Research article

Sleep deprivation induces oxidative stress in the liver and pancreas in young and aging rats

Karina Hernández Santiago [a], Ana Laura López –López [b], Fausto Sánchez-Muñoz [c], José Luis Cortés Altamirano [d], Alfonso Alfaro-Rodríguez [d], Herlinda Bonilla-Jaime [e,*]

[a] Maestria en Biologia de la Reproducción, Universidad Autónoma Metropolitana-Iztapalapa, CP 09340, Ciudad de Mexico, Mexico
[b] CINVESTAV sede sur, Departamento de Farmacología, Calz. de los Tenorios 235, Coapa, Rinconada de las Hadas, Tlalpan, Ciudad de México, 14330, México
[c] Departamento de Inmunología, Instituto Nacional de Cardiología (Ignacio Chávez), Juan Badiano No. 1, Col. Sección XVI, Delegación Tlalpan, C.P. 14080, Ciudad de Mexico, Mexico
[d] Departmento de Neurociencias, Instituto Nacional de Rehabilitación "Luis Guillermo Ibarra Ibarra" (INR) Secretaría de Salud (SSA), Ciudad de México, México
[e] Departamento de Biología de la Reproducción, Área de Biología Conductual y Reproductiva, Universidad Autónoma Metropolitana-Iztapalapa, CP 09340, Ciudad de Mexico, Mexico

ABSTRACT

The aging process is characterized by a gradual impairment generally caused by oxidative stress and, more specifically, sleep deprivation, which induces oxidative stress in the brain. The objective of this study was to assess the effect of three types of paradoxical sleep deprivation (PSD): 96 h of PSD (96PSD group); 192 h of PSD (192PSD group); 192 h of PSD followed by a recovery period of 20 days (192PSD + Recovery group) on an oral glucose tolerance test (OGTT), lipid peroxidation (LPO), and superoxide dismutase (SOD) and catalase (CAT) activities in the liver and pancreas of young (3-month-old) and adult (14-month-old) rats. The 96PSD and 192PSD groups of young rats showed lower glucose levels on the OGTT than the control group. In the adult rats, only the 96PSD group had lower glucose levels than the control group. However, the areas under the curve for the young and adult 192 and 192PSD + Recovery groups showed significant differences. Both LPO and SOD increased in the 192PSD and 192PSD + Recovery groups, but CAT decreased in the liver of young rats in the 192PSD group. Regarding the pancreas, LPO and SOD levels increased after 96 h of PSD. In adult animals, CAT decreased in the liver after 96 and 192 h of PSD, while LPO and SOD increased in the pancreas of the 192PSD and PSD + Recovery groups. Differences in the SOD and CAT activities in the liver and SOD activities in the pancreas were also observed between the young and adult rats and maintained across all the PSD groups. In conclusion, PSD induced differential responses that appeared to depend on the duration of the induced condition, the animals' age, and the tissue analyzed. It was found that adult rats were more susceptible to the effects of PSD than young rats.

1. Introduction

Aging is characterized by a gradual impairment of all physiological functions (De Luca d'Alessandro et al., 2011; López-Otín et al., 2013; Sohal et al., 2002). Oxidative stress, defined as an imbalance in the normal equilibrium between the formation of reactive oxygen species (ROS) and the antioxidant defense mechanisms of the body, seems to be a key factor in this process. Antioxidants belong to structurally heterogeneous groups that share the ability to scavenge free radicals and constitute the first line of defense against potential damage caused by ROS. Inside cells, ROS are neutralized by both enzymatic and non-enzymatic antioxidant defense systems. Glutathione reduction (GSH) is the

principal marker of non-enzymatic antioxidant mechanisms because it is widely distributed among organisms. Of all known enzymatic antioxidant mechanisms, superoxide dismutase (SOD) and catalase (CAT) have been studied extensively (Maritim et al., 2003). The general redox state of an organism is often used to represent its total antioxidant capacity, which denotes a balance in the generation of ROS, and when ROS production exceeds the antioxidant capacity, a condition that generates lipid peroxidation (LPO), protein oxidation, and DNA damage, resulting in cell death. Consequently, oxidative stress may lead to genomic instability and DNA damage while modifying oxidative proteins; these are two phenomena involved in the pathogenesis of age-related diseases, such as

* Corresponding author.
   E-mail address: bjh@xanum.uam.mx (H. Bonilla-Jaime).

neurodegenerative and cardiovascular diseases (Ballatori et al., 2009; Bohr et al., 2007; Maritim et al., 2003).

