Effects of Chow and Liquid Diet on Liver Integrity and Antioxidant Defense in Sleep-Deprived Male Rats

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Abstract

The influence of diet composition and availability on liver and antioxidant system function under sleep deprivation (SD) in rats is not completely known. We have previously demonstrated that chow-fed sleep-deprived rats lose weight and present a starvation-like metabolic and hormonal profile, accompanied by hyperphagia. On the other hand, liquid diet attenuates weight loss and the endocrine-metabolic effects associated to SD. It is widely known that energy metabolism is strongly correlated with the production of free radicals and that oxidative cell damage may occur. Our objective was to verify whether the two different diets offered during SD would also affect antioxidant defense system and liver integrity. Male rats were distributed into the following groups: control, sleep-deprived for 96 h by the platform technique (SD-96h) or SD-96h recovered for 24 h (Rebound). Rats were fed with chow pellets (CP) or a liquid diet (LD). Our results show that the CP rats showed changes in antioxidant defense parameters and liver damage markers after SD. However, such changes were attenuated in rats fed the liquid diet. We conclude that the attenuated effect of LD on studied liver damage and antioxidant defense markers lead us to suggest that these changes are at least partially linked to energy deficits induced by SD.

Keywords: Liquid diet; Liver damage markers; Oxidative stress; Sleep deprivation

Abbreviations: SD: Sleep Deprivation; SD-96h : Sleep Deprived for 96 h; CP: Chow Pellet; LD: Liquid Diet; AST: Aspartate-aminotransferase; ALT: Alanine-aminotransferase

Introduction

It is widely known that energy metabolism is strongly correlated with the production of free radicals and that oxidative cell damage may occur by the increased production of oxidative species as well as by the impairment of antioxidant defenses [1,2].

Sleep deprivation (SD) is thought to be a risk factor for several diseases. However, the physical and biochemical changes produced by SD and its health consequences are not completely known. Using an animal model, Rechtschaffen's group characterized the response to long-term SD and found that it includes a progressive increase in both food intake and energy expenditure [3,4]. Likewise, SD induced by the flowerpot or platform technique also increases food intake [5] as well as resting oxygen consumption and the expression of the uncoupling protein-1 gene in brown adipose tissue [6].

The higher energy expenditure characteristic of SD has been related to mitochondrial thermogenesis, which in turn attributed to high expression of the uncoupling protein-2 gene in the liver and muscle tissue of rats deprived of sleep using the disc-over-water technique [7].

Although some evidence exists that hyperphagia could be overestimated by the gnawing behavior [7].

The gnawing behavior that increases during SD [8]. This difference is likely due to the easier access to food and water. Animal care and use procedures were carried out by trained personnel (FELASA Category C) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals. The

Animals, housing conditions and ethical care

Male Wistar rats from a colony maintained by the Department of Psychobiology – UNIFESP were used. These animals were derived from the Charles River Laboratories Inc. (Wilmington, MA, USA) foundation colony. Throughout the experiment, all animals were maintained on a 12:12-h light-dark cycle (lights on at 0700 h) under controlled temperature (21°C- 24°C) conditions and with free access to food and water. Animal care and use procedures were carried out by trained personnel (FELASA Category C) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals. The

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Sleep deprivation procedure

The SD procedure was an adaptation of the classical model for use with rodents. This method consists of placing an animal on top of a narrow platform (6.5 cm in diameter) surrounded by water in a 23x23x35 cm container [for details, see Martins et al. [16]]. All animals were allowed to adapt to the platform for 30 to 40 min for three consecutive days, and SD was initiated at 0800 h after a day of washout. Members of the control group were individually placed in the same container as the sleep-deprived animals, but water was substituted for wood shavings. This substitution was also performed during the recovery period in the rebound group. Previous data from our laboratory indicate that this method results in a complete elimination of paradoxical sleep and a 37% decrease in slow-wave sleep. Moreover, sleep recovery after the 96-h SD protocol is characterized by an increased paradoxical sleep time (+184.7%) accompanied by a reduction in slow-wave sleep (-12.2%) [11].

