Theoretical Medical Sciences Ph.D. Program

EFFECTS OF FASTING AND OBESITY ON THERMOREGULATION

Ph.D. Thesis

Dr. Margit Solymár

Head of the Ph.D. School: Prof. Dr. Loránd Barthó

Head of the Ph.D. Program: Prof. Dr. Erika Pintér

Tutor: Prof. Dr. Zoltán Szelényi

UNIVERSITY OF PÉCS
FACULTY OF MEDICINE
Pécs, 2011

1. INTRODUCTION

The topic of this thesis is the examination of energetic regulation in the course of fasting and obesity. In developing countries the pathological loss of body weight/starvation, in modern society obesity is an important public health problem. International surveys show that in 2010 approximately one billion people starved worldwide (United Nations). In the last 25 years in the USA the prevalence of obesity increased by 75% (Flegal et al., 2002). In 2009, according to a Health Survey of Hungary issued by the Central Statistical Office, more than 50 percentage (53,7 %) of people older than 15 the body weight was higher than optimal, and every fifth adult had overweight.

Obesity and the pathological loss of body weight, or changes in the thermal balance (hypothermia, hyperthermia, and fever) indicate changes in energy balance. Components of the energy balance are in interaction with each other, where the metabolic rate (heat production) has a central role (Figure 1). In the complex regulation of energetics beside peripheral neural and humoral afferents central transmitters/mediators also play an important role. In case of normal energy balance body weight and core temperature remain in a normal range. Stability of body weight is the result of a prolonged balance between food intake and metabolic rate (heat production), while the short-term regulation of thermal balance depends on the relationship between heat loss and heat production/metabolic rate.

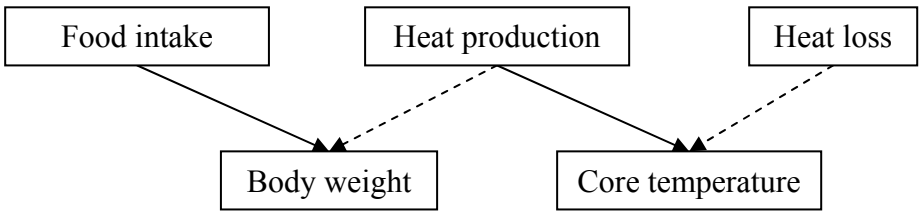

Figure 1: Components of regulation of energetics and their interactions
(→ Stimulation, ----▸ Inhibition)

Long-term energetic imbalances could lead either to obesity or to pathological loss of body weight. Continuous thermal regulation is characteristic of homeothermic species (birds, mammals). Active imbalances of central origin may result in regulated shifts of body temperature (fever, anapyrexia), while ineffective regulation due to failure of sufficient defense could lead to passive hyperthermia or hypothermia. Components of energy balance may influence both body weight and core temperature. Namely, primary alterations of thermoregulation also modify body weight, e.g. food intake is increased by cold exposure. Alternatively, primary changes of the feeding status by influencing metabolic rate may influence also the thermoregulatory state. For example, during fasting the changes of food intake initiate energetic changes. In particular, the resulting reduced metabolic rate may lead to a higher tendency for hypothermia. On the one hand, in the long-term metabolic rate (or heat production) should be adjusted to the general nutritional and actual feeding status, on the other hand, increased metabolic rate should counterbalance the heat loss to avoid hypothermia in cold in the short-term.

2. OBJECTIVES

Mice – because of their small body mass – have a relatively higher body surface area, so they have a more dynamic body temperature fluctuation than rats have, so this makes them ideal to study changes in core temperature. It was described earlier that during starvation heterothermia develops in mice, i.e. core temperature shows progressive falls during the day, while night core temperature remains largely normal (Williams et al., 2002; Overton and Williams, 2004; Gelegen et al., 2006). Using total fasting seemed to be an ideal procedure to assess the possible strategies protecting the mice against excessive, life-threatening hypothermia during fasting and to see how they can still conserve energy.

A standardized fasting protocol was applied in mice to induce heterothermia under free moving conditions while body core temperature and locomotor activity were monitored by biotelemetry. The role of shivering and non-shivering thermogenesis in the manifestation of fasting-induced heterothermia was assessed by administration of specific inhibitors of these processes (mephenesin and guanethidine,

respectively). Furthermore, the possible role of opiate mechanisms was assessed by administration of naloxone. It was hypothesized that inhibition of any of the mechanisms that are known to play a role in cold defense (Gordon, 1993) might also limit increased thermogenesis and affect the level of fasting-induced hypothermia. At the same time, the role of these thermogenic pathways during fasting-induced effects on core temperature and during recovery from fasting could also be ascertained.

Body core temperature and locomotor activity were monitored in TRPV1-KO and wild mice during fasting and on re-feeding by biotelemetry to reveal the possible role of this ion channel in the energetics of fasting state. Although temporary hypothermia as a response to fasting has been known for some time (Williams et al., 2002; Overton and Williams, 2004), the possible energetic background of night normothermia has not been studied so far. The monitoring of locomotor activity was believed to shed some light on the possible role of physical activity in body temperature regulation, an issue having been debated in animal studies (Gordon, 1993; Girardier et al., 1995).

Total fasting was applied also in mice made obese by feeding a fat-rich diet and monitored core temperature and locomotor activity before, during and after fasting. It appeared worthwhile to study, whether they respond to total fasting with a similar change of body core temperature and its daily oscillations as observed in control lean mice and whether they reduced their body mass to the same extent as controls did by the end of fasting. It was also expected to gain information about the maximal duration of total fasting tolerated by the animals and to see whether their original body weight and characteristic circadian rhythm of core temperature and locomotor activity was returning after re-feeding.

To study the role of ciliary neurotrophic factor (CNTF) in body weight regulation and temperature regulation, central CNTF infusion was applied in mice made obese with fat-rich diet. The aim of our study was to investigate effects of intracerebroventricular (icv) infusion of CNTF on changes of nocturnal rhythm and energy parameters such as day-time and night-time core temperature and locomotor activity beside the expected long-term reduction of body weight.