Sleep is critical for the repair and rejuvenation processes of the body, including muscle repair and hormone regulation. Growing evidence suggests that sleep plays a crucial role in metabolic and energy recovery. Sleep deprivation (SD) has been hypothesized to represent an oxidative challenge for the brain, and sleep may play a protective role against oxidative damage (Gopalakrishnan et al., 2004; Singh et al., 2008). Experimental sleep deprivation studies are of interest because they provide information on the deleterious effects of sleep loss, which has been shown to result in adverse metabolic consequences, reduce anabolic hormones (Brianza-Padilla et al., 2016), and increase susceptibility to infection (Knutson et al., 2007). In humans, sleep architecture changes with age (Kryger et al., 2004), and lack of adequate sleep is common in older adults, but sleep loss may also induce oxidative stress in the brain (Hipólide et al., 2002). A previous study that measured oxidative stress in whole-brain homogenates of sleep-deprived rats showed changes in specific brain regions related to oxidative stress, such as decreased glutathione levels in the hippocampus and thalamus (D';Almeida et al., 1998), while total SD induced a decrease in SOD activity in the hippocampus and brainstem (Ramanathan et al., 2002). A systemic elevation of ROS levels has been reported in non-human animals after SD (Villafuerte et al., 2015), but few studies have reported the effect of sleep deprivation on the antioxidant system of the liver and pancreas. Sleep deprivation and aging disturb brain functions (Gosselin et al., 2005; Prinz, 2004; Spiegel et al., 1999; Urrila et al., 2004), and oxidative stress induced by SP may contribute to aging. SD potentiates certain aging effects, affecting the brains of young, mature, and older subjects. In a study by Andersen et al. (2004), SD reduced blood viscosity in the aged but not young rats; therefore, SD studies related to aging are of great interest (Prinz, 2004). To the best of our knowledge, no study has directly tested the effects of SD on oxidative stress in the liver and pancreas of aged animals, even though these two organs play fundamental roles in regulating the body's energy balance, an essential process that has a specific impact on health. In previous studies conducted in our laboratory, we observed that paradoxical sleep deprivation (PSD) for 24, 96, or 192 h induced basal hypoglycemia, and the areas under the curve (AUC) from those experiments showed progressive hypoglycemia after 96 and 192 h of PSD. These studies also found reduced concentrations of insulin and leptin, with an increase in ghrelin, suggesting that PSD induces alterations in the metabolism of organisms (Brianza-Padilla et al., 2016). Although several studies have measured oxidative stress under SD conditions in the central nervous system (CNS) using animal models, no previous studies have focused on measuring the effects of SD on changes in the oxidative-antioxidant system at the tissue level in the liver and pancreas of rats aged 3 and 14 months. It has been suggested that the liver does not undergo significant changes with aging (Bonnet and Arand, 1995), but it demonstrates several characteristics consistent with oxidative injury. ROS levels can affect liver functions, and they are closely linked to most age-associated diseases (Rahal et al., 2014) and metabolic alterations (Brianza-Padilla et al., 2016). Thus, the objective of this study was to (a) test whether paradoxical sleep deprivation for 96 h, 192 h, and 192 h followed by a recovery period induces oxidative stress in the liver and pancreas of young and adult rats, (b) evaluate the effect of PSD on the oral glucose tolerance test (OGTT), and (c) assess whether these responses are age-dependent. We hypothesized that PSD induces a greater impact on antioxidant activity and glucose metabolism in adult subjects than in younger subjects.

2. Materials and methods

In this study, male Wistar rats aged 3 and 14 months were bred in a vivarium at UAM-Iztapalapa. The animals were maintained under standard laboratory conditions following Mexico's Official Norm NOM-062-ZOO-1999 and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH, revised 1996). The experimental protocol was approved by the Ethics Committee of the Division of Biological Sciences of the Universidad Autónoma Metropolitana Iztapalapa with number CBS.310.18. The animals were kept on a 12-hour light-dark cycle (9 am–9 pm) with food (Harlan, Indianapolis, IN, USA) and water *ad libitum*. At the appropriate ages, the rats were separated into a control group and a PSD group and housed in cages ($50 \times 30 \times 20$ cm) with five in each group. The young (3-month-old) and adult (14-month-old) PSD rats were subdivided into the following groups based on a previous study (Brianza Padilla et al., 2016): 96 h of PSD (96PSD), 192 h of PSD (192PSD), and 192 h of PSD followed by a recovery period of 20 days (192PSD + recovery) (n = 5 in each group). The multiple platform model was used for sleep deprivation (Suchecki et al., 2000). After PSD, the rats were subjected to an OGTT, and they were sacrificed 24 h later to dissect their livers and pancreases for the analyses of SOD, CAT, and LPO.

