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Tesi di Dottorato

REGULATION OF WAKE-SLEEP STATES AND STATE-DEPENDENT CARDIOVASCULAR FUNCTION IN DIET-INDUCED OBESITY RATS

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Dedication

To the soul of my father sheikh Yasein Aljahmany (Abu Yarub)

To my Damascene poem: my daughter Shaam and my beloved wife Dr.Eng. Dima Alkadri ; my small family, I have been justly questioned by you, whether there would ever be a completion date for this project? Now, that I have come this far. I would like to thank you for being with me all along and in every step; I want to write different words for you, to invent a language for you alone to fit the size of my love.

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LIST OF ABBREVIATIONS

BAT: Brown adipose tissue

BL: Baseline

BMI: Body mass index

BP: Blood pressure

DIO: Diet-induced obesity

EEG: Electroencephalogram

EMG: Electromiogram

diEMG: diaphragmatic EMG

nuEMG: nuchal EMG

EOG: Electrooculogram

HC: Hypercaloric

HR: Heart rate

LD: Light-Dark

NC: Normocaloric

NREMS: non-Rapid Eye Movement sleep

MS: Metabolic syndrome

OP: Obesity prone

OR: Obesity resistant

OSA: Obstructive sleep apnea

R0: Recovery, Day 0

R1: Recovery, Day 1

REMS: Rapid Eye Movement sleep

SD: Sleep deprivation

SEM: Standard error of the mean

SINGLE REMS: REMS occurring in the form of Single episodes

SEQUENTIAL REMS: REMS occurring in the form of Sequential episodes

SWA: Slow-wave activity

Ta: Ambient temperature

Thy: Hypothalamic temperature

WAT: White adipose tissue

W-S: Wake-Sleep

SUMMARY

Obesity often predisposes to coronary heart disease, heart failure, and sudden death. Also, several studies suggest a reciprocal enhancing interaction between obesity and sleep curtailment. Aim of the present study was to go deeper in the understanding of sleep and cardiovascular regulation in an animal model of diet-induced obesity (DIO). According to this, Wake-Sleep (W-S) regulation, and W-S dependent regulation of cardiovascular and metabolic/thermoregulatory function was studied in DIO rats under normal laboratory conditions and during either wake or sleep enhancement, during sleep deprivation and in the following recovery period, respectively.

After 8 months of the delivery of a hypercaloric (HC) diet, treated animals were heavier than those fed a normocaloric (NC) diet (NC: 441 ±17g; HC: 557±17g). HC rats slept more than NC ones during the activity period (Dark) of the normal 12h:12h light-dark (LD) cycle (Wake: 67.3±1.2% and 57.2 ±1.6%; NREM sleep (NREMS): 26.8±1.0% and 34.0±1.4%; REM sleep (REMS): 5.7±0. 6% and 8.6±0.7%; for NC and HC, respectively; $p<0.05$ for all). HC rats were hypertensive throughout the W-S states, as shown by the mean arterial blood pressure values across the 24-h period (Wake: 90.0±5.3 and 97.3±1.3; NREM: 85.1±5.5 and 92.2±1.2; REM: 87.2±4.5 and 96.5±1.1, mmHg for NC and HC, respectively; $p<0.05$ for all). Also, HC rats appeared to be slightly bradycardic compared to NC ones (Wake: 359.8±9.3 and 352.4±7.7; NREM: 332.5±10.1 and 328.9±5.4; REM: 338.5±9.3 and 334.4±5.8; bpm for NC and HC, respectively; $p<0.05$ for Wake). In HC animals, sleep regulation was not apparently altered during the sleep rebound observed in the recovery period following sleep deprivation, although REMS rebound appeared to be quicker in NC animals.

In conclusion, these data indicate that obesity interferes with W-S and cardiovascular regulation and that DIO rats as a suitable model to be used for a better understanding of obesity comorbidities.

EXTENDED SUMMARY

Introduction. The prevalence of obesity has risen dramatically worldwide. Obesity represents one of the most serious public health challenges since it tends to cluster with hypertension, insulin resistance, and dyslipidemia, which are documented risk factors for coronary heart disease and heart failure. In particular, in obese humans, arterial blood pressure (BP) is higher than in lean controls and decreases less than in lean controls on passing from the diurnal activity period to the nocturnal rest period. The consequences of these alterations on cardiovascular function still have to be fully clarified. In humans and animal models, the development of obesity is associated with alterations in the Wake-Sleep (WS) structure. In obese humans, excessive daytime sleepiness is associated with poor quality of sleep at night, partly because of the occurrence of sleep apneas which disrupts sleep. The tight link between sleep and obesity has also been stressed by recent data showing that sleep curtailment and sleep disturbances would lead to weight gain and to the development of insulin resistance and type-2 diabetes.

The circadian distribution and quality of W-S states are major determinants of the circadian arterial BP rhythm because they contribute to differences in BP between activity and rest periods. During non-Rapid Eye Movement sleep (NREMS), arterial BP decreases compared to Wake in humans and rodents, while during Rapid Eye Movement sleep (REMS), an apparent re-patterning of sympathetic activity to cardiovascular effectors causes changes in arterial BP, the direction and magnitude of which are highly sensitive to genetic and pathological factors in different species.

In general, obesity and other metabolic syndrome traits in humans ensue from interactions between polygenic susceptibility and an obesogenic environment. The understanding of the mechanisms of these interactions can be therefore hastened by studying animal models of diet-induced obesity (DIO), which present critical features of obesity in humans. In particular, about 50% of Sprague-Dawley rats fed a hyperlipidic/hypercaloric (HC) diet develops obesity in few weeks with derangements of glucose metabolism and reduced glucose

tolerance, hypercholesterolemia, hypertriglyceridemia, hyperleptinemia, and hypoghrelinemia. However, it is still unclear whether DIO rats develop hypertension. The relationship between obesity and sleep has been studied in several rodent models of obesity. These models develop obesity following the administration of a HC diet or through genetic manipulations aimed at interfering with the leptinergic system. All these studies showed the trend in obese animal to increase the amount of NREMS, while variable results have been seen on REMS and the daily distribution during L or D periods of the W-S states. However, no long-term studies on the effects of DIO on W-S and cardiovascular regulation have been carried out until now in the rat, which is currently the most widely used animal model in experimental biology.

The aim of this research was to study the structure of the W-S cycle and the possible state-dependent changes of brain temperature and cardiovascular function in rats made obese by the chronic administration of a HC diet. This experiment has also been conducted with the aim of identifying possible changes made by the prolonged administration of a HC diet to sleep regulation in response to a previous sleep deprivation. Particular care has been placed in the analysis of the possible modifications of the fine architecture of REMS, whose occurrence is strongly influenced by the degree of activation of thermoregulatory/metabolic processes and is under the control of central nervous structures at hypothalamic level that are known to be also involved in the regulation of body temperature/metabolism and food intake.

Methods. Male Sprague-Dawley rats were divided into two groups: a control group was fed a normocaloric (NC) diet while the other group was fed a hypercaloric (HC) diet (35% fat). Both groups were kept at an ambient temperature (Ta) of 25°C ± 0.5 °C, under a 12:12 h light-dark (LD) cycle, and had free access to food and water. At the 8th week form diet differentiation, 8 NC rats and 8 among the higher weight gained-rat from the HC group underwent surgery. Animals were deeply anaesthetized (diazepam, 5mg/kg, i.m., followed by ketamine-HCl, 100 mg/kg, i.p) and surgically implanted with: i) two epidural

electrodes for electroencephalographic (EEG) recording; ii) two electrodes for nuchal electromiographic (EMG) recording; iii) electrodes for the measurement of the diaphragmatic myoactivity. iv) a hypothalamic thermistor implanted above the left anterior hypothalamus for the determination of hypothalamic temperature (Thy); v) a catheter placed into the femoral artery for the telemetric recording of arterial BP and heart rate (HR).

Animals recovered from surgery for at least one week in a Plexiglas cage within a thermoregulated, sound-attenuated chamber where they were kept throughout the experiment. For both groups, recordings were carried out for four consecutive days: the first and second days were taken as the baseline (BL)**,** during the third day rats were sleep deprived (SD) by gentile handling along the entire 12-h L period and then they were allowed to recover for the entire 12-h D period of the third day (R0) and for the whole following day (fourth day, R1). Sleep stages were scored offline according to standard criteria based on EEG, EMG and Thy signals. Particular care was placed in the detection and the separation of Single and Sequential REMS episodes, since this partition has been shown to be critical in describing the processes underlying REMS regulation. In particular, sequential REMS episodes are those separated by short time intervals (<= 3min) and occurring in rapid sequence, while single REMS episodes are those separated by long time intervals (> 3min). EEG power spectra analysis in the Delta (0.5-4.0 Hz), Theta (5.5-9.0) and Sigma (11.0-16.0 Hz) band for the different wake-sleep states was also carried out offline.

Results and conclusions. After 8 weeks of treatment the weight was higher in the HC group than in the NC group (HC, 557±17g; NC, 441±17g; $p<0.05$). Under BL conditions, the 24-h total sleep amount and the amount of both NREMS and REMS was significantly larger in the HC group compared to the NC one, although for REMS the statistical significance was not reached. The analysis carried out on a 12-h time scale showed that HC animals slept significantly more than the NC animals during the D period, leading to the disappearance of the normal LD distribution of REMS. The analysis of the partition in Single and

Sequential REMS showed that in both group the increase of REMS during the D hours was substantially due to a significant increase in Sequential REMS, confirming that REMS regulation is mostly made through the modulation of Sequential REMS. Major changes were also observed on a 24-h basis on cardiovascular parameters. In particular, HC animals showed to be significantly hypertensive and bradycardic when compared to NC ones. In particular, mean arterial BP was significantly higher in each of the three W-S states in HC than in NC animals. No relevant changes were observed in average Thy levels.

The analysis of W-S parameters during the recovery period which followed sleep deprivation showed that the majority of the expected sleep rebound occurred during R0 in NC animals. This pattern was reproduced in the HC group. The analysis of the partition in Single and Sequential REMS clearly indicated that REMS rebound occurred under the form of Sequential REMS in both NC and HC animals. No substantial differences were observed in the dynamics of the accumulation of NREMS in the two groups, while that if REMS was slightly faster in the NC animals. Also, the dynamics of Delta Power, which is an index of the intensity of NREMS rebound, followed a similar pattern in NC and HC animals. An increase in arterial BP levels was observed during SD in the NC animals in both Wake and NREMS. These values returned to baseline levels in R0 and even to levels lower than those of the baseline during R1 in each of the three W-S states Interestingly, this arterial blood pressure drop during R1 was not present in HC animals. On the contrary, heart rate fell in both groups during R1.

Thus, it may be concluded that the results of the present experiment indicate that in the rat the development of obesity deeply interfere with both W-S and cardiovascular regulation and that diet-induced obesity rats represent a very good model for further studies aimed at going deeper in the understanding of the disturbances in the W-S activity and of the cardiovascular comorbidities which accompany the development of obesity in humans.

1. Introduction

OBESITY AND OVERWEIGHT

Obesity is now so common within the world's population that it is starting to replace undernutrition and infectious diseases as the most significant contributor to ill health. Either independently or in association with other diseases it causes or exacerbates many health problems, (Kopelman, 1994). The generally accepted view is that being overweight causes, to a lesser degree, similar health problems to obesity.

Obesity and overweight are considered the most important risk factors beside high blood pressure, high concentrations of cholesterol in the blood, inadequate intake of fruit and vegetables, physical inactivity and tobacco use which cause what is known as noncommunicable diseases. The Build and Blood Pressure Study pointed out that the side effects of overweight tend to be delayed, sometimes longer than ten years (Society of Actuaries, 1980). Obesity is established well now as an independent risk factor for type 2 diabetes, dyslipidemia, and cardiovascular diseases (CVD) (Bastard *et al*., 2006). According to the World Health Organization (World Health Organization, WHO) in the data published in 2003 by the WHO as part of the "Global Strategy on Diet, Physical Activity and Health", the worldwide burden of noncommunicable diseases has rapidly increased. Noncommunicable diseases caused in 2001 almost 60% of the 56 million deaths annually and 47% of the global burden of disease. On the light of these figures and the predicted growth of the figures in the future of disease burden which are expected to reach to 73% and 60%, respectively, by 2020 a major challenge arises to the global public health in order to prevent this risk. Among the causative factors of the major noncommunicable Diseases, namely; cardiovascular disease, type 2 diabetes, coronary heart disease (CHD), an increased incidence of certain forms of cancer, respiratory complications (obstructive sleep apnoea, OSA) and osteoarthritis of large and small joints, unhealthy diets and physical inactivity are considered key determinants and principally contribute to the worldwide burden of disease, disability and death. It

was noticed that in developing countries, and even in low-income groups in richer countries, the prevalence of overweight and obesity is increasing.

1.1.1 DEFINITION

The concept of body mass index (BMI, Body Mass Index, BMI = weight (kg) / height (m) squared high) which is used as a measure of the weight gain, was firstly introduced by the Belgian scientist Adolphe Quetelet (Eknoyan, 2008) in 1832 until 1972. It is assumed that it took the present name from the suggestion of Ancel Keys. It allows meaningful comparisons of weight status within and between populations and the identification of individuals and groups at risk of morbidity and mortality.

1.1.2 OVERWEIGHT vs. OBESITY

On this basis, it is conventionally considered overweight individuals who have a BMI greater than 25 kg/m2 and obese those who have a BMI greater than 30 kg/m2 but this does not take into account the morbidity and mortality associated with more modest degrees of overweight, nor the detrimental effect of intra-abdominal fat. In the context of this work, obesity is considered synonymous with pathological weight gain. By this definition, Najjar *et al*. (1987) mentioned that over 50% of adults' populations in the United States are overweight.

Obesity cases have tripled since 1980 in the WHO European basin which consist of 53 countries (WHO, 2007, 2009), (currently overweight varies in different countries of the WHO European region, between a minimum of around 30% and maximum around 80% of the adult population, it is estimated that of these individuals, at least 30% is obese) and are continuing alarming increase in youth. The trend of overweight in the WHO European region has shown an average increase of 0.1 percentage points during the '70s, by 0.4 percentage points in the '80s, by 0.8 percentage points in the early 90s and, in some countries, by 2

percentage points in the year 2000. The Task Force on International Obesity predicts that about 38% of school-age children in the European region of WHO will be overweight by 2010 and that more than a quarter of them will be obese. This alarmed recall from WHO is justified by the expectation that individuals with overweight in childhood redirected much more easily to the adult obesity (Barker, 2006; Venn *et al*., 2007).

The issue of overweight moved from the individual to the more purely social by the aid of the systematic approach to the problem. In fact, the use of sophisticated tools of analysis has led us to consider the association between overweight with other diseases not only as an expression of co-morbidity, but also as one of the major risk factors for the health of the population. Furthermore, the continuous refinement analysis allows now to follow not only the effects of changes in individual income, but also those of other health interventions. E.g. overweight is considered as an important risk factor not only in the developed regions, but also in the underdeveloped and, in particular, in those of the population that show a level of mortality which could be reduced mostly by the improvement of the state of general nutrition and treatment of infectious diseases (Ezzati *et al*., 2002).

In this context it should be emphasized the fact that the weight gain and, particularly, obesity, are characterized by a practically absolute co-morbidity with diabetes type 2 and more relative, but still significant, with the type of hypertensive cardiovascular disease and thrombus-embolic (same reference).

1.1.3 OBESITY AND LIFE EXPECTANCY

The International Bank for Reconstruction and Development (The World Bank) in 1990 has further developed the index of life expectancy by introducing the index DALY (disability-adjusted life years), also generically assignable to each individual (The World Bank, 1993). It is a measure of overall disease burden, expressed as the number of years lost due to ill-health, disability or early death. On other words, the DALY is the sum of years of life lost to premature

death or disability occurred due to illness or accident, the calculation of the index requires you to weigh the degree of disability and to define the average duration of the disease.

The weight gain, pathological or less, represents a well defined risk factor as it is possible easily and accurately evaluates its distribution in a particular population. Overweight and obesity are the fifth risk factor for death worldwide (the third in the middle and high-income countries) and the tenth in generating DALY (the fourth in middle-income countries and the third in high-income countries). However, when you take into account the food intake as the fundamental cause of overweight and obesity are associated with this and other risk factors correlated to dietary imbalances (hypertension, hyperglycemia, reduced physical activity, hypercholesterolemia, low intake of fruits and vegetables), you reach a set which is the highest cause of death and generation of DALYs. Fontaine *et al*.(2003) reported that even fifty years ago, overweight and especially obese men and women taking out insurance policies were known die earlier than the lean one. Other studies had mentioned that obesity shortens life expectancy by 7 years at the age of 40 years (Peeters *et al*., 2003). The positive relation between the increase in BMI and the increase in risk of death declines progressively with age but remains substantial until the age-group of 75 years (Stevens *et al*., 1998). Thus, the UK government now estimates that English man with BMI of 25·0 kg/m2 has less life expectancy by 2 years and the expected value would reach 5 years by 2050 (Haslam and James , 2005). In USA, each year, an estimated 300,000 US adults die of causes related to obesity (Allison *et al*., 1999). The rapid growth in the number of overweight and obese individuals found in the world has been defined by WHO as epidemic (obesity Epidemics), a term introduced in 1999 in an analysis regarding the dissemination of overweight in the United States from 1991 to 1998 (Mokdad *et al*., 1999).

In this context, this consideration of the obesity and overweight as disease cases create an opinion that the treatment of the obesity and overweight could be covered by the health insurance.

The movement in this direction was implemented in a country with high economic development such as the USA and was indirectly imprinted by the simultaneous discovery, by two different research groups, the existence of a peptides neurotransmitter (De Lecea *et al*., 1998) exclusively localized in neurons of the lateral hypothalamus (Peyron *et al*., 1998) and acting on the control of food intake (Sakurai *et al*., 1998). These neuropeptides, hypocretin (HCRT) 1 and 2, also called orexin 1 and 2 appointed that the main effect was obtained following its administration, which was to increase food intake. Few years after their discovery, it was shown that HCRT neurons degenerate in patients suffering from Narcolepsy (Peyron *et al*., 2000) Narcolepsy with cataplexy (NC) is an hypersomnia of central origin characterized by loss of clear boundaries between sleep and wakefulness leading to severe sleepiness. NC patients can quickly enter in REM sleep at any time of the day and also experience REM sleep like episodes intruding into wakefulness, such as loss of muscle tone triggered by emotions while awake (cataplexy). This suggest the existence of a strong link in the neural processes underlying wake-sleep regulation and the regulation of food-intake (Sakurai *et al*., 2011).

1.1.4. HUMORAL FACTORS INVOLVED IN THE INTERACTION BETWEEN OBESITY AND CARDIOVASCULAR (DYS) FUNCTION

There is now clear evidence that for a given adiposity, a large heterogeneity in the metabolic and cardiovascular risk mainly linked to the distribution and position of excessive adipose tissue. While central or visceral accumulation of fat is an important predictive factor of lipid, glucose or atherogenic disturbances, the peripheral one is not associated with increased alterations at the metabolic level.

A special attention should be paid to the relationship between fat cells and the immune system due to the strikingly and strong discovery which pointed out the association between obesity with a low-grade inflammation process in adipose tissue. In animal models (rodents), obesity is associated with a chronic

inflammatory reaction, which characterized by activation of some pro-inflammatory signaling pathways and abnormal adipokine production which in turn result in occurrence of a cascade of several biological markers of inflammation (Bastard *et al*., 2002). Nowadays, any protein that can be synthesized and secreted by adipocytes can be given the name adipokine (Trayhurn and Wood, 2004). In contrary, these biological parameters are diminished or at least normalized by the loss of BMI. Several animal models supported the idea of a causal relationship between these inflammatory processes with obesity and its co-morbidities such as insulin resistance, type-2 diabetes and CVD.

Leptin, is the product of the ob gene. It is involvement in regulation of energy homeostasis is documented by several authors e.g. (Zhang *et al*., 1994). It is almost exclusively expressed and produced by white adipose tissue (WAT), in particular, mature adipocytes. In obesity, the circulating levels and adipose tissue mRNA expression of leptin show significant correlation with BMI and fat mass. There is a relationship between leptin and the low-grade inflammatory state in obesity despite the fact that the main action centre of leptin is at the central nervous system (CNS) in regulate food intake and energy expenditure which created a suggestion that leptin could have peripheral biological effects due to its cytokine-like structure. Leptin is caopable to regulate tumor necrosis factor-α (TNF-α) production and activation by macrophages (Loffreda *et al*., 1998)

TNF-α is a pro-inflammatory cytokine produced mainly by lymphocytes and macrophages however, a variety of cell-types could produce it also. Adipose tissue can produce TNF-α although in human, this production is weak. It is thought that TNF-α plays a major role in the pathophysiology of insulin resistance in rodents via the phosphorylation of the insulin receptor substrate-1 (IRS-1) protein on serine residues (Hotamisligil *et al*., 1993). Moreover, plasma TNFa concentrations are significantly elevated in obese animals, and its level is positively correlated with insulin resistance and massive obesity.

Interleukin-6 is multifunctional cytokine acting on many cells and tissues. IL-6 is produced by many cell types (fibroblasts, endothelial cells, monocytes) in

addition to other tissues including fat tissue. It is now well established the increment of IL-6 production by adipose tissue in obesity (Bastard *et al*., 2002). Ali *et al*. (1997) mentioned that in the absence of an acute inflammation, a percentage of 15 to 30 % of circulating IL-6 levels derives from adipose tissue. Furthermore, at the level of the central nervous system, it may induce energy expenditure (including thermogenesis) and inhibit feeding behaviour (Wallenius *et al*., 2002). One of the main effects of IL-6 is the induction of hepatic C-reactive protein (CRP) production and the strong relation between IL-6 levels in adipose tissue with the circulating IL-6 and CRP is well established now (Ridker , 2003). In addition, IL-6 has been recently proposed to play a central role in the link between obesity, inflammation and coronary heart diseases (Yudkin *et al*., 2000).

However the mechanism is a matter of debate, it has now been clearly demonstrated that cytokines such as TNF-α and IL-6 are capable to inhibit insulin action (Rieusset *et al*., 2004). Therefore, the chronic increase in circulating cytokine levels could contribute to insulin resistance in addition to the aggravation of the cardiovascular risk linked to inflammation.

Also, IL-6 and TNFa were highest in the sleep apnea group, which had the highest BMI among the patient groups studied. Interestingly, even in the absence of sleep apnea, obesity is more frequently associated with subjective complaints of fatigue, EDS and nocturnal sleep disturbance. The results obtained by Vgontzas *et al*. (1997) presented that inflammatory cytokines especially, IL-6, might be related with the enhancement of fatigue and sleepiness exhibited by obese subjects.

In recent studies in animals and human models, the positive regulation of IL-6 production was found to be via catecholamines through beta adrenergic receptors (DeRijk *et al*., 1994; Papanicolaou *et al*., 1996). Stimulation of peripheral sympathetic activity in sleep apnea and obesity is known (Landsberg, 1999). Furthermore, circulating IL-6 stimulates the hypothalamic–pituitary–adrenal (HPA) axis, and this stimulation is associated with this activation of which is associated with hypertension, central obesity and insulin resistance. F

An important role of insulin in the relationship between dietary intake and sympathetic nervous system (SNS) activity was reported previously. Insulin mediates glucose uptake in central hypothalamic neurones responsible about the SNS activity in response to dietary intake, this links the hyperinsulinemia of obesity to sympathetic stimulation, the latter exerting a prohypertensive effect mediated by the kidney, the heart, and the vasculature.

The glucose uptake from neurons of ventromedial hypothalamus is decreased during fasting due to small fall in glucose and the larger fall in insulin. This decrease in glucose metabolism suppresses the sympathetic output by an inhibitory pathway between the hypothalamus and brainstem sympathetic centers. In contrast, the small increase in glucose and the greater rise in insulin, either in the presence of insulin resistance or increased carbohydrate intake, facilitates insulin-mediated glucose metabolism in these hypothalamic neurons, which leads to suppression of the inhibitory pathway and disinhibition of chronically active brainstem sympathetic centers and the result is increment in sympathetic outflow (Landsberg, 1999).

1.2 THE WAKE-SLEEP CYCLE

1.2.1 DEFINITION

Sleep is usually defined by sustained quiescence in a species-specific posture whereas the responsiveness to external stimuli is reduced, but the definition regarding mammals sleep requires several additional criteria, such as characteristic changes in electroencephalogram (EEG) and reversibility to wakeful state which distinguish sleep from coma and hypothermic states e.g. hibernation (Zepelin *et al*., 2005).

In the Wake-Sleep cycle (W-S cycle), these two states namely; wake and sleep are considered a consecutive behevioral states that are defined precisely by the recording of electrical potentials produced by the cerebral cortex EEG, the extrinsic muscles of the eye (electrooculogram, EOG) and skeletal muscle

(electromyogram , EMG). The WS cycle is constituted of the wake state in addition to two basic sleep states namely; rapid eye movement sleep (REMS) and non-REM sleep (NREMS).

1.2.2 WAKE AND WAKE ACTIVATING SYSTEM

In the early 1900, many physiologists believed that wakefulness and consciousness were maintained by ongoing sensory inputs to the brain. In 1940 Moruzzi and Magoun suggested that the disappearance of waking parameters is due to the interruption of input of the brainstem's netlike core of neurons, the reticular formation. They showed also that electrical stimulation of the reticular formation, but not the sensory pathways, initiated a long lasting and widespread activation of the cortex accompanied by substitution of the cortical slow waves with fast activity (Moruzzi and Magoun, 1949). Moreover, the lesions induced in the reticular formation, but not the sensory pathways, produced a loss of cortical activation which replaced by cortical slow waves and an immobility state resembled coma. The locations of the most marked lesions were in the oral pontine, midbrain reticular formation, posterior hypothalamus and subthalamus where ascending pathways reach into the forebrain. According to the electrophysiological and neuroanatomical studies, collateral input from, somatic, visceral and special sensory systems is received by the reticular formation to be projected in turn dorsally to the thalamus or ventrally to the basal forebrain. The impulses are then re-projected from thalamus and forebrain to be relayed in widespread manner to the cerebral cortex. This system called the ascending reticular activating system and is considered necessary and sufficient for the behavioral arousal of wakefulness and tonic maintenance of the cortical activation.

The origin of the ascending arousal system is largely from a series of well-defined cell groups with known neurotransmitters (Saper *et al*., 2001). This pathway has two major branches: the first ascending pathway branch to the thalamus activating the crucial part for the transmission of information to the

cortex, namely thalamic relay. The pedunculo-pontine and laterodorsal tegmental nuclei (PPT/LDT) (Hallanger *et al*., 1987), a pair of acetylcholine-producing cell groups is considered as the major upper brainstem source of input to the thalamic relay as well as the thalamic reticular nucleus. During the cortical activation i.e. during Wake and REMS states, PPT/LDT neurones fires most rapidly. In contrast, during NREMS, these neurones are much less active. As the reticular nucleus is positioned between the thalamic-relay nuclei and the cerebral cortex, their input is believed as gating mechanism capable to block transmission between thalamus and cortex which is important for wakefulness McCormick, 1989). The second branch of the ascending arousal system activates neurons in the lateral hypothalamic area and basal forebrain and throughout the cerebral cortex (Jones, 2003). The origin of this pathway is the different monoaminergic nuclei in the upper brainstem and caudal hypothalamus: including serotoninergic dorsal (DR) and median raphe nuclei, the noradrenergic locus coeruleus (LC), dopaminergic ventral periaqueductal grey matter and histaminergic tuberomammillary neurons.
The lateral hypothalamic peptidergic neurons (containing melanin-concentrating hormone (MCH) or orexin/hypocretin), and BF neurons (containing acetylcholine or GABA) serve to augment the input to the cerebral cortex (Saper *et al*., 2005). Posterior hypothalamus has been thought as a waking center due to its role in regulating of the sympathetic division of the autonomic nervous system. Collectively, the essential activating system had to be widened to include, in addition to the reticular formation, the posterior hypothalamus, hypothalamus-subthalamus as well as the basal forebrain. These ventral extrathalamic relay which receive ascending input from the reticular formation to be projected in turn to the cerebral cortex , had shown to be able to keep activation of the cortical activation of the forebrain even in the absence of the signal's input from the brainstem reticular formation and function thus independently as activating system.

1.2.3 SLEEP GENERATING SYSTEM

Between 1940s and 1950s, many physiologists believed in an idea that sleep was a result of a decrease and fatigue in the activity of the reticular activating system. Nonetheless, sleep dimensioned by transections through different areas in the brain. Particularly, total insomnia resulted by transections of the brainstem behind the oral pontine tagmentum. This fact indicated that lower brainstem has important sleep generating system with capacity to antagonize the ascending reticular activating system in the upper brainstem. The clinicians also notice that in clinical cases, the disappearance or diminishment of the slow wave sleep accompanied lesions in the lower pons or medulla. This state was referred to as (alpha coma), which characterized by the predominance of alpha activity on EEG typically to wakefulness, while the subject showed lacking of the behavioral alertness and responsiveness.

The finding that low frequency electrical activation of the medullary reticular formation, especially the dorsal medullary reticular formation and the solitary tract nucleus , in production of cortical slow wave activity in awake animals as well as the lesions of these structures which produce fast activity of the EEG in sleeping animals indicated the presence of neurons that could generate sleep. Solitary tract nucleus receive afferent fiber projections from 9^{th} and 10^{th} cranial nerves, glossopharyngeal and vagus respectively, which transmit input from baroreceptors and chemoreceptors of the abdominal and thoracic viscera. Many of the ascending projections from solitary tract nucleus and dorsal medullary reticular formation reach and terminate in the parabrachial nuclei. These nuclei in turn project rostrally to the thalamus, preoptic areas, hypothalamus, amygdala and orbitofrontal cortex, areas commonly belonging to visceral limbic forebrain. The solitary tract nucleus project lightly forward to all these areas excluding the cortex. This data pointed out that the role of solitary tract nucleus may not only by the inhibition of the reticular activating system but also by an action on the structures of the forebrain which had implicated in the generation of sleep. Studies of Bremer with *cerveau isole* had shown that

forebrain could locate a crucial synchrogenic structures because cortical slow wave activity still appears in absence of the brainstem influence. Applying low frequency electrical stimulations in acute experiments studies of midline thalamus induced a slow cortical activity. Moreover, in chronic studies of thalamic stimulation, this initiated a natural sleep defined by both behavioral and EEG criteria. Such evidences led to consider thalamus as (head ganglion of sleep) (Jones, 2005). This conclusion was also supported from clinical case of (fatal familial insomnia), in which a selective degeneration of thalamic nuclei is associated with it. However, lesion studies showed that although thalamus may be necessary for the production of cortical spindles, it is not necessary for the creation of behavioral and cortical slow waves sleep as clarified by its complete removal (Villablanca *et al*., 1972). Since 1900s, anterior hypothalamus proposed as a center of sleep due to several cases of (encephalitis lethargica) in which the lesions were concentrated in this area and the patients were suffering of insomnia.

This made Von Economo to posit that this area is in opposition to and normally in balance with waking center in posterior hypothalamus. Lesions studies in animals had confirmed the existence of sleep facilitatory regions in preoptic area and anterior hypothalamus. The ventrolateral preoptic area VLPO is a small neuronal core (radius 300 μm) located in the ventral POA, it was found to send outputs to all major brainstem and hypothalamic cell groups participating in the arousal (Sherin *et al*., 1996). The VLPO neurones contain the inhibitory neurotransmitters, galanin and GABA and they are primarily active during sleep (Gaus *et al*., 2002). These VLPO neurons form dense cluster and a more diffuse extended part of the nucleus. The main output to the LC and DR are provided by VLPO extended neurones, which are thought to be important in gating REM sleep (Lu *et al*., 2002). On contrary, the VLPO cluster more heavily innervates the histaminergic neurons, which are important to transitions between arousal and NREMS (John *et al*., 2004). On the other hand, each of the major monoaminergic systems sends afferent inhibitory input to the VLPO. However, VLPO does not have histaminergic receptors, but tuberomammillary neurons contain GABA also, which inhibit VLPO (Saper *et al*., 2005).