3. MATERIALS AND METHODS

3.1. Experimental animals and their housing

C57BL/6 wild type (WT) male and female and TRPV1-KO male mice were used in our experiments. Mice were kept individually in plastic cages with approximately 3-5 cm wood shaving bedding. Food pellets were available ad libitum with the exception of fasting periods, when only tap water was provided. Besides standard rodent food pellets, for some groups of animals high-fat diet was given (TestDiet 58Y1, IPS Product Supplies Ltd.) containing 60% fat-derived calories. A 12/12 hour light/darkness schedule was used (lights on at 6 a.m.). Ambient temperature in the animal house was set to 24-26 °C (cool) or 27-28 °C (just thermoneutral, only used when the duration of fasting was three-day long) volt. The mice were habituated to daily body mass measurements. The experiments were run according to the general rules set in the Hungarian law on animals and the experimental protocols used were approved by the Ethical Committee of the Pécs University (BA 02/2000-20/2001 and BA 02/2000-13/2006).

3.2. Surgeries

Mice were operated under ketamine/xylazine anesthesia (Calypsol (Richter) + Rometar (Spofa), 78mg/13 mg kg^{-1}). To prevent infections, 0.2 mg gentamycin was administered intraperitoneally (ip). The anesthesia was always the same regardless from the type of the surgery (ip radiotransmitter implantation, transmitter implantation or icv cannula implantation). Most procedures (food deprivation, another surgery, e.g., icv cannula insertion after ip radiotransmitter implantation, etc.) were executed at least one whole week after the (first) surgery.

3.3. Biotelemetry

Core temperature and horizontal locomotor activity were registered continuously with the help of the biotelemetry method. Biotelemetry transmitters (ER-

4000 model VMFH, Minimitter, Sunriver, OR) were implanted ip into the abdominal cavity of mice under the same anesthesia. Abdominal temperature and horizontal locomotor activity was measured at 5 min intervals throughout the experiments. For further data sampling and analysis the VitalView software supplied by the manufacturer (Minimitter Co., Ltd., Sunriver, OR) was used.

3.4. Total fasting duration

At least 7 days following transmitter implantation, the mice were exposed to complete fasting initiated at 9 a.m. The duration of fasting was set either to 2 days (in a cool environment, 23-24 °C), or to 3 days (at a temperature of 27-28 °C). Extension of the duration of fasting to 3 days allowed a longer time course to follow changes in animal energetics, while the extent of fall in body mass remained in the same order by the end of fasting. In an other experiment, fasting period was set to 40 hours at 24-26 °C.

Also in obese mice, core temperature was closely monitored during the course of the longer periods of fasting. On the basis of earlier experience in total fasting was continued as long as daytime body core temperature approached a value around 30-31°C in mice belonging to either group. Thereafter, fat-rich food was given back to the animals. In other words, the duration of fasting could not be exactly the same in every obese mouse exposed to long fasting periods but ranged between 24 and 30 days.

No attempt has been made in the present study to investigate the survival of mice, in other words, not even one mouse was lost as a result of total fasting. After re-feeding every animal looked like normal, re-feeding led to a fast body weight gain.

3.5. Osmotic minipump operation

An icv cannula (Brain-infusion kit, ALZET) was implanted into the right lateral cerebral ventricle of mice fed on DIO diet using a stereotaxic frame for mice (Narishige, Japan). The icv cannula was fixed by dental cement and screws to the skull and attached by a connecting tube to an ALZET osmotic minipump inserted underneath the skin of the nape. Osmotic minipumps of 100 or 200 µl capacity having a mean pumping rate of 1.0 µl/h (ALZET micro-osmotic pump, model 1003D or

2001D) were used, allowing infusions for 3 or 7 days, respectively. Intraperitoneal implantation of the osmotic minipump secured the ip infusion of the solution.

3.6. Substances applied

CNTF (Sigma-Aldrich): 720 ng/hour, 7 day-long icv infusion;
Guanethidine (Sigma-Aldrich): 10 mg/kg/day daily ip injections;
Control salt infusion: 0,9 % NaCl solution;
Mephenesin (Sigma-Aldrich): 42 mg/kg/day 3 day-long ip infusion;
Naloxone (Sigma- Aldrich): 20mg/kg/day 3 day-long sc infusion

3.7. Statistical analyses

Based on the design of the actual experiment, for statistical analyses ANOVA repeated measures, one-way ANOVA with *post hoc* test was used, as appropriate. All results are presented as means ± S.E.M. The level of significance was set at $p<0.05$.

4. RESULTS

4.1. Energetics of fasting heterothermia in TRPV-KO and wild type mice

The overall response of core temperature to 2-day-long or 3-day-long complete fasting carried out at cool or neutral ambient temperature, respectively, consisted of progressive fall of day minima with maintenance of night maxima at or close to pre-fasting values. On re-feeding core temperature returned to normothermia within one hour.

When comparing average responses to two-day fasting of WT mice with those of TRPV1-KO ones, during the first fasting day there was no significant core temperature difference between the two groups. During the whole second fasting day, however, core temperature decreased significantly more in WT mice than in TRPV1-KO ones. Rises of activity in TRPV1-KO mice were significantly higher than that of WT-mice.

When fasting was extended to 3 days in mice exposed to a warmer ambient temperature, similar differences could be observed between the two groups as when

fasting lasted for two days. In particular, decreases of core temperature on the second and third days were less severe in TRPV1-KO mice, than in WT ones with minimum values showing progressive decreases in the course of the three days of fasting. When fasting lasted for three days, TRPV1-KO mice exhibited progressive increased activity rises on consecutive fasting nights, while in WT mice activity could not be enhanced on the third night of fasting.

A further phenomenon is related to the timing of increased activity and temperature during fasting. In TRPV1-KO mice the rising phases of night temperature and activity occurred about the same time just before the start of the dark period. In WT mice, however, there occurred a progressive advance in the appearance of rises in temperature and activity well before the start of the dark period of the day. Mean duration of daily cycles of core temperature were 23-25 hours in the fed state, but were shortened to 17 hours during the third day of fasting in WT mice only.

