Paradoxical Sleep Deprivation and Sleep Recovery: Effects on the Hypothalamic–Pituitary–Adrenal Axis Activity, Energy Balance and Body Composition of Rats

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Sleep deprivation and/or fragmentation are common features of several pathologies, including those directly related to sleep

- insomnia
- obstructive sleep apnoea

Although several theories have been proposed, the physiological and behavioural implications of sleep loss remain unclear

Animal models

- short-term sleep deprivation
- long-term sleep deprivation
  - disk-over-water method
    - induce total sleep deprivation or paradoxical sleep deprivation
  - flower pot method
    - Produce selective suppression of paradoxical sleep (PS) and a significant reduction of slow wave sleep

common outcome of sleep deprivation

indicating

- sleep deprivation induces augmented energy expenditure
  (Everson et al., 1995; Erol, 1997; Coenen, 1985; Kash, Bergmann, & Rechtschaffen, 1989; Everson, Bergmann, & Rechtschaffen, 1994; Brock et al., 1994; Suchecki & Tufik, 2003)

Studies in human beings have shown that metabolic alterations can also occur in sleep pathologies

- Insomniac patients
  - metabolic rate (measured by maximum oxygen use) (Bonnet & Arand, 1996)
- Sleep apnoea patients
  - levels of anabolic hormones
    - growth hormone (GH) & testosterone
  - energy expenditure (Grunstein, 1996)
- Sleep-deprived volunteers (72 h)
  - level of urea
    - suggesting protein catabolism & gluconeogenesis (Kant et al., 1984)

total or PS deprivation

- ↑ food intake
- ↓ body weight
- without nutritional waste or changes in intermediary metabolism
  - accelerated use of some nutrients
    (Everson, Bergmann & Rechtschaffen, 1989; Brock et al., 1994)
- fat or sucrose diet supplement do not prevent these changes (Everson & Wehr, 1993; Suchecki, Antunes & Tufik, 2003)
Pilcher et al. (1990) demonstrated that the sympathetic nervous system plays a pivotal role in energy expenditure. Guanethidine-induced blockade of noradrenaline release produces catecholamine plasma adrenaline concentrations. Guanethidine-treated sleep-deprived rats show elevated energy expenditure, suggesting a shift from noradrenaline to adrenaline participating in this metabolic phenomenon.

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Chronically sleep-deprived rats

- Anabolic hormones
  - GH & prolactin
  - Thyroid hormones
  - Testosterone
  - Leptin
- Catabolic hormones
  - Adrenocorticotropic hormone (ACTH)
  - Corticosterone
- Resting oxygen consumption

PS-deprived rats

Leptin suggests fat tissue

Prolonged elevation of corticosterone suggests proteolysis

Moreover, because control (yoked) rats in these experiments are also sleep-deprived to some extent, the differences in energy expenditure between the groups, although significant, may not reflect the real magnitude of the changes in plasma concentrations of some hormones, such as ACTH, corticosterone, and adrenaline, which are augmented in both yoked and sleep-deprived groups above baseline.

The present study aimed to examine whether changes in energy expenditure and body composition, possible mediators (insulin, ACTH, corticosterone), can take place during shorter periods of sleep deprivation. Whether sleep recovery might reverse possible changes in energy expenditure and body composition.

Materials and methods
3 month-old male Wistar rats

The animal facility of the Department of Psychobiology

- N = 39
- T 21 ± 2°C
- 12:12 h light/dark cycle (lights on at 07:00 h)
- access to rat chow and tap water

paradoxical Sleep deprivation (PSD)

All procedures and methods with the animals were carried in accordance with protocols that were approved by the Ethical Committee of the Universidade Federal de Sao Paulo (UNIFESP-EPM) and are in line with the NIH guidelines for care and use of animals.

Control rats

Water chamber 22 x 22 x 35 cm

Ø 7 cm

This method is based on the loss of muscle tonus that occurs during PS.

paradoxical Sleep deprivation (PSD)

- single platform technique

Water chamber

Ø 7 cm

22 x 22 x 35 cm

Control and PSD rats were kept inside the chambers for 4 days and had free access to rat chow and water.

- All rats were habituated to their experimental environment for 1 h per day on the 2 days preceding the onset of the experiment.