POA has beside VLPO, median preoptic nucleus (MnPN) which has sleep active neurones with a majority of MnPN neurons activated during sleep contain GABA as proved by the finding that a majority of its neurones expressing c-Fos-immunoreactivity (IR) during sleep are GABAergic. Similar to VLPO, anatomical tracer studies reveal projections from MnPN to multiple arousal-regulatory systems in the posterior and lateral hypothalamus and the rostral brainstem to promote sleep onset and sleep maintenance by inhibitory modulation of these systems (Szymusiak *et al*., 2007). Furthermore, the electrical stimulation of the basal forebrain and preoptic areas resulted in appearance of drowsiness followed by behavioral and EEG patterns of natural sleep.

Thus, the three areas i.e. preoptic area , hypothalamus, basal forebrain, together with lower brainstem were shown to be clearly important for the sleep generation. Subsequent evidences had shown that although the importance of these structure, but they were not sufficient for slow wave sleep and thus cerebral cortex and basal ganglia could contribute to onset of sleep also.

1.2.4 Non-REM SLEEP

Generally, in mammals the onset of sleep is associated with slowing of EEG activity, a rising of the EEG amplitude and decrease of muscle activity which followed in most species with appearance of the spindles (7 to 14 Hz). A shorthand definition of NREMS is “a relatively in active yet actively regulating brain in a movable body” (Carskadon and Dement, 2009). The alternation between NREM and REMS occurs in human in cyclic fashion. Usually, REMS became longer across the night. Stages 3 and 4 occupy less time as the sleep cycles progress i.e. occupy less time in the second cycle and may disappear in the later cycles and stage 2 expands to occupy the whole NREMS. Approximately, the mean duration of the first NREMS-REMS cycle lies between 70 to 100 minutes, whereas the second and later cycles is approximately 90 to 120 minutes. So, the average NREMS-REMS cycle across the night in normal subjects is 90 to 110 minutes. Slow waves and spindling are the prominent hallmarks of the

mammalian NREMS or quiet sleep. Slow wave activity (SWA; 0.5 to 4 Hz) differs in its peak frequency between different species as it is concentrated at lower frequency in some species as human and rat. NREMS could be further subdivided into light and deep sleep depending on the amount of delta wave activity.

The classical stages of NREMS in humans are four however the new classification has made them three stages by merging the third and fourth stages in one stage. These four stages defined along one measurement, EEG. By passing from the first till the fourth stage, sleep being deeper and awakening is more difficult. Thus, one can consider that these four stages roughly parallel a depth of sleep continuum, with lowest thresholds of awake in stage one and highest in the fourth. Onset of sleep is accompanied with reduce of body temperature (Tb) and the Tb is actively regulated at lower level in sleep than during wakefulness. This idea came after the observations that in napping children, the decline in rectal temperatures coincided with elevated skin temperature as well as increasing of the evaporative water loss. It is commonly observed that in animal studies, the brain temperature (Tbr) falls during NREMS in comparison to the wake state, but in REMS it is higher than NREMS (Parmeggiani, 1980). The change in Tbr can be influenced by the change in blood temperature which perfusing it, change in the metabolism of the cerebrum or changes of the cerebral blood flow. Shivering in NREMS in cool environment occurs in stage 1 and 2 but not during the rest NREMS stage or the whole REMS indicating the marked inhibition of thermoregulatory response during REMS and intact thermoregulatory mechanisms in NREMS. The cause of this is the state dependent changes of the functions of preoptic hypothalamic thermostat neurons.

NREMS is characterized by a down-regulation of cardiovascular activity of variable intensity depending on the species and the previous level in quiet wake state. Blood pressure declines in cat but not in rabbit whereas this decrement is not consistent in rat (Lacombe *et al*., 1988). In human, this decrease in arterial pressure was noticed but to a varying intensity between different individuals with increase in the sensitivity of the baroreceptors. On the other hand, heart rate

shows significant decrease in rat but this was not statistically significant in rabbit. On the whole, cardiovascular changes in NREMS are consistent with the respiratory and thermoregulatory changes in a condition of postural and motor quiescence.

1.2.5 REM SLEEP

REMS was discovered by Aserinsky and Kleitman in 1953. This state of sleep was first identified by most obvious behavior: rapid eye movement during sleep. Most adult mammals' neocortex shows low voltage EEG during this phase while the hippocampus has regular high-voltage theta (4 to 8 Hz) waves throughout REMS. They noticed that EEG pattern during REMS closely resembled that of alert waking and they found that the subjects awakened from this state reported vivid dreams. Jouvet reported this observation and found in addition a loss of muscle tone (atonia) in REMS and called REMS as paradoxical sleep (Siegel, 2009). In addition to the above mentioned patterns of REMS, other classic criteria were also noticed during this type of sleep; erection tends to occur in men and clitoral engorgement in women. The pupil constriction (miosis), reflecting a parasympathetic dominance in the iris control. All these changes which are distinguished throughout REMS have been termed its tonic features.

The brainstem is believed to be the key brain structure for generation of REMS, in particular the pons and the neighboring portions of the midbrain. These areas in addition to hypothalamus contain neuronal cells that are maximally active during REMS and hence it called REMS-on cells and cells that are minimally active during REMS which called REMS-off cells. Subgroups of REMS-on cells use the transmitters; gamma-aminobutyric acid (GABA), acetylcholin, glutamate, or glycine. On the other hand, subgroups of REM-off cells use the transmitters; adrenaline, noradrenaline, seretonine, and histamine. The interaction between these two types of cells controls the key phenomena of REMS. The entire destruction of the areas of pons and midbrain can prevent the occurrence of REMS while the partial damage to portions of brainstem can lead to abnormalities

of certain aspects of REMS especially the loss of muscle tone. Lesions in medulla and pons cause REMS to occur in the animal without atonia. During this abnormality, the animal exhibits during REMS locomotor activities and appear to attack imaginary objects. This syndrome shares with some commonalities in REMS behavior disorders seen in human. In contrary, the activation of REMS controlling areas creates muscle loss in antigravity and respiratory muscles.

Collectively, the dorsal part of pontis oralis (PnO) and caudalis (PnC) nuclei contain the neurons responsible for REMS onset i.e. REMS-on neurones (Webster and Jones 1988). Another achievement in the regulatory mechanisms of REMS was the finding that raphe nuclei serotonergic neurons and noradrenergic neurons from LC cease firing specifically during REMS, i.e, show a REMS-off firing activity, reciprocal to that of REMS-on neurons (Aghajanian and Vandermaelen, 1982). Recently, it was found that tuberomamillary nucleus-histaminergic neurons and hypocretinergic neurons from the lateral hypothalamus depict a REMS-off firing activity (Mileykovskiy *et al*., 2005; Takahashi *et al*., 2006). Gating REMS occurrence by mutual interactions between REMS-on and REM-off neurons namely, reciprocal inhibitory interactions between cholinergic REMS-on and monoaminergic REMS-off neurons for REMS onset and maintenance is the well-accepted hypothesis.

A very small area of the dorsolateral pontine tegmentum which is called sublaterodorsal nucleus (SLD) has the ability to induce a long-lasting REMS-like hypersomnia after injection of two GABAA receptor antagonists: bicuculline or gabazine, (Boissard *et al*., 2002). Furthermorem, glycinergic neurons from the ventral and alpha gigantocellular (GiV) and raphe magnus nuclei were found to receive direct projection from SLD to generate atonia during REMS by direct projections to cranial and spinal motoneurons. GABAergic neurons within the ventrolateral part of the periaqueductal gray (vlPAG) and the dorsal deep mesencephalic reticular nuclei (dDpMe) gate REMS by inhibiting tonically REMS-on neurons from the SLD during SWS and waking (Fort *et al*., 2009). Melanin-concentrating hormone (MCH) has a role in REMS due to the finding that MCH neurons activation is selective for REMS (Hanriot *et al*., 2007). Luppi

et al. (2006) proposed that REMS increases induced by MCH might be as the result of the inhibitory direct effect of the GABAergic dDpMe and vlPAG neurons gating REMS onset while inputs to these areas from the HCRT neurons would be excitatory to prevent REMS. A relevant role for neurons localized in the lateral hyopthalamus in the regulation of REMS occurrence came from a recent paper (Clement *et al.*, 2012) in which it has been shown that the inhibition of the lateral hypothalamus by the local microinjection of muscimol suppressed REMS occurrence in the rat. The results have been interpreted as being the effect of the tonic suppression of the activity of the MCH neurons which are intermingled and in close functional relationship with the HCRT ones. Moreover, a role in the promotion of REMS occurrence has been attributed to the peptide Nesfatin-1 which is co-expressed in MCH neurons in the tuberal and lateral zone of the hypothalamus and has been shown to be also involved as a satiety factor in appetite regulation (Jego *et al.*, 2012)

From the point of view of physiological regulation, the two sleep states are very different (Parmeggiani, 2005), since while NREMS is characterized by a substantial stability of the autonomic parameters which are typically regulated in accordance with Walter Cannon's homeostatic paradigm, REMS is characterized by a large autonomic instability, with sudden surges in arterial blood pressure and heart rate and profound irregularities in breathing. In particular, during REMS thermoregulation is depressed or suppressed in different species and the animal body temperature shift toward the environmental temperature as reptiles (Parmeggiani, 2003). This led to define REMS as a poikilostatic state, in contrast to NREMS (Parmeggiani, 2005).

The interest in deepening the relationship between sleep and thermoregulation comes from the fact that these two functions influence each other. The thermoregulatory regulation changes during sleep, also the structure of sleep changes during the thermoregulatory activity. This close interaction was probably driven by the fact that these two functions share some of the regulatory structures at the central level, in particular, the basal preoptic regions and ventral nuclei of the hypothalamus. As already said, the main thermoregulatory difference

which is observed during the wake-sleep cycle is the thermal homeostasis suspension during REMS, which has been observed in many species, including humans, and has been postulated that this impairment generally depends on insufficiency in the hypothalamic integration of autonomic function (Heller, 2005; Parmeggiani, 2005). During REMS, animals exposed to high or low ambient temperatures were missing the normal thermoregulatory responses such as shivering, the thermal polypnea and vasomotor responses. Experimental animal had shown highest total sleep time at thermonutral ambient temperature and decreases both in the cold to hot and that REMS is the one most affected by the variation of the ambient temperature. Amici *et al*., (1994); Cerri *et al*. (2005) confirmed this in the rat, where they observed that the amount of REMS is reduced almost to zero in extreme environmental conditions. However, Luppi and colleagues (2010) reported that osmoregulation, is not impaired during REMS, so, we can now suppose that the link between REMS and suspended homeostasis is prevalently (or exclusively) linked to suspended thermoregulation and therefore REMS function could be precisely related to the regulation of body temperature and metabolism.

Furthermore, blood pressure in human, rat and rabbit show an increase from NREMS to REMS but this rise was not always accompanied with primary cardiovascular changes namely; heart rate and vascular conductance. In rabbit, decrement during REMS was noticed in renal and vascular conductance. The weak correlation between regional and systemic variables proves that the central integration of cardiovascular functions is altered during REMS. The variability in heart rate and blood pressure is a prominent and an important feature of REMS in rabbit, rat, cat and human being and it is loosely associated with bursts of rabid eye movements, myoclonic twitches and more often probably with breathing irregularities. The main causes of such instability of cardiovascular regulation in REMS are due to the interaction between the central variabilities of visceral control and the central effects of activated reflexes. In rat, during REMS, the arterial blood pressure (BP) increases (hypertension) while in sinoaortic denervated counterparts, hypotension occurs as in cat.

There is a mode of description of the succession of episodes of different REMS which is based on the possibility to describe the succession of episodes of REMS regardless of the phase of the sleep cycle interposed between two successive episodes of REMS, but simply taking into account the duration of the time interval which separates the two mutually adjacent episodes. The study of the frequency distribution of the duration of the intervals between two consecutive REMS episodes, namely the intervals of time after the end of an episode of REMS and the beginning of the next, showed a bimodal characteristic appearance that varies in a species-specific. In rats, 3 minutes has been shown to be the time interval that identifies the limit of separation between short and long episodes of REMS (Amici *et al*., 1994). As mentioned above, one can distinguish individual episodes of REMS, which are separated by intervals of duration greater than 3 minutes (single REMS episodes), and sequential episodes, which are separated by intervals of less than 3 minutes (sequential REMS episodes). More episodes of REMS sequential occur in groups known as REMS clusters, in which the first and the last episode are separated from the previous and next respectively by long REMS intervals. Amici and colleagues (1994) had reported that during the normal circadian rhythm of light-dark in rat, about half of the REMS is executed in the form of sequential episodes. Moreover, it has been shown that REMS occurrence is mainly regulated through changes in sequential REMS episodes either under thermal challenges (Amici *et al*., 1994, 1998; Cerri *et al*., 2005) or in response to changes in environmental light (Zamboni *et al*., 2001), while the amount of single REMS episodes is kept almost constant, if possible. The partition between single and sequential REMS episodes is therefore considered to be a good index describing the interaction between REMS pressure (increasing sequential REMS episode) and the activity of the autonomic nervous system dedicated to the conservation of bodily homeostasis (depressing sequential REMS episodes) (Amici *et al*.,1994; Zamboni *et al*., 2004).

In this thesis, the amounts of REMS under the form of sequential REMS episodes or single REMS episodes will be addressed as Sequential REMS or Single REMS, respectively.

1.2.6. SLEEP HOMEOSTASIS

Both the amount of sleep and the dynamics of its recovery after deprivation suggest a kind of homeostatic regulation for sleep occurrence. In the 80s, Borbély was the first to address the issue of sleep regulation in term of intensity and the duration (Borbély, 1980). According to his model, the propensity to sleep state is the result of the interaction of two processes, called process C and process S. The process C, or circadian, describes the control of the timing of episodes of sleep and sleep propensity during the day, and derives from the supra-chiasmatic nucleus of the hypothalamus (SCN). The process S, or homeostatic, describes how the sleep propensity increases proportionally to the accumulation of the time spent in wakefulness and decreases, instead, after periods of sleep, especially during NREMS.

Therefore, it can be said that sleep is a behavioral state during which, in the NREMS phase, physiological variables are homeostatically regulated, but it is also true that the same state of sleep is subjected to a homeostatic regulation. An indicator of the intensity of NREMS, and, therefore, used to describe the homeostatic process below, consists of the electroencephalography slow-wave activity (SWA), with a meaning equivalent to that of Delta waves. The reason why SWA serves as an index of the intensity of NREMS is due to the fact that it is particularly intense at the beginning of a phase of sleep, precisely parallel to the period in which the threshold of awakening organism is the most high and, therefore, the sleep becomes deeper. After this first phase, the need for sleep is reduced and, simultaneously, SWA becomes much less intense also. Furthermore, in many studies it has been observed that periods of deprivation are followed by intense SWA in the recovery phase of sleep (Borbély and Achermann, 2005). The SWA is not, therefore, only an epiphenomenon of NREMS, but it reflects important regulatory mechanisms, the meaning of which is still matter of debate.

To date, the most accepted theory to explain the homeostasis of NREMS SWA is synaptic homeostasis (Tononi and Cirelli, 2003). This theory relates the

synaptic potentiation during wakefulness, with the SWA during sleep. Specifically, the different activities during wakefulness (such as, reactions to sensory stimuli, motor activity, mental associations, thoughts, acquire new experiences) lead to an increase in the number and strength of synaptic connections between neurons (synaptic potentiation); this phenomenon is directly correlated with the increase of the SWA during the next sleep phase, the function of which would be an elimination of synaptic connections considered superfluous. From this point of view the progressive decline of the SWA during rest reflect the corresponding re-modulation of synaptic weights in order to optimize the functionality of neuronal connections. The function of the slow-wave activity during NREMS, according to this theory, would therefore be to avoid overload of synaptic connections on nerve cells, thinning the density of connections and at the same time improving their efficiency.

In contrast to NREMS, REMS does not present dynamics of recovery based on the intensity, but rather on the duration of the phenomenon (Parmeggiani *et al.,* 1980b; Cerri *et al*., 2005). In fact, it is not yet clear whether the REMS recovery is also based on an increase in the intensity of theta waves, typical of this stage of sleep (Cerri *et al*., 2005). A procedure that has allowed a detailed study of the dynamics of recovery of REMS consisted of exposure to low temperatures which is a powerful inhibitory stimulus against the appearance of REMS. After exposure to cold, in the rat the recovery process of REMS appears to depend only on the amount of REMS lost during the previous deprivation, following a dynamic seems that regardless of the temperature of exposure (Amici *et al*. 2008). After 24-h deprivations of REMS of different intensities, this process leads to a 100% recovery of the REMS loss in few days (Amici *et al*. 2008). From studies of deprivation and recovery, it is clear that REMS behavior is a finely regulated on the homeostatic base and how the chronic deprivation of this phase of sleep represents a severe stress for the organism so as to lead to an organic deterioration such as to induction of death (Rechtschaffen *et al,* 2002). As mentioned earlier, the chronic deprivation of REMS is accompanied by a dysmetabolic syndrome characterized by the reduction of the production of leptin followed by appearance

of hyperphagia, contrary to what the body weight is reduced gradually, while the metabolic activity of fat brown increases (Koban and Swinson, 2005). This suggests that REMS is involved in the maintenance of the metabolic activity of the organism and that behavior is essential for the biological economy of the individual and his own life. In support of the hypothesis of a link between REMS and metabolic function, it has been shown that the intensity of the REMS rebound following REMS deprivation, and therefore the REMS need, apparently decreases in proportion with the increase in the body mass (and therefore with the decrease of the basal metabolic rate per Kg) of the different species, being more urgent in rats than in cats, and reaching a lower level in humans (Amici *et al*. 2008).

1.3 CARDIOVASCULAR PHYSIOLOGY AND SLEEP

Cardiovascular autonomic system is a highly network integrates to keep visceral functions under control, which in a short timescale (within second to hours), can adjust the circulation in keeping with, environment, emotions and behavior. Ensuring adequate cardiac output to the vital organs through continuous and rapid adjustment of HR, arterial BP, and redistribution of blood flow, is counted as the primary and main role of this system. This neural circulatory regulation seems to be in the longer term unfolds the coupling with the circadian rhythms, W-S cycle, and some ultradian rhythms ; including REMS and NREMS stages, in addition to hormones implicated in the long term with BP control.

The perturbations of the regulation of cardiovascular system during the nocturnal sleep is crucial for the public health and this is underscored by the estimated annual sleep related cardiac events which accounts for 20% of myocardial infarctions or (250,000) and 15% of sudden cardiac deaths or (48,750) in United states (Eckberg and Sleight, 1992). Typically, during night's sleep, a wide spectrum of autonomic patterns uncovers which provides cardiovascular system with respite or stress. These effects are due to the fine toned or harmonic changes in physiology of Central Nervous System (CNS) as the periodic

reexcitation of the brain during transition from relative tranquility in NREMS to REMS.

1.3.1 SLEEP STATE-CONTROL OF CARDIOVASCULAR FUNCTION

Blood pressure undergoes wide physiological changes between Wake-Sleep states (Silvani, 2008). During NREMS, arterial pressure substantially decreases with respect to Wake in humans (Silvani *et al*., 2008) and rodents (Silvani *et al.*, 2009) because of decreases in cardiac output and vascular resistance (Silvani, 2008). During REMS, an apparent re-patterning of sympathetic activity to cardiovascular effectors causes changes in arterial pressure, the direction and magnitude of which are highly sensitive to genetic and pathological factors (Silvani, 2008).

The initial stage of sleep cycle, namely, NREMS is characterized as a period of relative autonomic stability with dominance of vagal nerve and hightened baroreceptors gain. Normal respiratory sinus arrhythmia is a term used to describe a near sinusoidal modulation of heart rate (HR) variation occurs as a result of a coupling with respiratory activity and cardiorespiratory centers in the brain during NREMS stage. Inspiration induces a brief accelerations of HR in order to accommodate increased venous return, resulting in increased cardiac output , while progressive deceleration ensues during expiration. This variability in HR, particularly in NREMS, is considered normal and it is generally indication of cardiac health whereas, the absence of this phenomenon is related to aging and cardiac pathology. Also, during breathing, a reflexive cardiovascular alterations manifested as cyclical differences in HR which also have an inverse relationship as a transient increase in arterial blood pressure results in slowing, interruption or decrement of the breathing efforts. During sleep, this effect is enhanced when even a small reduction in BP induces increase in respiratory rate (Lombardi and Parati, 2000). These pauses or increasing heart rates needed as compensatory mechanisms and to normalize the arterial BP. The absence of these normal pauses and dimensioned breathing variation in addition to reductions in respiration

induced HR difference, are a characteristic finding in infants who later suffer from sudden infant death syndrome (SIDS). Furthermore, reduced HR variability is typically associated with another infant syndrome which called congenital central hypoventilation syndrome, in which the respiratory drive is lost during sleep.

Obstructive sleep apnea (OSA) is accompanied with extreme HR variation. Thus, loss of normal vagal nerve function is the common factor of cardiac risk associated with suppressed HR variability. Bernardi *et al*. (1990) reported that sympathetic nerve activity tends to be relatively stable during NREMS and from wakefulness to stage four of NREMS, the cardiac output reduced by more than 50%. This stability of autonomic functions of NREMS, with hypotension, bradycardia, and reduction in cardiac output and systemic vascular resistance, support the body with relatively healthy neurohumoral background during which the heart has a chance for metabolic restoration. These two phenomena; bradycardia and hypotension are believed to be caused mainly by increase parasympathetic nerve activity and sympathetic vasomotor tone reduction, respectively (Pagani *et al*., 1986). Bursts of vagal nerve activity during the transitions from NREMS to REMS are accompanied with the occurrence of pauses in heart rhythm and frank asystole. Particular attention should be given to the cardiovascular regulation during REMS as the instability and perturbations occur due to loss of integration between forebrain structures and brainstem which makes these pronounced changes to be attributable to distinct mechanisms related to specific brain sites rather than demonstrating an autonomic change continuum. REMS which started at 90 minutes intervals can disrupt cardiorespiratory homeostasis. The increased activity of brain during REMS causes the major surges in cardiac sympathetic nerve innervation to the coronary vessels.

Baroreceptors gain was found to be suppressed during REMS phase also. Obvious fluctuations of HR, with marked episodes of both bradycardia and tachycardia were reported. As the individual inters the REMS, the efferent vagal tune to the heart is, in general, suppressed and subsequent high irregularity in respiratory rhythmicity initiates which can lead to reduction in the oxygen level that affects in particular those patients with pulmonary or cardiovascular diseases

(Mancia, 1993). Neurons responsible about activating principal diaphragmatic muscles are normally escape from this generalized suppression although the upper airway and the accessory muscles show inhibition of their activity. This inhibition is prominent in can be noticed in infants abdominal and thoracic muscles during REMS. Sleep apnea, a case which is known to exaggerate in obesity, might be accompanied with interruption of central respiratory activity or peripheral obstruction hundred times each night, with the possible terrible consequences for cardiorespiratory activity. The pontine and suprapontine structures have shown a capability to alter cardiorespiratory patterns during sleep and wake states. The important of pontine area as a key player in REMS activation is well known and demonstrates a preferential activation of limbic and paralimbic regions in forebrain compared with NREMS and wake (Nofzinger *et al*., 1997). Furthermore, the serotonergic neurons which exist in midline raphe of the pons have an important role in vascular control as in cases of heart failure, patients demonstrate loss of these neurons which it is likely as a results of hypoxia and impaired perfusion during impaired breathing while sleep in this condition (Woo *et al*., 2009). Other cerebral structures are frequently included among the forebrain structures which ruling the cardiorespiratory patterns in addition to affective behavior as orbital frontal cortex are hypothalamic structures as well as portions of the hippocampal formation. The central nucleus of the amygdala is a cornerstone in regulating cardiac and respiratory functions due to its extensive projections to other brain structures known to have a significant influence on cardiac action namely; barabrachial pons and the nucleus of the solitary tract, the dorsal motor nucleus and the periaqueductal gray region. These structures are recruited by inspiratory and expiratory loading that takes place during the impairment of breathing through sleep. The insular cortex deserves special interest over other areas responsible of the cardiovascular control during sleep and wakeful. The importance of this area is being the area both sympathetic and parasympathetic actions in both animal and human studies. However, there is an interaction between right and left sides of insular cortices, the right side is responsible about the control of sympathetic outflow while, the left side controls

the parasympathetic one (Verrier *et al.*, 2009). Under conditions with sleep disordered respiration and high sympathetic tone i.e. heart failure and OSA, marked deficits were noticed in the insula. Moreover, Cerebellum has a particular role in controlling the cardiovascular and respiratory functions, although it is not classically considered as a component of either respiratory or cardiac control. A part of this role in regulation BP is via cerebellar-vestibular mechanisms. Vestibular mechanism has a role in adjusting BP during a fast change in the posture which is normally seen as syncope in hypotensive patients when rise from horizontal position. Ineffective compensatory responses to hypotension with subsequent death were accompanied with lesions in the cerebellar fastigial nucleus. OSA and heart failure cases were found to be accompanied with cerebellar damage with prominent loss of gray matter in its cortex and deep nuclei.

1.3.2 SYMPATHETIC VS PARASYMPATHETIC CONTROL

Circulation is under the neural control which appears via vagal nerves effect on the heart and sympathetic nerves efferents operate to heart, blood vessels, kidneys as well as adrenal medulla. Cardiovascular stimulation is occurred primarily via vagus nerve by activation of the muscarinic receptors resulting in bradycardia. In contrast, the sympathetic stimulation of the heart is induced by activation of β1 adrenoceptors at the cardiac pacemaker i.e. sinoatrial node (SA node) and the cardiac muscles (myocardium) which increased contractility and results in tachycardia. Moreover, the sympathetic activation on the vascular beds entrains several effects demonstrated as vasoconstrictions by activation α1 adrenoceptors in the skin and splanchnic districts but in contrary, vasodilatation by stimulation β2 receptors in the heart and skeletal muscles. Both efferent's activity of sympathetic and parasympathetic to the heart might also alter electrophysiological properties of the heart which can in turn create several types of arrhythmias, especially in the existence of proarrhythmic substrates.

1.4 THE RECIPROCAL INTERACTION BETWEEN SLEEP DISTURBANCES AND OVERWEIGHT

In fact, the association between sleep disturbance and excessive body weight had been known since the sixties of the last century, when it was described the occurrence of episodes of sleep apnea in severely obese patients (Gastaut *et al*., 1965). The obstructive sleep apnea (OSA) is classified as part of the intrinsic hypnic disorders characterized by disturbances of breathing (sleep-related breathing disorders; Vgontzas *et al*., 1998). The OSA can be interpreted as an early symptom of the so-called metabolic syndrome (metabolic syndrome, MS), as it is often characterized by the appearance of insulin resistance independent from overweight which can continue to the emergence of obesity and the framework of the full-blown MS (Vgontzas *et al*., 2005).

The MS is manifested by the appearance of a set of symptoms that contribute to increase the probability of occurrence of a type 2 diabetes and cardiovascular disease namely; obesity, arterial hypertension, high level of triglycerides, low HDL cholesterol level and hyperglycemia (Ryden *et al*., 2007). The definition of MS is continuously subjected to review and refresher courses, either for new addition to the collection of symptoms parameters, or for the elaboration of clinical data by different organizations or professional associations.

The current accepted definitions are five, and they differ mainly for the threshold level established for pathological metabolic parameters (Graham *et al.* 2007). The association between obesity, OSA and hypnic disorders remained limited for a long period in a field of research and the main goal was for refining the definition of the various comorbidities. Webb and Agnew (1975) were the first in separation the hypnic disorders by the possibility of a reduction of sleep time in relation to a socially determined lifestyle. This observation was followed sporadically by others until the first systematic relationship to the insufficiency of sleep in a large population sample (Broma *et al*., 1996). This study, conducted in a Swedish community, reported that regardless of gender and age, about 6% were

suffering of sleep curtailment attributed to social reasons rather than to sleep disorders as insomnia. In 1999, a study by Spiegel *et al*. was decisive step in determining the relationship between this reduction in sleep and metabolic status of individuals. These authors had shown how forced sleep deprivation in young during six consecutive nights related to reduction of glucose tolerance, a reduction in blood levels of Thyroid-stimulating hormone (TSH) , an increase of cortisol in the afternoon and evening and a shift of sympatho-vagal tone to the sympathetic tone. The key idea in this study is the appearance of one of the cardinal symptoms of the evolution of the MS, such as impaired glucose tolerance following a partial sleep deprivation for a short period. Although the relation between chronic reduction of sleep and the development of obesity is not clearly shown, one could infer that the simultaneous appearance of early signs of metabolic disorder in a sleep curtailment subjects for a short period was able to lead to a causal relationship simply turning partial deprivation in a prolonged state.

This topic could not be addressed experimentally in human due to ethical reasons as in order to study this point, we need to modify the life style for long period. In order to overcome this problem we can progress in two ways: 1) the animal model, although this will give us a partial solution as it is not possible to change the behavior of the animal chronically without a very stressful constraint, and for that the animal model can be excellent in studies related to partial sleep deprivation; 2) human being, in which we can conduct retrospective epidemiological studies on large and well defined human populations.

In 2002, and particularly on North American adolescents, was the first human defined cohort on whom it was studied the relationship between sleep reduction and the pathological weight gain (Gupta *et al*., 2002). It was concluded that poor sleep quality proved to be significantly related to obesity. This finding was also confirmed by another study performed by Hasler *et al*. (2004) on a population of young adults lasted for 13 years. These conclusions were certainly in line with the alarm of obesity (WHO, 2003), but the epidemiological approach more accurately determined sleep curtailment and the appearance of glucose metabolism disorder were confirmed by the critical review of Spiegel *et al*. (2009)

who put into consideration the relationship between the prolonged sleep reduction and the appearance of insulin resistance, the early symptom which possibly develops toward type 2 diabetes. Also, the authors highlighted the role of sleep in the regulation of food intake and appetite and overthrew the relationship between obesity and OSA assuming that sleep fragmentation, primitively induced the OSA, to explain the association with obesity in patients suffering from this respiratory disorder. In this work relationship existed between control of food appetite and control of sleep. Such hypothesis of the relationship was first made in a study for a couple of years in adult subjects in both sexes, determining the concentration of two satiety hormones which regulate the food appetite and their roles in sleep curtailment and how this relation was highly correlated to the increase of BMI (Taheri *et al*., 2004); another relevant and new data of this work was the finding of U-shaped pattern of the relationship between BMI and sleep duration. In persons sleeping less than 8 h, increased BMI was proportional to decreased sleep. Similarly, in their study to explore the relationship between sleep duration and diabetes incidence over an 8- to 10-year follow-up period, Gangwisch *et al*. (2007) found that subjects with sleep durations of 5 or fewer hours (odds ratio = 1.47, 95% confidence interval 1.03-2.09) were significantly more likely to have incident diabetes over the follow-up period after controlling for covariates. They reported also that subjects who slept 5 or fewer hours were almost twice as likely as those who slept 7 hours to have incident diabetes over the follow-up period. Another study by Gangwisch and colleagues (2006) mentioned that subjects between the ages of 32 and 59 years who reported averaging 5 hours of sleep per night were at an increased risk for developing hypertension over the follow-up period (8- to 10-year).The impact of recurrent sleep curtailment had reported to lead to weight gain and obesity by compromising insulin sensitivity and by increasing appetite by decreasing leptin levels and increasing ghrelin levels. Other study performed on the data of 4222 Korean participants revealed that in subjects under 60 years old the prevalence of abdominal obesity and hypertension were highest in subjects who slept <5h per night whilst those who slept 7 hours have the lowest prevalence for MS (P=0.006)

(Choi *et al*., 2008). On the other hand , the finding that the relatively few obese adults/children are short sleepers, and few short sleeping adults/children are obese or suffer obesity related disorders makes some researcher as Horne (2008) mention that many years of changing in sleeping manner are needed to show any weight or BMI differences between short, normal, and long sleepers. Furthermore, he reported that the a BMI difference of 2.5 units (about 7 kg) between otherwise comparable short (5 h) and normal (7-8 h) sleepers, developed slowly during 10 years of such sleep; that is, with hundreds or even thousands of hours difference accumulated in the daily sleep between the two groups over this 10 y period. According to the author this would show that the effects of poor sleep on the development of obesity have probably been overestimated.