Re-feeding has completely altered the parallel behavior of core temperature and activity prevailing both before and during fasting. Immediately after the return of food to the mice core temperature started to rise and reached normal values independent of the actual core temperature observed at the last fasting morning. In neither types of mice did increased activity accompany the sharp rise in core temperature on re-feeding, a unique phenomenon observed only very rarely in chronobiological studies in general and in the practice of the present authors, in particular. It should be emphasized that the speed of the rise in core temperature was very rapid in both types of mice and reached normothermia within 30 to 40 minutes.

4.2. Mechanism of fasting heterothermia and re-feeding normothermia in mice

In the control mice, complete fasting resulted in a progressive daytime hypothermia with night normothermia that was followed by rapid return of body core temperature to normal on re-feeding applied in the morning of the second fasting day. During administration of guanethidine, mephenesin or naloxone the basic course of core temperature remained essentially similar, while significant quantitative changes in these trends were observed.

Under the effect of guanethidine, core temperature was slightly lower on the first day of fasting, but was slightly higher during most of the second day and after re-

feeding. Under either condition, re-feeding led to rapid return of core temperature to normal with daily oscillations reappearing gradually.

Mephenesin decreased core temperature during fasting compared to the same animals tested under control conditions. In addition, during the second fasting night, mephenesin treatment resulted in a progressive fall of core temperature up to the time of re-feeding. The re-feeding recovery of core temperature was unaffected by the infusion of mephenesin.

Under the effect of naloxone core temperature was lower during the whole fasting period compared to controls. Re-feeding recovery of core temperature was as rapid with naloxone as was observed under control conditions.

None of the inhibitors of heat production applied had an effect on the dynamics of re-feeding normothermia.

4.3. A month-long reversible total fasting in mice with diet-induced obesity

Effects of total fasting were compared in mice originally on a conventional rodent diet (control) with those in mice made obese by feeding them a high-fat diet. Core temperature and locomotor activity and daily body mass were monitored.

Mice were fed with a fat-rich diet until body mass increased about 50% after two months. Body mass curves for control and DIO-fed (DIO) mice indicated a significant difference in favor of DIO mice from the 5^{th} weeks onwards. Daily core temperature excursions during control feeding were in the order of 2.5 to 3.0 °C and decreased within two days to 1.2 to 1.5 °C after switching to DIO chow with their night maxima remaining similar, while day minima increased significantly and stabilized rapidly.

In contrast to the 2-days fasting in mice with initial body weight of 24-26 g (aged 2-3 months), mice having a body weight of 50-55 g tolerated complete fasting for some 30-35 days, while their body weight reduced to 19-20 g, a value not different from those measured in non-obese animals at the end of fasting. In the whole period of long-term complete fasting body temperature and locomotor activity and their daily oscillations remained normal as long as body weight approached the low value mentioned above; these animals lost 64 per cent of their original body mass.

Re-feeding reversed obese body mass with a rapid rise leading to the original obese values by the end of five weeks 5. It was only during the fourth week of fasting

when daytime core temperature started to fall more progressively on consecutive days reaching values below 31 °C, and the re-feeding that followed led to a robust decrease of daily body temperature oscillations. The duration of fasting could not be exactly the same in every obese mouse exposed to long fasting periods, total fasting was continued as long as daytime body core temperature approached a value around 30-31°C.

Over a body weight range of 27-19 g the change in body weight as a function of daylight minimum core temperature was nearly linear with a coefficient of correlation that was 0.89 and 0.94 for the obese and the control groups, respectively. The two regression lines share a common point for a body mass of about 19 g, the daylight minimum core temperature is than about 30.5 °C.

Seven days before re-feeding daily excursions of core temperature started to rise significantly and reached a value of 4.1±0.4 °C on the last day of fasting. Re-feeding led to rapid significant decrease of daily body temperature excursions to 0.5±0.1 °C with a return to the control value by day 5 of re-feeding (1.5±0.3 °C).

4.4. Effect of central infusion of CNTF in freely moving mice with diet-induced obesity on the core temperature and locomotor activity

In this study core temperature and locomotor activity of obese mice has been followed during and after intracerebroventricular infusion of CNTF for 7 days. Body weight of the mice was measured manually. DIO mice infused icv with CNTF lost body mass and this tendency remained progressive beyond the time of infusion, while icv infusion of the solvent was without effect on body mass. A transient hypothermia developed after any surgery in the first couple of days.

Daily body temperature of DIO mice infused with CNTF increased immediately and remained high for the whole period of infusion. The rise of average daily values of core temperature proved to be even more marked as a result of CNTF infusion, in other words, core temperature excursions virtually disappeared and then returned only after the end of infusion. Locomotor activity decreased from the beginning of CNTF infusion and returned to higher values towards the end of infusion. Also, similar to the case of core temperature, daily activity excursions disappeared but

reappeared again from the next days of infusion onwards. Neither core temperature, nor activity or their daily excursions were affected by icv infusion of the solvent.

5. DISCUSSION

5.1. Energetics of fasting heterothermia in TRPV-KO and WT mice

It was M. Chossat who around the middle of the nineteenth century first described the phenomenon of decrease in body temperature during starvation (Chossat, 1843). Indeed, depending on the body mass and the severity of food restriction, core temperature shows progressive falls during the day, while night temperature remains largely normal in nocturnal species (Overton and Williams, 2004; Gelegen et al, 2006; Abe et al, 2007). The present data confirmed these basic results and extended them to mice lacking functional TRPV1 receptor. Furthermore, evidence has been presented in favor of physical activity as an important source of heat during fasting allowing normothermia at night.

To look for the possible tendency of changes in core temperature and activity, complete fasting was applied not only for two days in mice exposed to a cool ambient temperature (23-25 ^{o}C), but also for three days in mice exposed to slightly warmer neutral ambient temperature (26-28 ^{o}C). In particular, the application of a longer fasting period allowed one to observe progressive falls of day core temperature followed by repeated rises observed at night.