Experimental procedures

The SD procedure was an adaptation of the classical model for rodents [7,10,11]. Thirty rats were housed individually for 3 days and adapted to sleep deprivation procedures during this period, followed by a washout day. Thereafter, the animals were weighed (362.5 ± 39.8 g) and weight-matched cohorts were distributed into the control, sleep-deprived for 96 h (SD-96 h), and SD-96 h recovered for 24 h (Rebound) groups. The sleep-deprived and Rebound groups were submitted to 96 h of sleep deprivation while the control rats were maintained in isolated home-cages. After 96 h of sleep deprivation, the Rebound group was permitted to sleep for 24 h before sample collection. Chow pellets (25.3% calories as protein, 11.6% as fat, and 63.1% as carbohydrate; 3.485 Kcal/g; Nuvilab-CR1, Colombo, Brazil) were accessible during all experiments. Other thirty rats (379.9 ± 37.6 g) were adapted, weight-matched and assigned to groups as described above but instead of receiving chow pellets, they consumed a liquid diet (20.8% protein, 11.9% fat, and 7.3% carbohydrate; 1.0 Kcal/mL; cat. #F1268; Bio-Serv; French-town, NJ, USA) delivered via feeding tubes (cat. #9011, Bio-Serv; French-town, NJ, USA), accessible during all experiments from wall feeders.

After SD and rebound periods, all animals were euthanized by decapitation between 0700 h and 0930 h for blood and liver sample collection.

Analytical procedures

Blood was collected in tubes containing pre-chilled heparin or no anti-coagulant (Becton Dickinson, New England, UK); the tubes were centrifuged at 4°C for 10 min at 3,000 rpm to extract plasma and serum aliquots, respectively. Liver and blood antioxidant defense markers were assessed as described elsewhere [12,13]. Aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) were measured using colorimetric automated procedures (ADVIA 1650, BAYER Diagnostics Corporation) routinely performed in clinical laboratories. Fibrinogen was determined using an automated blood coagulation system (Dade Behring, New York) that assessed plasma collected in sodium-citrate tubes. Interleukin-6 was determined using a rat-specific ELISA kit (Behring, New York) that assessed plasma collected in sodium-citrate tubes.

Statistical analysis

The results are presented as mean ± SE. Because there was no protocol difference between the sleep-deprived and Rebound groups except for a day of recovery in the Rebound group after 96 h of sleep deprivation, we considered both groups together as one sleep-deprived group to compare the body weight and food intake. Analysis of variance (ANOVA) was followed by the Tukey post-hoc test with the alpha value set at 0.05.

Results

Chow pellet experiment

Sleep deprivation affected serum markers of liver damage, including the enzymes aspartate- and alanine-aminotransferase. Although the difference in AST level did not reach statistical significance [F(2,27)=2.21, p=0.12], the ALT levels were increased [F(2,27)=13.59, p<0.001] in the SD-96h and Rebound groups (Table 1). Hepatic fibrinogen [F(2,27)=6.87, p<0.01] was increased in the SD-96h group, although the levels of the pro-inflammatory cytokine interleukin-6 were decreased [F(2,27)=6.40, p<0.01] in the SD-96h and the Rebound groups (Table 1).

Blood antioxidant defense markers revealed no changes in catalase [F(2,27)=1.10, p=0.34], glutathione peroxidase [F(2,27)=1.57, p=0.22] and superoxide dismutase [F(2,27)=0.66, p=0.52] activities of red blood cells to all groups. However, these cells had a reduced concentration of total glutathione [F(2,27)=5.16, p<0.05] in the SD-96h and Rebound groups (Table 2). Reduced antioxidant defenses were observed in the liver in terms of lower catalase activity [F(2,27)=11.27, p<0.001] and total glutathione concentration [F(2,27)=12.18, p<0.001]. Hepatic glutathione peroxidase [F(2,27)=0.81, p=0.45] and superoxide dismutase [F(2,27)=0.30, p=0.73] activities were not changed by SD (Table 3). Correlation analyses indicated that the changes in antioxidant defenses were associated with changes in antioxidant enzymes in liver. The antioxidant enzymes activities were not affected by SD in red blood cells (Table 3). Thus, the SD procedure was an adaptation of the classical model for use with rodents.

**Table 1:** Blood liver damage markers and inflammatory response in the control, sleep-deprived for 96 h (SD-96 h) and recovery (Rebound) rats fed with regular chow pellets.

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<th>Parameters</th>
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<th>SD-96 h</th>
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<td>Total Glutathione (umol/g Hb)</td>
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<td>Superoxide Dismutase (U/g Hb)</td>
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**Table 2:** Blood oxidative stress markers in the control, sleep-deprived for 96 h (SD-96h) and recovery (Rebound) rats fed with regular chow pellets.