1.5 STATE OF THE ART

1.5.1 THE OBESITY EPIDEMIC IS A THREAT TO HEALTH CARE

The prevalence of obesity has risen dramatically worldwide (WHO, 2006). In Europe, an estimated 150 million adults are overweight or obese, thus, obesity represents one of the most serious public health challenges (WHO, 2007). Obesity also predisposes to coronary heart disease, heart failure, and sudden death (Poirier *et al*., 2006). Abdominal obesity tends to cluster with glucose intolerance, insulin resistance, dyslipidemia, and hypertension, which are documented risk factors for cardiovascular disease, leading to a constellation of metabolic abnormalities known as the metabolic syndrome (Eckel *et al*., 2005). In particular, obesity is associated with hypertensive derangements of arterial BP, which are most prominent during the rest period of the daily rest-activity cycle. This phenomenon and its underlying mechanisms are of great clinical interest because a blunted nocturnal decline in arterial BP (non-dipping) and high nocturnal arterial BP values are recognized as powerful predictors of mortality in patients referred for ambulatory arterial BP monitoring and the general population.

1.5.2 ANIMAL MODELS ALLOW MECHANISTIC INSIGHT ON THE PATHOPHYSIOLOGY OF OBESITY

In general, obesity and other metabolic syndrome traits in humans ensue from interactions between polygenic susceptibility and an obesogenic environment (Lusis *et al*., 2008). The understanding of the mechanisms of these interactions can be hastened by studying animal models of diet induced obesity (DIO), which present critical features of obesity in humans. Interestingly, it has been shown that when Sprague-Dawley rats are fed an energy-dense diet, rich in saturated fat, only about 50% of them develop obesity (obesity-prone, OP), while the others appear to be resistant to DIO (Levin *et al*., 1983, 1997). In OP rats, DIO is associated with derangements of glucose metabolism and reduced glucose tolerance (Levin *et al*., 1997), hypercholesterolemia (Dobrian *et al*., 2000), hypertriglyceridemia (Dobrian *et al*., 2000), hyperleptinemia (Levin *et al*., 2003), and hypoghrelinemia (Levin *et al*., 2003).

1.5.3 HYPERTENSION IN DIET INDUCED OBESITY

In obese humans, arterial BP is higher than in lean controls and decreases less than in lean controls on passing from the diurnal activity period to the nocturnal rest period (Kotsis *et al*., 2005). The risk of developing frank hypertension is 6 time larger in obese subjects than in lean ones (Poirier *et al*., 2006). During the period with light on (i.e., the rest period in nocturnal mice), hypertension is also enhanced in mouse models of morbid obesity because of genetically impaired leptin signaling (Swoap , 2001; Silvani *et al*., 2009) as well as in C57BL/6J mice with DIO (Williams *et al*., 2003). The mechanisms underlying hypertension during the rest period in obesity are of great clinical interest because a blunted nocturnal decline in arterial BP and high nocturnal BP values are powerful predictors of mortality (Ohkubo *et al*., 2002; Dolan *et al*., 2005). High values of arterial BP during sleep may result from sleep

fragmentation (Carrington and Trinder, 2008, Matthews *et al*., 2008) and sleep apneas (Parati *et al*., 2007; Hla *et al*., 2008). The circadian distribution and quality of wake-sleep episodes are major determinants of the circadian arterial BP rhythm because they contribute to differences in arterial BP between activity and rest periods (Kerkhof *et al*., 1998; Smolensky *et al*., 2007).

At present, however, it is still unclear whether OP rats are susceptible to or protected from obesity-associated hypertension, since while the development of hypertension have been shown after 8-10 weeks of administration of a hypercaloric (HC) diet (Dobrian *et al*., 2000), this observation has not been subsequently confirmed (Carroll *et al*., 2006). However, while in the first study arterial BP determination was made by tail-cuff method, which is considered quite obsolete and not fully reliable, in the second study the determination was made by the state of the art method, i.e. by means of a telemetric transmitter implanted in the abdominal aorta. It is worth noting, that, at present, no W-S state dependent determinations of arterial blood pressure have been conducted in obese rats.

1.5.4 OBESITY ENTAILS ALTERATIONS IN WAKE-SLEEP STRUCTURE AT ULTRADIAN AND CIRCADIAN TIME SCALES

In human subjects and animal models, the development of obesity is associated with alterations in wake-sleep structure. The consequences of these alterations on cardiovascular function remain poorly understood. In obese humans, excessive daytime sleepiness is associated with poor quality of sleep at night (Vgontzas *et al*., 1994,1998) particularly because of sleep apneas (Vgontzas *et al*., 1994). However, sleep apneas are not the only causative factor of excessive daytime sleepiness in obese subjects (Vgontzas *et al*., 1998). The tight link between sleep and obesity has also been stressed by recent data showing that sleep deprivation and chronic sleep loss are related to weight gain and to the development of insuline resistance and type-2 diabetes (Spiegel *et al*., 2008).

The relationship between obesity and sleep has been studied in several animal models of obesity. These models develop obesity following the

administration of a high-calorie diet or through genetic manipulations aimed at manipulating the leptinergic system. In the second case, the animals have been deprived of the ability to produce leptin (ob/ob mice) or have become resistant to the action of the hormone itself through the induction of a mutation of the hypothalamic receptor for leptin (db/db mice) or after being rendered incapable of expressing the same receptor (fa/fa Zucker rat). The absence or ineffectiveness of leptin that is normally produced from energy deposits in adipose tissue and signals to the hypothalamus of the magnitude of those deposits prevents the appearance of inhibition of food intake and body metabolism activation that normally follows an increase in the size of energy store. All these studies showed the trend in obese animal to increase the amount of NREMS, while variable results have been seen on REMS and the daily distribution during L or D periods of the Wake-Sleep states.

In particular, the ob/ob mice, shows an increase of NREM sleep and a significant attenuation of the amplitude of the normal LD distribution of NREMS and REMS compared to their controls (Laposky *et al*., 2008; Silvani *et al*. 2009), mostly due to an increase in the amount of NREMS during the daily activity period (Dark). Similar results were observed in db/db mice (Laposky, 2008), showing a concomitant reduction of the daily amount of REMS. In mice that are obese because of dysfunctional leptin signalling, a significant sleep fragmentation is observed as well (Laposky *et al*., 2006, 2008). Studies on Zucker fa/fa rats also showed an increase of NREMS, but no effects on REMS sleep and LD distribution of W-S states (Danguir *et al*., 1989; Megirian *et al*. 1998).

Studies on mice made obese by the administration for 4-8 weeks of a hypercaloric (HC) diet showed a consistent tendency to an increase in the amount of NREMS, and less consistently, of REMS, in the absence of any significant change of the LD distribution of the Wake-Sleep states (Jenkins *et al*., 2006; Guan *et al*, 2008). The only published study conducted on rats subjected to high-energy diet (Danguir *et al*., 1987) showed that after 10 days from the start of the administration, the animals, which were still not significantly overweight compared to controls, tended to increase the amount of NREMS and REMS,

without apparent changes of the LD distribution of the Wake-Sleep states. Finally, recent data from our laboratory (Laudadio, 2011), that still have to be published on the international literature, have shown that in OP rats fed a HC diet for 8 weeks REMS occurrence is increased compared to lean animals, but the normal increase in REMS occurrence which is usually observed when the albino rat is kept in the absence of environmental light (continuous darkness) is dampened.

2. Aims

The aim of this research is to study the structure of the W-S cycle and the possible state-dependent changes of brain temperature and cardiovascular function in rats made obese by the chronic administration of a hypercaloric diet.

This study goes to fill a gap in the scientific literature related to this topic, since, as previously pointed out, no studies of this kind have still been conducted in the rat, which is currently the most widely used animal model in experimental biology. At present, studies of this complexity have been carried out exclusively in a mouse model of obesity induced by a genetic modification leading to the lack of the hormone leptin, in which the syndrome develops due to functional lesions that are not usually present in human pathology. Thus, it would be very useful to have an animal model in which the development of obesity and of possible cardiovascular comorbidities are induced by the administration of a hypercaloric diet, since it would be very similar from a patho-physiological point of view to what normally occurs in humans.

This experiment has also been conducted with the aim of identifying whether and, if the answer is positive, what are the changes made by the prolonged administration of a hypercaloric diet to sleep regulation in response to a previous sleep deprivation. Particular care has been placed in the analysis of the possible modifications of REMS, whose occurrence is strongly influenced by the degree of activation of thermoregulatory/metabolic processes and is under the control of central nervous structures at hypothalamic level that are known to be also involved in the regulation of body temperature/metabolism and food intake.

3.Material and Methods

3.1 ANIMALS

The experiments were conducted using outbred CD Sprague-Dawley male rats (Charles River) of an age of 5 weeks and a weight comprised between 100 and 125 g at the time of purchase. The animals, after their arrival, spent a week in the animal house and have been adapted to normal laboratory conditions: ambient temperature (Ta) 25 ± 1 ° C, light-dark cycle (cycle LD) 12h: 12h (L 9:00-21.00), light intensity at the level of the cages 150 lux; food and water ad libitum. During the week of adaptation the animals were housed in pairs in transparent plastic cages (Techniplast) containing bedding depolverate that were changed every two days. All animals were weighed with an electronic scale twice a week, from the day of arrival in the laboratory until the end of the experimental procedure.

The animals were divided into two groups: control and treatment. Obesity was induced in the treatment group (n=24) by administering an obesogenic hypercaloric-hyperlipidic (HC) diet starting from the 5th week of age for 8 consecutive weeks (D12492: 35% fat, 60% calories from fat, Mucedola). Control group (n=16) was fed a standard normocaloric (NC) diet (D12450B: 3% fat, 10% calories from fat, Mucedola).

Starting from the seventh week of their arrival and, subsequently, in agreement with the experimental plan (see EXPERIMENTAL PLAN) the animals were subjected to surgery. The experiments were performed in accordance with European Union Directive (86/609/EEC) and under the supervision of the Veterinary Service Center of the University of Bologna and the National Health Authority.

After 7 weeks of the diet regime, the weight of the animals were (NC: 441 ±16g; HC: 556±17g): data are mean ± SEM. After surgery, the animals were allowed to recover for 4 days at least in the box used for recordings (Ta 25 ± 1 °C; 12h: 12h light-dark (LD) cycle (L 09:00 to 21:00); light intensity 150 lux; food and water ad libitum). In the morning of the fifth day were connected to the recording cable and allowed to adapt to the system of acquisition for the next

three days, during which recordings were made of the test to verify the correct operation of the sensors and to choose the derivation electroencephalography to acquire.

3.1 SURGERY

3.1.1 PREPARATION OF ELECTRODES

Before each surgical session, electrodes for the chronic recording of the electroencephalogram (EEG), nuchal electromyogram (nuEMG), diaphragmatic electromyogram (diEMG) and a thermistor for the recording of the hypothalamic temperature (Thy) were assembled. For the EEG electrodes, two copper wires with a length of 2 cm and a diameter of 0.3 mm coated with an insulating film were used. The insulating film on each free-end of each copper wires was removed for 1 mm. The EMG electrodes were constructed from pairs of wires of stainless steel (model AS 632, Cooner Wire Inc., Chatsworth (CA), USA) coated with an insulating sheath of polyethylene, with a length of 15cm (diEMG) or 8cm (nuEMG), to which were removed 3mm insulating sheath at the ends, and 2mm of sheath at mid-length of the cable.

3.1.2 THERMISTORS

For the measurement of the hypothalamic temperature (Thy), thermistors embedded in glass gob (NTC Thermometrix) diameter of 0.3mm were used. The thermistors have been inserted into the tip of a needle 21 G and connected to a spinet two-pin placed in the neck of the needle connection; the whole was insulated with several layers of paint for electrodes.

On the day preceding the surgery the thermistors were subjected to a calibration procedure during which it was evaluated the constancy of the physical characteristics of the transducer, and it proceeded to the same linear calibration. To do this, the thermistor was immersed in a large container of water (thermo

bath) brought to a temperature of 39 ° C, measured by means of a mercury thermometer (scale 34 ° C-42 ° C), and connected to current amplifier that would be used during the following days to the animal.

To evaluate the constancy in time of the physical characteristics of the thermistor, it the mass of water was let to cool spontaneously. To compare the operation of the thermistor to different times of use, the impedance was measured at three temperatures: 38.5 ° C, 37.5 ° C and 36.5 ° C.

3.1.3 SURGICAL INTERVENTION

After 7 weeks of adaptation to the laboratory conditions and feeding animals which were fed either the HC or the NC diet were selected for the experimental procedures. Animals underwent surgery under general anesthesia (Preanesthesia: Diazepam, Valium Roche, 5 mg / kg intramuscularly; Anesthesia: Ketamine-HCl, Parke-Davis, 100 mg / kg intraperitoneally.) For implantation of the apparatuses for recording physiological parameters, the animal were shaved on the head, the chest area corresponding to the xiphoid process and the abdominal area along the line formed between the abdominal muscles and the hind leg.

All the shaved areas have been disinfected with Betadine for surgical use in order to avoid bacterial contamination during the operations. In the abdominal area, between the abdominal wall and the hind leg, a cut of about 2 cm of the skin and the subcutaneous tissue has been practiced to expose the femoral artery, which has been detached from the connective tissue surrounding the femoral vein that runs adherent to it. A small incision was then made by a scissors in the artery where the catheter was inserted in order to measure the changes in the arterial BP in the abdominal aorta. The telemetric BP transducer (TA11PA-C40, DSI) connected to the catheter was housed and fixed subcutaneously in the abdominal wall. Before the operation of catheterization, the catheter has been suitably sterilized by placing it for about ten minutes in a sterilizing solution (NU-CIDEX NCX010, Johnson&Johnson).

For the implantation of electrodes for recording of diaEMG, two incisions have been practiced, one at chest level of the xiphoid process, the other at the level of the skull; the xiphoid process was then grasped with forceps and folded to upward in order to expose the underlying diaphragm: there, by means of a suture needle, electrode wires were inserted into the muscle. At this point by means of a lead plastic tube, electrode wires were passed subcutaneously in the chest area up to the skull, where the two ends of each of the two wires were joined and connected to a connector. The incision made at the level of the skull was also used to access the nuchal muscles, passes through which, by means of a suture needle, were inserted into the muscle and scroll to bring the central part, unsheathed, in direct contact with the muscle tissue.

The animal was then placed on a stereotaxic apparatus (Kopf Instruments) (bar stops snout 3.8 mm) and after removing the periosteum were charged in the following order: four craniotomies (0.5mm diameter) at the periphery of the operative field that are served for insertion of fastening screws, a craniotomy (diameter 0.5) adjacent to the bregma for the insertion of the hypothalamic thermistor, two craniotomies (diameter 0.3mm) one on the frontal bone (-3mm anteroposterior (AP) 2mm Latero Lateral (LL) from bregma) and one parietal bone (AP 4mm, 2mm LL from bregma) for the insertion of two electrodes aimed to determining the EEG . All connectors were finally anchored to the skull with acrylic resin (ResPal cold). At the end of the surgical procedures, for disinfection of abdominal wounds, Betadine (10% Betadine gel Meda Pharma Milan) for surgical use was applied followed by intramuscular administration of broad-spectrum antibiotic (108,000 IU Benzilpennicillina, 2.4 mg Neobicina), to prevent post-surgical infections, and subcutaneous administration of 5ml of saline solution, in order to rehydrate the animal. Finally, the animal was kept under observation until the appearance of the first signs of recovery from general anesthesia and then was placed in its cage allowing for a week to recover from surgery. Two days before the experimental session each animal was connected to the cables for recording of physiological variables in order to allow their adaptation to the experimental conditions.

3.2 APPARATUS FOR THE RECORDING

3.2.1 RECORDING BOX

The cage containing the animal was placed inside a freezer box that has been modified to be able to control efficiently the ambient temperature. This control takes place by means of a thermostat which is connected to the compressor of the freezer and a heater placed inside the latter. When the temperature deviates from the one set by the operator, the thermostat switches on the compressor or the heater (Vortex Microsol 600) for correcting the variation of temperature. The box is also equipped with: a ventilation system, an illumination system by means of optical fibers (100 lux at the level of the cage), and a telemetry receiver for recording of blood pressure of the animal, a video system that allows the study of the animal behavioural and a swivel for recording physiological variables.

3.2.2 AMPLIFIERS AND SIGNAL ACQUISITION

All bioelectrical signals recorded from the animal were amplified (Grass mod. 7P511L, Astro-Med, West Warwick (RI), USA) and filtered, respectively, for the low-pass and high-pass filter, with the following values for each variable: EEG 0.3 Hz / 30 Hz, nuEMG 10 Hz / 3000 Hz, diEMG 100 Hz / 1000 Hz and 0.5 Hz Thy. Following all the signals have undergone an analog-digital conversion to 12-bit (CED Micro 1401 MK II) to be stored on a computer-readable form (PC ASUS) with a sampling frequency of 500Hz for the EEG, of 1KHz for the nuEMG and of 50 Hz for Thy. The cages, at their top, were also equipped with a holder for passive infrared motion detector (PID20, Siemens). This sensor was oriented for maximum sensitivity of the movements of the animal. During the experimental sessions, was possible to monitor the behaviour of the animals thanks to a closed-loop system (Philips), consisting of two monitors in black and

white connected to a camera positioned inside the box of recording in correspondence of the two cages.

The electroencephalographic signal has been subjected to a spectral analysis using the algorithm of the Fourier transform (FFT) on a sliding (1 second) window of 4 seconds in order to obtain the values of power density for the bands Delta (0. 5-4 Hz), theta (5.5-9 Hz) and Sigma (11-15 Hz). The signal of the PA was recorded telemetrically, amplified (DSI Phyiotel PA-C40, DataSciences) and incorporated digitally on a PC with an acquisition frequency of 500 Hz.

3.3 EXPERIMENTAL DESIGN

3.3.1 STUDY OF WAKE-SLEEP BEHAVIOR AND AUTONOMIC FUNCTION IN ANIMALS FED WITH HYPERCALORIC DIET FOR 8 WEEKS

At their arrival, animals were randomly assigned to the NC or the HC experimental group. At the seventh week of the treatment, the selection of the NC diet-fed animals which underwent surgery and entered the experiment (n=8) was made randomly. Since about 50% of Sprague-Dawley rats fed a HC diet are apparently resistant to obesity and obesity comorbidities development (Levin *et al*., 1983), the selection of animals (n=8) for the HC experimental group was made randomly among those whose weight, at the moment of the selection, was over the median value of the population.

After at least seven days of recovery from surgery and of adaptation to the recording chamber, animals were recorded for four consecutive days: Day 1 and 2 for the baseline (BL1 and BL2, respectively); Day 3, which was divided in two halves; 12-h sleep deprivation (SD), from 09:00h till 21:00h (sleep deprivation was carried out manually by the gentle handling of animals, immediately after EEG signs of NREMS were detected) and 12-h recovery, for the rest of the day

(R0, 21:00h – 09.00). The whole Day 4 was aimed at monitoring the completion of the recovery from sleep deprivation (R1).

In all groups the baseline recordings under normal laboratory conditions (BL1 and BL2) were preceded by a day of trial recording, aimed at verifying the good functionality of the recording apparatus. The beginning of each day's record (09.00 h) was made to coincide with the light-on time of the normal light-dark cycle. In every day, 15 minutes after illumination of the lamp in the box have been constantly employed for the cleaning of the cages and the control of correct acquisition of data relating to the previous day. The animals were recorded in pairs, and had been used two boxes of recording. For each group the experiment was conducted always in parallel between two animals NC and two animals HC.

Every couple underwent the recording session individually in a separated lab to prevent any effect on the results of the counterpart animal. The recordings in the two different boxes alternated between NC and HC animals.

Due to e major problem in the EEG signal, one NC animal has been excluded from the experiment. Therefore data relative to only 7 NC animals will be presented.

3.3.2 DATA COLLECTION

Scoring of S-W episodes and analysis of the arterial blood pressure signal were conducted as hereafter: a visual scoring of wake-sleep states were performed on all consecutive 4-s epochs based on EEG and EMG signals. Inter-peak interval between two consecutive pressure pulse was used to derive the heart rate (HR).

Extreme attention was placed in the determination of the sleep microstructure, especially in the discrimination of REMS episodes between single REMS episodes (separated by long REMS intervals, > 3 min) and sequential REMS episodes (separated by short REMS intervals, <= 3 min), according with previously published methods (Amici *et al*., 1994).

Differentiation between the different wake-sleep stages were done offline using Spike-2 (CED) software based on the measured parameters. The data were

then filtered using custom software developed in LabView 6i (National Instruments) in order to remove the artefacts. By this software we were able to average each parameter in 30 minutes windows.

Through this type of analysis it has been possible to study the following variables: i) The total amount of Wake, NREMS, REMS, Sequential and Single REMS; ii) Number and duration of Sequential and Single REMS episodes; iii) Power density of the Delta and Sigma bands in NREMS, and the Theta band in REMS; iv) Hypothalamic temperature during the different wake-sleep states v) Systolic, diastolic and mean arterial BP and HR during the different W-S states.

The data collected from the diaphragmatic EMG are still under analysis and will not be shown in this thesis.

3.3.3 STATISTICAL ANALYSIS

Statistical analysis was carried out by ANOVA (SPSS 9.0). A number of pre-planned orthogonal and non-orthogonal contrasts were made by means of the modified t-test (Winer, 1971). For the non-orthogonal contrast the alpha level was adjusted by the "sequential" Bonferroni correction (Holm, 1979).

Two-way ANOVA for repeated measures on one factor was used, with either a 24-h, or a 12-h, or a 2-h resolution according to the different parameters analyzed.

In particular, for the 24-h resolution analysis were considered as Main Factors: i) Factor "Time", which was considered for the repeated measures, with four levels (Day 1 -4); ii) Factor "Diet" with two levels (NC, HC). The following orthogonal contrast [NC-BL1; NC-BL2] vs. [HC-BL1; HC-BL2], aimed at comparing NC to HC was carried out.

For the 12-h resolution analysis were considered as Main Factors: i) Factor "Time", which was considered for the repeated measures, with eight levels (BL1-L, BL1-D, BL2-L, BL2-D, SD-L, R0-D, R1-L, R1-D); ii) Factor "Diet" with two levels (NC, HC). Orthogonal contrasts were carried out aimed at comparing NC to HC values, while non-orthogonal contrast were carried out aimed at comparing: i)

L to D values within the Baseline (BL1, BL2) and within R1; ii) each 12-h L or D value of Day 3 or Day 4 to the corresponding L or D level of the Baseline (Day 1-2). In particular, the following orthogonal contrasts: i) [NC-BL1-L, NC-BL2-L] vs. [HC-BL1-L, HC-BL2-L]; ii) [NC-BL1-D, NC-BL2-D] vs. [HC-BL1-D, HC-BL2-D]; iii) [NC-SD-L] vs. [HC-SD-L]; iv) [NC-R0-D] vs. [HC-R0-D]; v) [NC-R1-L] vs. [HC-R1-L]; vi) [NC-R1-D] vs. [HC-R1-D]; and the following non-orthogonal contrasts: i) [NC-BL1-L; NC-BL2-L] vs. [NC-BL1-D, NC-BL2-D]; ii) [HC-BL1-L; HC-BL2-L] vs. [HC-BL1-D, HC-BL2-D]; iii) [NC-R1-L] vs. [NC-R1-D]; iv) [HC-R1-L] vs. [HC-R1-D]; v) [NC-BL1-L; NC-BL2-L] vs. [NC-SD- L]; vi) [HC-BL1-L; HC-BL2-L] vs. [HC- SD- L]; vii) [NC-BL1-D; NC-BL2-D] vs. [NC- R0- D]; viii) [HC-BL1-D; HC-BL2-D] vs. [HC- R0- D]; ix) [NC-BL1-L; NC-BL2-L] vs. [NC- R1- L]; x) [HC-BL1-L; HC-BL2-L] vs. [HC-R1-L]; xi) [NC-BL1-D; NC-BL2-D] vs. [NC-R1-D]; xii) [HC-BL1-D; HC-BL2-D] vs. [HC- R1- D], were carried out.

For the 2-h resolution analysis were considered as Main Factors: i) Factor “Time”, which was considered for the repeated measures, with 48 levels (2h * 4 Days); ii) Factor “Diet” with two levels (NC, HC). Orthogonal contrasts were carried out aimed at comparing NC to HC values for each 2-h time interval, while non-orthogonal contrast were carried out aimed at comparing: i) L to D values within the Baseline (BL1, BL2) and within R1 for each 2-h time interval; ii) each 2-h value of Day 3 and Day 4 to the corresponding 2-h level of the Baseline (BL1, BL2).

The weights of the animals were statistically analyzed by one-way ANOVA.

Throughout all analyses, differences were considered statistically significant when $P<0.05$.

4.Results

4.1. BODY WEIGHT

As shown in Fig. 3, after 8 weeks of treatment the weight of the animals was higher in the HC group than in the NC group (HC, 557±17 g; NC, 441±17 g; $p<0.05$). At the seventh week, the weight of the animals that have been selected in the NC group was 420±12g and it was not different from that of those that were not selected, i.e. 436±15. On the contrary, the weight of the animals that were selected in the HC group was 516±18g, significantly different from that of those that were not selected, i.e. 442±10. The latter rats were not heavier than those of the NC group, confirming that only about 50% of animals fed a HC diet develop obesity.

4.2 ANALYSIS OF THE WAKE-SLEEP STATES UNDER BASELINE CONDITIONS

As shown in Fig. 4, the proportion between the amount of Wake and Sleep was different in the two experimental groups when analyzed on a 24-h time scale. In particular, in the NC group the amount of Wake was largely over the 50% of total time and it was significantly higher than in the HC group ($p<0.05$). Therefore, reversely, the amount of total sleep was lower in the NC than in the HC group. In particular, the amount of both NREMS and REMS was significantly larger in the HC group compared to the NC one, although only for NREMS the statistical significance was reached ($p<0.05$).

The analysis of this parameter was also carried out on a 12-h time scale and is shown in Fig. 6. The distribution of the W-S states followed the normal Light-Dark (LD) pattern, since in the NC group the amount of Wake (and, reversely, the amount of total sleep) was significantly larger during the D period ($p<0.05$) than during the L period, confirming that the rat is more active during the D hours. This pattern was maintained in the HC group, although it was largely dampened due to a specific significant depression of Wake occurrence during the

D hours ($p<0.05$), with no differences with the NC group in the L hours. The reverse was observed for both NREMS and REMS, since the HC animals slept more than the NC animals during the D hours ($p<0.05$, for both) In particular, the increase of REMS occurrence was so large that the normal LD distribution of this sleep stage disappeared.

The analysis of the partition of REMS in Single REMS (episodes which are both preceded and followed by a long REMS interval, > 3min) and Sequential REMS (episodes which are preceded and/or followed by a short REMS interval, <= 3min) showed that the increase of REMS during the D hours was mostly due to a significant increase in Sequential REMS ($p<0.05$). This effect was accompanied by the disappearance of the normal LD distribution of Single REMS).

A more detailed analysis on the number and duration of Single and Sequential REMS episodes is shown in Fig.7. Still, the effect of HC diet delivery was on Sequential REMS, leading to an increase in the number of Sequential REMS episodes ($p<0.05$) during the D hours, with almost no effects on the duration of the episodes. Also, the normal LD distribution of Sequential episodes disappeared in the HC group.

4.3 ANALYSIS OF THE AUTONOMIC PARAMETERS UNDER BASELINE CONDITIONS

As shown in Fig.9, major changes were observed on a 24-h basis on the cardiovascular parameters which were taken into account. In particular, the HC animals showed to be hypertensive when compared to the NC ones, since the average 24-h mean arterial BP significantly ($p<0.05$) increased from 88.7±5.2 mmHg to 95.9 ±1.2 mmHg. The opposite was observed for average HR, which was slightly but significantly ($p<0.05$) lower in the HC group (350 ± 9) than in the NC group (343 ± 7 bpm). No relevant changes were observed in the average Thy levels.

These changes were substantially confirmed by the 24-h state-dependent analysis of the three parameters (Fig.10). In particular, mean arterial BP was significantly higher ($p<0.05$) in each of the three W-S states in HC than in NC animals. HR was also lower in each of the three states in the HC animals, but the difference reached the statistical significance only during Wake ($p<0.05$). Again, non changes were observed in Thy.

A more detailed analysis of the autonomic parameters was carried out on a 12-h LD time scale. In NC animals, Thy levels showed the normal LD cyclic pattern, with higher values during the D hours in each W-S state. This oscillation was confirmed in HC animals, but for REMS, where the oscillation was not statistically significant. It is worth noting that this effect was concomitant with the large aforementioned increase in REMS occurrence during the D period.

Average systolic, diastolic and mean arterial BP were significantly higher ($p<0.05$ for all comparisons) in HC animals compared to NC ones in each of the three W-S states. Furthermore, a significant LD oscillation appeared in HC animals in systolic, diastolic and mean arterial BP in Wake and in systolic and diastolic arterial BP in NREMS, with higher levels during the D period, which was not observed in NC animals. Concomitantly, HR levels were significantly lower ($p<0.05$) during Wake and REMS in the D period only in the HC group, while a not significant decrease was also observed in NREMS.

4.4 ANALYSIS OF THE WAKE-SLEEP STATES DURING THE 12-h PERIOD OF TOTAL SLEEP DEPRIVATION AND IN THE FOLLOWING RECOVERY PERIOD

The analysis of the W-S states during the 12-h period of total sleep deprivation by gentle handling and in the following period of recovery (R0, which corresponds to the D period of the same experimental day (Day 3) in which the deprivation has been carried out, and R1, which is the following experimental day (Day 4) is shown with a 12-h time scale in Fig. 16.

The analysis of the results showed that during the period of sleep deprivation Wake didn't reach the 100%, as may be expected. This depend on the fact that in order to minimize the stress of the animal due to the manipulation to keep it awake, the intervention of the experimenter only follows the first signs of NREMS. On this basis, as expected a few NREMS, but no REMS, was observed during the SD period. REMS was not observed since, of course, some NREMS is necessary for REMS to occur, and the intervention of the experimenter was always quick enough after the start of a NREMS episode to prevent the occurrence of REMS.

The results showed that, as expected, in the NC group the amount of Wake was lower during R0 than during the corresponding D period of BL ($p<0.05$), while no significant post effects were observed during R1, suggesting that the most of the expected sleep rebound occurred during R0. This pattern was reproduced in the HC group, in which the amount of Wake was significantly lower ($p <0.05$, for all comparisons) than in the NC group not only during the D period of BL (as already described), but also during both R0 and the D period of R1. Reciprocal results were observed for both NREMS and REMS, although the REMS rebound during R0 was not significantly larger in HC than in NC.

The analysis of the partition of REMS in Single and Sequential REMS (Fig. 17) clearly indicated that REMS rebound occurred under the form of Sequential REMS in both NC and HC animals, while no rebound of Single REMS was observed. The Sequential REMS rebound was large and significant during R0 in both groups ($p<0.05$, for both), but reached the statistical significance during the L period of R1 for NC animals only, while the amount of Sequential REMS amount was significantly lower ($p <0.05$) in the HC group compared to the NC group in the L period of R1.