In both types of mice applied in this study body temperature rises were accompanied by increased activity at night, in TRPV1-KO mice the activity raises were even progressively greater during the second and third night. This phenomenon may be regarded as an evidence for the possible role of physical activity in thermoregulatory heat production (Gordon 1993). In fact, during fasting skeletal muscle UCP3 is upregulated and may play a role in increased thermogenesis that may coincide with increased locomotor activity (Argyropoulos, 2002). The behavior of locomotor activity during food restriction was variable in different published studies depending on the severity and duration of food restriction (complete or partial) and on the method of measuring physical activity (spontaneous natural or running-wheel)

applied. In the present study complete fasting has resulted in increased activity only at night with a tendency of further rise during third day of fasting.

The phenomenon of day hypothermia followed by night normothermia can be regarded as heterothermia as opposed to torpor, the latter lasting the whole day (Schleucher et al., 2006).

The difference between the responses of TRPV1-KO mice and the WT ones were twofold: on the one hand, the extent of decrease in core temperature on fasting was significantly greater in wild type mice when compared to that of TRPV1-KO ones. On the other hand, during fasting there appeared an advance in the rises of core temperature and activity in WT mice well ahead of the dark period, a phenomenon not observed in the TRPV1-KO mice exposed to the same fasting. The advance in the appearance of increases in core temperature and activity during fasting can be explained as a sign of resetting of the circadian pacemaker caused by the anticipation activity otherwise occurring in connection with food intake. The robust shortening of the activity-temperature rhythm during fasting has been known in rat and mice but shown only either in constant light or in constant darkness (Chalet et al, 1997). In the present experiment the phase-advance caused by fasting occurred in spite of the maintenance of the 12:12 hours light-darkness schedule. In other words, the rapidly developing energetic insufficiency induced by complete fasting might have induced a strong speeding up of the need for food and thus masking the effect of the main pacemaker stimulus, that is, the darkness cue.

The idea of comparing WT mice with TRPV1-KO ones in their responses to fasting has originated from results of earlier studies from the authors laboratory showing that TRPV1-KO mice had a significantly less effective defense response to heat exposure than WT ones (Szelényi et al., 2004b). In the present study TRPV1-KO mice tolerated fasting much better than their WT counterparts in terms of stability of core temperature.

In both types of mice refeeding was followed by a steep rise in core temperature either without or with only a small rise or no rise in activity. It should be emphasized that the parallel behavior of core temperature and activity generally observed by a number of authors (Gelegen, 2006; Murphy, 1996 and Weinert, 1998) and also in the present studies both under conditions of food intake and activity disappeared for several days following refeeding. As observed both in WT and TRPV1-KO mice, following the rapid rise of core temperature at the first hour of

refeeding circadian cycles of core temperature and activity disappeared with a gradual return of the daily rhythms only after several days. It should be emphasized that in the present study refeeding led to a rise of core temperature within a couple of ten minutes that could have started well before absorption of the ingested food.

5.2. Mechanism of fasting heterothermia and re-feeding normothermia in mice

Fasting has been known to influence the strategy of temperature regulation in small rodent. In laboratory rodents such as the rat and mouse, homeothermia is maintained for several days of fasting only at night, presumably a response that allows for effective search for food. Opposed to this, progressive hypothermia during the daytime develops during several days of fasting as long as the extent of hypothermia will not prevent the animal from achieving normothermia during the next active nocturnal period (Overton and Williams, 2004). In other words, small rodents develop hypothermia only during their inactive period, while night normothermia has to be maintained by heat produced by progressively increased locomotor (physical) activity.

The heat generated in the nocturnal period by increased motor activity could contribute to the maintenance of normothermia at night. However, we hypothesized that the heat from shivering and non-shivering thermogenesis would have a major role in the maintenance of nocturnal normothermia in the fasted mouse.

In the present studies, repeated injections of guanethidine did not cause reductions in core temperature either before or during fasting. In fact, serial injections of guanethidine led to a slight improvement in the tendency to reach normothermia at night and led to a higher body temperature during the daytime period. Although the dose of the drug corresponded to that used by other authors in rats calculated on a body weight basis (Johnson et al., 1975; Mory et al., 1982; Tordoff et al., 1984), it cannot be excluded that significantly higher doses could have influenced daily body temperature oscillations in the expected way (i.e. decrease of core temperature).

Even when applied in the dose used here, guanethidine could probably inhibit NST as part of the sympathetically mediated thermogenic response induced by cold exposure (Griggio, 1982; Lowell and Spiegelman, 2000; Morrison et al., 2008), but in the present studies neutral ambient temperature was applied without the need for an increased thermoregulatory heat production.

Peripheral infusion of mephenesin led to a significant reduction in body temperature of fasted mice during the day time. This would suggest that fasted mice employ shivering thermogenesis to thermoregulate during the daytime when they are normally inactive.

The general course of core temperature was similar during fasting whether naloxone was infused or not, but core temperature was lower under the effect of opiate antagonist during the whole fasting period. In addition, the fall of core temperature during the second fasting day was markedly accentuated in these mice by the application of the opiate receptor blocker. These effects of naloxone may speak for the role of opiate mechanisms in protecting the mice against excessive day hypothermia and in maintaining normothermia at night.

It is concluded that normothermia during the first fasting night is mainly supported by increased locomotor activity, since none of the inhibitors of heat production applied in the present study had a major effect on it. Effects of mephenesin indicate that shivering could play a role in keeping temperature normal at the second night and in mitigating daylight hypothermia during fasting. Similarly, an opiate mechanism appears to have also some a role in the adaptation of temperature regulation to fasting.

However, none of the inhibitors of heat production applied had an effect on the dynamics of re-feeding normothermia. The mechanism of re-feeding rise of core temperature, therefore, still awaits clarification.

