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

D. C. Hipolide,* D. Suchecki,* A. Pimentel de Carvalho Pinto, E. Chiconelli Faria, S. Tufik* and J. Luz

Universidade Federal de Sao Paulo, Brazil . University of California, Riverside, USA.

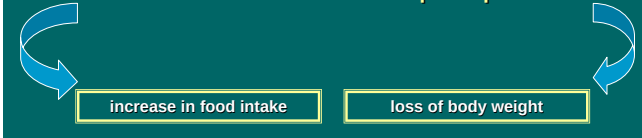
Journal of Neuroendocrinology, 2006, Vol. 18, 231–238

- 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; Elomaa,1979; Coenen& van Luijtelaar,1985; Kushi, Bergmann & Rechtschaffen, 1989; Everson, Bergmann & Rechtschaffen,1989; Brock et al., 1994; Suchecki & Tufik, 2000)

- 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

→ 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
    - ↑ energy expenditure

suggesting a shift from noradrenaline to adrenaline participating in this metabolic phenomenon

7

Chronically sleep-deprived rats

- ↓ anabolic hormones
  - GH & prolactin
  - Thyroid hormones
  - Testosterone
  - leptin
- ↑ catabolic hormones
  - adrenocorticotropic hormone (ACTH)
  - corticosterone
- ↑ resting oxygen consumption

8

PS-deprived rats

↓ Leptin suggest ↓ fat tissue

(Papakonstantinou, Ryn & Harrys, 2003)

prolonged elevation of corticosterone

suggest ⇨ proteolysis

9

- 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
  - plasma concentrations of some hormones, such as
    - ACTH
    - corticosterone
    - adrenaline

which are augmented in both yoked and sleep-deprived groups above baseline

10

The present study aimed to examine

→ whether changes in
- energy expenditure
- 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 & body composition

Materials and methods

12

3 month-old male Wistar rats

The animal facility of the Department of Psychobiology

- T 21 ± 2 °C
- 12 : 12 h light/dark cycle (lights on at 07.00 h)
- access to rat chow and tap water

N = 39

Control

paradoxical Sleep deprivation (PSD)

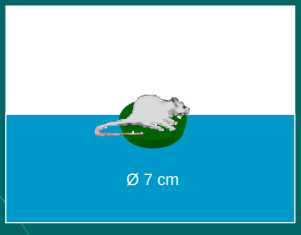
All procedures and methods with the animals were carried in accordance with protocols that were approved by the ethical committee of the Universidade Federal de São Paulo (UNIFESP-EPM) and are in line with the NIH guidelines for care and use of animals

13

paradoxical Sleep deprivation (PSD)

- single platform technique

Water chamber



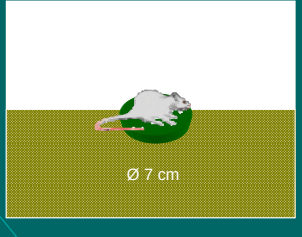
22 x 22 x 35 cm

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

14

Control rats

Water chamber

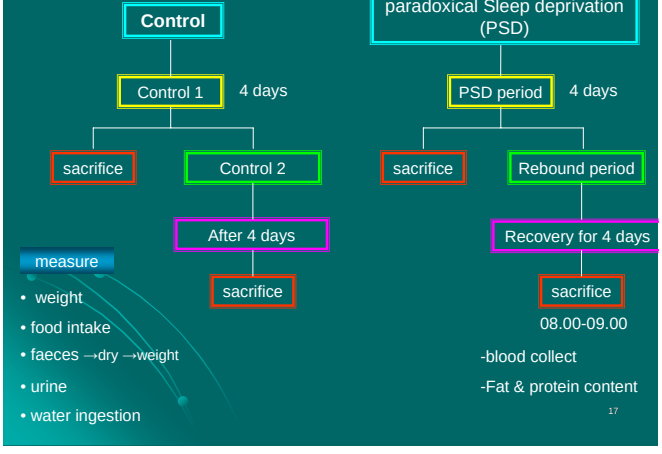


22 x 22 x 35 cm

15

- All rats were habituated to their experimental environment for 1 h per day on the 2 days preceding the onset of the experiment
- Control and PSD rats were kept inside the chambers for 4 days and had free access to rat chow and water

16



17

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) × 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**

18

Energy expenditure (kJ)	metabolisable energy intake − body energy gain
Gross food efficiency (%)	(energy gain / metabolisable energy intake) × 100
Percentage of water in the carcass (%)	(wet − dry weight/wet weight) × 100