The detailed analysis of the number and duration of Single and Sequential REMS episodes (Fig. 18 and 19, respectively) showed that the REMS rebound occurred substantially through and increase in the number of Sequential REMS episodes in both experimental groups, while no substantial changes in the number of Single REMS episodes were observed. The pattern of changes in the number of

Sequential REMS episodes overlapped even in statistical terms that of the amount of Sequential REMS. Minor changes, although statistically significant ($p < 0.05$), in the duration of Sequential REMS episodes were observed in both groups during R0 only, while a little increase in the duration of Single REMS episodes was observed in NC animals only.

The analysis of the dynamics of the sleep rebound process is shown for NREMS and REMS in Fig. 20 and 21, respectively, in which the cumulative amount of both sleep states across the experimental sessions is shown. Data are shown as the accumulation of 12-h period amounts which are expressed as the percent of the 24-h baseline value (which is therefore taken as 100%). No substantial differences in the accumulation of NREMS were observed in the two groups. The actual loss of NREMS during SD was 53.4% of daily amount for NC animals and 52.4% of daily amount for HC animals, while the cumulative amount of the SD_R0 and R1 Days, which was expected to be 200% in the absence of any deprivation, was actually 162.3 ± 5.3% for NC animals and 165.4 ± 3.8 for HC animals, showing a weak NREMS rebound in both groups.

For what concerns REMS, the loss was of 59.6% and 49.1% for NC and HC animals, respectively. For both the cumulative amount of the SD_R0 and R1 Days, which was expected to be 200% in the absence of any deprivation, was actually close to 200%, since it was 197.7 ± 11.0 % for NC and 191.5 ± 11.9% in NC and HC animals respectively, showing an almost complete REMS rebound in both groups. The dynamics of the process underlying the REMS rebound appeared to be faster in the NC group, since a statistically significant ($p<0.05$) larger accumulation of REMS was observed in NC at R1_L.

The study of the dynamics of the sleep rebound has been completed by the analysis of the time course of Delta and Sigma Power in NREMS (Fig. 22) and Theta Power in REMS (Fig. 23). The dynamics of Delta Power followed a similar pattern in NC and HC animals. As it may be expected, Delta Power increased largely over the baseline values in the NC group in the few NREMS episodes which occurred during the last two hours ($p<0.05$) of the sleep deprivation process, when a sleep debt had already been accumulated. The peak in Delta

power occurred during the first two-four hours of the recovery period ($p>0.05$) and progressively decreased to normal levels at the end of R0, returning to baseline levels in R1. A similar pattern was observed for HC animals, even if the increase in Delta Power during sleep deprivation was observed two hours in advance and was significantly different form baseline levels only in the first two hours of R0. No significant differences in the dynamics were observed between the two groups.

Similarly, the profile of Sigma Power, which typically showed a clear LD oscillation in the baseline, was clearly shifted towards higher level with the progression of the deprivation and during the initial part of the recovery. However, apparently due to the large variability of data, the difference with the baseline was significant only during the third 2-h interval on R0 in the HC group. Still, no significant differences in the dynamics of the process were observed in the two experimental groups.

Also for Theta Power in REMS, no significant difference were observed between the two groups. The expected increase in Theta Power during the initial R0 was not large enough to reach a statistically significant level.

4.5 ANALYSIS OF THE AUTONOMIC PARAMETERS DURING THE 12-h PERIOD OF TOTAL SLEEP DEPRIVATION AND IN THE FOLLOWING RECOVERY PERIOD

Hypothalamic temperature levels were similarly affected by the manipulation of the animals in both groups, since Thy was largely increased during the induced Wake in SD ($p<0.05$, for both) and even in the few NREMS episodes which occurred during SD ($p<0.05$, for both). However, the effect of the manipulation on this parameter appeared to be more intense in the HC group than in the NC group during NREMS ($p<0.05$). The pattern of Thy levels was similar

in the two groups also during the Recovery period, however Thy in NREMS remained larger during R1_D in the HC group ($p<0.05$).

For what concerns the mean arterial BP levels, an increase in this parameter was observed during SD in the NC animals in both Wake and NREMS compared with the L period of the baseline ($p<0.05$, for both). These values returned to baseline levels in R0 and even to levels lower than those of the baseline during R1_D in each of the three W-S states ($p<0.05$, for all comparisons). The increase in mean arterial BP levels in Wake and NREMS during SD was observed also in the HC group ($p<0.05$ for both). Furthermore, HC animals showed higher arterial blood pressure values than NC animals throughout the experiment and in each of the W-S states ($p<0.05$, for all comparisons). Interestingly, the aforementioned arterial blood pressure drop during R1_D was not present in HC animals.

Finally, in NC animals the pattern of changes in HR practically overlapped with that observed for arterial BP, although the fall during R1_D compared to the D period of the baseline was significant only during NREMS and REMS ($p<0.05$ for both). Differently from what observed for mean arterial BP, the fall in HR was also observed in HC animals in NREMS and REMS ($p<0.05$ for both). Furthermore, heart rate was significantly lower in HC animals than in NC animals during R0 throughout the W-S states ($p<0.05$, for all). The statistical significance of the difference between NC and HC levels disappeared during R1, possibly due to the evident drop of HR observed in the NC group.

5.Discussion

The results of the present study indicate that, in the rat, the long-term administration of a high-energy hypercaloric diet leading to the development of a frank diet-induced obesity (DIO) produces relevant changes in the W-S pattern and leads to a consistent increase in the arterial BP levels which persisted in each of the three W-S states (Wake, NREMS, REMS).

The selection of the animals may have introduced some bias, since it is not possible to distinguish among obesity prone (OP), which will become obese when fed a HC diet, and obesity resistant (OR) rats, which will not develop obesity, before the long-term delivery of the HC diet (Levin *et al.*, 1983, 1997). On this basis, the selection of NC animals was made randomly, and, possibly, a 50%/50% of obesity prone (OP) and OR rats may have been selected. On the contrary, the HC animals which were selected for the study were among those over the median weight of the HC population after 8 weeks of treatment, and therefore within those which could be actually classified among DIO rats. In spite of this, since the authors who studied the DIO rat model stated that clear differences in metabolic and brain functions between OP and OR rats only emerged after a long-term delivery of the HC diet or after the development and perpetuation of the genetic traits of the two populations following selective inbreeding (Levin *et al.*, 1998), it can be assumed that NC animals that were used in the present study constituted a reasonably good control group for obese animals. Also, the possibility to use animals that didn't gain weight after HC diet delivery as a control group was discarded due to the fact that it couldn't be excluded that the absence of weight gain came from the sufferance of the animal from any kind of injury different from being OR Furthermore one major determinant of the development of DIO is the increase in energy intake that was observed in OP rats compared to OR ones when exposed to the HC diet, which is much more palatable (Levin *et al.*, 1983). Although other autonomic and metabolic determinants has been indicated, in particular after selective inbreeding (Levin *et al.*, 1997), the largest difference between OP and OR animals comes from the amount of HC food ingested. Therefore, OR animals couldn't fully match HC animals.

The results of the present study clearly indicate that the 24-h total sleep time is increased of about 10% in HC animals compared to NC ones. The effect is mostly due to a large increase in both NREMS and REMS during the activity period (that is the D period) of the normal LD cycle, leading to a disappearance of the normal LD oscillation of REMS occurrence.

An effect on the W-S pattern, mostly consistent in an increase in sleep occurrence has been observed in several rodent models of obesity previously studied. However, the outcome from different studies are slightly different, in particular when results obtained in rats are compared to those in mice and when the effects observed in animals which have been made obese by a HC diet delivery are compared to those observed in animals made obese by interfering with the normal function of the leptinergic system.

The W-S pattern after the development of obesity following the administration of a HC diet have been consistently studied in mice only, which were made obese by a 2-10 week HC diet administration (Jenkins *et al*., 2006; Guan *et al*, 2008). In the study by Jenkins, total sleep was observed to be increased mostly during the D period of the LD cycle, but this was due to an increase in NREMS without any effect on REMS. The weak or absent effects on REMS were confirmed by Guan, since a significant, but transient, increase in REMS occurrence accompanied the consistent increase in NREMS only in one of the two experimental groups studied. On the overall, some dampening in the normal LD distribution of activity and rest was observed, but not in that of REMS.

The only two studies in which a HC diet has been delivered to a rat are a pioneering research by Danguir (1987) and a more recent study by Laudadio (2010). In the first study, an increase in both NREMS and REMS were observed after just 10 days of HC diet delivery, well before the development of a frank obesity, while in the second (which was part of a PhD thesis; data have still not been published on the international literature) an increase in the total amount of sleep was observed after 8 weeks of HC diet delivery, that was mostly explained by an increase in the amount of REMS. An overall tendency to sleep enhancement during the D period was observed.

In other studies, the W-S states have been analysed in animals in which obesity was developed by genetic manipulations aimed at interfering with the leptinergic signalling. With respect to this, mice have been deprived of the ability to produce leptin (*ob/ob* mice) or have become resistant to the action of the hormone itself through the induction of a mutation of the hypothalamic receptor for leptin (*db/db* mice). The *ob/ob* mice, showed an increase in NREMS amount, mostly during the D period, but no changes in the total amount of REMS, with a significant attenuation of the amplitude of the normal LD distribution of NREMS and REMS compared to their controls (Laposky *et al.,* 2008; Silvani *et al*. 2009), due to a redistribution of sleep states between L and D. Similar results were observed in *db/db* mice (Laposky *et al.,* 2008), which even showed a concomitant reduction of the 24-h amount of REMS. Studies on rats have been carried out on Zucker *fa/fa* animals, which lack leptin receptors, which showed either an increase in NREMS (Danguir *et al.,* 1989), with no effects on REMS sleep and LD distribution of W-S states, or even no effects on the amount of sleep (Megirian *et al.* 1998).

Thus, from one side it seems that, in mice, the development of obesity has prevalent effects on NREMS enhancement, mostly during the D period, and is accompanied by a weak enhancing effect on REMS in HC-diet delivered animals only. No major effects on REMS amount was observed in leptin-absent or leptin-resistant animals, in which the only effect on REMS was the dampening of the daily REMS oscillation. From the other side, in the rat, studies on HC diet-delivery leading to obesity are missing or still unpublished, but suggest an enhancing effect of DIO on sleep occurrence, and in particular on REMS, while studies on leptin-resistant animals indicate an inconsistent increase in NREMS only.

Therefore, the present study supports the studies on mice, which showed the tendency of the animal to sleep more during the normal activity period after the development of obesity, but also shows that, differently from what observed in *ob/ob* and leptin-resistant mice, the increase in REMS occurrence during the D period was not accompanied by a decrease in REMS occurrence during the L

period and therefore, even if in the present study the increase in REMS did not reach the significance level on a 24-h time scale, it can be concluded that REM sleep enhancement during the activity D period is not explained by its depression during the rest L period.

The results of the present study therefore support those from studies in obese humans (Vgontzas *et al.* 1998), which suggest that the tendency of obese humans to sleep more during the day cannot be only explained on the basis of the sleep disruption during the night (which usually depends on the development of an obstructive sleep apnea syndrome in the obese, leading to sleep fragmentation), but appears to be more related to a metabolic and/or circadian abnormality due to the disorder. This finding underlines the need for the development of a reliable animal model of DIO, in order to develop further physiological studies aimed at addressing and clarifying these issues.

The analysis of the partition of REMS in Single and Sequential REMS clearly indicated that REMS enhancement during the D period was the effect of an increase in Sequential REMS, due to an increase in the number of Sequential REMS episodes. This finding fully confirm that the occurrence of Sequential REMS is the modality through which REMS occurrence is physiologically modulated in the rat when the animal has to produce more REMS, e.g. to compensate for a previous REMS deprivation, or to cope with environmental conditions which are known to induce either a suppression or an enhancement of REMS occurrence (Amici *et al.,* 1994, 1998; Zamboni *et al.,* 2001; Cerri *et al.,* 2005). The production of Sequential REMS, in which the REMS episodes occur in rapid sequence within a cluster, is considered to be safer for the animal, since it allows him to produce longer REMS episodes with brief interruptions, in which the possibility to prolong the episode may be checked (Amici *et al*., 1994). In fact, physiological regulation during REMS has been shown to shift from a full homeostatic to a poikilostatic modality (Parmeggiani, 2005), during which the suspension of the homeostatic control of body temperature represents the main feature. On this basis, it may be assumed that the brief interruptions within REMS episodes allow the animal to check whether the level of the physiological

variables still fit the "safety" requirements for a further prolongation of the REMS episode. Thus, the fact that REMS enhancement in the obese rat is modulated through the modality which is commonly observed in this species suggests that such an enhancement represents a specific physiological need for the obese animal and that such REMS would be part of a quota that would be defended and, therefore, recovered following sleep deprivation.

The observed increase in NREMS and REMS in the obese animal is difficult to interpret, mostly due, of course, to the poor knowledge of sleep functions. For sure, it cannot be considered to be a mechanism aimed at energy saving, since this would go in the opposite direction with respect to the metabolic needs of the animal. More reliably, it may be assumed that metabolic signals coming from the energy deposits operate at hypothalamic level within the neural network where the control of W-S states and the regulation of food intake/metabolism overlap. With respect to this, possible targets of this overlap could be the hypocretinergic (HCRT) neurons of the lateral hypothalamus which are known to promote both active Wake and food intake (Sakurai *et al.,* 2011). The activity of HCRT neurons has been shown to be inhibited by both glucose (Burdakov *et al.,* 2006) and leptin (Wynne *et al.,* 2005). In other terms, it may be assumed that in the presence of a large energy store and, consequently, of high circulating leptin levels, there would be no need for the animal to be active for foraging.

The analysis of the sleep pattern during the recovery period which followed sleep deprivation showed the absence of major differences in the dynamics of both NREMS and REMS rebound between obese and lean animals. From one side, this indicates that the brain mechanisms underlying sleep homeostasis are not altered in obese animals. Since the control of these mechanisms is largely attributed to different hypothalamic structures mostly at preoptic level (Szymusiak *et al.,* 2007), it appears that the functional alteration in the metabolic function of the obese animals doesn't interfere with these structures. With respect to this, it has to be reminded that the REMS rebound which follows REMS deprivation has been shown to be largely depressed when the cellular

activity is functionally impaired at preoptic-hypothalamic level, in regions involved in the regulation of both sleep and body temperature/metabolism (Zamboni *et al,* 2004).

From the other side, the presence in the HC animals of a sleep rebound the intensity of which substantially overlap that observed in NC animals also in terms of the amount of sleep recovered calculated as the percent of sleep produced under baseline conditions, clearly indicate that sleep produced by the HC animals is defended and normally recovered following a challenge which leads to its loss. It is known that REMS is precisely homeostatically regulated in terms of its amount in different species (Parmeggiani *et al*., 1980; Cerri *et al*, 2005; Amici *et al*, 2008). In particular, in the rat a fast rebound is observed following deprivation in which the urgent need of REMS is satisfied, which is followed by a slow (probably less urgent) rebound, which leads in few days to a 100% recovery of the REMS loss. In the present experiment, the fact that both NC and HC animals practically fully recover the REMS loss in less than two days, suggests that the apparent excess of REMS produced during the D period by the HC animal when compared to the NC one cannot be "used" by the animal to buffer the previous loss. In other words, this excess of REMS is not part of what has been previously described as "facultative" REMS in the cat (Parmeggiani *et al.,* 1980).

Also the dynamics of the NREMS rebound, which usually occurs in terms of an increase of NREMS intensity marked by an increase in the intensity of the Delta Power of the EEG, is not different in the two groups. It may be reminded that the absolute levels of Delta Power cannot be compared in the two groups, since the amplitude of the EEG signal may be largely influenced in different animals by physical factors not linked to the real activity of the source (e.g., the position and deepening of the electrodes on the scalp). However, the fact that the proportion between the Delta Power intensity during the NREMS rebound which followed enhanced Wake induced by gentle handling and that observed in the baseline was maintained at the same level in the two groups suggests that the quality of Wake under baseline conditions, which is known to largely influence

Delta Power in the following NREMS (Tononi and Cirelli, 2003; Borbély and Achermann, 2005), was not substantially different between HC and NC animals.

The analysis of the autonomic parameters in the two groups has shown that DIO rats develop a hypertensive state, with an increase in systolic, diastolic and mean arterial BP that is maintained across the different W-S states during both the L and the D period of the LD cycle.

Data shown in Fig 10 confirm that in the rat, as it occurs in humans, mean arterial BP levels changes through the different W-S states, reaching a maximum during Wake and a minimum during NREMS, while during REMS an increase towards Wake levels is observed, the degree of which is in accordance with the average duration of REMS episodes and the degree of phasic cardiovascular activations (Sei *et al,* 1997; Amici *et al.,* 2013). The presence of variable changes, in accordance to their genetic background, in mean arterial BP in the transition from NREMS to REMS has also been shown in mice (Silvani *et al.,* 2009; Campen *et al.,* 2002). This state dependent oscillation was apparently maintained in HC animals, during both the L and the D period of the LD cycle. It needs also to be stressed that the apparently low absolute values of mean arterial BP that have been found in the present study in NC animals are compatible with the fact that, particularly in small rodents, arterial BP levels are largely influenced by the ambient temperature (Ta), and largely decrease when animals are kept at Tas ranging from 25° to 30° C (Sei *et al*., 1996).

Previous observations on the arterial BP levels in obese rats are inconsistent. In fact, while the development of hypertension has been shown after 8-10 weeks of administration of a hypercaloric (HC) diet (Dobrian *et al*., 2000), this observation has not been subsequently confirmed (Carroll *et al*., 2006). However, in the first study, arterial BP determination was made by the tail-cuff method, which is not fully reliable, since the animal is disturbed during the BP determination, and this doesn't allow the experimenter to make determinations during either quite wakefulness or sleep. In the second study, where the determination was made more reliably by means of a chronically implanted telemetric transmitter, determinations were made randomly largely independently

from the W-S states. The consistent increase in mean arterial BP in each W-S-state that have been shown in HC animals in the present study, which is also characterized by the presence of a small variability of the parameter within obese subjects, makes the present observation quite reliable and suggests that frank hypertension develops in DIO rats, as already observed in DIO mice (Rahmouni *et al.,* 2005a).

The determinants of the increase in arterial BP in obese subjects are multiple and are still matter of debate (Rahmouni *et al.*, 2005b). The excess of leptin signalling in the obese subject is considered to be a possible determinant, since it has been shown that: i) leptin acts centrally as an activator of sympathetic nervous activity and metabolic expenditure, through brown adipose tissue activation (Rahmouni *et al*., 2005b); ii) chronic leptin i.c.v. administration induce hypertension and tachycardia (Shek *et al.,* 1998). However, although it has been shown that DIO mice don't develop resistance to the cardiovascular effects of leptin, differently form what happens for the effects on metabolism and food intake (Rahmouni *et al., 2*005a), it has also been shown that, after developing obesity, leptin deficient *ob/ob* mice are hypertensive during both Wake and NREMS (Silvani *et al.*, 2008). Among other possible hypertension-inducing factors in the obese are: i) the activation of the renin-angitensin system by the release of adipocyte-derived angiotensinogen; ii) a possible excess of aldosterone release; iii) a decrease in the vascular responsiveness to nitric oxide, due to the development of endothelial lesions; these changes would lead, on the overall, to vasoconstriction and to an increase in renal water and sodium retention (Rahmouni *et al., 2*005b). Interestingly, since, as previously discussed, heart rate has been shown to increase in mice after chronic leptin administration (Shek *et al.,* 1998) and mice have been shown not to become resistant to the cardiovascular effects of leptin, the mild but significant decrease in heart rate that was found in the present study would indicate that the at least part of the cardiovascular effects which have been observed in the present study were not leptin-dependent, or even that the observed increase in arterial BP was not due to a generalized activation of the sympathetic outflow.

Such an arterial BP dysregulation appeared to be more evident during the recovery day 1, since while an overall decrease of mean arterial BP accompanied the restoration of the normal daily activity pattern animals after the prolonged period of sleep deprivation and manipulation in the NC, this decrease was not observed in obese animals. Since, on the contrary, a decrease of HR rate during R1 was observed in both groups, it may be assumed that while HR mirrors the potential decrease in sympathetic activity which would reasonably accompany the processes of restoration in both groups, still, the (dys)regulation of arterial BP appears to be relatively independent from this supposed decrease in sympathetic activity in the HC group and, therefore, would be determined by factors other than a generalized increase in sympathetic tone.

Interestingly, no significant changes were observed in the level of hypothalamic temperature in the different W-S states between NC and HC animals. This suggests that in obese animals, in which passive thermal dissipation is much lower than in NC animals due to both the larger body mass and the presence of a higher fat content, either the basal metabolism is kept at a lower level or thermal dissipation is enhanced by, e.g., an enhanced vasodilation of the heat exchangers (tail, paws).

In conclusion, the results of the present experiment indicate that in the rat the development of obesity deeply interfere with both W-S and cardiovascular regulation and that diet-induced obesity rats represent a very good model to be used in order to go deeper in the understanding of the disturbances of the W-S activity and of the cardiovascular comorbidities which accompany the development of obesity in humans.

6. Figures

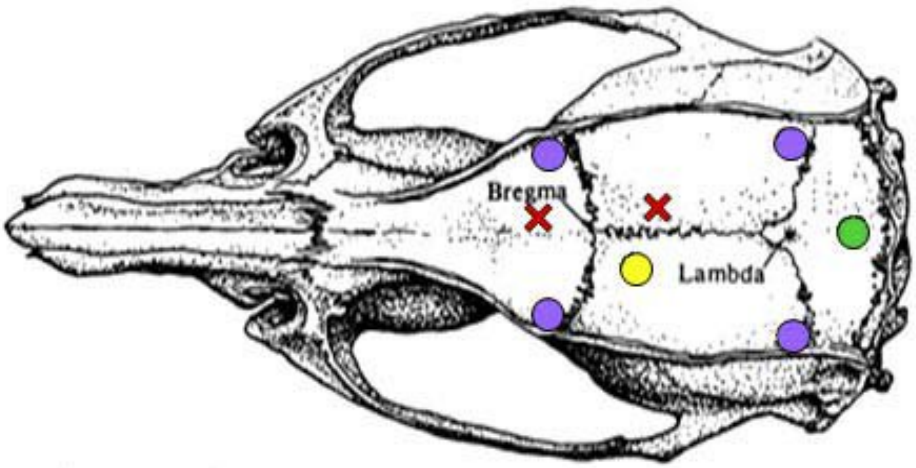


Figure 1. Schema of surgical cranial implant. Dorsal view of the rat's skull that shows the points where craniotomies were made during surgery on animals under general anesthesia. Four screws have been placed (purple) to anchor the implant, two in the anterolateral portion of the frontal bones, two in the posterolateral portion of the parietal bones; Two electrodes for detection of the Electroencephalographic signal (EEG; corss), one at + 3.00 mm anterior and at + 3.00 mm lateral to bregma, one in the parietal bone at -4.00 mm posterior and 1.00 mm lateral to bregma; a thermistor (yellow) for the detection of the hypothalamic temperature was placed -1.00 mm posterior and 1.00 mm lateral to bregma.

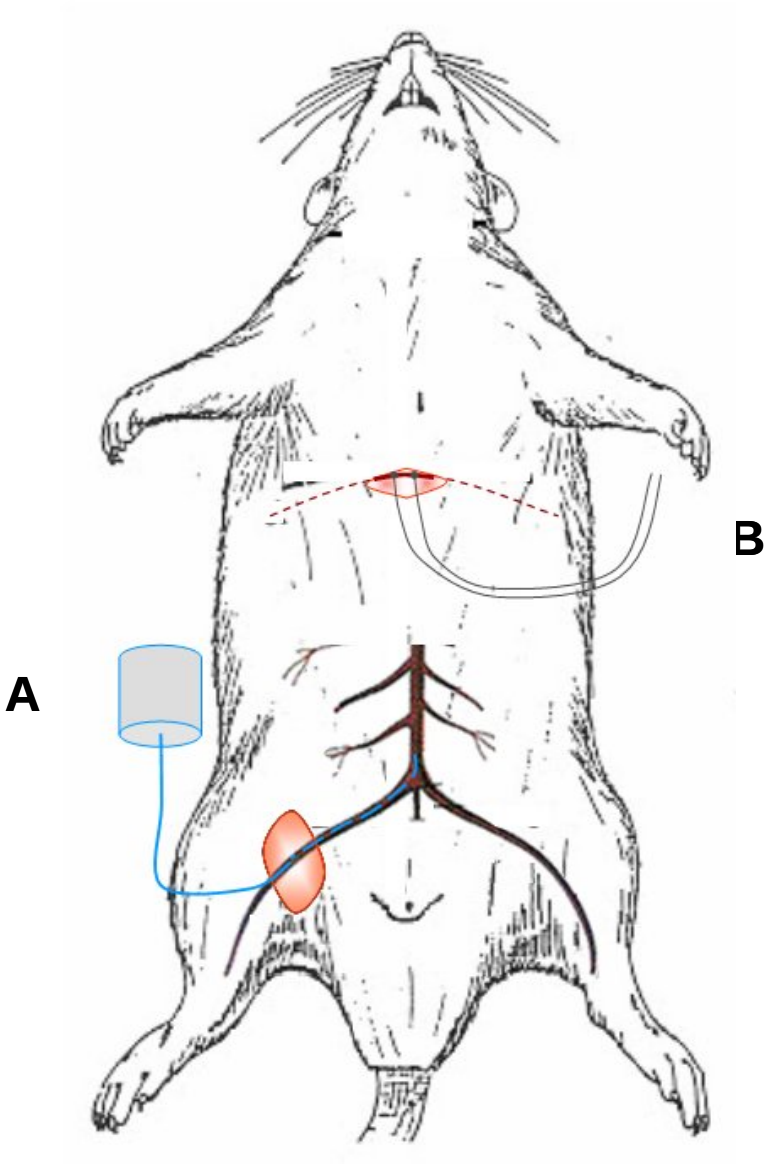


Figure 2. The figure shows a schematic illustration of the surgical implantation of the catheter for telemetric measurement of arterial pressure in the femoral artery (A) and the electrodes for recording of diaphragmatic electromyogram (B).

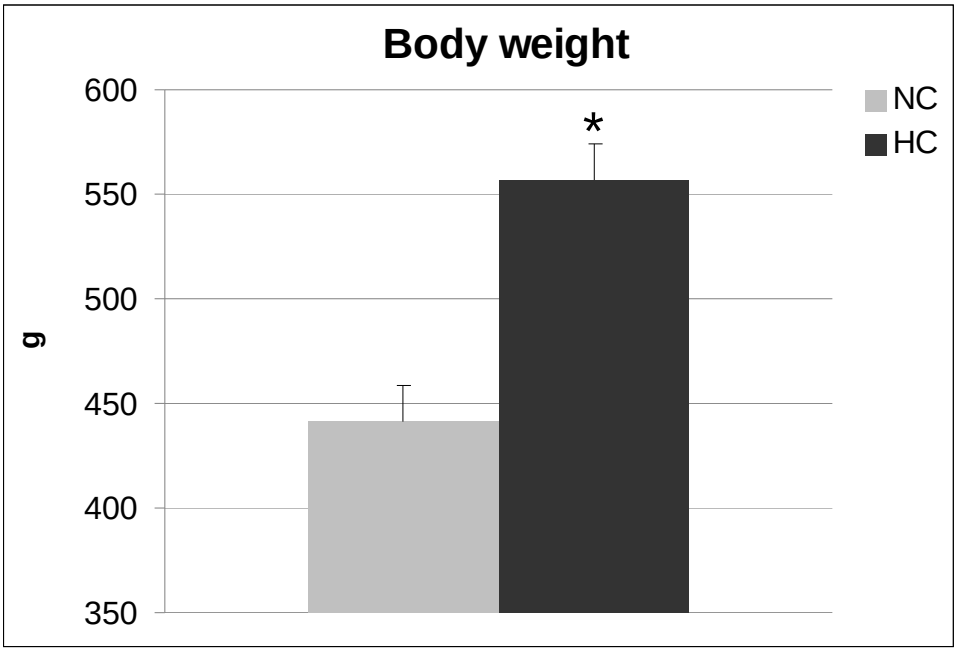


Figure 3. The weight of rats kept for 8 weeks under either a normocaloric (NC, n=7) or a hypercaloric (HC, n=8) diet is shown. * NC vs. HC, $p<0.05$

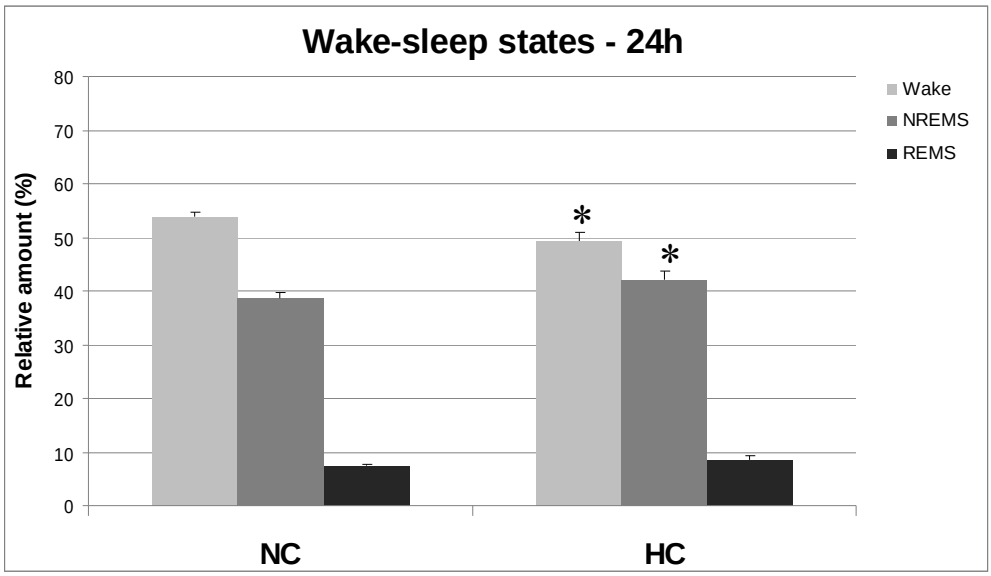
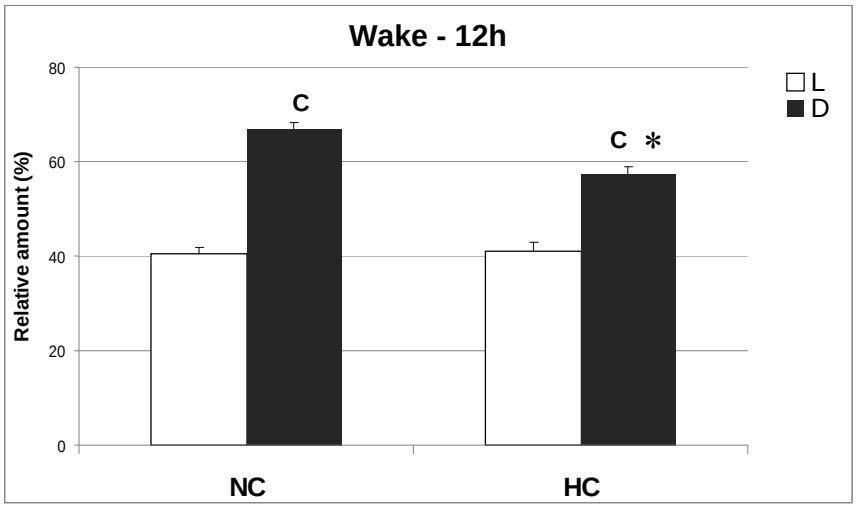
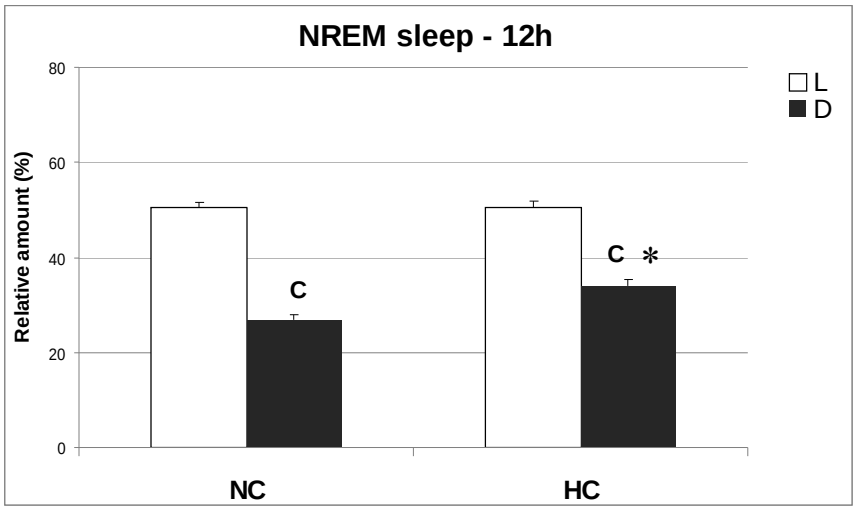


Figure 4. The relative amount (mean ± S.E.M.) of Wake, NREM sleep (NREMS) or REM sleep (REMS) during a 24-h period in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Amount is expressed as the percent of the 24-h period. Data represent the average of two consecutive days of baseline recording. * NC vs. HC, $p<0.05$.