5.3. A month-long reversible total fasting in mice with diet-induced obesity

Total fasting as a way to reduce grossly obese body mass of humans has been applied since the 60's of the last century (Bray et al. 1972) and was also tested in rats and in mice to learn changes of body composition. In earlier studies carried out either in humans or animals no clear threshold symptom or physiological parameter could be defined for stopping total fasting before irreversible pathological changes occur, although limitations such as the size of remaining protein pool for gluconeogenesis or adverse changes in different plasma electrolytes affecting cardiac rhythmicity have long been known. As an alternative signal of severe depletion of energy stores for basic life processes, hypothermia has been observed in previous studies carried out during total fasting in mice previously fed a normal chow.

It was therefore logical to apply biotelemetry also in the present study in obese mice to follow the time-course of changes in body core temperature during total fasting to see if there was any major difference in the endpoint of body mass that was still compatible for survival.

Under natural circumstances, small rodents have been known to enter torpidity or daily heterothermia when food sources are severely limited and/or ambient temperature decreases (Geiser, 2004). Still, no relevant data have been published so far that compared effects of long-term total fasting on body mass, core temperature and locomotor activity in normal or obese mice. In fact, the use of biotelemetry in the present study furnished some evidence for the close link between the loss of body mass on the bases of a daytime hypothermia utilized as an indicator.

As demonstrated by the present data, obese mice previously kept on a fat-rich diet could survive total fasting as long as day body temperature reached a value just below 31 °C which was in the same range as in mice previously kept on conventional chow and exposed to total fasting. It should be emphasized that no attempt has been in the present study to investigate the survival of mice, in other words, not even one mouse was lost as a result of total fasting. In fact, extensive experience gained from earlier studies served as a safeguard to avoid any mortality also in the present study by carefully monitoring core temperature with biotelemetry and hence interrupting fasting if daytime core temperature approached threshold values of 30-31 °C.

As opposed to the control mice able to withstand only 2 to 3 days of total fasting with daily core temperature oscillations increasing from the first day onwards, the obese mice showed normal daily body temperature oscillation for some three weeks and it was only about four weeks of fasting, after which time daylight body temperature approached values below 31 °C, while night core temperature was maintained around normothermia. In particular, daily oscillation of core temperature increased from 1.5 °C to 4 °C by the end of fasting. Hence, the duration of reversible total fasting seems to depend on a threshold low body mass that was indicated by a progressive decrease of daylight body temperature both in obese and in non-obese mice.

Metabolic consequences of 16 days long total fasting in ob/ob mice losing some 40 per cent of their body mass were reported several decades ago; in these studies mice became slightly hypoglycemic and all substrates for gluconeogenesis remained high up to the end of fasting (Cuendet et al. 1975) but body core temperature

was not monitored. It would be interesting to learn the way cerebral glucose supply must have maintained sufficient during a still longer fasting applied in the present study carried out in mice with dietary obesity.

The present authors are not aware of any study carried out so far in mice with diet-induced or any other types of obesity, in which body mass decreased as much as in the present study (by 64 per cent) and the animals survived after 4 weeks of fasting and re-feeding with the final body mass ending up the original obese value measured at the beginning of the experiments. It is remarkable that a body mass of about 19 g proved around the low threshold at which daytime hypothermia became severe enough to necessitate re-feeding to prevent fatal outcome irrespective of the original body mass before fasting. It can be speculated that a hormonal and/or metabolic signal activated by the normal adult body mass could act like a ponderostat (Cabanac and Richard, 1996), in this case acting as a fail-safe leading to hypothermia that could lead to a metabolic depression and later to death of hypothermia. This mechanism still awaits clarification.

5.4. Effect of central infusion of CNTF in freely moving mice with diet-induced obesity on the core temperature and locomotor activity

The dynamics of increase of body mass induced by the DIO diet was similar to that observed by an earlier study carried on in the same mouse strain (Kokoeva et al., 2005). The present studies have revealed a robust decrease in the daily body core temperature excursions at the start of switching to DIO diet caused by a rise of daylight core temperature with night values remaining virtually unchanged. At the same time, the excursions of locomotor activity have remained unchanged. This modification of daily core temperature is difficult to explain, but may be connected to disruption of the circadian system observed in mice with diet-induced obesity studied under free-running conditions (Kohsaka et al, 2007; Mendoza et al., 2008). In the present study standard light/darkness schedule was applied that may have masked a real disruption of circadian changes observed in the studies just quoted and the reduction of rhythmic changes of core temperature may be interpreted as an indication of weakened effect of the light/darkness cue on application of the fat-rich diet applied.

The dose of CNTF infused icv in the present study was the same as that published in an earlier paper (Kokoeva et al., 2005), accordingly, the decrease of body

mass proved to be also in the same order of magnitude in our study as in the one quoted. In the present study monitoring changes in body core temperature and locomotor activity has provided additional information on the energetics of the effects of centrally administered CNTF.

In particular, CNTF-induced decrease in body mass could partly accounted for by a rise in body core temperature probably due to an increased metabolic rate, an idea supported by a reciprocal relationship between body mass and metabolic rate in a variety of mammals (Lambert et al., 2001; Janoschek et al., 2006).

The significant decrease of locomotor activity together with a rise of core temperature observed may be regarded as two components of a febrile response known to accompany infections, and if coupled with decreased food intake, these three factors together may represent sickness behavior (Szelényi and Székely, 2004). In fact, this fever-like response induced by icv infusion of CNTF resembles that observed in rats during icv infusion of either CCK-8 or PGE1 (Szelényi et al, 2004). Our finding of a fever-like effect of centrally infused CNTF in mice is a novel one, the only similar effect having been earlier shown in rabbit on peripheral administration of the peptide without information on other components of sickness behavior (Shapiro et al., 1993). These results are compatible with the idea that chronic decrease of obese body mass on central infusion of CNTF may have been the result of hypothalamic neurogenesis, but the short-term fever-like syndrome may be a result of the peptide's effect on gp130 receptors (Schuster et al., 2003).