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

- **Plasma concentrations of ACTH & corticosterone**
  - **ACTH**
  - →sequential chemiluminescence immunometric method
  - →Monoclonal mouse antibody specific for ACTH
  - **Corticosterone**
  - →double antibody radioimmunoassay method
  - →Commercial kit

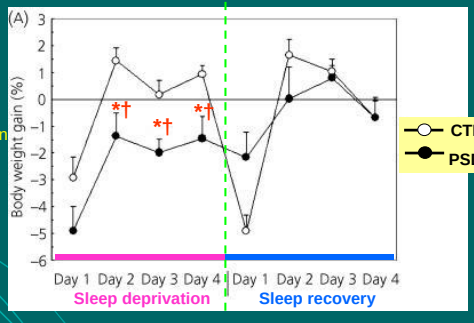
Statistical analysis

- two-way ANOVA
- → main factors group (Control, PSD) and period (deprivation, recovery)
- Three-way ANOVA with repeated measures
- → percentage of weight gain and food intake (days 1–4 of deprivation and days 1–4 of recovery)
- a post-hoc analysis was performed using the Newman–Keuls test
- →In the case of interaction between the factors,
- $P < 0.05$ was considered statistically significant

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

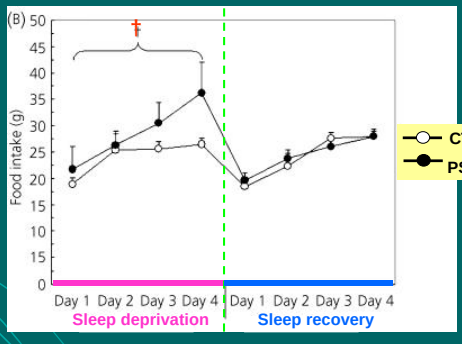


Mean ± SEM of 10 animals/group

* Different from control rat

† Different from rats in the recovery period

Fig 1 B The results of food intake



Mean ± SEM of 10 animals/group

* Different from control rat

† Different from rats in the recovery period

Fig. 2

energy gain

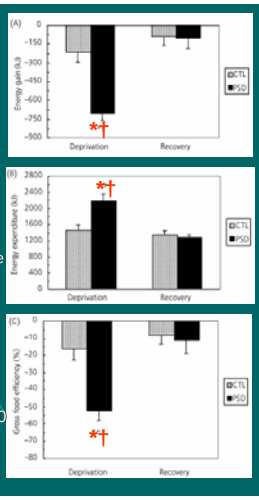
= energy in the carcasses - initial body energy

energy expenditure

= metabolisable energy intake – body energy gain

Gross food efficiency

$= \frac{\text{energy gain}}{\text{metabolisable energy intake}} \times 100$



* Different from control rat

† Different from rats in the recovery period

Mean ± SEM of 10 animals/group

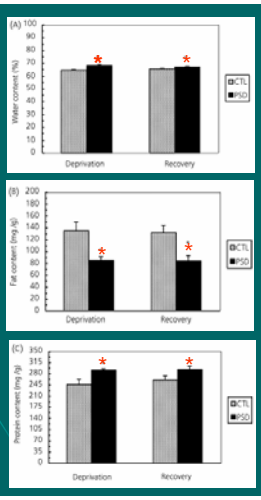
Fig.3

The results of body composition

Water content

Fat content

Protein content



* Different from control rat

Mean ± SEM of 10 animals/group

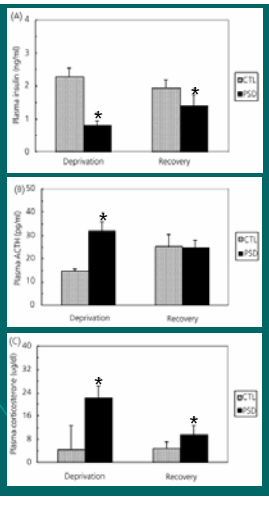
Fig.4

The results of hormone plasma concentrations

Insulin

adrenocorticotroic (ACTH)

Corticosterone



* Different from control rat

Mean ± SEM of 10 animals/group

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)*

In rats which were sleep deprived for very long periods

↓ leptin concentrations

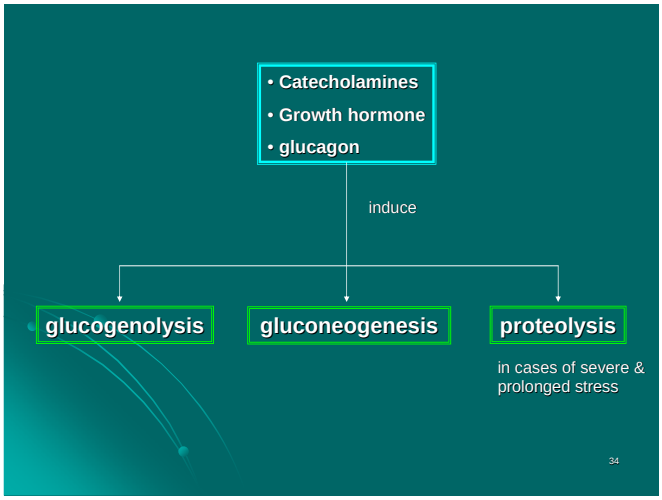
(Everson & Crowley WR,2004; Patchev , Felszeghy & Koranyi L,1991)