2.1. Sleep deprivation

The rats in the SD groups were placed in a tank ($12 \times 45 \times 45$ cm) with water that contained 15 circular platforms (6 cm in diameter $\times$ 3 cm high) separated by approximately 10 cm. The surfaces of the platforms were maintained 1 cm above the water level so that the rats could move around freely inside the tank by jumping from one point to another. Upon reaching the REM phase during their attempts to sleep, the rats suffered muscular atony and fell into the water. This woke them up brusquely, forcing them to repeat the sleep-wakefulness cycle. The separation of the platforms impedes the formation of groups, making it difficult for the rats to enter the phase of REM sleep (Machado et al., 2005). Each group of sleep-deprived rats was placed for one period at a time, with food and water always freely accessible in the upper section of the tank. The cages were cleaned thoroughly before each SD period. Upon the completion of treatment, the rats were housed in a simple cage ($50 \times 30 \times 20$) for later evaluation.

2.2. Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was initially conducted on 3- and 14-month-old rats before they were subjected to PSD. The test was performed after 12 h of fasting and an intragastric administration of dextrose (2 g/kg weight). Glucose measurements were obtained at the time of administration (t = 0) at intervals of 30, 60, 90, 120, and 150 min. All glucose readings were obtained from blood samples drawn from the caudal vein. The samples were placed on reactive strips, and the glucose dehydrogenase method (Accu-Chek Performa, Roche, Basel, Switzerland) was used for measurements. The AUC was calculated using Tai's model (Ricart-Jané et al., 2002; Tai, 1994).

2.3. Tissue analyzes

The liver and pancreas of the rats were dissected, washed in saline solution at 4 °C, and homogenized in 600 μL of 0.067 M phosphate buffer (pH 7.8 with a stock protease inhibitor (Roche, Indianapolis, IN). The homogenized tissue was re-suspended three times, incubated for 5 min at 4 °C, and centrifuged at $164 \times g$ for 20 min at 4 °C. The resulting supernatant containing the cytoplasmic protein was separated into small volumes and stored at -70 °C.

2.4. SOD quantification

SOD activity was determined using the method of Winterbourn et al. (1975), which is based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium (NBT) by the superoxide generated by the photoreduction reaction of riboflavin and oxygen. Samples were read at 560 nm and enzyme activity was expressed as U of SOD/mg of protein.

2.5. CAT quantification

CAT enzyme activity was determined using the Beers and Sizer method (1952), which measures the disappearance of hydrogen peroxide (H_2O_2) using catalase. CAT enzyme activity is expressed as mU/mg of protein per min.

2.6. Lipoperoxidation determination

Lipoperoxide levels in the tissues were determined using a commercial kit (Calbiochem, Gibbstown, NJ, USA). To extract the lipoperoxides, 1 g of liver and pancreatic tissues was homogenized with a vortex in 500 μL of deionized, sterile water, 500 μL of the R extract (provided by the supplier), and 1 mL of deoxygenated chloroform. This mixture was centrifuged at $1500 \times g$ for 10 min at 0 °C, and the chloroform fraction with hydroperoxides was collected. Lipoperoxidation produces hydroperoxides, which are measured using reduced reactions with ferrous ions. The presence of lipoperoxides was detected at 500 nm, and the data are presented as nmol of LPO/mg of protein.

2.7. Determination of protein content

Protein extraction was performed by re-suspending the tissue homogenate three times before incubating for 5 min at 4 °C. The homogenate was centrifuged at $8000 \times g$ for 20 min at 4 °C, and the supernatant containing the cytoplasmic proteins was aliquoted into small volumes and stored at -70 °C. Colorimetric determinations were performed in duplicate, and the data were expressed as mg of protein. The protein concentration was determined using the Bradford assay (Bradford, 1976).