6. SUMMARY

In TRPV1-KO mice, the fasting-induced hypothermia is attenuated compared to their wild type counterparts. Furthermore during food deprivation their circadian core temperature and activity rhythms are shifted. In TRPV1-KO mice the rising phases of night temperature and activity occurred about the same time just before the start of the dark period. In WT mice, however, there occurred a progressive advance in the appearance of rises in temperature and activity well before the start of the dark period of the day.

Fasting small rodents develop hypothermia only during their inactive period, while night normothermia has to be maintained by heat produced from locomotor

activity and with the help of shivering, non-shivering thermogenesis and opiate-mechanism.

After fasting, on re-feeding core temperature is normalized within a couple of ten minutes without increased locomotor activity. This rapid return of core temperature was unaffected by guanethidine, mephenesin or naloxone, and it was the same in TRPV1-KO animals.

As opposed to the control mice able to withstand only 2 to 3 days of total fasting with daily core temperature oscillations increasing from the first day onwards, the obese mice showed normal daily body temperature oscillation for some three weeks and it was only about four weeks of fasting, after which time daylight body temperature approached values below 31 °C, while night core temperature was maintained around normothermia. In particular, daily oscillation of core temperature increased from 1.5 °C to 4 °C by the end of fasting. Locomotor activity showed a rise in both groups upon fasting. It is remarkable that a body mass of about 19 g proved around the low threshold at which daytime hypothermia became severe enough to necessitate re-feeding to prevent fatal outcome irrespective of the original body mass before fasting. Re-feeding reversed the body mass of the animals.

Right after switching to fat-rich diet core temperature minima values increased significantly, while maxima remained similar, so it led to prompt decrease in circadian core body temperature excursions.

Icv infusion of CNTF resulted in a reduction of body mass of obese mice beyond the period of infusion. This response was accompanied by a rise in day-time (passive period) core temperature and a fall in night-time (active period) locomotor activity, so this means a fever-like response.

Acknowledgements

I would like to thank Professor Dr. Szelényi Zoltán, my supervisor for tutoring, mentoring and funding this research, from whom I have learned scientific work. My whole hearted thanks are due to colleagues in the Department of Pathophysiology and Gerontology for their continuous technical help and valuable advice.

REFERENCES

Abe H, Honma S, Honma K-i, Daily restricted feeding resets the circadian clock in the suprachiasmatic nucleus of CS mice. Am J Physiol 2007, 292:R607-R615

Argyropoulos G, Marper M-E, Invited review, Uncoupling proteins and thermoregulation. J Appl Physiol 2002, 92:2187-2198

Bray GA, Davidson MB, Drenick EJ, Obesity: a serious symptom. Ann Int Med 1972, 77:779-795

Cabanac M, Richard D, The nature of the ponderostat: Hervey's hypothesis revisited. Appetite 1996, 26:45-54

Chalet E, Pévet P, Lakhdar-Ghazal N, Malan A, Ventromedial nuclei of the hypothalamus are involved in the phase advance of temperature and activity rhythms in food-restricted rats fed during daytime. Brain Res Bull 1997, 43:209-218

Chossat C, Recherces Experimentales sur l'inanition. Paris Impr. Royale, 1843

Cuendet GS, Loten EG, Cameron DP, Renold AE, Marliss EB, Hormone-substrate responses to total fasting in lean and obese mice. Am J Physiol 1975, 228:276-283

Flegal KM, Carroll MD, Ogden CL, Johnson CL, Prevalence and trends in obesity among US adults, 1999-2000 JAMA 2002, 288:1723-1727

Geiser F, Metabolic and body temperature reduction during hibernation and daily torpor. Annu Rev Physiol 2004, 66:239-227

Gelegen C, Collier DA, Campbell IC, Oppelaar H, Kas MJH, Behavioral, physiological, and molecular differences in response to dietary restriction in three inbred mouse starins. Am J Physiol 2006, 291:E574-E581

Girardier L, Clark MG, Seydoux J, Thermogenesis associated with spontaneous activity: an important component of thermoregulatory needs in rats. J Physiol 1995, 488:779-787

Gordon CJ, Temperature regulation in laboratory rodents. Cambridge University Press 1993, 107-108

Griggio MA, The participation of shivering and nonshivering thermogenesis in warm and cold-acclimated rats, Comp Biochem Physiol 1982, 73:481-484

Handler CM, Price R, Baumgart M, Law E, Geller EB, Adler MW, Effect of selective and non-selective opioids on body temperature in warm- and cold-acclimated rats. J Therm Biol 2001, 26:351-356

Heldmaier G, Ortmann S, Elvert R, Natural hypometabolism during hibernation and daily torpor in mammals. Respir Physiol Neurobiol 2004, 141:317-329

Janoschek R, Plum L, Koch L et al, gp130 signaling in proopiomelanocortin neurons mediates the acute anorectic response to centrally applied ciliary neurotrophic factor. PNAS 2006, 103:10707-10712

Johnson EM Jr, Cantor E, Douglas JR Jr, Biochemical and functional evaluation of the sympathectomy produced by the administration of guanethidine to newborn rats. J Pharmacol Exp Ther 1975, 193:503-512

Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshu C, Kobayashi Y, Turek FW, Bass J, High-fat diet disrupts behavioral and molecular circadian rhythms in mice. Cell Metab 2007, 9:414-421

Kokoeva MV, Yin H, Flier JS, Neurogenesis in the hypothalamus of adult mice: potential role in energy balance. Science 2005, 310:679-683

Lambert PD, Anderson KD, Sleeman MW, Wong V, Tan J, Hijarunguru A, Corcoran TL, Murray JD, Thabet KE, Yancopoulos GD, Wiegand SJ, Ciliary neurotrophic factor activates leptin-like pathways and reduces body fat, without cachexia or rebound weight gain, even in leptin-resistant obesity. PNAS 2001, 98:4652-4657

Lowell BB, Spiegelman BM, Towards a molecular understanding of adaptive thermogenesis. Nature 2000, 404:652-660

Mendoza J, Pévet P, Challet E, High-fat feeding alters the clock synchronization to light. J Physiol 2008, 586:5901-5910