during deprivation & recovery periods

↑ corticosterone concentrations

- purpose

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



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

- 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 , Griggio & Vieira,2003)*

resulting

→ ↓ fat content

→ ↑ water percentage

(Le Blanc et al.,1975)

long-term sleep deprivation → ↑energy expenditure

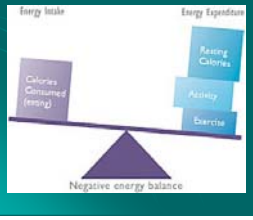
Direct & Indirect method — Means of Oxygen consumption

shorter periods of sleep deprivation → ↑energy expenditure

~ 50 %

↓Body weight

"**negative energy balance**"



PS-deprived rats

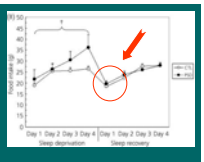
↓ **Gross food efficiency**

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

↓ food intake

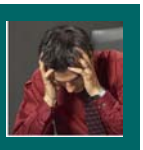
explain

→ 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

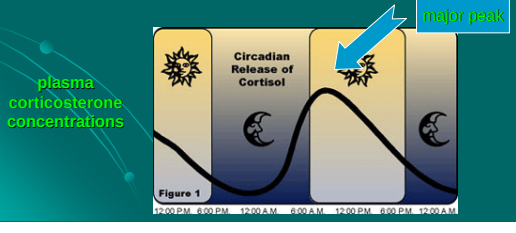
PS deprivation

→ 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

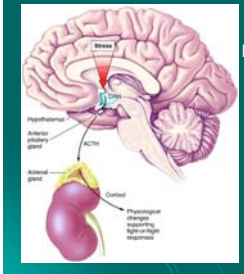
- sleep deprivation produces a marked effect on the circadian secretion pattern of corticosterone



PS-deprived rats

plasma corticosterone concentrations are increased throughout the whole circadian period

(Retana-Márquez et al.,2003)



Hypothalamic–pituitary–adrenal axis

In the morning

↑ Responses to stress *(Hanlon et al.,2005)*

at the time when rats were sampled in the present study

PS-deprived rats

consumed more chow in the dark than in the light period

indicating → manipulation does not appear to shift the rhythm

stressful situation

catecholamines & glucocorticoids

↓

↑ glycaemia

Glucocorticoid actions generally oppose but sometimes synergise with those of insulin

opposite actions

- glucose transport
- protein synthesis
- lipogenesis
- fat deposition in adipose tissue

Insulin ↑

- on blood glucose levels
- on appetite
- gluconeogenesis
- muscle wastage
- lipolysis

Insulin ↓

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

- 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.

- 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

sleep deprivation

catabolic state

Defense mechanism

↓ 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)

50

- 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)

51

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

recovery sleep } normal

suggesting

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

52

- 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 *(Dubiela 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

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-Zuconi ,1997)*

54

In summary

- direct measurements of metabolism

our results showed that in rats,

4 days of paradoxical sleep deprivation results

- ↑ metabolic rate
- ↑ food intake
- ↓ body weight

paradoxical sleep deprivation

weight loss

due to reduction of fat and not protein content.

rebound period

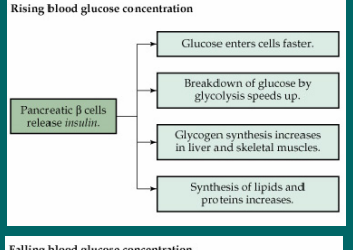
- normalisation in parameters related to energy expenditure, but not in those related to body composition and plasma corticosterone concentrations

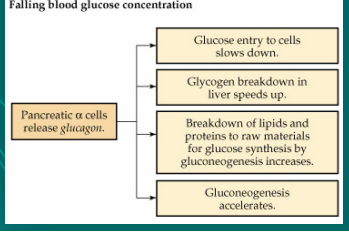
longer periods of recovery are necessary to replace the fat tissue that is lost during 4 days of PS deprivation