2.8. Statistical analyzes

Results are presented as the mean ± standard error of the mean (SEM). An analysis of variance (ANOVA) was performed to detect differences between the distinct PSD and the control groups. To analyze the OGTT data, AUC, enzymatic activity, and lipoperoxidation, a post-hoc Tukey-Kramer test was conducted with $p < 0.05$. All data were analyzed using the NCSS statistical program (Number Cruncher Statistical System).

3. Results

3.1. Effect of PSD on carbohydrate metabolism

Glucose development in the control group of young rats was demonstrated by an increase in the OGTT at 30 and 60 min, followed by a progressive decrease after 150 min. For the group of young rats, the OGTT showed lower glucose levels several times in the 96PSD and 192PSD groups than in the control and PSD + Recovery groups (Figure 1A). Conversely, in adults, only the 96PSD group showed lower glucose levels (Figure 1A). The AUCs of glucose levels also showed differences between the controls and the PSD 96 and 192 h groups (Figure 1B). Finally, the AUCs for the young and adult rats showed differences in the 192PSD and 192PSD + Recovery groups (Figure 1B).

3.2. Effect of PSD on lipoperoxidation levels in the liver and pancreas

Lipoperoxide levels in the liver of young rats in the 192PSD and 192PSD + Recovery groups were higher than those in the controls (Figure 2A). In addition, higher lipoperoxide levels were found in the livers of young rats in the 192PSD and 192PSD + Recovery groups (Figure 2A). In contrast, higher lipoperoxide levels were found in the pancreas of young rats in the 96PSD and 192PSD + Recovery groups than in the control animals (Figure 2B). Finally, the pancreas of the 192PSD

adult group had higher lipoperoxide levels than the corresponding young rats (Figure 2B).

3.3. Effect of PSD on antioxidant enzymatic activity

The enzymatic activity of SOD in the liver of young rats in the 192PSD and 192PSD + Recovery groups was lower than that in the controls (Figure 3A). In addition, SOD enzymatic activity in the liver of all groups of young rats was higher than that in adult animals (Figure 3A). Conversely, the enzymatic activity of SOD in the pancreas of young rats was higher in the 96PSD group than in the control group (Figure 3B). The enzymatic activity of SOD was higher in the pancreas of adult rats in the 192PSD and 192PSD + Recovery groups than in the control animals (Figure 3B). Finally, the enzymatic activity of SOD in the pancreas of all young rat groups was higher than that in the adult rats (Figure 3B).

The enzymatic activity of CAT in the liver of the young rats after 192 h of PSD was lower than that of the controls (Figure 4A), whereas the livers of adult rats subjected to 96 h and 192 h of PSD also showed lower levels of CAT activity than their respective control groups (Figure 4A). In addition, differences between the CAT enzymatic activities in the livers of young and adult rats were found in the control and the 96PSD and 19PSD + Recovery groups (Figure 4A). Conversely, CAT enzymatic activity in the pancreas in the controls and any of the PSD groups did not differ (Figure 4B). However, the pancreases of all adult groups showed higher CAT enzymatic activity than those of the young rats (Figure 4B).

4. Discussion

Several studies have shown that physiological aging can influence the responses of β-pancreatic cells, tissue sensitivity to insulin, and metabolic processes that play a crucial role in the progressive deterioration of all organs and are associated with chronic and metabolic diseases such as obesity, impaired glucose tolerance, and type 2 diabetes (Knutson et al., 2007; Mariño and López-Otín, 2008; Rajawat et al., 2009). In this study, the results of OGTT in the control rats showed the development of glucose with increases at 30 and 60 min, followed by a progressive decrease after 150 min. In young rats, however, PSD induced hypoglycemia after 96 h and 192 h. The adult rats showed adequate development during the OGTT, with hypoglycemia induced by PSD after 96 and 192 h. However, the AUC showed hyperglycemia in adult rats compared with young animals in the 192PSD group, with observations of an inverse effect during the recovery period. Studies on metabolism and glucose regulation under SD conditions in a controlled experimental setting are scarce (Barf et al., 2010; Spiegel et al., 1999), but OGTT and AUC studies performed in our laboratory revealed progressive hypoglycemia after 24, 96, and 192 h of PSD, with reduced insulin concentrations in young rats (Brianza-Padilla et al., 2016). Another study showed that chronic sleep disturbance for 8 days led to hyperglycemia and a concomitant reduction in the insulin response in an intravenous glucose tolerance test (Barf et al., 2010). Meanwhile, Naidoo et al. (2014) found that young rats (10-weeks-old) subjected to chronic SD were sensitized to insulin and showed improved glycemic control, whereas aged animals (22–27 months) became hyperglycemic and failed to maintain appropriate concentrations of plasma insulin. Moreover, short-term SD with nocturnal light exposure altered the secretion of insulin and glucagon-like peptide-1, a peptide determinant of insulin secretion in enteroendocrine L-cells regulated by circadian rhythms in rodents (Gil-Lozano et al., 2016). Thus, the alterations in glucose metabolism caused by the effect of PSD may have been due to modifications in insulin levels, which seemed to depend on the duration of PSD and the ages of the animals.