Morrison SF, Nakamura KI, Madden CJ, Central control of thermogenesis in mammals. Exp Physiol 2008, 93:773-797

Mory G, Ricquier D, Nechad M, Hemon P, Impairment of trophic responses of brown fat to cold in guanethidine-treated rats. Am J Physiol 1982, 242:C159-C165

Murphy PJ, Campbell SS, Physiology of the circadian system in animals and humans. J Clin Neuophysiol 1996, 13:2-16

Overton JM, Williams TD, Behavioral and physiologic responses to caloric restriction in mice. Physiol Behav 2004, 81:749-747

Schleucher E, Prinziger R, Heterothermia and torpor in birds: highly specialized physiological ability or just deep "nocturnal hypothermia"? – The limitations of terminology. Acta Zool Sin 2006, 52:S393-S396

Schuster B, Kovaleva M, Sun Y, Regenhard P, Matthews V, Grötzinger J, Rose-John S, Kallen K-J, Signaling of human ciliary neurotrophic factor (CNTF) revisited. The interleukin-6 receptor can serve as an α-receptor for CNTF. J Biol Chem 2003, 278:9528-9535

Shapiro L, Zhang X-X, Rupp RG, Wolff SM, Dinarello CA, Ciliary neurotrophic factor is an endogenous pyrogen. Proc Natl Acad Sci. USA 1993, 90:8614-8618

Szelényi Z, Hummel Z, Szolcsányi J, Davis JB, Daily body temperature rhythm and heat tolerance in TRPV1 knockout and capsaicin pre-treated mice. Eur J Neurosci 2004b, 19:1421-1424

Szelényi Z, Székely M, Sickness behavior in fever and hyperthermia. Front Biosci 2004, 9:2447-2456

Székely M, Szelényi Z, Regulation of energy balance by peptides: a review. Curr Prot Pept Sci 2005, 6:327-353

Székely M, Pétervári E, Pákai E, Hummel Z, Szelényi Z, Acute, subacute and chronic effects of central neuropeptide Y on energy balance in rats. Neuropeptides 2005, 39(2):103-115

Tordoff MG, Glick Z, Butcher LL, Novin D, Guanethidine sympathectomy does not prevent meal-induced increases in the weight or oxygen consumption of brown fat. Physiol Behave 1984, 33:975-979

Vidal C, Suaudeau C, Jacob J, Hyper- and hypothermia induced by non-noxious stress: effects of naloxone, diazepam and γ-acetylenic GABA. Life Sci 1983, 33:587-590

Weinert D, The temporal order of mammals. Evidence for multiple central and peripheral control mechanisms and for endogenous and exogenous components: some implications for research on aging. Biol Rhythm Res 2005, 36:293-308

Williams TD, Chambers JB, Henderson RP, Rashotte ME, Overton JM, Cardiovascular responses to caloric restriction and thermoneutrality in C57BL/6J mice. Am J Physiol 2002, 282:R1459-R1467

LIST OF PUBLICATIONS

Impact factor of publications in peer-reviewed journals: **12,726**

Impact factor of abstracts: **15,329**

PUBLICATIONS RELATED TO THIS THESIS

Published in peer-reviewed journals:

1. Kanizsai P, Garami A, **Solymár M**, Szolcsányi J, Szelényi Z: Energetics of fasting heterothermia in TRPV1-KO and wild type mice.
Physiol. Behav. 96 (2009)149-54 **IF: 3,295**

Independent citations:
Liu DL et al. Research progress in transient receptor potential vanilloid 1 of sensory nervous system, Neurosci. Bull. 25, 221-227 (2009)

Glanville EJ, Seebacher F, Advantage to lower body temperatures for a small mammal (Rattus fuscipes) experiencing chronic cold, Journal of Mammalogy 91, 1197-1204 (2010)

Steiner A. et al. The hypothermic response to bacterial lipopolysaccharide critically depends on brain CB1, but not CB2 or TRPV1, receptors, J. Physiol. 589, 2415-2431 (2011)

Maloney SK. Et al. Minimum daily core body temperature in western grey kangaroos decreases as summer advances: a seasonal pattern, or a direct response to water, heat or energy supply? J. Exper. Biol. 214, 1813-1820 (2011)

2. **Solymár M**, Kanizsai P, Pétervári E, Garami A, Szelényi Z: Mechanism of fasting heterothermia and re-feeding normothermia in mice *J. Therm. Biol.* 35 (2010) 280-283
IF: 1,273

3. **Solymár M**, Szelényi Z, Pétervári E: A fever-like effect of central infusion of CNTF in freely moving mice with diet-induced obesity. *J. Molec. Nerusosci.* 45 (2011) 212-5
IF: 2,922

4. **Solymár M**, Pétervári E, Szelényi Z: A month-long total fasting followed by re-feeding in mice with diet-induced obesity: is daily torpor regulated by remaining body mass?
J. Comp. Physiol. B (submitted)

Published abstracts:

1. **Solymár M**, Kanizsai P, Pétervári E, Garami A, Szelényi Z:
Mechanism of fasting heterothermia and refeeding normothermia in mice.
Acta Physiol. Hung. 96 (2009) 125-126 **IF: 0,750**

2. **Solymár M**, Szelényi Z: Effects of central infusion of CNTF on the energetics of obese mice. Biotelemetric studies.
Frontiers in Systems Neuroscience. Conference Abstract: 12th Meeting of the Hungarian Neruroscience Society. (2009) doi: 10.3389/conf.neuro.01.2009.04.019

3. **Solymár M**, Szelényi Z: Effects of intracerebroventricular infusion of ciliary neurotropic factor on the energetics of obese mice. *Acta Physiol. Hung.* 97 (2010) 133-34
IF: 1,226

4. **Solymár M**, Szelényi Z: Effects of central infusion of CNTF on the energetics of obese mice. Biotelemetric studies. *Neuropeptides* 44 (2010) 536
IF: 1,917