Wake - 12h
Relative amount (%)
80
60
40
20
0
C
C *
L
D
NC
HC


NREM sleep - 12h
Relative amount (%)
80
60
40
20
0
C
C *
L
D
NC
HC

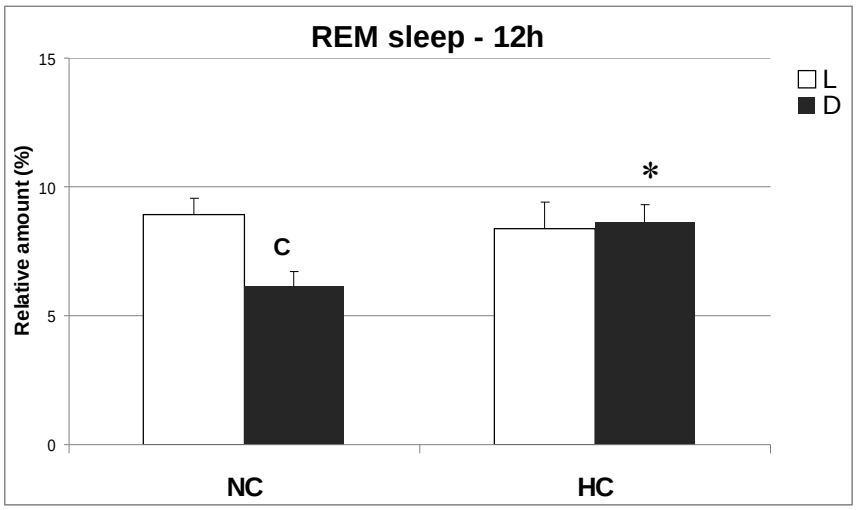

REM sleep - 12h
Relative amount (%)
15
10
5
0
C
*
L
D
NC
HC

Figure 5. The relative amount (mean ± S.E.M.) of Wake, NREM sleep or REM sleep during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Amount is expressed as the percent of the 12-h period. Data represent the average of two consecutive days of baseline recording. * NC vs. HC, $p<0.05$. ^{C} L vs. D, $p<0.05$.

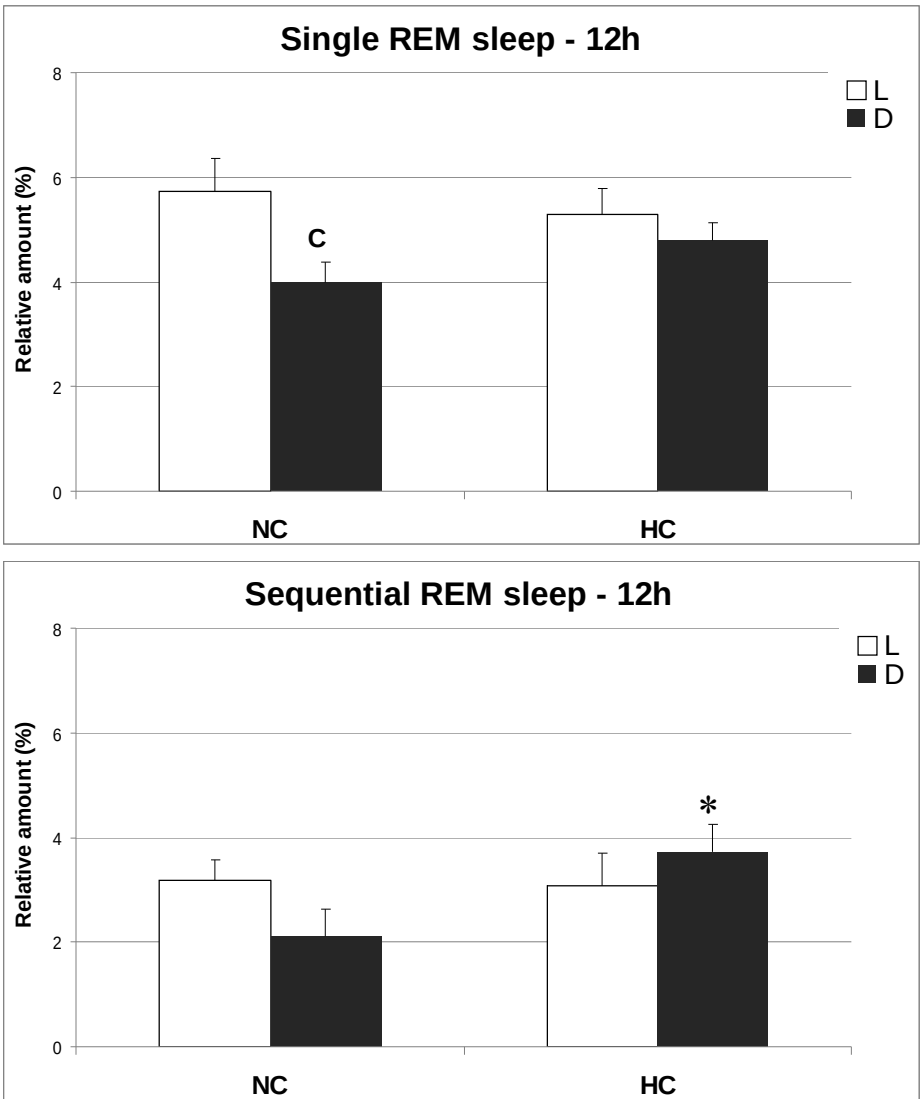


Figure 6. The relative amount (mean ± S.E.M.) of Single REM sleep or Sequential REMS sleep during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Amount is expressed as the percent of the 12-h period. Data represent the average of two consecutive days of baseline recording. * NC vs. HC, $p<0.05$. ^{C} L vs. D, $p<0.05$.

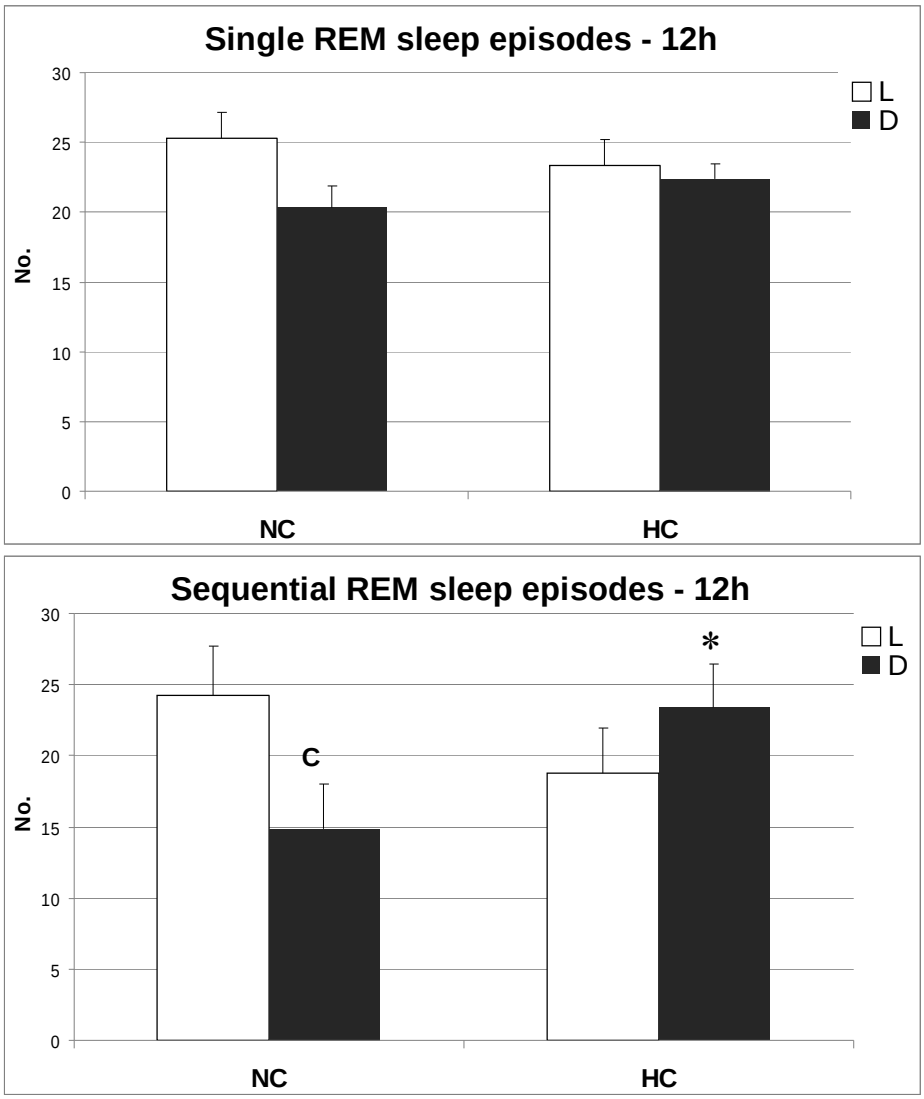


Figure 7. The number (No., mean ± S.E.M.) of Single REM sleep episodes or Sequential REMS sleep episodes during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Data represent the average of two consecutive days of baseline recording. * NC vs. HC, $p<0.05$. ^{C} L vs. D, $p<0.05$.

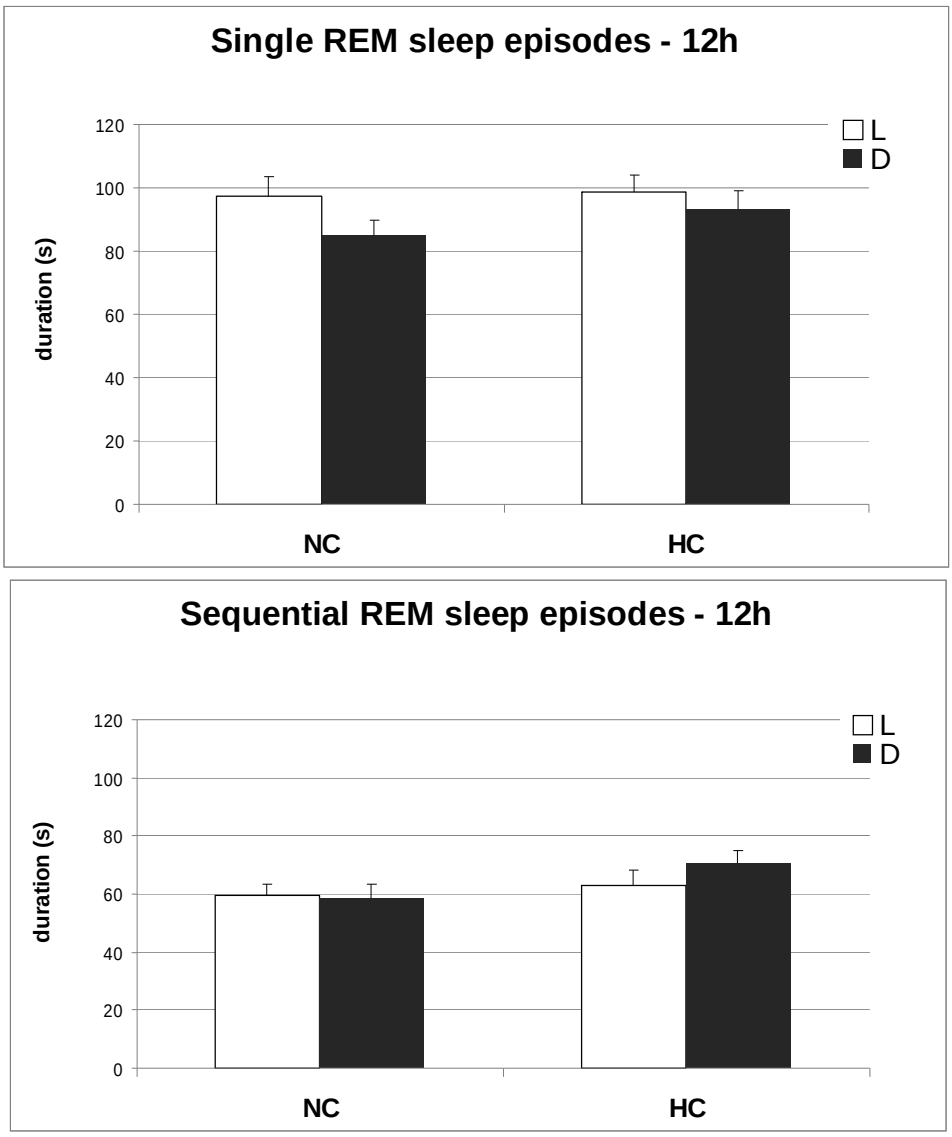
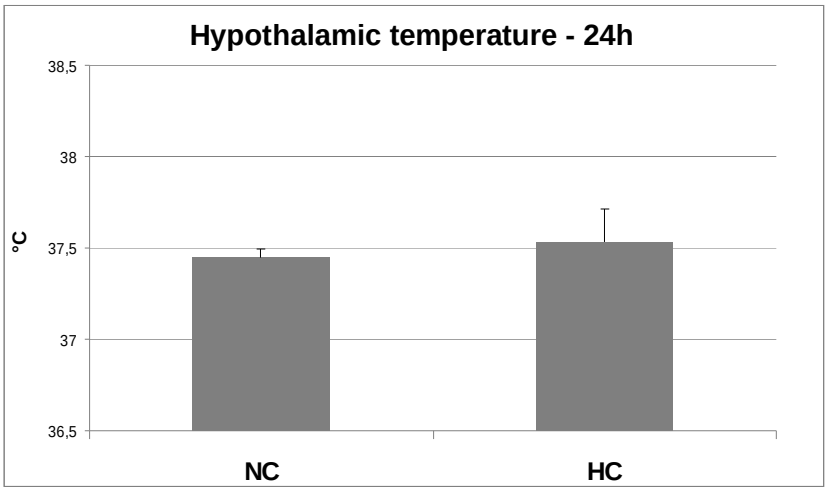
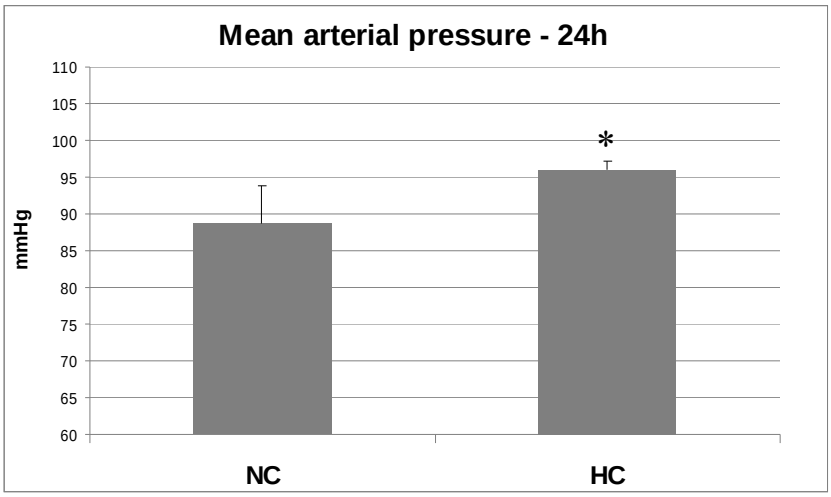


Figure 8. The average duration (s, mean ± S.E.M.) of Single REM sleep episodes or Sequential REMS sleep episodes during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Data represent the average of two consecutive days of baseline recording. * NC vs. HC, $p<0.05$. ^{C} L vs. D, $p<0.05$.


Hypothalamic temperature - 24h
38,5
38
37,5
37
36,5
°C
NC
HC


Mean arterial pressure - 24h
110
105
100
95
90
85
80
75
70
65
60
mmHg
*
NC
HC

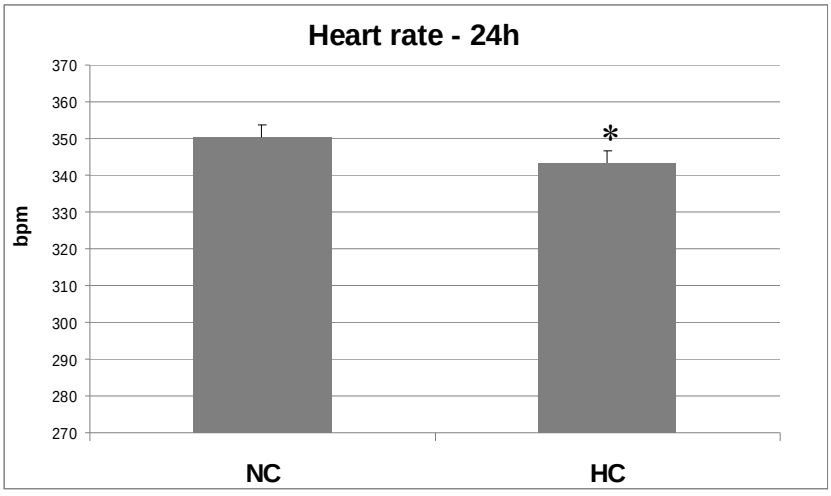
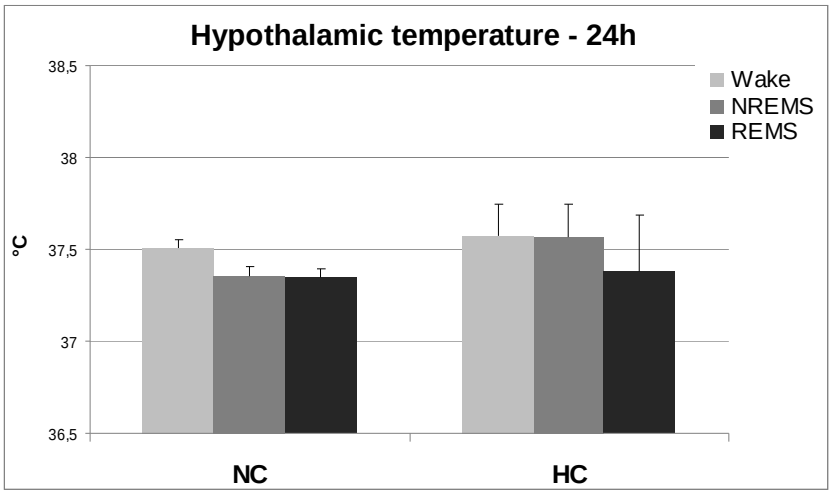
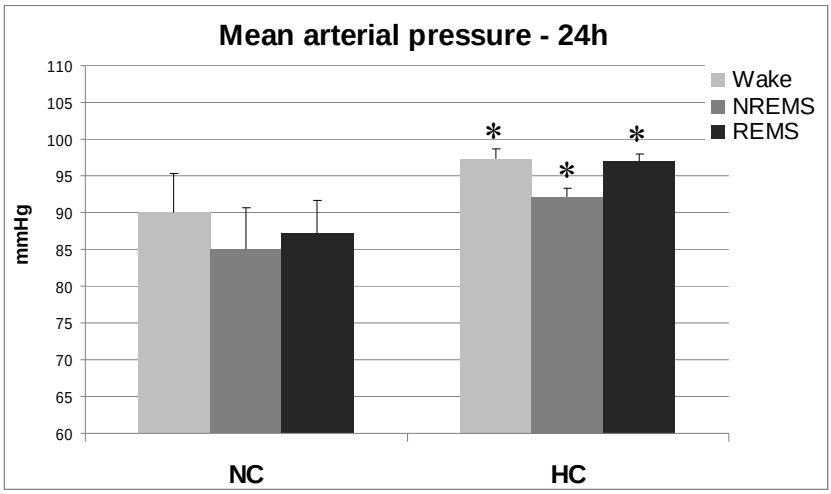

Heart rate - 24h
370
360
350
340
330
320
310
300
290
280
270
bpm
*
NC
HC

Figure 9. The average hypothalamic temperature (°C), mean arterial blood pressure (mmHg) and heart rate levels (bpm) (mean ± S.E.M.) during a 24-h period in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet are shown. Data represent the average of two consecutive days of baseline recording. * NC vs. HC, $p<0.05$.


Hypothalamic temperature - 24h
38,5
38
37,5
37
36,5
°C
Wake
NREMS
REMS
NC
HC


Mean arterial pressure - 24h
110
105
100
95
90
85
80
75
70
65
60
mmHg
*
*
*
Wake
NREMS
REMS
NC
HC

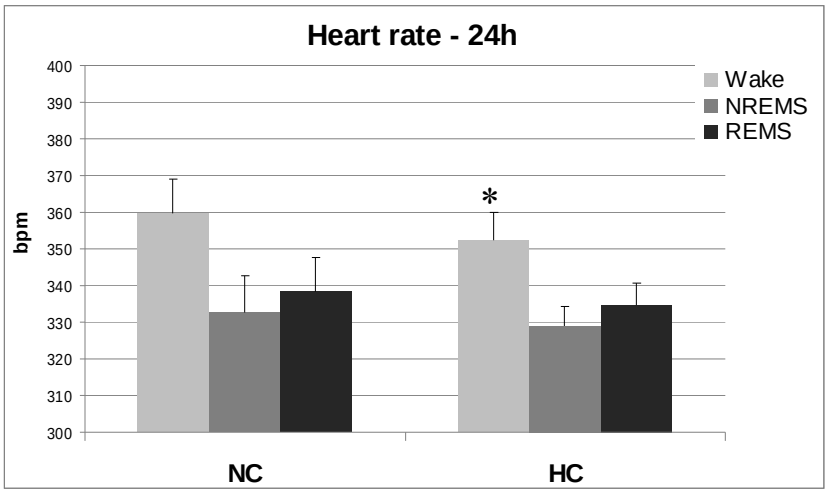
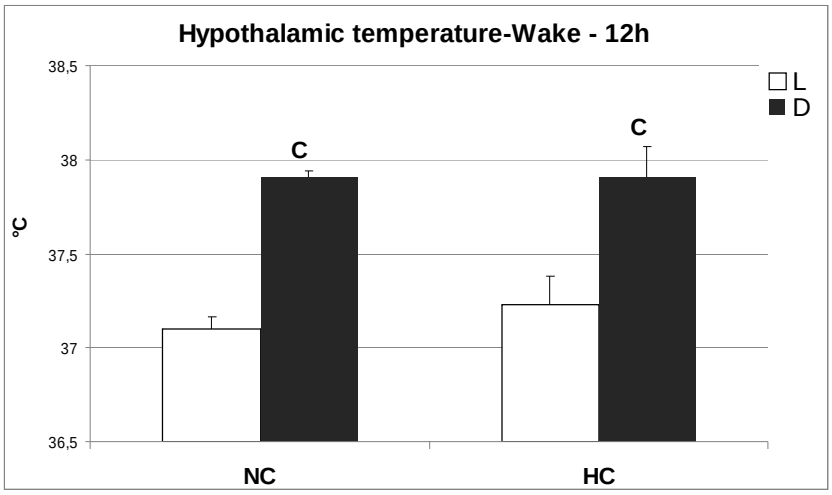
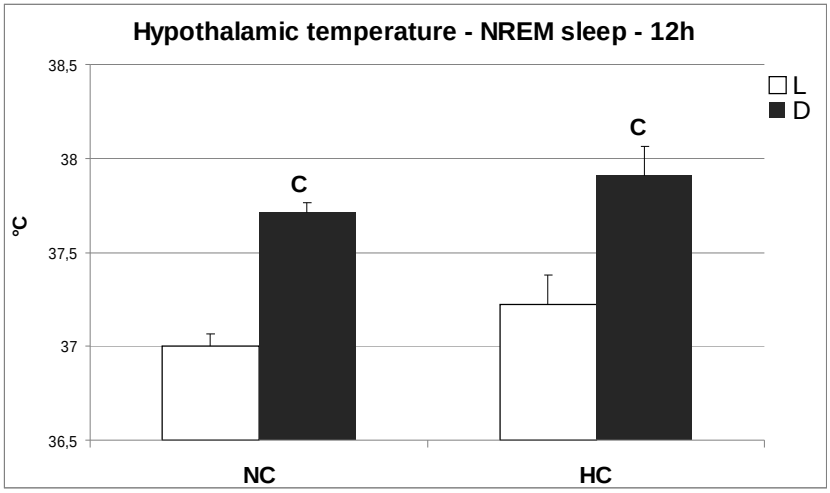

Heart rate - 24h
400
390
380
370
360
350
340
330
320
310
300
bpm
*
Wake
NREMS
REMS
NC
HC

Figure 10. The average hypothalamic temperature (°C), mean arterial blood pressure (mmHg) and heart rate levels (bpm) (mean ± S.E.M.) in either Wake, NREM sleep (NREMS), or REM sleep (REMS), during a 24-h period in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet are shown. Data represent the average of two consecutive days of baseline recording. * NC vs. HC, $p<0.05$.


Hypothalamic temperature-Wake - 12h
38,5
38
37,5
37
36,5
°C
L
D
C
C
NC
HC


Hypothalamic temperature - NREM sleep - 12h
38,5
38
37,5
37
36,5
°C
L
D
C
C
NC
HC

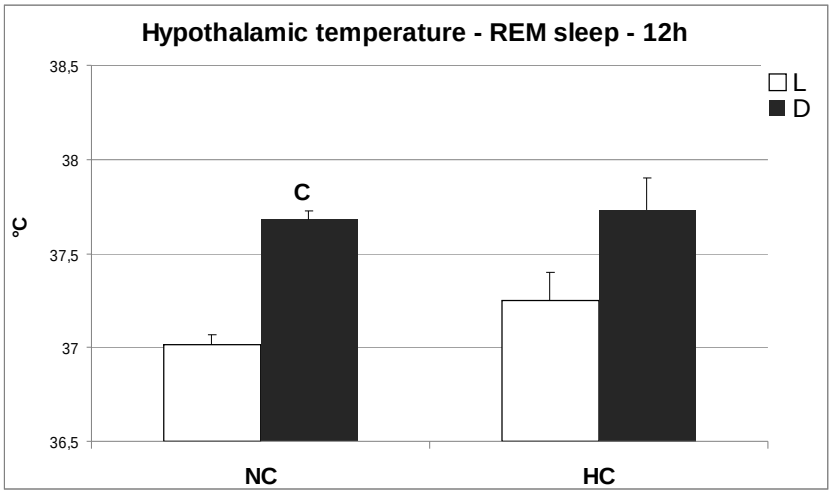
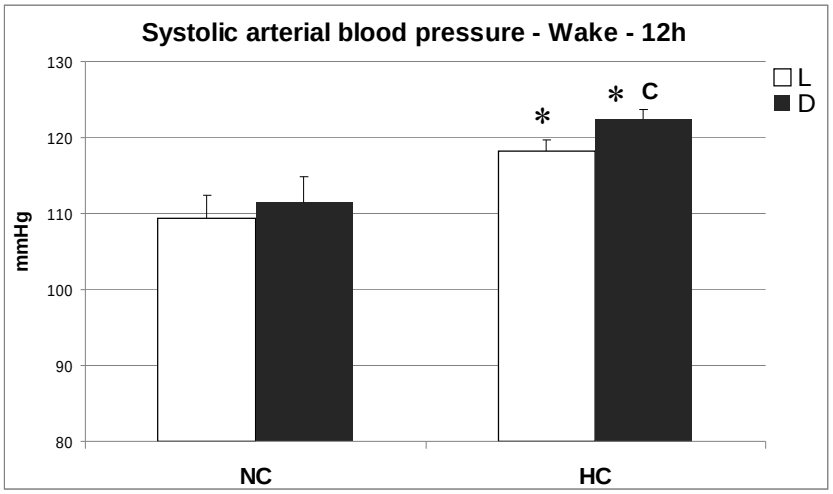
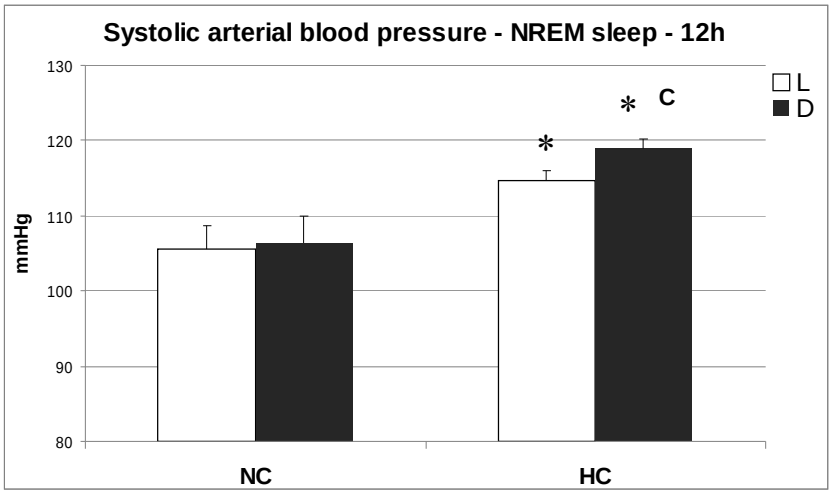

Hypothalamic temperature - REM sleep - 12h
38,5
38
37,5
37
36,5
°C
L
D
C
NC
HC

Figure 11. The average hypothalamic temperature levels (°C, mean ± S.E.M.) during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in either Wake, NREM sleep, or REM sleep, in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet are shown. Data represent the average of two consecutive days of baseline recording. * NC vs. HC, $p<0.05$. ^{C} L vs. D, $p<0.05$.


Systolic arterial blood pressure - Wake - 12h
130
120
110
100
90
80
mmHg
L
D
*
* C
NC
HC


Systolic arterial blood pressure - NREM sleep - 12h
130
120
110
100
90
80
mmHg
L
D
*
* C
NC
HC

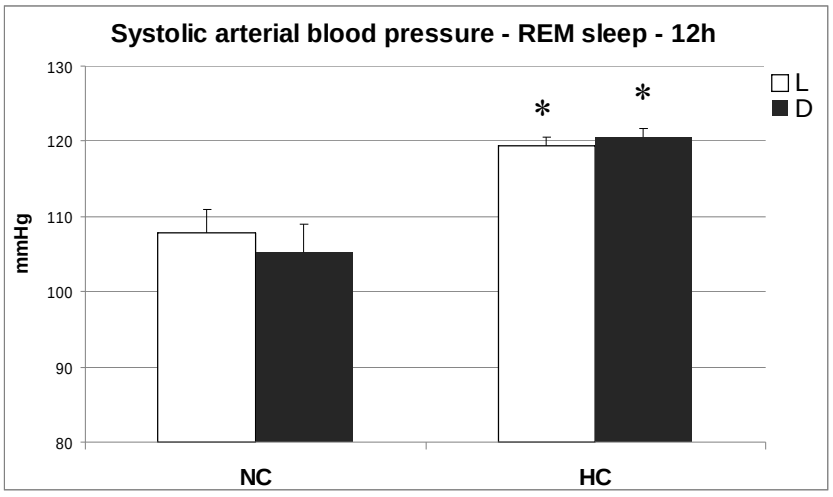

Systolic arterial blood pressure - REM sleep - 12h
130
120
110
100
90
80
mmHg
L
D
*
*
NC
HC

Figure 12. The average systolic arterial blood pressure levels (mmHg, mean ± S.E.M.) during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in either Wake, NREM sleep, or REM sleep, in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Data represent the average of two consecutive days of baseline recording. * NC vs. HC, $p<0.05$. ^{C} L vs. D, $p<0.05$.