During aging, the pancreas undergoes morphological and metabolic changes that contribute to the inappropriate regulation of glucose levels. These changes mainly affect the insulin-producing β-cells, with only a few modifications observable in other cell types (Brown, 2012). The pancreatic β-cell mass, which represents the islet mass in rodents,
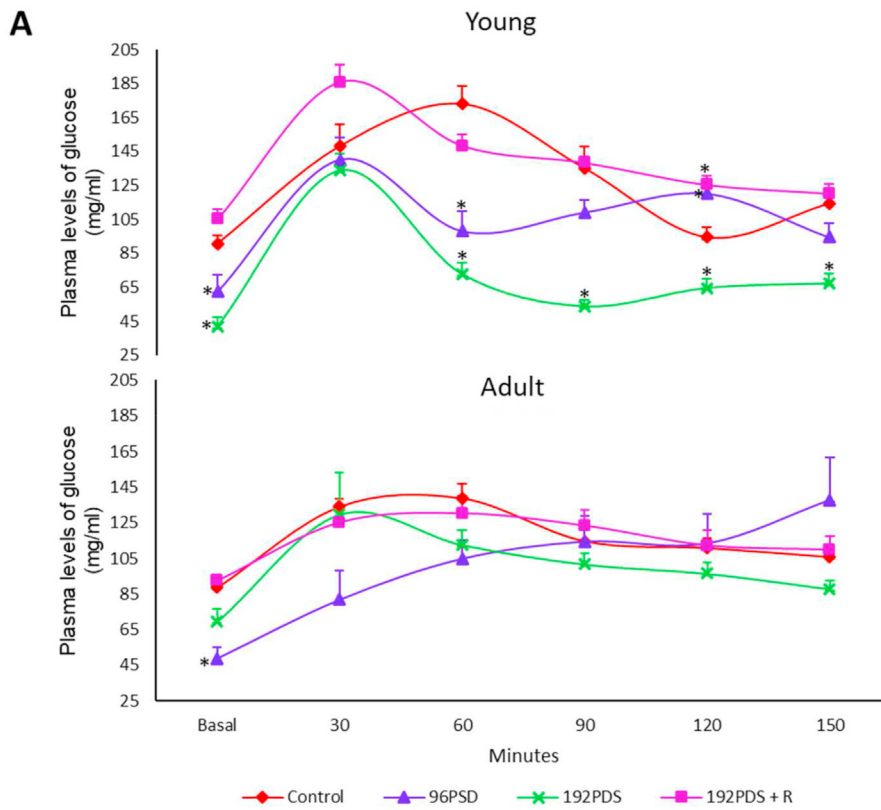
Figure 1. Glucose tolerance test (A) and area under the curve (B) for young and adult rats with different durations of paradoxical sleep deprivation (PSD) and PSD followed by a recovery period. Young rats show hypoglycemia in the 192 and 192PSD + Recovery groups, whereas the adult rats only show baseline hypoglycemia in the 96PSD group. However, the areas under the curve for the young and adult rats show significant differences between the 192 and 192PSD + Recovery groups. Values are presented as mean ± SEM. t-Students. (n = 5). *Significant differences compared with its control. + Significant differences compared with the control in 14-month-old animals.
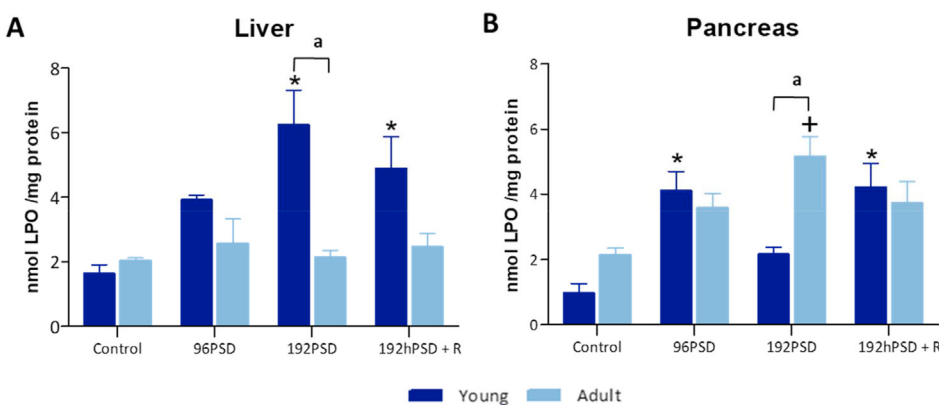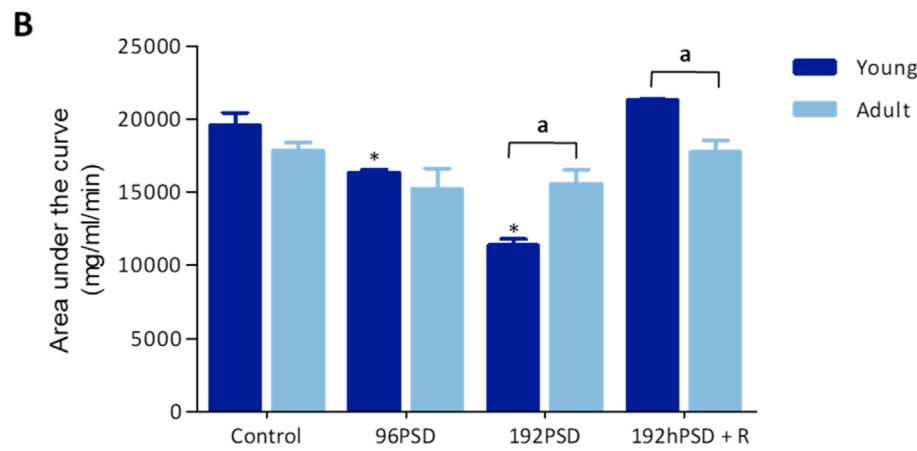