5. **Solymár M**, Pétervári E, Szelényi Z: Complete fasting for several weeks in obese mice.
Acta Physiol. Hung. 97/4 (2010) 472 **IF: 1,226**

6. **Solymár M**, Párniczky A, Pétervári E, Szelényi Z: One-month long total fasting in ob/ob mice – a biotelemetric study. *Acta Physiol.* 202/S684 (2011) 106 **IF: 3,138**

FURTHER PUBLICATIONS

Published in peer-reviewed journals:

1. Kanizsai P, Vámos Z, **Solymár M** , Garami A, Szelényi Z: Effects of repeated surgical stress on daily changes of body core temperature in mice.
Acta Physiol. Hung. 97 (2010) 203-10 **IF: 1,226**

2. Garami A, Balaskó M, Székely M, **Solymár M**, Pétervári E: Fasting hypometabolism and refeeding hyperphagia in rats: effects of capsaicin desensitization of the abdominal vagus.
Eur. J. Pharmacol. 644 (2010) 61-66 **IF: 2,737**

Independent citation:
Scott V, Brown CH: Kisspeptin activation of supraoptic nucleus neurons in vivo. Endocrinology 152(2011) 3862-70

3. **Solymár M,** Párniczky A, Hamar J, Koller Á: A plazma magas homocysteinszintje és a kardiovaszkuláris betegségek. A terápia eredményességének és hatástalanságának rejtélye.
Kardiovaszkuláris prevenció és rehabilitáció I/61 (2011) 27-31

4. Pétervári E, Balaskó M, **Solymár M**, Párniczky A, Székely M, Szelényi Z: CCK-8 induces fever-like regulated hyperthermia and symptoms of sickness behavior in mice: A biotelemetric study. *J. Therm. Biol.* doi:10.1016/j.jtherbio.2011.07.013
IF: 1,273

Published abstracts:

1. **Solymár M**, Garami A, Pákai E, Szelényi Z: Development of cold-acclimation in the rat and mouse. Biotelemetric study of core temperature and activity.
Acta Physiol. Hung. 94 (2007) 390 **IF: 0,453**

2. **Solymár M,** Garami A, Pákai E, Szelényi Z: Comparison of energetics of short-term cold-acclimation in the rat and mouse biotelemetric studies.
Clin. Neurosci. / Ideggyógy. Szle. 61 (2008) (S1): 58

3. Vámos Z, Vinagre JM, **Solymár M**, Balaskó M, Pétervári E, Székely M: Factors of energy balance during central leptin infusion.
Clin. Neurosci. / Ideggyógy. Szle (2008) 61/1

4. **Solymár M**, Garami A, Pákai E, Szelényi Z: Comparison of energetics of short-term cold-acclimation in the rat and mouse. Biotelemetric studies.
FENS Abstr. Vol 4 (2008) 194.13

5. Balaskó M, Vinagre JM, Pétervári E, **Solymár M**, Vámos Z, Székely M: Relationship between the effects of a central leptin infusion and the feeding state of rats.
Acta Physiol. Hung. 96 (2009) 55-56 **IF: 0,750**

6. Cséplő P, Garami A, **Solymár M**, Balaskó M, Pétervári E, Székely M: Feeding pattern after intraperitoneal capsaicin desensitization in rats.
Frontiers in Systems Neuroscience. Conference Abstract: 12th Meeting of the Hungarian Neuroscience Society. (2009) doi: 10.3389/conf.neuro.01.2009.04.006

7. Székely M, Bartha ZL, Garami A, **Solymár M**, Balasko M: Thermoregulatory effects of central CRF administration.
Frontiers in Systems Neuroscience. Conference Abstract: 12th Meeting of the Hungarian Neuroscience Society. (2009) doi: 10.3389/conf.neuro.01.2009.04.003

8. **Solymár M**, Hamar J, Koller Á: Comparison of antioxidant effects of superoxide dismutase (SOD) and hydrogen sulphide (H2S) in isolated small veins
Cardiol. Hung. 40 (2010) G48

9. **Solymár M**, Tanai E, Hamar J, Koller Á: Comparison of antioxidant effects of hydrogen sulphide and superoxide dismutase in isolated small veins
Kidney Blood Press. Res. (2010) 33:432 DOI: 10.1159/000321436 **IF: 1,50**

10. Soós S, **Solymár M**, Balaskó M, et al.: The role of somatostatin type 4 receptors in cholecystokinin fever. *Acta Physiol. Hung.* 97/4 (2010) 473 **IF: 1,226**

11. Tanai E, **Solymár M,** Hamar J, Koller Á: Comparison of antioxidant effects of hydrogen sulphide (H_2S) and superoxide dismutase (SOD) in isolated small veins.
Acta Physiol. Hung. 97/4 (2010) 481 **IF: 1,226**

12. Barcza Zs, **Solymár M**, Helyes Zs, Pétervári E, Szelényi Z, Székely M: Somatostatin receptor type 4 in the regulation of energy metabolism.
Neuropeptides 44 (2010) 53 **IF: 1,917**

13. Párniczky A, **Solymár M**, Miseta A, Lenkey Zs, Szabados S, Cziráki A, Koller Á. A perikardiális folyadék összetétele koszorúér revaszkularizációs műtéten (CABG) és műbillentyű beültetésen átesett (VR) betegekben.
Érbetegségek XVIII/Suppl.1 (2010) 21

14. Párniczky A, **Solymár M**, Vigh É, Miseta A, Németh Á, Lenkey Zs, Szabados S, Cziráki A, Koller Á. A perikardiális folyadék összetétele koszorúér-revaszkularizációs műtéten (CABG) és műbillentyű-beültetésen átesett (VR) betegekben.
Cardiol. Hung. 39 (2011) F39

15. **Solymár M**, Tanai E, Párniczky A, Porpáczy A, Hamar J, Koller Á. Comparison of antioxidant effects of hydrogen sulphide (H2S) and superoxide dismutase (SOD) in isolated carotid arteries.
Acta Physiol. 202/S684 (2011) 107 **IF: 3,138**