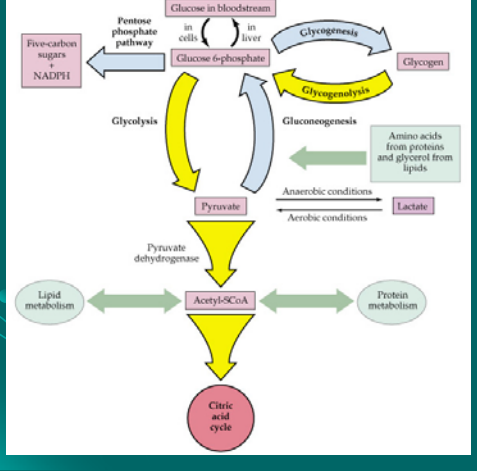
THANK YOU

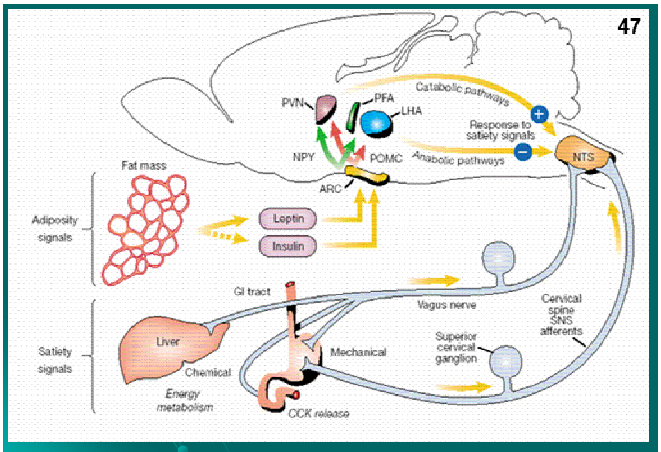
Metabolic Pathways of Glucose

Name	Derivation of Name	Function
Glycolysis (Section 23.3)	*glyco-*, glucose (from Greek, meaning "sweet" *-lysis*, decomposition	Conversion of glucose to pyruvate
Gluconeogenesis (Section 23.11)	*gluco-*, glucose *-neo-*, new *-genesis*, creation	Synthesis of glucose from amino acids, pyruvate, and other noncarbohydrates
Glycogenesis (Section 23.10)	*glyco(gen)-*, glycogen *-genesis*, creation	Synthesis of glycogen from glucose
Glycogenolysis (Section 23.10)	*glycogen-*, glycogen *-lysis*, decomposition	Breakdown of glycogen to glucose
Pentose phosphate pathway (Section 23.12)	*pentose*, a five-carbon sugar *phosphate*	Conversion of glucose to five-carbon sugar phosphates

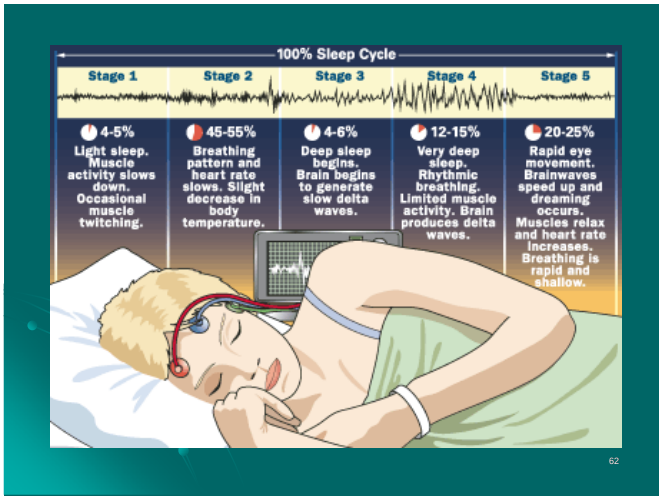




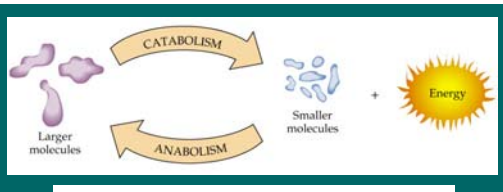


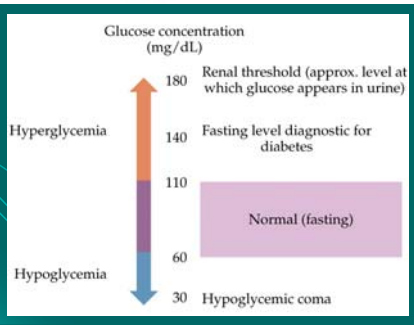


Neuroanatomical model of pathways by which adiposity signals (leptin and insulin) interact with central autonomic circuits regulating meal size 61



62





63