Figure 2. Effect of different periods of paradoxical sleep deprivation (PSD) and PSD + Recovery treatments on the levels of lipoperoxides (LPO) in the liver (A) and pancreas (B) in young and adult rats. The 192 and 192 PSD + R groups show an increase in LPQ in young rats; in the adult rats, the increase is observed in the 96 and 192 PSD + R groups. Values are presented as mean ± SEM. ANOVA followed by Newman Keuls tests (n = 5): * Significant differences compared with the controls in 3-month-old animals. + Significant difference compared with the controls in 14-month-old animals.
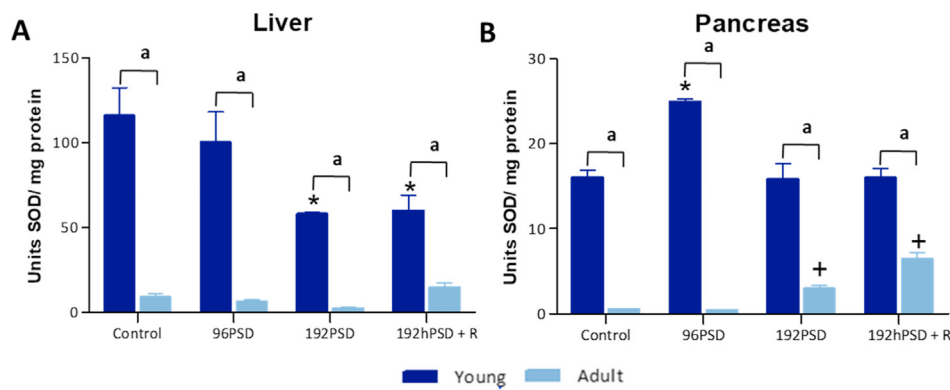
Figure 3. Effect of different periods of paradoxical sleep deprivation (PSD) and PSD + Recovery on the enzymatic activity of superoxide dismutase (SOD) in the liver (A) and pancreas (B) in young and adult rats. PSD for 192 h and 192 h followed by a recovery period increases SOD activity in the liver and pancreas of young and adult rats. Values are presented as mean ± SEM. ANOVA followed by Newman Keuls tests (n = 5): *Significant differences compared with the controls in young animals. +Significant differences compared with the control in adults.
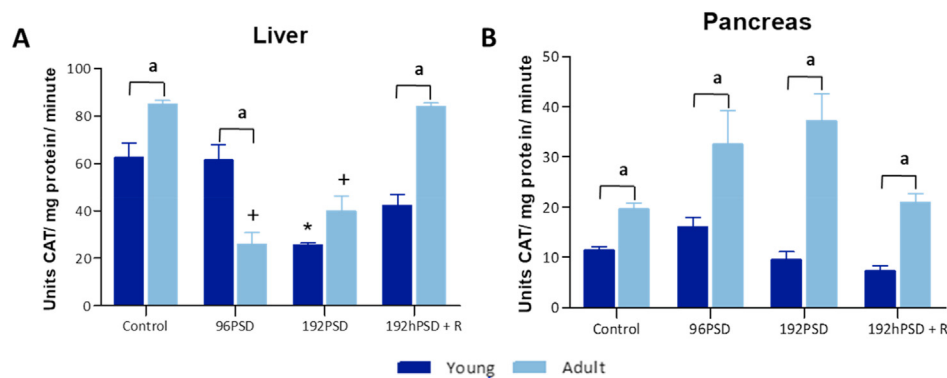