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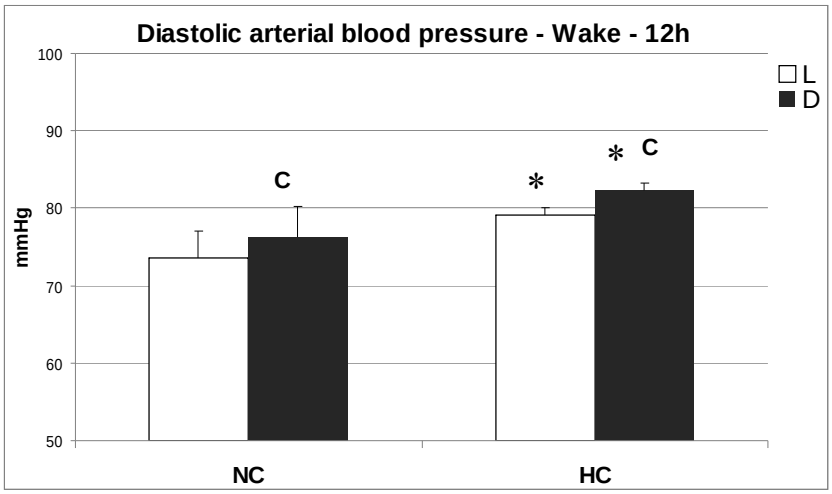
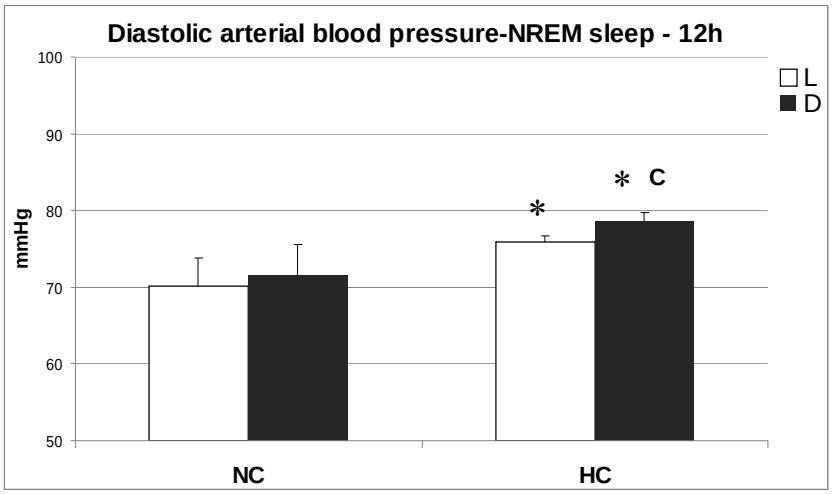

Diastolic arterial blood pressure - Wake - 12h
100
90
80
70
60
50
mmHg
L
D
C
*
* C
NC
HC


Diastolic arterial blood pressure-NREM sleep - 12h
100
90
80
70
60
50
mmHg
L
D
*
* C
NC
HC

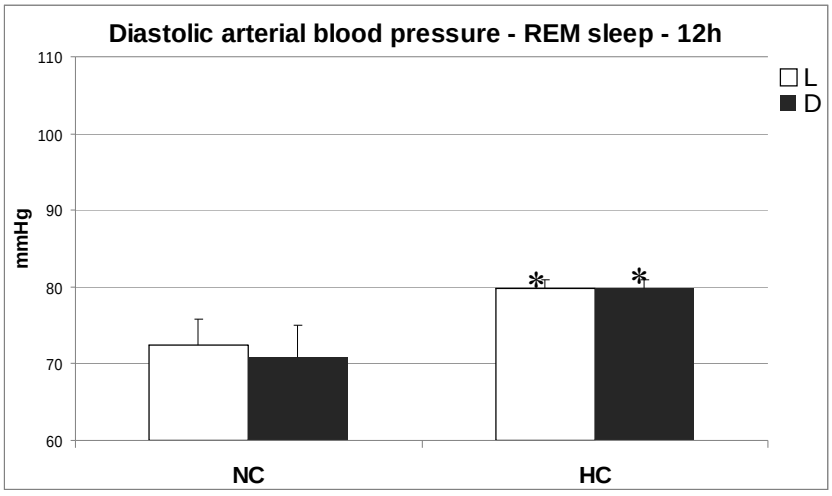
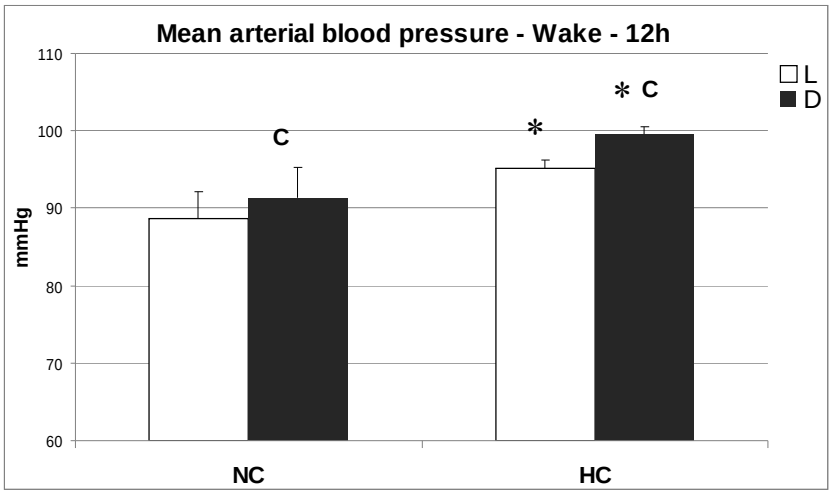
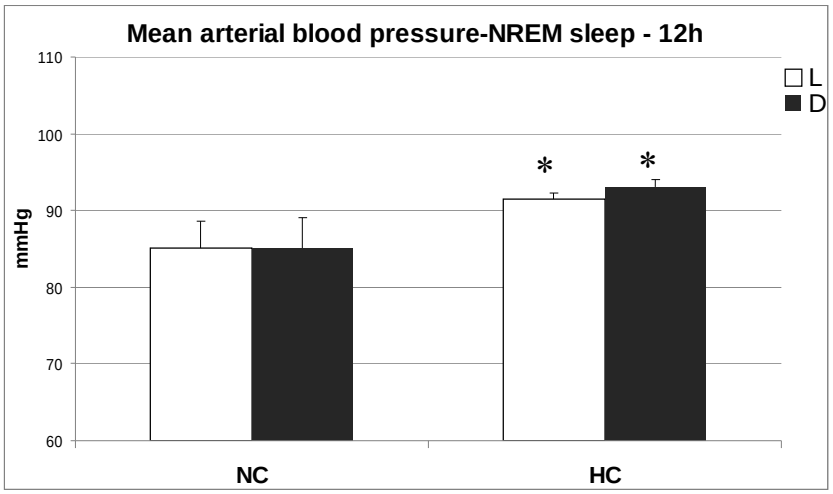

Diastolic arterial blood pressure - REM sleep - 12h
110
100
90
80
70
60
mmHg
L
D
*
*
NC
HC

Figure 13. The average diastolic arterial blood pressure levels (mmHg, mean ± S.E.M.) during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in either Wake, NREM sleep, or REM sleep, in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Data represent the average of two consecutive days of baseline recording. * NC vs. HC, $p<0.05$. C L vs. D, $p<0.05$


Mean arterial blood pressure - Wake - 12h
110
100
90
80
70
60
mmHg
C
* C
*
L
D
NC
HC


Mean arterial blood pressure-NREM sleep - 12h
110
100
90
80
70
60
mmHg
*
*
L
D
NC
HC

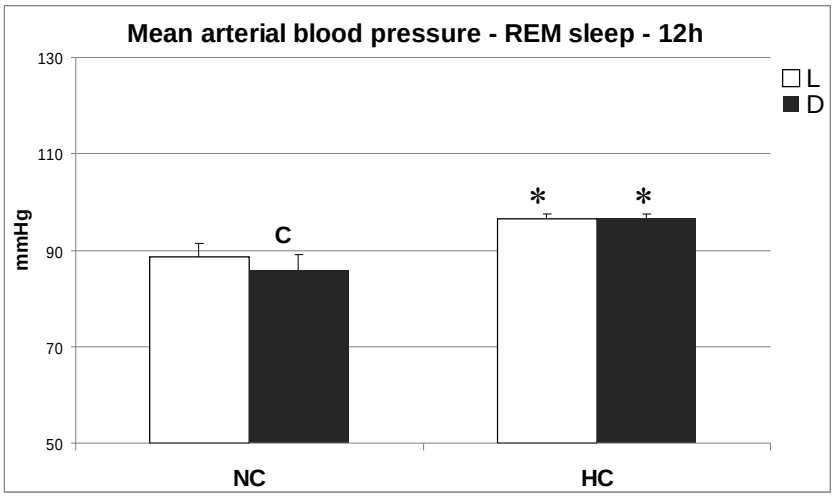
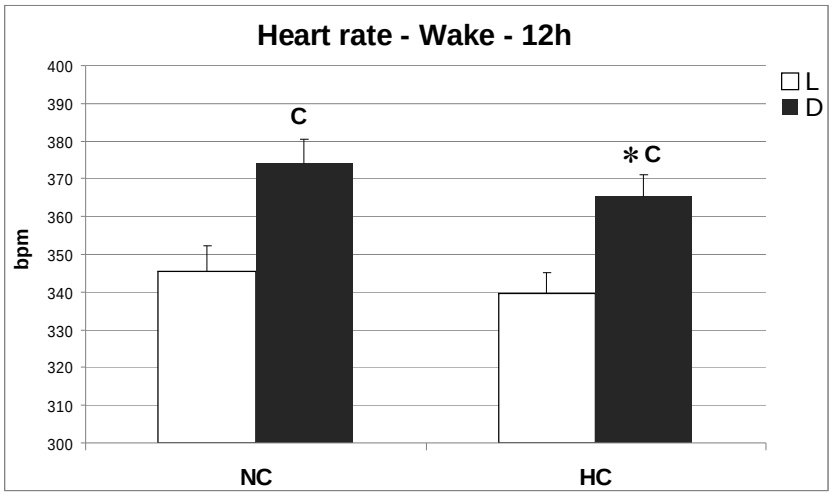
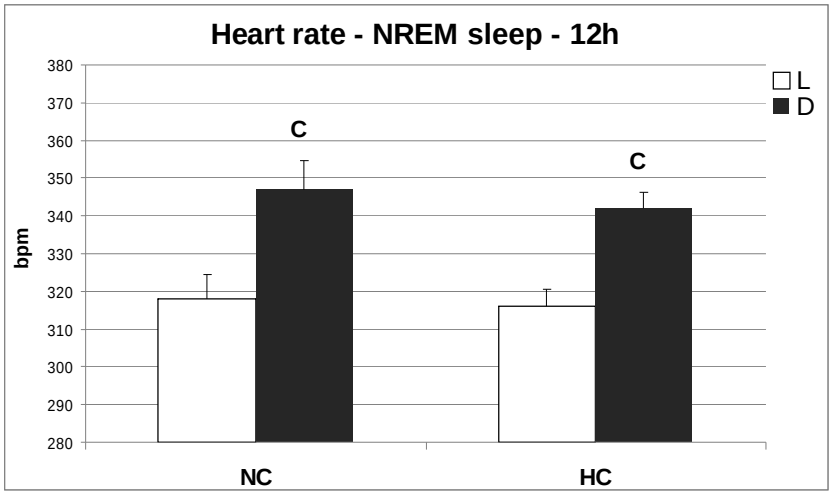

Mean arterial blood pressure - REM sleep - 12h
130
110
90
70
50
mmHg
C
*
*
L
D
NC
HC

Figure 14. The average mean arterial blood pressure levels (mmHg, mean ± S.E.M.) during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in either Wake, NREM sleep, or REM sleep, in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Data represent the average of two consecutive days of baseline recording. * NC vs. HC, $p<0.05$. ^{C} L vs. D, $p<0.05$.


Heart rate - Wake - 12h
bpm
400
390
380
370
360
350
340
330
320
310
300
C
* C
L
D
NC
HC


Heart rate - NREM sleep - 12h
bpm
380
370
360
350
340
330
320
310
300
290
280
C
C
L
D
NC
HC

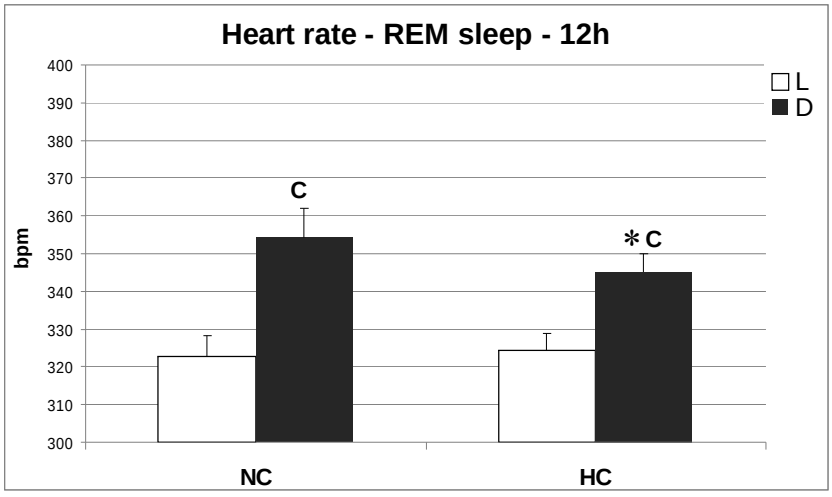
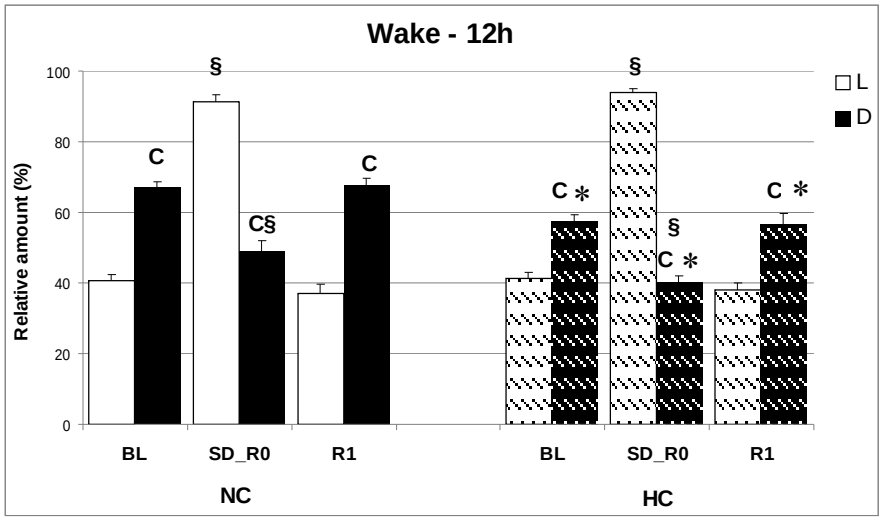
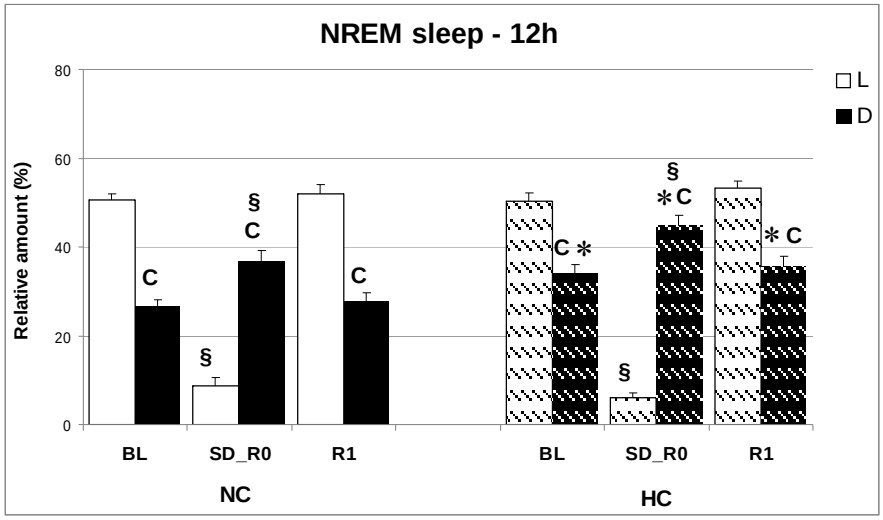

Heart rate - REM sleep - 12h
bpm
400
390
380
370
360
350
340
330
320
310
300
C
* C
L
D
NC
HC

Figure 15. The average heart rate levels (bpm, mean ± S.E.M.) during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in either Wake, NREM sleep, or REM sleep, in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Data represent the average of two consecutive days of baseline recording. * NC vs. HC, $p<0.05$. C L vs. D, $p<0.05$


Wake - 12h
Relative amount (%)
100
80
60
40
20
0
L
D
C
§
C§
C ∗
§
C ∗
BL
SD_R0
R1
NC
HC


NREM sleep - 12h
Relative amount (%)
80
60
40
20
0
L
D
C
§
C ∗
§ ∗C
∗ C
BL
SD_R0
R1
NC
HC

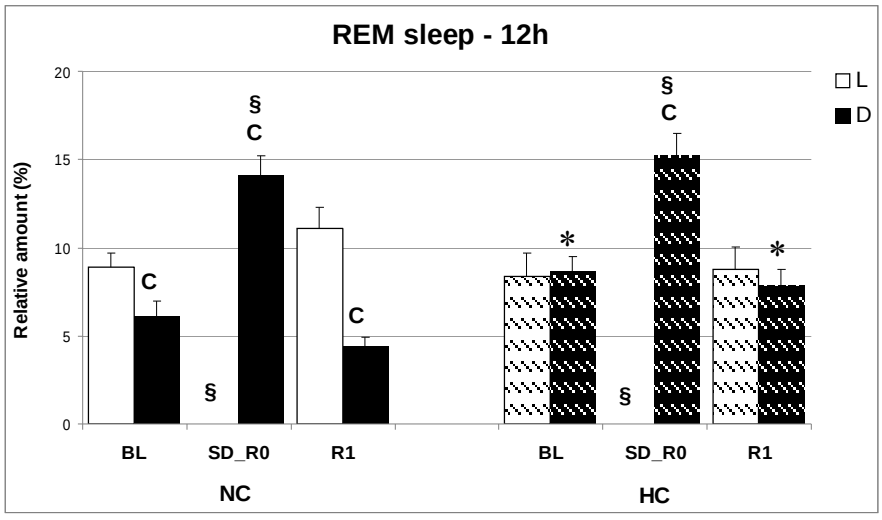
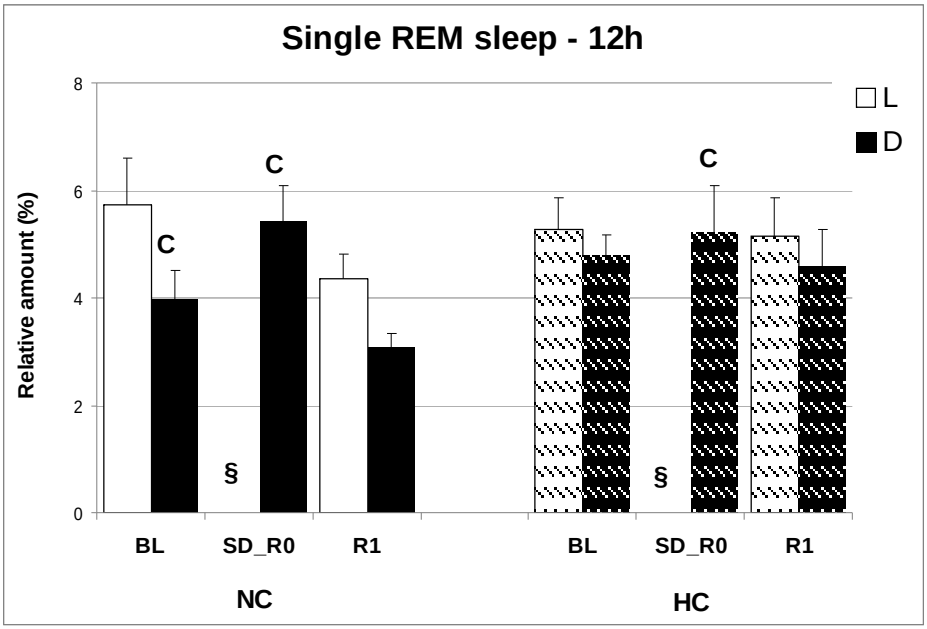

REM sleep - 12h
Relative amount (%)
20
15
10
5
0
L
D
C
§
∗
BL
SD_R0
R1
NC
HC

Figure 16. The relative amount (mean ± S.E.M.) of Wake, NREM sleep or REM sleep during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Amount is expressed as the percent of the 12-h period. Animals were kept under baseline (BL) conditions for two consecutive days. During the third day they were totally sleep deprived by gentle handling for 12 hours (SD) and then allowed to recover for 12 hours (RD). During the fourth day the recovery was completed (R1). Data of the two days of the baseline recording have been averaged. ***** NC vs. HC, $p<0.05$. ^{C} L vs. D, within the same experimental Day, $p<0.05$. § SD, R0, or R1 vs. BL.


Single REM sleep - 12h
Relative amount (%)
8
6
4
2
0
L
D
C
C
C
§
§
BL
SD_R0
R1
BL
SD_R0
R1
NC
HC

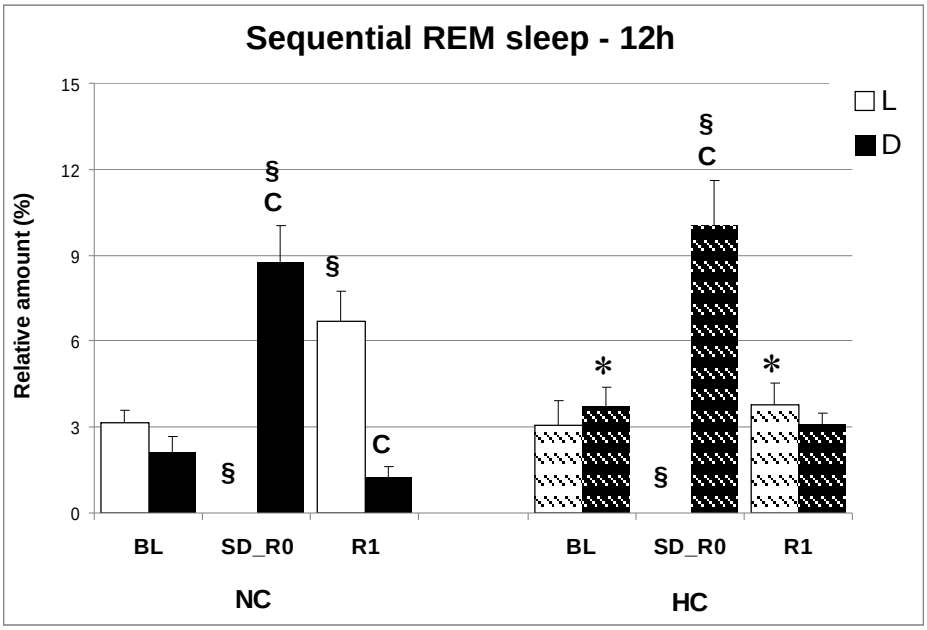
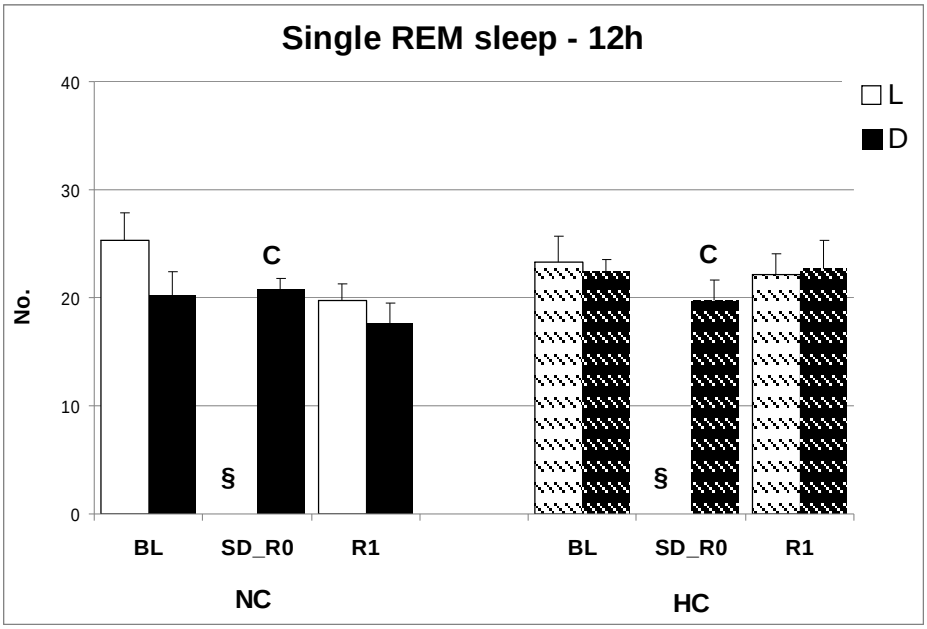

Sequential REM sleep - 12h
Relative amount (%)
15
12
9
6
3
0
L
D
§
C
§
C
§
C
*
*
§
§
BL
SD_R0
R1
BL
SD_R0
R1
NC
HC

Figure 17. The relative amount (mean ± S.E.M.) Single REM sleep or Sequential REM sleep during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Amount is expressed as the percent of the 12-h period. Animals were kept under baseline (BL) conditions for two consecutive days. During the third day they were totally sleep deprived by gentle handling for 12 hours (SD) and then allowed to recover for 12 hours (RD). During the fourth day the recovery was completed (R1). Data of the two days of the baseline recording have been averaged. * NC vs. HC, $p<0.05$. ^{C} L vs. D, within the same experimental Day, $p<0.05$. § SD, R0, or R1 vs. BL.


Single REM sleep - 12h
No.
40
30
20
10
0
L
D
C
C
§
§
BL
SD_R0
R1
BL
SD_R0
R1
NC
HC

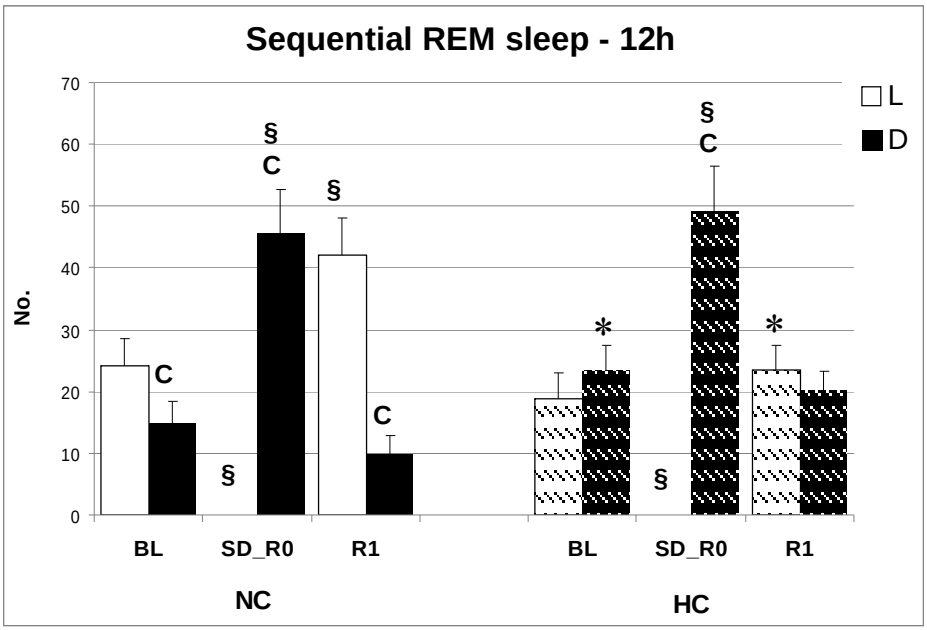
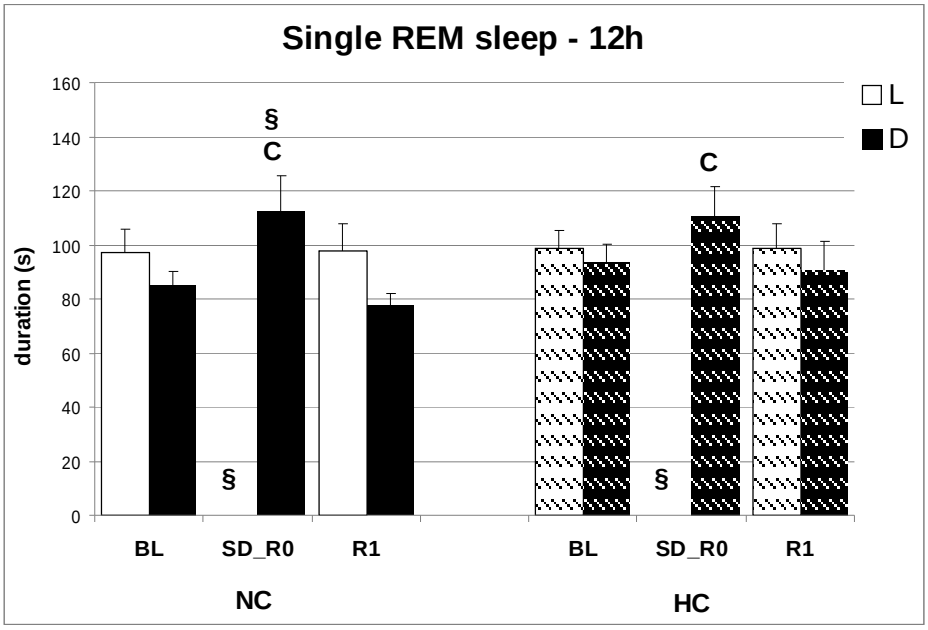

Sequential REM sleep - 12h
No.
70
60
50
40
30
20
10
0
L
D
§
C
§
C
§
C
*
*
§
§
BL
SD_R0
R1
BL
SD_R0
R1
NC
HC

Figure 18. The number (mean ± S.E.M.) of Single REM sleep episodes or Sequential REM sleep episodes during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Animals were kept under baseline (BL) conditions for two consecutive days. During the third day they were totally sleep deprived by gentle handling for 12 hours (SD) and then allowed to recover for 12 hours (RD). During the fourth day the recovery was completed (R1). Data of the two days of the baseline recording have been averaged. * NC vs. HC, $p<0.05$. ^{C} L vs. D, within the same experimental Day, $p<0.05$. § SD, R0, or R1 vs. BL.