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**Table 3:** Liver oxidative stress markers in the control, sleep-deprived for 96 h (SD-96h) and recovery (Rebound) rats fed with regular chow pellets.

**Table 4:** Parameters of liver oxidative stress markers.

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### Inflammation produced by 96 h of sleep deprivation

Because of its location in the circulatory system, the liver functions as a center of metabolism and a center of defense. Therefore, it is not surprising that both functions were disturbed in association with the energy deficit induced by SD. The liver is a major source of acute phase proteins whose secretion is driven by inflammatory cytokines released in response to tissue damage, infection and stress. Some studies in humans have found increased levels of interleukin-6 after sleep restriction or deprivation [22,23]. However, it was observed that levels of interleukin-6 were decreased after 40 h of SD [24]. In our experiments, we found that chow-fed rats presented increased levels of fibrinogen after 96 h of SD (Table 1). Moreover, the increased levels of fibrinogen and the decreased levels of interleukin-6, which is the most potent and broadly effective stimulant of acute-phase proteins [25], suggest that another metabolic factor may account for higher acute protein levels during SD. Previous reports suggest that the metabolic, endocrine and nutritional statuses can influence the blood levels of acute phase response and inflammatory markers.

### Obesity association

Obesity is associated with an increased inflammatory response and high levels of pro-inflammatory cytokines and acute phase proteins, and weight loss programs reduce the levels of interleukin-6, among others [26]. It has already been shown that fat tissues are an important source and contribute approximately one-third of the total circulating amount of interleukin-6 [27]. Accordingly, animal models have also shown that weight loss associated with moderate stress reduces the peripheral expression of interleukin-6 in mice [28]. Similar to our chow-fed sleep-deprived rats, a previous study found decreased circulating levels of tumor necrosis factor-α, which is another pro-inflammatory cytokine, after 96 h of SD using the platform technique [21]. Nevertheless, the stimulation of acute-phase protein synthesis requires other mediators because the infusion of catabolic hormones increases hepatic protein synthesis in vivo, but not in perfused livers [29]. Raj and colleagues [30] showed that amino acid infusion during hemodialysis increased the fractional synthesis rate of fibrinogen significantly more than without amino acid infusion. Therefore, because such changes to the fibrinogen and interleukin-6 levels were avoided in the liquid-fed sleep-deprived rats (Table 4), the increased production of acute phase proteins in the SD chow-fed rats might be the consequence of increased liver amino acid availability. This may be the result of increased proteolysis, as shown by the large weight loss and carcass protein content [21].

### Repercussions in the antioxidant system after 96 h of sleep deprivation

There was a decrease in erythrocyte total glutathione after 96 h of SD, and this level remained low after recovery (Table 2). Furthermore, although liver total glutathione was decreased in the SD-96h and Rebound groups, we found no changes in the cytosolic glutathione peroxidase and superoxide dismutase activities in the chow-fed rats (Table 3).

Our group was the first to show that SD decreases total glutathione concentrations in the rat hypothalamus and thalamus without changes in antioxidant enzymes [31,32]. Thereafter, other researchers showed...
decreased activity of the antioxidant enzyme superoxide dismutase in the hippocampus and brainstem [33] as well as reduced liver total glutathione and catalase activity in the livers of sleep-deprived rats [1].

These data suggest that rather than inducing oxidative damage, SD suppresses the antioxidant defense system, which is strongly linked to weight loss. Reduced intakes of vitamins and amino acids from food can affect both enzymatic and non-enzymatic components of the antioxidant system [34]. This hypothesis is also partially supported by our observations that liquid diet-fed rats showed no changes in antioxidant defense markers (Table 4).

Conclusion

This study is the first to demonstrate that LD attenuates alterations in liver integrity and some antioxidant defense markers leading us to suggest that LD favored food intake, probably by inhibiting the gnawing behavior, consequently lessening the energy deficit induced by SD.

Acknowledgements

This work was supported in part by grants from FAPESP, Brazil (CEPID #98/14303-3) and AFIP. Brazil. P.J.F. Martins, S. Tuftk, and V. D’Almeida are recipients of fellowships from CNPq, Brazil. We also thank CNPq for our technical staff fellowships (Proc. # 501248/2005-6, 500256/2008-0 and 501343/2010-5).

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