Figure 4. Effect of different periods of paradoxical sleep deprivation (PSD) and PSD + Recovery on the enzymatic activity of catalase (CAT) in the liver (A) and pancreas (B) of young and adult rats. CAT activity only decreases in rats subjected to 192 h of PSD. Values are presented as mean ± SEM. ANOVA followed by the Newman Keuls test (n = 5): *Significant differences compared with the controls in young animals. +Significant differences compared to the control in young rats.

declines with age, and it is induced by an imbalance in β-cell turnover accompanied by an increase in β-cell dysfunction, which together leads to an overall reduction in the functional β-cell mass, which can be aggravated by sleep deprivation (Cerf, 2013; Kushner, 2013; Maedler et al., 2006; Rankin and Kushner, 2010; Swenne, 1983; Teta et al., 2005). Despite the heterogeneity of study designs, partially sleep-deprived subjects have demonstrated impairments in numerous parameters of glucose tolerance and insulin sensitivity (Buxton et al., 2010; Schmid et al., 2011; Spiegel et al., 2004), as well as pancreatic β-cell dysfunction (Buxton et al., 2010) caused by excessive production of reactive oxygen species (Lee et al., 2013), inflammation, and increased cortisol levels (Brianza-Padilla et al., 2016), all of which affect the functionality of organs such as the pancreas (Briançon-Marjollet et al., 2015; Periasamy et al., 2015). In this regard, our results indicate, for the first time, that PSD induces oxidative stress in the pancreas, increasing LPO and SOD activity in young and adult rats. These findings provide additional evidence that sleep abnormalities are causally linked to impairments in glucose homeostasis, metabolic syndrome, insulin resistance, and type 2 diabetes mellitus (Briançon-Marjollet et al., 2015; Gil-Lozano et al., 2016; Periasamy et al., 2015). Several studies have reported differences in oxidative stress after prolonged waking in the liver, spleen, nodose ganglion, heart, brain, and plasma (Babkoff et al., 2005; Periasamy et al., 2015; Reimund, 1994; Villafuerte et al., 2015). Sleep deprivation in animals and obstructive sleep apnea syndrome in humans are associated with increased oxidative stress (Barcelo, 2006; McEwen, 2006). Thus, SD induced by the disc-over-water (DOW) method for 5 and 10 days showed significant decreases in liver GSH and CAT activity (Everson et al., 2005), contrary to the results found by Chang et al. (2008), where liver LPO increased. In the present study, PSD induced oxidative stress and

increased LPO in the liver of young rats, with an increase in SOD activity but a decrease in CAT activity. In adult rats, CAT levels decreased after 96 h and 192 h of PSD. The liver is the body's principal detoxifier, and it eliminates several toxic substances, including ROS. An increase in ROS induces LPO activity. It has been proposed that ROS accumulate during wakefulness, and sleep directly promotes an increase in the antioxidative systems in the liver. Therefore, the intrinsic antioxidative defenses of the liver may be overwhelmed by the increased concentration of ROS induced by PSD. In addition, under SD conditions, an enhancement is prevented and the liver becomes incapable of coping with normal ROS concentrations (Reimund, 1994; Villafuerte et al., 2015), which requires time to re-establish itself when dealing with the stress induced by PSD.