Single REM sleep - 12h
duration (s)
160
140
120
100
80
60
40
20
0
L
D
§
C
C
§
§
BL
SD_R0
R1
BL
SD_R0
R1
NC
HC

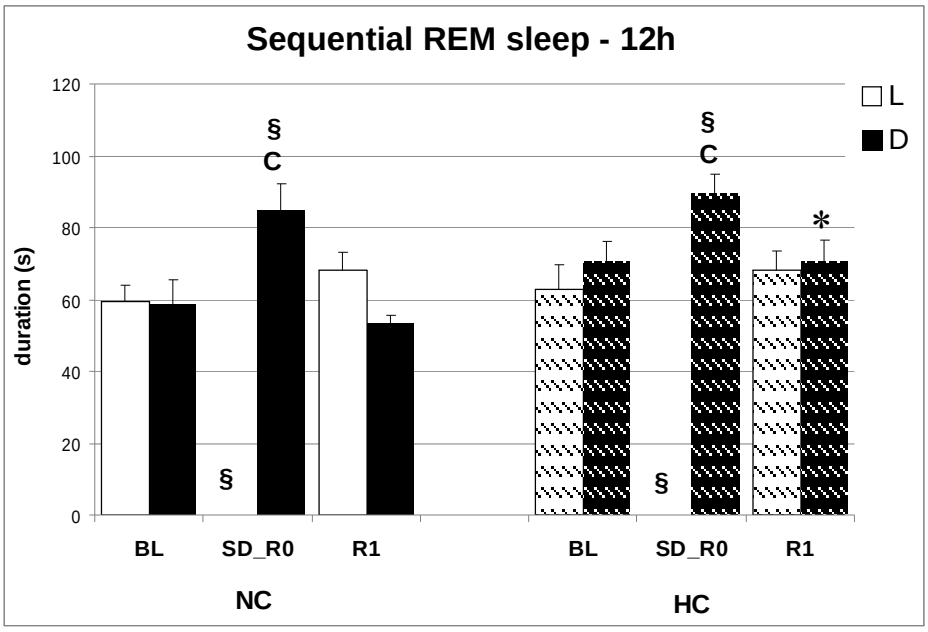

Sequential REM sleep - 12h
duration (s)
120
100
80
60
40
20
0
L
D
§
C
§
C
*
§
§
BL
SD_R0
R1
BL
SD_R0
R1
NC
HC

Figure 19. The average duration (s, mean ± S.E.M.) of Single REM sleep episodes or Sequential REM sleep episodes during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Animals were kept under baseline (BL) conditions for two consecutive days. During the third day they were totally sleep deprived by gentle handling for 12 hours (SD) and then allowed to recover for 12 hours (RD). During the fourth day the recovery was completed (R1). Data of the two days of the baseline recording have been averaged. * NC vs. HC, $p<0.05$. ^{C} L vs. D, within the same experimental Day, $p<0.05$. § SD, R0, or R1 vs. BL.

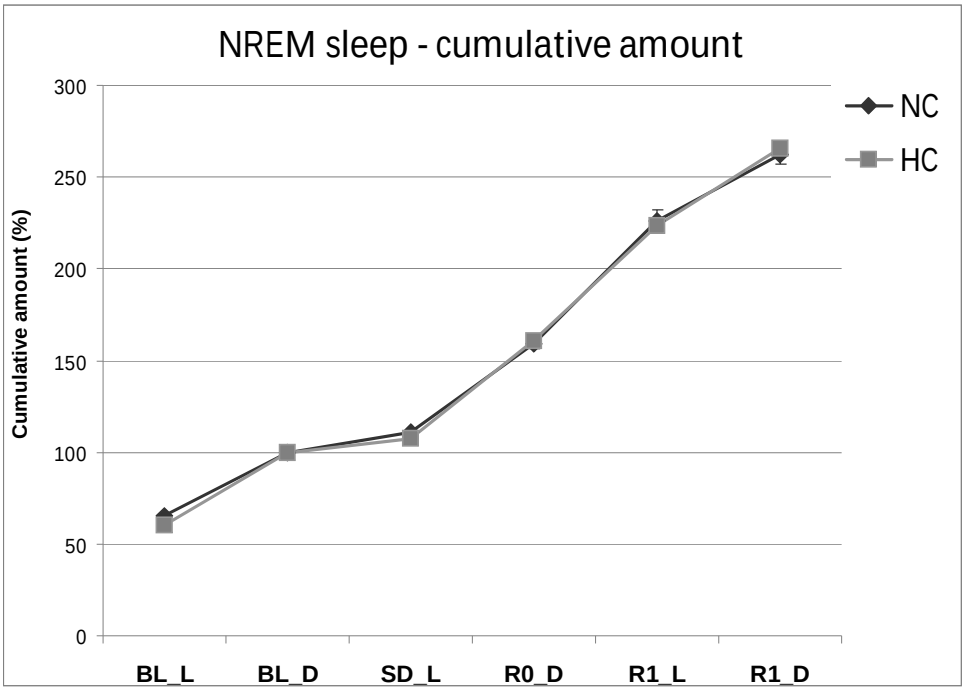


Figure 20. The relative cumulative amount of NREM sleep (mean ± S.E.M.) in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown with a 12-h resolution, according to the normal 12h:12h Light-Dark (LD) cycle. Data are expressed as the percent of the baseline levels (baseline=100%). Animals were kept under baseline (BL_L, BL_D) conditions for two consecutive days. During the third day they were totally sleep deprived by gentle handling for 12 hours (SD_L) and then allowed to recover for 12 hours (R0_D). During the fourth day the recovery was completed (R1_L, R1_D). Data relative to the two days of the baseline recording have been averaged.

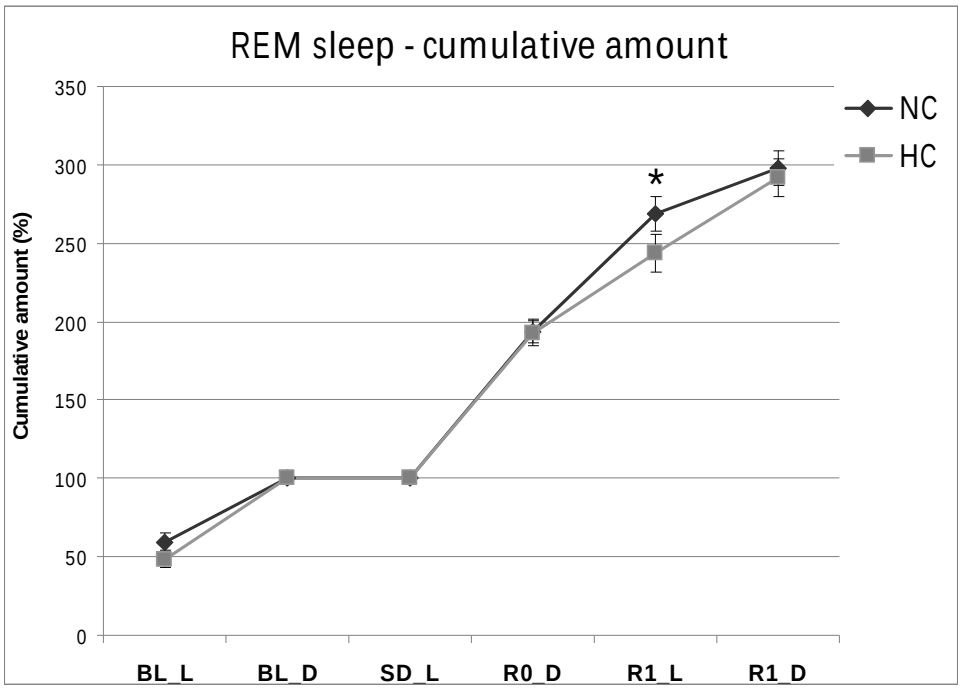
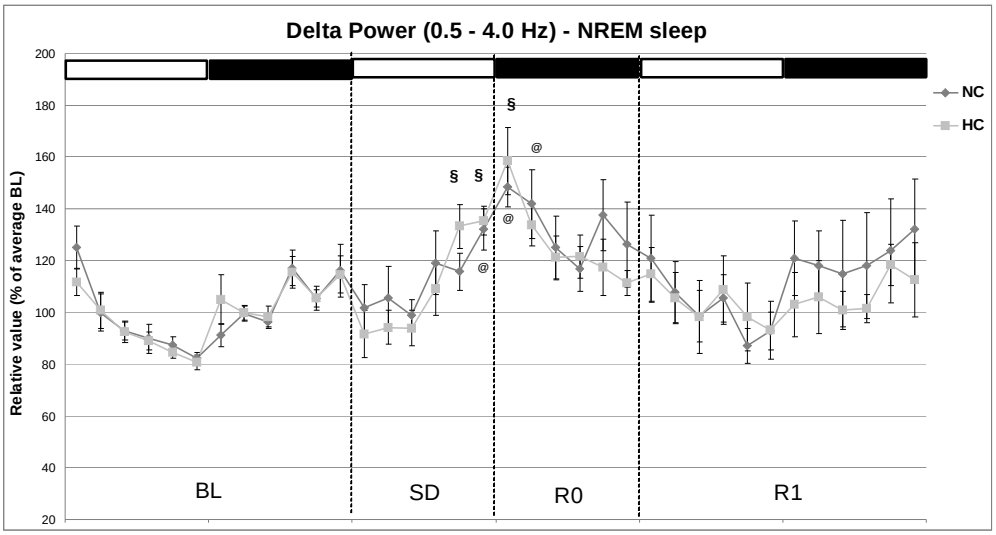


Figure 21. The relative cumulative amount of REM sleep (mean ± S.E.M.) in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown with a 12-h resolution according to the normal 12h:12h Light-Dark (LD) cycle. Data are expressed as the percent of the baseline levels (baseline=100%). Animals were kept under baseline (BL_L, BL_D) conditions for two consecutive days. During the third day they were totally sleep deprived by gentle handling for 12 hours (SD_L) and then allowed to recover for 12 hours (R0_D). During the fourth day the recovery was completed (R1_L, R1_D). Data relative to the two days of the baseline recording have been averaged.


Delta Power (0.5 - 4.0 Hz) - NREM sleep
Relative value (% of average BL)
200
180
160
140
120
100
80
60
40
20
§
§ §
@
@
@
BL
SD
R0
R1
NC
HC

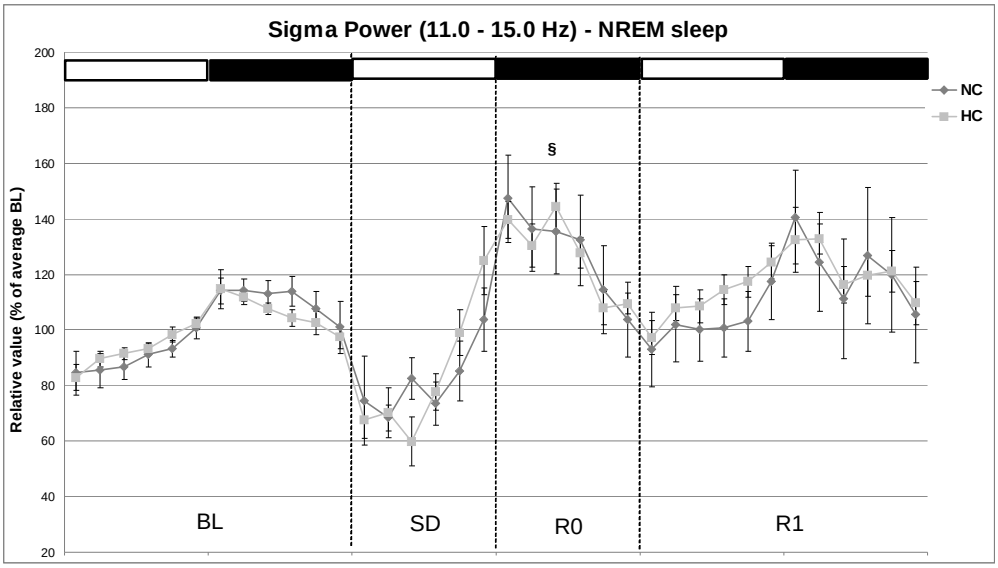

Sigma Power (11.0 - 15.0 Hz) - NREM sleep
Relative value (% of average BL)
200
180
160
140
120
100
80
60
40
20
§
BL
SD
R0
R1
NC
HC

Figure 22. The time course of Delta (0.5-4.0 Hz) and Sigma (11.0- 15.0 Hz) Power during NREM sleep in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Data expressed as the percent of the average 24-h baseline levels and are shown with a 2-h resolution. Animals were kept under baseline (BL) conditions for two consecutive days. During the third day they were totally sleep deprived by gentle handling for 12 hours (SD) and then allowed to recover for 12 hours (RD). During the fourth day the recovery was completed (R1). Data of the two days of the baseline recording have been averaged. $^{@}$ SD, RD, or R1 vs. BL, for NC**.** § SD, RD, or R1 vs. BL, for HC**.**

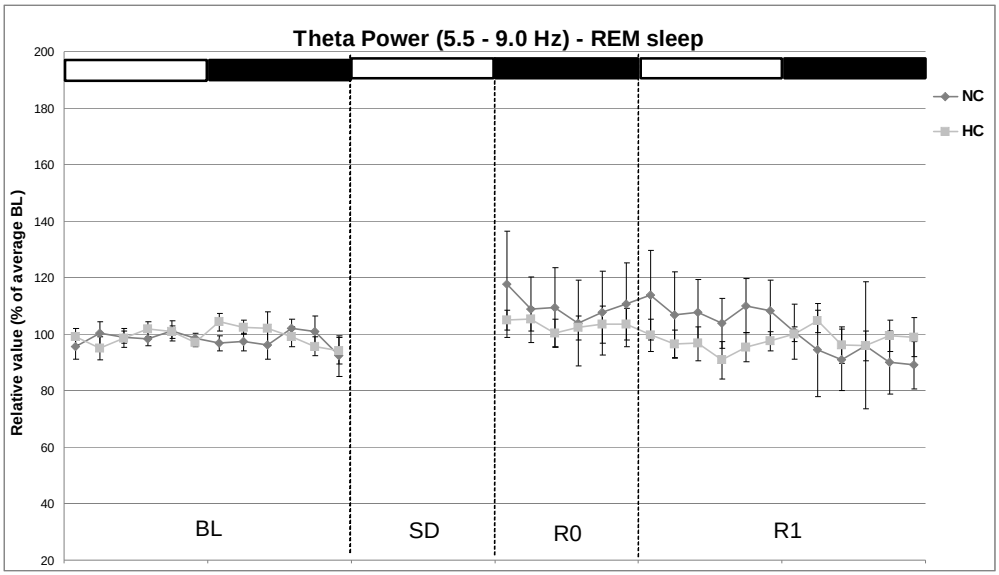
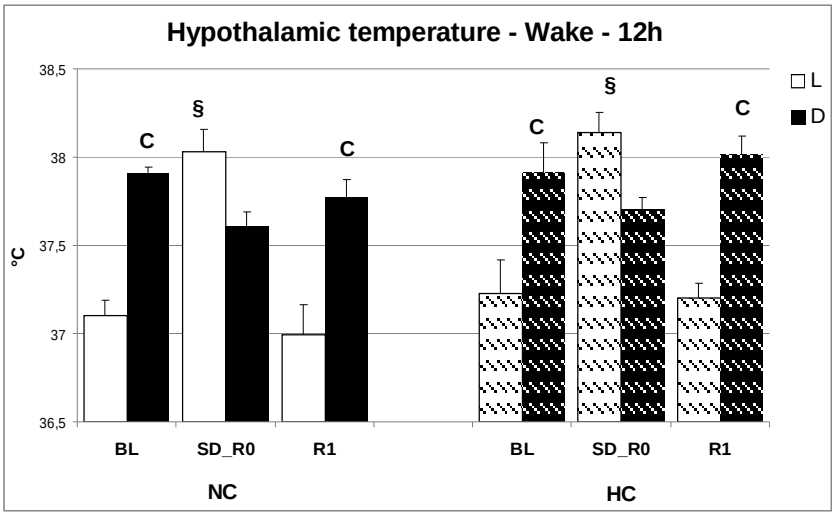
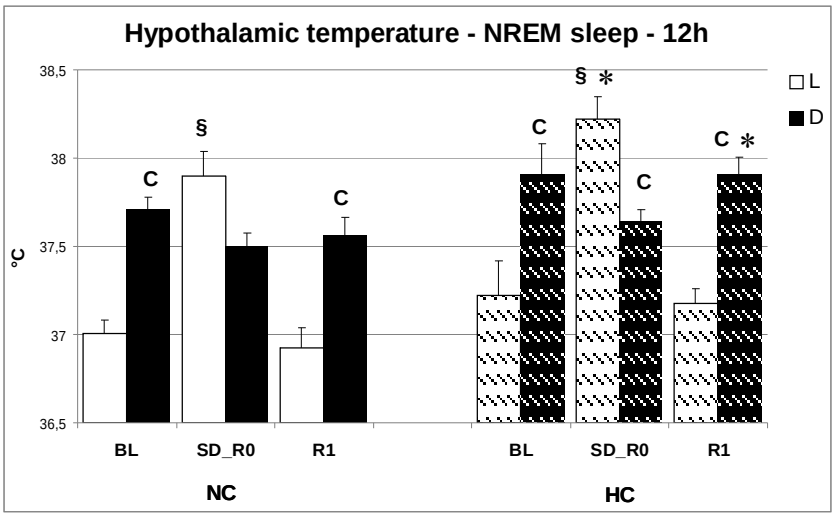


Figure 23. The time course of Theta (5.5-9.0 Hz) Power during REM sleep in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet is shown. Data are expressed as the percent of the average 24-h baseline levels and are shown with a 2-h resolution. Animals were kept under baseline (BL) conditions for two consecutive days. During the third day they were totally sleep deprived by gentle handling for 12 hours (SD) and then allowed to recover for 12 hours (RD). During the fourth day the recovery was completed (R1). Data of the two days of the baseline recording have been averaged.


Hypothalamic temperature - Wake - 12h
38,5
38
37,5
37
36,5
°C
L
D
C
§
BL
SD_R0
R1
NC
HC


Hypothalamic temperature - NREM sleep - 12h
38,5
38
37,5
37
36,5
°C
L
D
C
§
*
BL
SD_R0
R1
NC
HC

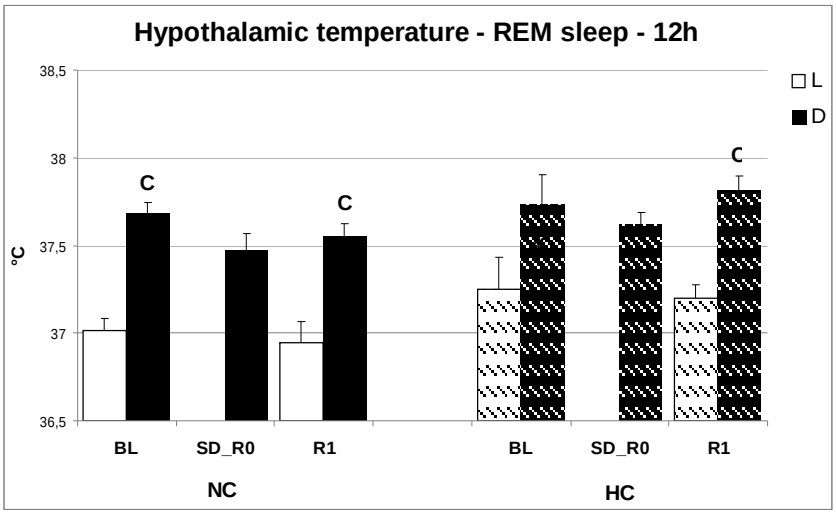
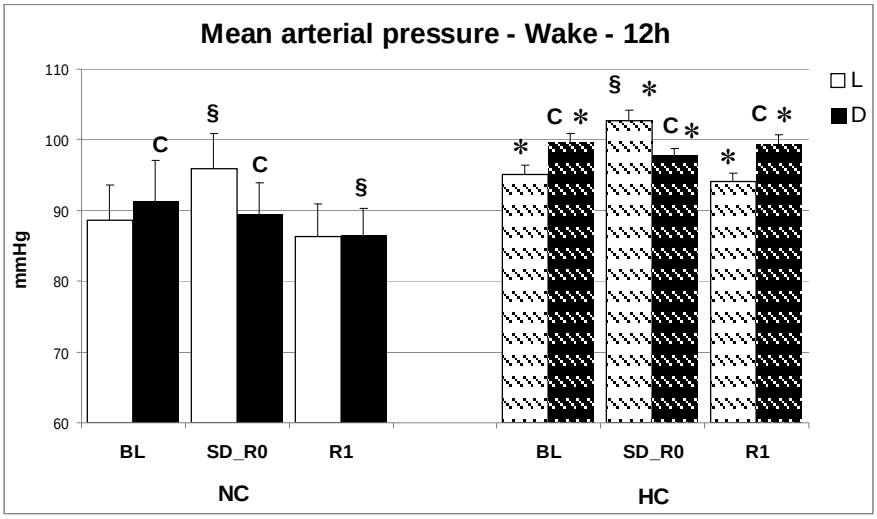
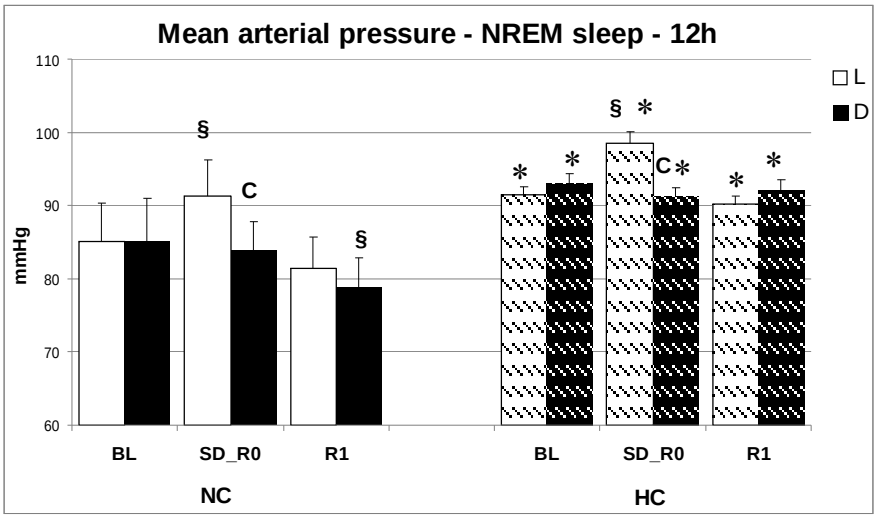

Hypothalamic temperature - REM sleep - 12h
38,5
38
37,5
37
36,5
°C
L
D
C
BL
SD_R0
R1
NC
HC

Figure 24. The average hypothalamic temperature levels (°C, mean ± S.E.M.) during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in either Wake, NREM sleep, or REM sleep, in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet are shown. Animals were kept under baseline (BL) conditions for two consecutive days. During the third day they were totally sleep deprived by gentle handling for 12 hours (SD) and then allowed to recover for 12 hours (RD). During the fourth day the recovery was completed (R1). Data of the two days of the baseline recording have been averaged. ***** NC vs. HC, $p<0.05$. ^{C} L vs. D, within the same experimental Day, $p<0.05$. § SD, RD, or R1 vs. BL.


Mean arterial pressure - Wake - 12h
mmHg
110
100
90
80
70
60
L
D
BL
SD_R0
R1
NC
HC


Mean arterial pressure - NREM sleep - 12h
mmHg
110
100
90
80
70
60
L
D
BL
SD_R0
R1
NC
HC

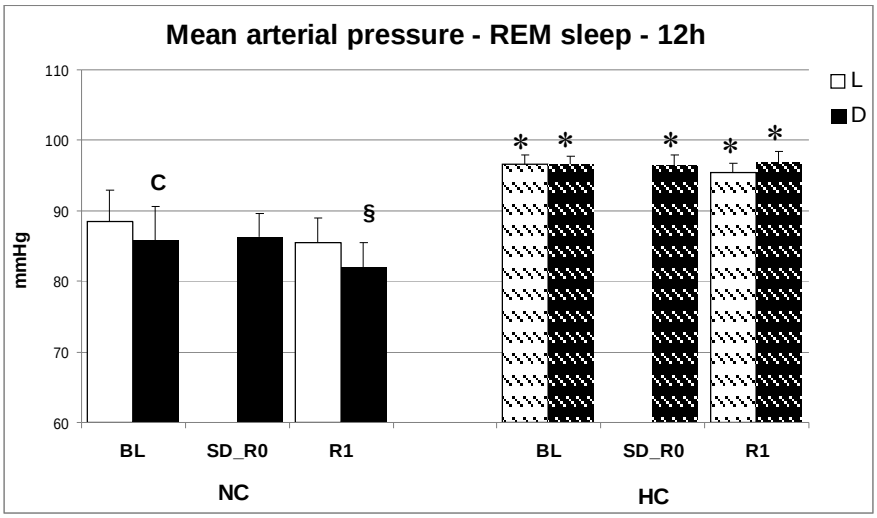
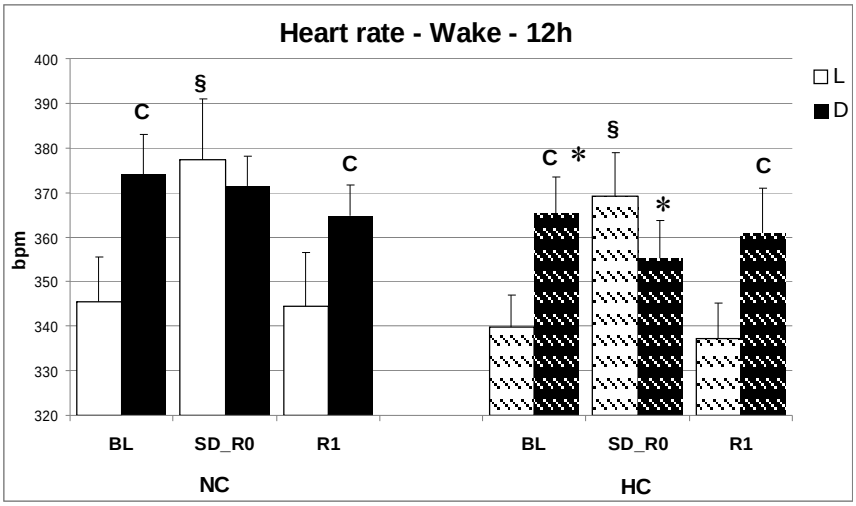
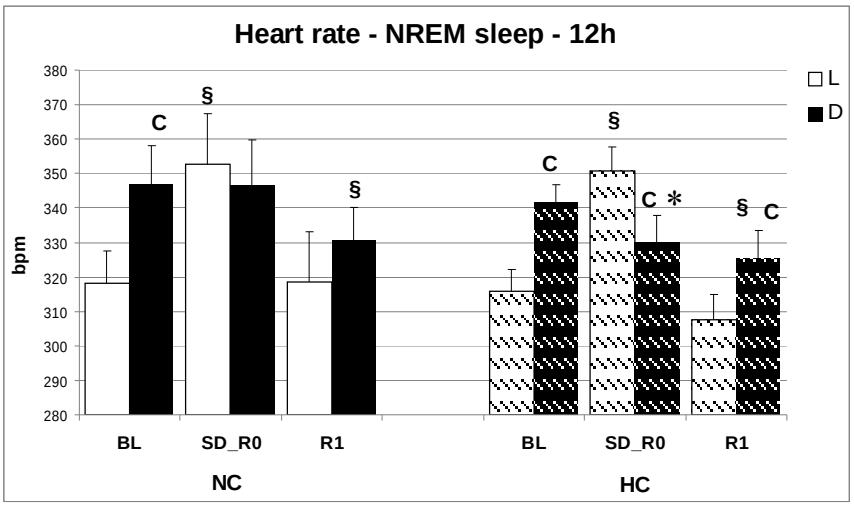

Mean arterial pressure - REM sleep - 12h
mmHg
110
100
90
80
70
60
L
D
BL
SD_R0
R1
NC
HC

Figure 25. The average mean arterial blood pressure levels (mmHG, mean ± S.E.M.) during either the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in either Wake, NREM sleep, or REM sleep, in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet are shown. Animals were kept under baseline (BL) conditions for two consecutive days. During the third day they were totally sleep deprived by gentle handling for 12 hours (SD) and then allowed to recover for 12 hours (RD). During the fourth day the recovery was completed (R1). Data of the two days of the baseline recording have been averaged. * NC vs. HC, $p<0.05$. ^{C} L vs. D, within the same experimental Day, $p<0.05$. § SD, RD, or R1 vs. BL.


Heart rate - Wake - 12h
bpm
400
390
380
370
360
350
340
330
320
L
D
C
§
C
C *
§
*
C
BL
SD_R0
R1
BL
SD_R0
R1
NC
HC


Heart rate - NREM sleep - 12h
bpm
380
370
360
350
340
330
320
310
300
290
280
L
D
C
§
§
C
§
C *
§
C
BL
SD_R0
R1
BL
SD_R0
R1
NC
HC

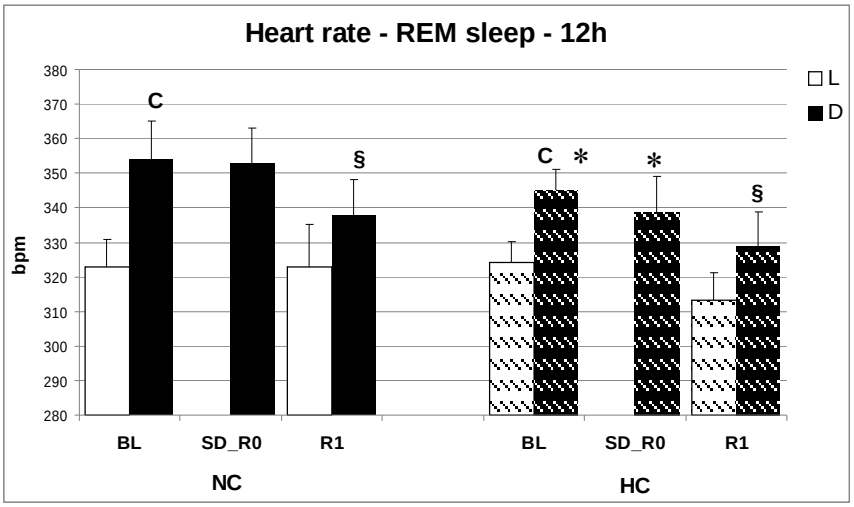

Heart rate - REM sleep - 12h
bpm
380
370
360
350
340
330
320
310
300
290
280
L
D
C
§
C *
*
§
BL
SD_R0
R1
BL
SD_R0
R1
NC
HC

Figure 26. The average heart rate levels (bpm, mean ± S.E.M.) during either a the Light (L) or the Dark (D) period of the normal 12h:12h LD cycle in either Wake, NREM sleep, or REM sleep, in rats kept under either a normocaloric (NC) or a hypercaloric (HC) diet are shown. Animals were kept under baseline (BL) conditions for two consecutive days. During the third day they were totally sleep deprived by gentle handling for 12 hours (SD) and then allowed to recover for 12 hours (RD). During the fourth day the recovery was completed (R1). Data of the two days of the baseline recording have been averaged. * NC vs. HC, $p<0.05$. ^{C} L vs. D, within the same experimental Day, $p<0.05$. § SD, RD, or R1 vs. BL**.**

7. References

Aghajanian, G.K. & Vandermaelen, C.P. (1982). Intracellular identification of central noradrenergic and serotonergic neurons by a new double labeling procedure. *J. Neurosci.*, 2, 1786–1792.

Allison, D. B., Fontaine, K. R., Manson, J. E., Stevens, J., & VanItallie, T. B. (1999). Annual deaths attributable to obesity in the United States. *JAMA,* 282(16), 1530-1538.

