One* 4:e5960
- Wilson PW, Anderson KM, Kannel WB (1986) Epidemiology of diabetes mellitus in the elderly. The Framingham study. *Am J Med* 80:3–9
- Yeung F, Hoberg JE, Ramsey CS et al (2004) Modulation of NFkappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J* 23:2369–2380
- Yoon KH, Ko SH, Cho JH et al (2003) Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. *J Clin Endocrinol Metab* 88:2300–2308
- Zanquetta MM, Seraphim PM, Sumida DH et al (2003) Calorie restriction reduces pinealectomy-induced insulin resistance by improving GLUT4 gene expression and its translocation to the plasma membrane. *J Pineal Res* 35:141–148