- Control rats

- Water chamber

Ø 7 cm

22 x 22 x 35 cm

Control and PSD rats were kept inside the chambers for 4 days and had free access to rat chow and water.

Frequent analysis of the energy content of stock diet

- caloric density = 17.03 ± 0.52 kJ/g

- Energy intake (kJ) = amount of chow ingested (g) x energy content of the diet (17.03 kJ)

- Absorbed energy (kJ) = energy intake - energy in the faeces

- Metabolisable energy intake (kJ) = 96% of the absorbed energy

- Body energy gain (kJ) = energy in the carcasses - initial body energy
### Statistical analysis

- Fat content
  - chloroform–methanol method
- Protein content
  - Lowry method
- Glycaemia
  - enzymatic colourimetric method
- Insulin
  - radioimmunoassay

### Results

- No differences in metabolisable energy and glycaemia were found between groups or periods (data not shown)
**Fig 1 A** The results of body weight gain

- **Body weight gain (%)**
  - Mean ± SEM of 10 animals/group
  - * Different from control rat
  - † Different from rats in the recovery period

**Fig 1 B** The results of food intake

- **Food intake (g)**
  - Mean ± SEM of 10 animals/group
  - * Different from control rat
  - † Different from rats in the recovery period

**Fig. 2**

- **energy gain** = energy in the carcasses - initial body energy
- **energy expenditure** = metabolisable energy intake - body energy gain
- **Gross food efficiency** = (energy gain - 100) metabolisable energy intake

**Fig. 3** The results of body composition

- **Water content**
- **Fat content**
- **Protein content**

**Fig. 4** The results of hormone plasma concentrations

- **Insulin**
- **adrenocorticotropic (ACTH)**
- **Corticosterone**

**PS-deprived rats**

- Body weight gain
- Energy gain
- Gross food efficiency
- Fat content
- Plasma insulin
- Food intake
- Energy expenditure
- Water content
- Protein content
- Plasma ACTH
- Plasma corticosterone
**Discussion**

**The effects of sleep deprivation on the metabolic profile of rats**

- body weight
  - reduction of fat (~37%) > protein content

- absence of body fat at the necropsy
  (Everson & Bergmann, 1989)

- PS deprivation produces a reduction of fat tissue, which is not reversed by a supplement with fat or fish oil
  (Papakonstantinou, Ryan & Harrys, 2003)

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**In rats which were sleep deprived for very long periods**

- leptin concentrations
  (Everson & Crowley WR, 2004; Patchev, Felszeghy & Koranyi L, 1991)

During deprivation & recovery periods

- corticosterone concentrations

**Purpose**

- provide readily usable energy
  (i.e. glucose to the central nervous system)

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**PS-deprived rats**

- protein content in the carcass

Indicates

- proteolysis did not take place during the 4-day period of manipulation

- Although highly speculative
  one possibility is that once the animals are prevented from entering paradoxical sleep
  maintain a sustained muscle tonus
  contributing in part to the increased protein content in the carcass

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**plasma corticosterone concentrations** remained somewhat elevated even after 4 days of sleep recovery

- explain the maintenance of altered body composition

- Similar alterations in body composition are described for cold-acclimated animals, in which fat deposits are mobilised to compensate for cold-induced thermogenesis
  (Luz, Griggo & Vieira, 2003)

Resulting

- fat content
- water percentage

(Le Blanc et al., 1975)
long-term sleep deprivation ➞ energy expenditure
Direct & Indirect method
Means of Oxygen consumption
shorter periods of sleep deprivation ➞ energy expenditure
- 50 %
Body weight
"negative energy balance"

PSP-deprived rats

Gross food efficiency
the amount of body energy gained (or spent for negative energy balance) per unit of metabolisable energy intake
→ confirms the catabolic state of these animals
→ defined as the incorporation of biomass dependent on maintenance requirements (Rothwell & Stock, 1986)

control rats

on the first day of the recovery period

explanation
→ animals were transferred to metabolic cages, which represent a novel environment
→ Although the animals were habituated, they remained inside the novel environments for only 1 h. in the morning, when feeding behavior is not expressive
→ This reduction in food intake might have contributed to the small loss of energy observed during the recovery period

An important confounding factor

stress → effects of sleep deprivation per se
- For example
  - restraint stress
  - footshock
  - emotional stress
  → food intake in both male and female rats
(Papakonstantinou, Ryan & Harrys, 2003; Kuriyama, Shibasaki, 2004)