Amici, R., Zamboni, G., Perez, E., Jones, C. A., Toni, I., Culin, F., & Parmeggiani, P. L. (1994). Pattern of desynchronized sleep during deprivation and recovery induced in the rat by changes in ambient temperature. *J. Sleep. Res.*, 3(4), 250-256.

Amici, R., Zamboni, G., Perez, E., Jones, C.A., Parmeggiani, P.L. (1998). The influence of a heavy thermal load on REM sleep in the rat. *Brain. Res.,* 781, 252-258.

Amici, R., Cerri, M., Ocampo-Garcés, A., Baracchi, F., Dentico, D., Jones, C. A., ... & Zamboni, G. (2008). Cold exposure and sleep in the rat: REM sleep homeostasis and body size. *Sleep,* 31(5), 708.

Amici R., Cerri M. and Parmeggiani P.L. (2013). Overview of Physiological Processes During Sleep.. In: Kushida C.A. (ed.) *The Encyclopedia of Sleep*, Vol. 1, pp. 385-389. Waltham, MA: Academic Press.

Barker, D. J. P. (2007). Obesity and early life. *Obes. Rev.*, 8, 45-49.

Bastard, J. P., Maachi, M., van Nhieu, J. T., Jardel, C., Bruckert, E., Grimaldi, A., ... & Hainque, B. (2002). Adipose tissue IL-6 content correlates with resistance to insulin activation of glucose uptake both in vivo and in vitro. *J Clin. Endocrinol. Metab.*, 87(5), 2084-2089.

Bastard, J. P., Maachi, M., Lagathu, C., Kim, M. J., Caron, M., Vidal, H., ... & Feve, B. (2006). Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur. Cytokine Netw.*, 17(1), 4-12.

Bernardi, L., Salvucci, F., Suardi, R., Soldá, P. L., Calciati, A., Perlini, S., ... & Ricciardi, L. (1990). Evidence for an intrinsic mechanism regulating heart rate variability in the transplanted and the intact heart during submaximal dynamic exercise?. *Cardovasc. Res.*, 24(12), 969-981.

Boissard, R., Gervasoni, D., Schmidt, M. H., Barbagli, B., Fort, P., & Luppi, P. H. (2002). The rat pontomedullary network responsible for paradoxical sleep onset and maintenance: a combined microinjection and functional neuroanatomical study. *Eur. J. Neurosci.*, 16(10), 1959-1973.

Borbély, A.A. (1980). Sleep: circadian rhythm versus recovery process. In: Functional states of the brain: their determinants (M Koukkou, D Lehmann, J Angst eds) pp. 151-161, Elsevier, Amsterdam.

Borbély, A.A., Achermann, P. (2005). Sleep Homeostasis and Models of Sleep Regulation. In: Priciples and practice of sleep medicine. Terza edizione (Kryger MH, Roth C, Dement WE, eds) pp 405-417. Philadelphia: Saunders.

Broman, J. E., Lundh, L. G., & Hetta, J. (1996). Insufficient sleep in the general population. *Neurophysiol. Clin.*, 26(1), 30-39.

Burdakov, D., Jensen, L.T., Alexopoulos, H., *et al*. (2006). Tandem-pore K+ channels mediate inhibition of orexin neurons by glucose. *Neuron*, 50, 711-722.

Campen. M.J., Tagaito, Y., Jenkins, T.P., Smith, P.L., Schwartz, A.R., O'Donnell, C.P. (2002). Phenotypic differences in the hemodynamic response during REM sleep in six strains of inbred mice. *Physiol. Genomics.* 11(3), 227-34.

Carrington, M. J., & Trinder, J. (2008). Blood pressure and heart rate

during continuous experimental sleep fragmentation in healthy adults. *Sleep*, 31(12), 1701.

Carroll, J. F., Zenebe, W. J., & Strange, T. B. (2006). Cardiovascular function in a rat model of diet-induced obesity. *Hypertension*, 48(1), 65-72.

Carskadon, M. and Dement, W. Normal Human Sleep: An Overview. In: Kryger MH, Roth T, Dement WC (eds). *Principles and Practice of Sleep Medicine,* Fourth Edition. Elsevier Saunders, Philadelphia. 2009, pp.13-90.

Choi, K. M., Lee, J. S., Park, H. S., Baik, S. H., Choi, D. S., & Kim, S. M. (2008). Relationship between sleep duration and the metabolic syndrome: Korean National Health and Nutrition Survey 2001. *Int. J. Obes.*, 32(7), 1091-1097.

Cerri, M., Ocampo-Garces, A., Amici, R., Baracchi, F., Capitani, P., Jones, C. A., ... & Zamboni, G. (2005). Cold exposure and sleep in the rat: effects on sleep architecture and the electroencephalogram. *Sleep*, 28(6), 694.

Clement, O., Sapin, E., Libourel, P.A., Arthaud, S., Brischoux, F., Fort, P., Luppi, P.H. (2012). The lateral hypothalamic area controls paradoxical (REM) sleep by means of descending projections to brainstem GABAergic neurons. *J Neurosci*. 32(47),16763-74.

Danguir, J. (1987). Cafeteria diet promotes sleep in rats. *Appetite*, 8, 49-53.

Danguir. J. (1989). Sleep patterns in the genetically obese Zucker rat: effect of acarbose treatment. *Am J Physiol - Reg Int Comp Physiol.*, 256, 281-283.

de Lecea, L., Kilduff, T. S., Peyron, C., Gao, X. B., Foye, P. E., Danielson, P. E., ... & Sutcliffe, J. G. (1998). The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc. Natl. Acad. Sci.*, 95(1), 322-327.

DeRijk, R. H., Boelen, A., Tilders, F. J., & Berkenbosch, F. (1994). Induction of plasma interleukin-6 by circulating adrenaline in the rat.

Psychoneuroendocrinol., 19(2), 155-163.

Dobrian, A. D., Davies, M. J., Prewitt, R. L., & Lauterio, T. J. (2000). Development of hypertension in a rat model of diet-induced obesity. *Hypertension*, 35(4), 1009-1015.

Dolan, E., Stanton, A., Thijs, L., Hinedi, K., Atkins, N., McClory, S., ... & O'Brien, E. (2005). Superiority of ambulatory over clinic blood pressure measurement in predicting mortality the Dublin outcome study. *Hypertension*, 46(1), 156-161.

Eckberg, D. L., & Sleight, P. (1992). *Human baroreflexes in health and disease* (pp. 78-299). Oxford: Clarendon Press.

Eckel, R. H., Grundy, S. M., & Zimmet, P. Z. (2005). The metabolic syndrome. *Lancet*, 365(9468), 1415-1428.

Eknoyan, G. (2008). Adolphe Quetelet (1796–1874)—the average man and indices of obesity. *Nephrol. Dial. Transpl.*, 23(1), 47-51.

Ezzati, M., Lopez, A. D., Rodgers, A., Vander Hoorn, S., & Murray, C. J. (2002). Selected major risk factors and global and regional burden of disease. *Lancet*, 360(9343), 1347.

Fontaine, K. R., Redden, D. T., Wang, C., & Westfall, A. O. (86). Allison, DB (2003). Year of life lost due to obesity. *JAMA*, 289, 187-193.

Fort, P., Bassetti, C. L., & Luppi, P. H. (2009). Alternating vigilance states: new insights regarding neuronal networks and mechanisms. *Eur. J. Neurosci.*, 29(9), 1741-1753.

Gangwisch, J. E., Heymsfield, S. B., Boden-Albala, B., Buijs, R. M., Kreier, F., Pickering, T. G., ... & Malaspina, D. (2006). Short Sleep Duration as a Risk Factor for Hypertension Analyses of the First National Health and Nutrition

Examination Survey. *Hypertension*, 47(5), 833-839.

Gangwisch, J. E., Heymsfield, S. B., Boden-Albala, B., Buijs, R. M., Kreier, F., Pickering, T. G., ... & Malaspina, D. (2007). Sleep duration as a risk factor for diabetes incidence in a large US sample. *Sleep*, 30(12), 1667.

Gastaut, H. (1965). Etude polygraphique des manifestations episodiques (hypniques et respiratoires), diurnes et nocturnes, du syndrome de Pickwick. *Rev. Neurol.*, 112, 568-579.

Gaus, S. E., Strecker, R. E., Tate, B. A., Parker, R. A., & Saper, C. B. (2002). Ventrolateral preoptic nucleus contains sleep-active, galaninergic neurons in multiple mammalian species. *Neuroscience*, 115(1), 285-294.

Graham, I., Atar, D., Borch-Johnsen, K., Boysen, G., Burell, G., Cifkova, R., ... & Hemingway, H. (2007). European guidelines on cardiovascular disease prevention in clinical practice: executive summary Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). *Eur. Heart J.*, 28(19), 2375-2414.

Guan, Z., Vgontzas, A. N., Bixler, E. O., & Fang, J. (2008). Sleep is increased by weight gain and decreased by weight loss in mice. *Sleep*, 31(5), 627-633.

Gupta, N. K., Mueller, W. H., Chan, W., & Meininger, J. C. (2002). Is obesity associated with poor sleep quality in adolescents?. *Am. J. Hum. Biol.*, 14(6), 762-768.

Hanriot, L., Camargo, N., Courau, A.C., Leger, L., Luppi, P.H. & Peyron, C. (2007). Characterization of the melanin-concentrating hormone neurons activated during paradoxical sleep hypersomnia in rats. *J. Comp. Neurol.*, 505, 147–157.

Haslam, D. W., & James, W. P. (2005). Obesity. *Lancet*, 366, 1197–209.

Hasler, G., Buysse, D. J., Klaghofer, R., Gamma, A., Ajdacic, V., Eich, D., ... & Angst, J. (2004). The association between short sleep duration and obesity in young adults: a 13-year prospective study. *Sleep*, 27(4), 661.

Hallanger, A. E., Levey, A. I., Lee, H. J., Rye, D. B., & Wainer, B. H. (1987). The origins of cholinergic and other subcortical afferents to the thalamus in the rat. *J. Comp. Neurol.*, 262(1), 105-124.

Hla, K. M., Young, T., Finn, L., Peppard, P. E., Szklo-Coxe, M., & Stubbs, M. (2008). Longitudinal association of sleep-disordered breathing and nondipping of nocturnal blood pressure in the Wisconsin Sleep Cohort Study. *Sleep*, 31(6), 795.

Heller, H.C. (2005)Temperature, Thermoregulation, and Sleep. In: *Principles and practice of sleep medicine*, Third Ed. (Kryger MH, Roth T, Dement WC, eds) pp:291-304. Philadelphia: Saunders Company.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scand. J. Stat.*, 6, 65–70.

Horne, J. (2008). Too weighty a link between short sleep and obesity?. *Sleep*, 31(5), 595.

Hotamisligil, G. S., Shargill, N. S., & Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*, 259(5091), 87.

Jego, S., Salvert, D., Renouard, L., Mori, M., Goutagny, R., Luppi, P.H., Fort, P. (2012). Tuberal hypothalamic neurons secreting the satiety molecule Nesfatin-1 are critically involved in paradoxical (REM) sleep homeostasis. *PLoS One*. 7(12):e52525. doi: 10.1371/journal.pone.0052525.

Jenkins, J.B., Takenori, O., Zhiwei, G., Vgontzas, A.N., Bixle, E.O., Fang, J. (2006). Sleep is increased in mice with obesity induced by high-fat food. *Physiol Behav.,* 87, 255-262

John, J., Wu, M. F., Boehmer, L. N., & Siegel, J. M. (2004). Cataplexy-active neurons in the hypothalamus: implications for the role of histamine in sleep and waking behavior. *Neuron*, 42(4), 619-634.

Jones, B. E. (2003). Arousal systems. *Front. Biosci.*, 8, s438-s451.

Jones, B.E. Basic mechanisms of sleep-wake sleep. In: Kryger MH, Roth T, Dement WC (eds). *Principles and Practice of Sleep Medicine,* Fourth Edition. Elsevier Saunders, Philadelphia. 2005, pp.136-150.

Kerkhof, G. A., Van Dongen, H. P., & Bobbert, A. C. (1998). Absence of Endogenous Circadian Rhythmicity in Blood Pressure? *Am. J. Hypertens.*, 11(3), 373-377.

Keys, A., Fidanza, F., Karvonen, M.J., Kimura, N., Taylor, H.L. (1972). Indices of relative weight and adiposity. *J. Chronic. Dis.*, 25, 329-343.

Koban, M., & Swinson, K. L. (2005). Chronic REM-sleep deprivation of rats elevates metabolic rate and increases UCP1 gene expression in brown adipose tissue. *Am. J. Physiol. - Endocr. Metabol.*, 289(1), E68-E74.

Kopelman, P. G. (1994). Causes and consequences of obesity. *Med. Int.* 22, 385–388.

Kotsis, V., Stabouli, S., Bouldin, M., Low, A., Toumanidis, S., & Zakopoulos, N. (2005). Impact of obesity on 24-hour ambulatory blood pressure and hypertension. *Hypertension*, 45(4), 602-607.

Lacombe, J., Nosjean, A., Meunier, J. &Laguzzi, R. (1988). Computer analysis of cardiovascular changes during the sleep wake cycle in Sprague-

Dawley rats. *Am. J. Physio l- Heart C*, 254: H217-H222.

Landsberg, L. (1999). Role of the sympathetic adrenal system in the pathogenesis of the insulin resistance syndrome. *Ann. N.Y. Acad. Sci.*, 892(1), 84-90.

Lane, R. D., McRae, K., Reiman, E. M., Chen, K., Ahern, G. L., & Thayer, J. F. (2009). Neural correlates of heart rate variability during emotion. *Neuroimage*, 44(1), 213-222.

Laposky, A. D., Shelton, J., Bass, J., Dugovic, C., Perrino, N., & Turek, F. W. (2006). Altered sleep regulation in leptin-deficient mice. *Am. J. Physiol.-Reg. Int. Comp. Physiol.*, 290(4), R894-R903.

Laposky, A. D., Bradley, M. A., Williams, D. L., Bass, J., & Turek, F. W. (2008). Sleep-wake regulation is altered in leptin-resistant (db/db) genetically obese and diabetic mice. *Am. J. Physiol.-Reg. Int. Comp. Physiol.* 295(6), R2059-R2066.

Laudadio S. (2011). *Studio del comportamento ipnico durante l'accrescimento ponderale patologico*. PhD Thesis in Neurophysiology. University of Bologna.

Levin, B. E., Trsicari, J., Sullivan, A.C. (1983). Relationship between sympathetic activity and diet-induce obesity in two rat strains. *Am. J. Physiol.-Reg. Int. Comp. Physiol.,* 245, R367-R371.

Levin, B. E., Dunn-Meynell, A. A., Balkan, B., & Keesey, R. E. (1997). Selective breeding for diet-induced obesity and resistant rats. *Am. J. Physiol-Reg. Int. Comp. Physiol.,* 273, R725-R730.

Levin, B. E., Keesey, R. E. (1998). Defense or differing body weight set points in diet-induced obese and resistant rats. *Am. J. Physiol.-Reg. Int. Comp. Physiol.,* 274, R412-R419.

Levin, B. E., Dunn-Meynell, A. A., Ricci, M. R., & Cummings, D. E. (2003). Abnormalities of leptin and ghrelin regulation in obesity-prone juvenile rats. *Am. J. Physiol.-Endocr. Metabol.*, 285(5), E949-E957.

Loffreda, S., Yang, S. Q., Lin, H. Z., Karp, C. L., Brengman, M. L., Wang, D. J., ... & Diehl, A. M. (1998). Leptin regulates proinflammatory immune responses. *The FASEB Journal*, 12(1), 57-65.

Lombardi, F., & Parati, G. (2000). An update on: cardiovascular and respiratory changes during sleep in normal and hypertensive subjects. *Cardiovasc. Res.*, 45(1), 200-211.

Lu, J., Bjorkum, A. A., Xu, M., Gaus, S. E., Shiromani, P. J., & Saper, C. B. (2002). Selective activation of the extended ventrolateral preoptic nucleus during rapid eye movement sleep. *J Nneurosci*, *22*(11), 4568-4576.

Luppi, P.H., Gervasoni, D., Verret, L., Goutagny, R., Peyron, C., Salvert, D., Leger, L. & Fort, P. (2006). Paradoxical (REM) sleep genesis: the switch from an aminergic-cholinergic to a GABAergic-glutamatergic hypothesis. *J. Physiol.* Paris, 100, 271–283.

Luppi, M., Martelli, D., Amici, R., Baracchi, F., Cerri, M., Dentico, D., ... & Zamboni, G. (2010). Hypothalamic osmoregulation is maintained across the wake–sleep cycle in the rat. *J. Sleep. Res.*, 19(3), 394-399.

Lusis, A. J., Attie, A. D., & Reue, K. (2008). Metabolic syndrome: from epidemiology to systems biology. *Nat. Rev. Genet.*, 9(11), 819-830.

Magnes, J., Moruzzi, G., Pompeiano, O. (1961). Synchronization of the EEG produced by low frequency electrical stimulation of the region of the solitary tract. *Arch. Ital. Biol.*, 99, 33-67.

Mancia, G. (1993). Autonomic modulation of the cardiovascular system during sleep. *N Engl J Med* 328:347–349.

Matthews, K. A., Kamarck, T. W., Hall, M. H., Strollo, P. J., Owens, J. F., Buysse, D. J., ... & Reis, S. E. (2008). Blood pressure dipping and sleep disturbance in African-American and Caucasian men and women. *Am.J. Hypertens.*, 21(7), 826-831.

McCormick, D. A. (1989). Cholinergic and noradrenergic modulation of thalamocortical processing. *Trends Neurosci*, 12(6), 215-221.

Megirian, D., Dmochowski, J., & Farkas, G. A. (1998). Mechanism controlling sleep organization of the obese Zucker rats. *J. Appl. Physiol.*, 84(1), 253-256.

Mileykovskiy, B.Y., Kiyashchenko, L.I. & Siegel, J.M. (2005). Behavioral correlates of activity in identified hypocretin / orexin neurons. *Neuron*, 46, 787–798.

Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S., ... & Coppack, S. W. (1997). Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-α, in vivo. *J. Clin. Endocr. Metabol.*, 82(12), 4196-4200.

Mokdad, A. H., Serdula, M. K., Dietz, W. H., Bowman, B. A., Marks, J. S., & Koplan, J. P. (1999). The spread of the obesity epidemic in the United States, 1991-1998. *JAMA*, 282(16), 1519-1522.

Moruzzi, G., & Magoun, H. W. (1949). Brain stem reticular formation and activation of the EEG. *Electroencephal. Clin. Neurophysiol.*, 1(1), 455-473.

Najjar, M., Rowland, M. (1987). For the National Center for Health Statistics: Anthropometric reference data and prevalence of overweight: United States, 1976–80. Washington, DC, Government Printing Office, Department of Health and Human Services Publication (PHS) 87-1688.

Nofzinger, E. A., Mintun, M. A., Wiseman, M., Kupfer, D. J., & Moore, R.

Y. (1997). Forebrain activation in REM sleep: An FDG PET study. *Brain Res.*, 770(1), 192-201.

Ohkubo, T., Hozawa, A., Yamaguchi, J., Kikuya, M., Ohmori, K., Michimata, M., ... & Imai, Y. (2002). Prognostic significance of the nocturnal decline in blood pressure in individuals with and without high 24-h blood pressure: the Ohasama study. *J. Hypertens.*, 20(11), 2183-2189.

Papanicolaou, D. A., Petrides, J. S., Tsigos, C., Bina, S., Kalogeras, K. T., Wilder, R., ... & Chrousos, G. P. (1996). Exercise stimulates interleukin-6 secretion: inhibition by glucocorticoids and correlation with catecholamines. *Am. J. Physiol.-Endocr. Metabol.*, 271(3), E601-E605.

Pagani, M., Lombardi, F., Guzzetti, S., Rimoldi, O., Furlan, R., Pizzinelli, P., Sandrone, G., Malfatto, G., Dell'Orto, S., Piccaluga, E. (1986). Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ. Res*., 59(2), 178-193.

Parati, G., Lombardi, C., & Narkiewicz, K. (2007). Sleep apnea: epidemiology, pathophysiology, and relation to cardiovascular risk. *Am. J. Physiol.-Reg. Integr.,* 293(4), R1671-R1683.

Parmeggiani, P. (1980a). Temperature regulation during sleep: a study in homeostasis. In: H Orem and Ch. Barnes (Eds), Physiology in Sleep, Academic Press, 97-134.

Parmeggiani, P.L. Cianci,T., Calasso, M., Zamboni, G., Perez, E. (1980b). Quantitative analysis of short term deprivation and recovery of desynchronized sleep in cats. *Electroencephal. Clin. Neurophysiol.*, 50, 293-302.

Parmeggiani, P. L. (2003). Thermoregulation and sleep. *Front. Biosci*, 8, s557-s567.

Parmeggiani P. Physiologic Regulation in Sleep. In: Kryger MH, Roth T,

Dement WC (eds). *Principles and Practice of Sleep Medicine,* Fourth Edition. Elsevier Saunders, Philadelphia. 2005, pp.185-191.

Peeters, A., Barendregt, J. J. M., Willekens, F., Mackenbach, J. P., Al Mamun, A., & Bonneux, L. G. A. (2003). Obesity in adulthood and its consequences for life expectancy: a life-table analysis. *Ann. Intern. Med.*, *138*(1), 24-32.

Peyron, C., Tighe, D. K., van den Pol, A. N., de Lecea, L., Heller, H. C., Sutcliffe, J. G., & Kilduff, T. S. (1998). Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J. Neurosci.,*18(23), 9996-10015.

Peyron C, *et al* (2000). A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat. Med.,* 6, 991-7.

Poirier, P., Giles, T. D., Bray, G. A., Hong, Y., Stern, J. S., Pi-Sunyer, F. X., & Eckel, R. H. (2006). Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation*, 113(6), 898-918.

Rahmouni, K., Morgan, D.A., Morgan, G.M., Mark, A.L., Haynes, W.G. (2005a). Role of selective leptin resistance in diet-induced obesity hypertension. Diabetes, 54(7), 2012-8.

Rahmouni, K., Correia, M.L., Haynes, W.G., Mark, A.L. (2005b). Obesity-associated hypertension: new insights into mechanisms. Hypertension, 45(1),,9-14.

Rechtschaffen A, Bergmann BM (2002). Sleep deprivation in the rat: an

update of the 1989 paper. *Sleep*, 25, 18-24.

Ridker, P. M. (2003). Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation*, 107(3), 363-369.

Rieusset, J., Bouzakri, K., Chevillotte, E., Ricard, N., Jacquet, D., Bastard, J. P., ... & Vidal, H. (2004). Suppressor of cytokine signaling 3 expression and insulin resistance in skeletal muscle of obese and type 2 diabetic patients. *Diabetes*, 53(9), 2232-2241.

Ryden, L., Standl, E., Bartnik, M., Van den Berghe, G., Betteridge, J., De Boer, M. J., ... & Wood, D. (2007). Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). *Eur. Heart J.*, 28(1), 88-136.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., ... & Yanagisawa, M. (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*, 92(4), 573.

Sakurai T, *et al* (2011). Connectomics of orexin-producing neurons: interface of systems of emotion, energy homeostasis and arousal. *Trends Pharmacol. Sci.*, 32, 451-62.

Saper, C. B., Chou, T. C., & Scammell, T. E. (2001). The sleep switch: hypothalamic control of sleep and wakefulness. *Trends Neurosci.*, 24(12), 726-731.

Saper, C. B., Scammell, T. E., & Lu, J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature*, 437(7063), 1257-1263.

Sei, H., Morita, Y. (1996). Effect of ambient temperature on arterial

pressure variability during sleep in the rat. *J Sleep Res.*, 5(1), 37-41.

Sei, H., Furuno, N., Morita, Y. (1997). Diurnal changes of blood pressure, heart rate and body temperature during sleep in the rat. *J Sleep Res.*, 6(2),113-9.

Shek, E.W., Brands, M.W., Hall, J.E. (1998). Chronic leptin infusion increases arterial pressure. *Hypertension*, 31(1 Pt 2), 409-14.

Sherin, J. E., Shiromani, P. J., McCarley, R. W., & Saper, C. B. (1996). Activation of ventrolateral preoptic neurons during sleep. *Science*, 271(5246), 216.

Siegel M. Mammalian Sleep. REM sleep. In: Kryger MH, Roth T, Dement WC (eds). *Principles and Practice of Sleep Medicine*, Fourth Edition. Elsevier Saunders, Philadelphia. 2009, pp.120-130.

Silvani, A. (2008). Physiological sleep-dependent changes in arterial blood pressure: central autonomic commands and baroreflex control. *Clin. Exp. Pharmacol.*, 35(9), 987-994.

Silvani, A., Grimaldi, D., Vandi, S., Barletta, G., Vetrugno, R., Provini, F., ... & Cortelli, P. (2008). Sleep-dependent changes in the coupling between heart period and blood pressure in human subjects. *Am. J. Physiol.-Reg. Integr.*, 294(5), R1686-R1692.

Silvani, A., Bastianini, S., Berteotti, C., Franzini, C., Lenzi, P., Martire, V. L., & Zoccoli, G. (2009). Sleep modulates hypertension in leptin-deficient obese mice. *Hypertension*, 53(2), 251-255.

Smolensky, M. H., Hermida, R. C., Castriotta, R. J., & Portaluppi, F. (2007). Role of sleep-wake cycle on blood pressure circadian rhythms and hypertension. *Sleep. Med.*, 8(6), 668-680.

Society of Actuaries. Build Study of 1979. (1980). Recording and

Statistical Corporation.

Spiegel, K., Leproult, R., & Van Cauter, E. (1999). Impact of sleep debt on metabolic and endocrine function. *The Lancet*, 354(9188), 1435-1439.

Spiegel, K., Tasali, E., Leproult, R., & Van Cauter, E. (2009). Effects of poor and short sleep on glucose metabolism and obesity risk. *Nat. Rev. Endocrinol.*, 5(5), 253-261.

Stevens, J., Cai, J., Pamuk, E. R., Williamson, D. F., Thun, M. J., & Wood, J. L. (1998). The effect of age on the association between body-mass index and mortality. *New Engl. J. Med.*, 338(1), 1-7.

Swoap, S. J. (2001). Altered leptin signaling is sufficient, but not required, for hypotension associated with caloric restriction. *Am. J. Physiol.-Heart Circul.*, 281(6), H2473-H2479.

Szymusiak, R., Gvilia, I., & McGinty, D. (2007). Hypothalamic control of sleep. *Sleep. Med.*, 8(4), 291-301.

Taheri, S., Lin, L., Austin, D., Young, T., & Mignot, E. (2004). Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. *PLoS Med.*, 1(3), e62.

Takahashi, K., Lin, J.S. & Sakai, K. (2006). Neuronal activity of histaminergic tuberomammillary neurons during wake-sleep states in the mouse. *J. Neurosci*., 26, 10292–10298.

Tononi, G., & Cirelli, C. (2003). Sleep and synaptic homeostasis: a hypothesis. *Brain Res. Bull.*, 62(2), 143-150.

Trayhurn, P., & Wood, I. S. (2004). Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Brit. J. Nutr.*, 92(3), 347-355.

Verrier R. and Harper R. Cardiovascular physiology: Central and autonomic regulation. In: Kryger MH, Roth T, Dement WC (eds). *Principles and Practice of Sleep Medicine,* Fourth Edition. Elsevier Saunders, Philadelphia. 2009, pp.215-225.

Venn, A. J., Thomson, R. J., Schmidt, M. D., Cleland, V. J., Curry, B. A., Gennat, H. C., & Dwyer, T. (2007). Overweight and obesity from childhood to adulthood: a follow-up of participants in the 1985 Australian Schools Health and Fitness Survey. *Med. J. Austral.*, 186(9), 458.

Villablanca, J., Marcus, R. (1972). Sleep-wakefulness, EEG and behavioural studies of chronic cats without neocortex and striatum: the 'diencephalic' cat. *Arch. Ital. Biol.*, 110, 348-82.

Vgontzas, A. N., Tan, T. L., Bixler, E. O., Martin, L. F., Shubert, D., & Kales, A. (1994). Sleep apnea and sleep disruption in obese patients. *Arch. Intern. Med.*, 154(15), 1705.

Vgontzas, A. N., Papanicolaou, D. A., Bixler, E. O., Kales, A., Tyson, K., & Chrousos, G. P. (1997). Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. *J. Clin. Endocr. Metab.*, 82(5), 1313-1316.

Vgontzas, A. N., Bixler, E. O., Tan, T. L., Kantner, D., Martin, L. F., & Kales, A. (1998). Obesity without sleep apnea is associated with daytime sleepiness. *Arch. Intern. Med.*, 158(12), 1333.

Vgontzas, A. N., Bixler, E. O., & Chrousos, G. P. (2005). Sleep apnea is a manifestation of the metabolic syndrome. *Sleep Med. Rev.*, 9(3), 211-224.

Wallenius, V., Wallenius, K., Ahrén, B., Rudling, M., Carlsten, H., Dickson, S. L., ... & Jansson, J. O. (2002). Interleukin-6-deficient mice develop mature-onset obesity. *Nat. Med*., 8(1), 75-79.

Waxman, A. (2004). WHO global strategy on diet, physical activity and health. *Food Nutr. Bull.*, 25(3), 292-302.

Webb, W. B., & Agnew Jr, H. W. (1975). The effects on subsequent sleep of an acute restriction of sleep length. *Psychophysiology*, 12(4), 367-370.

Webster, H.H. & Jones, B.E. (1988). Neurotoxic lesions of the dorsolateral pontomesencephalic tegmentum-cholinergic cell area in the cat. II. Effects upon sleep-waking states. *Brain. Res.*, 458, 285–302.

Williams, T. D., Chambers, J. B., Roberts, L. M., Henderson, R. P., & Overton, J. M. (2003). Diet induced obesity and cardiovascular regulation in C57BL/6J mice. *Clin. Exp. Pharmacol.,* 30(10), 769-778.

Winer, B. J. *Statistical principles in experimental designs* (2nd ed.) New York: McGraw-Hill. 1971.

Woo, M. A., Kumar, R., Macey, P. M., Fonarow, G. C., & Harper, R. M. (2009). Brain injury in autonomic, emotional, and cognitive regulatory areas in patients with heart failure. *J. Card. Fail.,* 15(3), 214-223.

World Health Organization. (2003). Controlling the global obesity epidemic. *Online: http://www. who. int/nut/obs. htm. Updated September*, 3.

World Health Organization. (2006). Fact sheet n. 311: Obesity and overweight.

World Health organization. (2007). *The challenge of obesity in the WHO European Region and the strategies for response.* Branca F, Nikogosian H, Lobstein T (Eds). (ISBN 978 92 890 1409 0).

World Health Organization. (2011). *"The Global Burden of Disease concept.".*

Wynne K, Stanley S, McGowan B, Bloom S. (2005). Appetite control. *J Endocrinol.* 184, 291-318.

Yudkin, J. S., Kumari, M., Humphries, S. E., & Mohamed-Ali, V. (2000). Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis,* 148(2), 209-214.

Zamboni, G., Amici, R., Perez, E., Jones, C.A., Parmeggiani, P.L. (2001). Pattern of REM sleep occurrence in continuous darkness following the exposure to low ambient temperature in the rat. *Behav. Brain Res. ,* 122, 25-32.

Zamboni, G., Ann Jones, C., Domeniconi, R., Amici, R., Perez, E., Luppi, M., ... & Luigi Parmeggiani, P. (2004). Specific changes in cerebral second messenger accumulation underline REM sleep inhibition induced by the exposure to low ambient temperature. *Brain. Res.*, 1022(1), 62-70.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372(6505), 425-432.

Zepelin H, Siegel M, Tobler I. Mammalian Sleep. In: Kryger MH, Roth T, Dement WC (eds). *Principles and Practice of Sleep Medicine,* Fourth Edition. Elsevier Saunders, Philadelphia. 2005, pp.91-100.