In the present study, LPO activity was maintained in young rats after 20 days of recovery. It is important to mention that although the adult rats did not show an increase in LPO, they seemed to respond efficiently. It is interesting to observe the reductions in SOD activity, in the liver and pancreas, and CAT activity, in the liver of adult animals (14-month-old) and young animals, which suggested a decrease in antioxidant enzymes because of the effect of senescence (Kassem et al., 2017). Oxidative stress increases in the liver during aging, and it may contribute to oxidative damage, which plays an important role in this process (Harman, 1992). SD can exacerbate this effect, considering that adult rats showed a reduction in SOD and CAT activity compared with young rats, which could have repercussions on the response of the antioxidant system.

In the liver, there have been reports that the levels of malondialdehyde, protein carbonyl, and advanced oxidized protein products, following ROS formation, are all higher in the serum and liver tissues of older rats (20 months) than in young rats (3 months) (Bingül et al., 2017), and the concentrations of reactive substances to thiobarbituric

acid (TBARS) increase at 18 and 24 months of age (Mármol et al., 2010). Similar findings reveal that normal aging is associated with a significant decrease in the activities of antioxidant enzymes (SOD, GST, and CAT) in the livers of animals at 10 and 24 months, with an increase in lipid peroxidation in the same organ (Samarghandian et al., 2015). Contrary to our results, where no modifications were observed in lipoperoxidation in the 14-month-old rats, we cannot rule out liver damage due to the reduction in antioxidant activity in adult rats although this has been utilized as an indirect marker of cellular lesions induced by oxidative stress (Samarghandian et al., 2014). Although we did not evaluate the indicators of liver damage, there is evidence from male C57BL/6J mice sleep-deprived for 24, 48, and 72 h that LPO was not altered in the liver, but histology revealed mild-to-moderate liver injuries in the sleep-deprived mice (Periasamy et al., 2015). In addition, Ilan et al. (1992) reported that human male volunteers sleep-deprived for 72 h had increased plasma AST and ALT levels, which are indicators of liver injury; increased levels of oxidative stress combined with insufficient antioxidant activity may result in liver cell lesions (Everson et al., 2005; Lima et al., 2014). Studies in humans and animals suggest that an aged liver has a much higher risk of failing than a young liver (Gagliano et al., 2007). Aged livers have a greater susceptibility to oxidative stress induced by substance abuse (Singh et al., 2002) and sleep deprivation, which can induce damage to the peripheral cell membrane as an early consequence of SD; SD has been associated with advanced morbidity and mortality (Brown and Naidoo, 2010; Everson et al., 2005; Lima et al., 2014; Ramanathan et al., 2002). This effect can also occur in adult rats. However, in adult rats subjected to PSD, mortality reached 50% in the 192PSD and PSD + Recovery groups, unlike in young rats that survived after being subjected to PSD. Therefore, high indices of oxidative stress and insufficient antioxidant activity are associated with injuries to the liver cells (Everson et al., 2005; Lima et al., 2014) and the pancreas (Armstrong et al., 2018; Curran et al., 2000; Tsai et al., 1998).

5. Conclusion

Sleep deprivation induces a differential response that seems to be dependent on its duration, the age of the animals, and the specific tissue analyzed. Thus, adult rats in our study were more susceptible to SD, as it altered the activity of the antioxidant system; this contrasted with the findings in young animals, where the pancreas seemed to be more susceptible to the harmful effects of PSD. Our results show that chronic sleep loss triggers distinct responses; it also demonstrates that oxidative stress in the liver and pancreas are causal effects of sleep deprivation.

Declarations

Author contribution statement

Karina Hernández Santiago: Performed the experiments.
Ana Laura López –López: Performed the experiments.
Fausto Sánchez-Muñoz: Analyzed and interpreted the data.
José Luis Cortés Altamirano: Performed the experiments; Contributed reagents, materials, analysis tools or data.
Alfonso Alfaro-Rodríguez: Contributed reagents, materials, analysis tools or data.
Herlinda Bonilla-Jaime: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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