By contrast
PS deprivation has been reported to either increase or have no effect on food intake, regardless of whether the food is offered as pellets or a liquid diet

PS deprivation

a form of mixed physical/psychological stressor with unique metabolic effects

One other factor that could at least partly explain the effects of PS deprivation on the hormones

a shift in the circadian rhythm of hormone secretion
- sleep deprivation produces a marked effect on the circadian secretion pattern of corticosterone

PS deprivation

...
PS-deprived rats
plasma corticosterone concentrations are increased throughout the whole circadian period

Responses to stress (Hanlon et al., 2005)

Hypothalamic–pituitary–adrenal axis in the morning

PS-deprived rats consumed more chow in the dark than in the light period indicating manipulation does not appear to shift the rhythm

Glucocorticoid actions generally oppose but sometimes synergise with those of insulin

**opposite actions**
- glucose transport
- protein synthesis
- lipogenesis
- fat deposition in adipose tissue

**synergise action**
- stimulating hepatic glycogen deposition
- lipogenesis
- acting as a preparative factor for the next stressful situation

The lack of significant differences in blood glucose between sleep-deprived and control animals due to a synergistic effect between insulin and glucocorticoids towards increased glycogen storage

Thus, 6 h of gentle handling decreases glycogen levels in the cerebellum and hippocampus, but not in the cortex or brain stem (Kong et al., 2002) suggesting a regional variability in metabolic rate or glycogen metabolism

The effects of sleep deprivation appear to be influenced by multiple factors including
- age
- genotype
- brain region
- duration of sleep deprivation

Although we have not measured either peripheral or brain glycogen levels, it has been shown that glycogen levels in brain homogenates decrease as sleep deprivation period increases (6, 12 or 24 h) (Kong et al., 2002)

Further studies indicate an anatomical specificity for short-term sleep deprivation-induced brain glycogen changes.
**sleep deprivation**

Defense mechanism

- catabolic state

- insulin

independent glucose transporter, GLUT1

keep adequate glucose supply to the central nervous system

(Kumagai, 1999)

**total-or paradoxical sleep deprived rats**

- lack of hyperglycaemia, glucose in the urine

- ↑ glucose clearance

indicate → augmented glucose utilisation

(Kushida, Bergmann & Rechtschaffen, 1989)

**recovery sleep**

returns

- energy
- temperature
- hormone measurements to baseline levels (Everson et al., 1989)

paradoxical sleep deprivation induced

- ↑ energy expenditure
- body weight gain
- ↓ energy gain
- gross food efficiency

suggesting

→ 4 days of recovery is an appropriate period for animals to normalize energy balance, but not to reverse the alterations in body composition

**sleep**

The first step for homeostatic regulation

because recovery of sleep precedes those of metabolic and cognitive variables

- These findings appear to be in agreement with Marie Manaceine's original idea that

→ sleep is a physiological process far more important to be fulfilled than any other process, including feeding (Bentivoglio & Grassi-Zucconi, 1997)

- These results are in conflict with human data prolonged sleep restriction

- hyperglycaemia

- slower glucose clearance

- ↑ insulin resistance

- These alterations are concurrent with

- ↑ sympathetic activity

- ↑ plasma cortisol concentrations

(Spiegel, Leproult & Van Cauter, 1999)

- A period of 4 days of recovery was chosen because previous data from our laboratory showed

24 h of sleep rebound

→ sufficient to completely normalise sleep parameters after four days of PS deprivation (Machado et al., 2004)

→ insufficient to completely reverse sleep deprivation-induced learning deficit (Chubela et al., 2005)

Therefore, the compensatory effect (i.e. the rebound effect) is observed for sleep, but not for physiological variables that return during recovery period to near baseline
In summary

- direct measurements of metabolism

Our results showed that in rats, 4 days of paradoxical sleep deprivation results in:

- metabolic rate
- food intake
- body weight

paradoxical sleep deprivation

- weight loss
due to reduction of fat and not protein content.

- normalisation in parameters related to energy expenditure, but not in those related to body composition and plasma corticosterone concentrations

Longer periods of recovery are necessary to replace the fat tissue that is lost during 4 days of PS deprivation.

THANK YOU