CENTRAL METABOLIC EFFECTS OF THE CORTICOTROPIN SYSTEM IN THE COURSE OF AGING

Doctoral (Ph.D.) Thesis

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LIST OF ABBREVIATIONS

Act  spontaneous horizontal locomotor activity
ACTH  adrenocorticotropic hormone
AgRP  agouti-related protein
alpha- MSH  alpha- melanocyte- stimulating hormone
ARC  arcuate nucleus
BW  body weight
CART  cocaine and amphetamine-regulated transcript
CRF  corticotropin-releasing factor
CRF-BP  corticotropin-releasing factor binding protein
F  female
FI  food intake
GI  gastrointestinal
HLI  heat loss index
HPA axis  hypothalamo-pituitary-adrenal axis
HR  heart rate
ICV  intracerebroventricular
IP  intraperitoneal
LHA  lateral hypothalamic area
M  male
MCH  melanin- concentrating hormone
MCR4  melanocortin receptor type 4
MR  metabolic rate
NPY  neuropeptide Y
NTS  nucleus of the solitary tract
PFS  pyrogen- free saline
POMC  pro-opiomelanocortin
PVN  paraventricular nucleus of hypothalamus
qRT-PCR  quantitative real-time polymerase chain reaction
Ucn  urocortin
Ta  ambient temperature
Tc  core temperature
TRH  thyrotropin-releasing hormone
Ts  tail skin temperature
VO$_2$  oxygen consumption
VMN  ventromedial nucleus of hypothalamus
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1. INTRODUCTION

During the past decades, prevalence of metabolic disorders increased dramatically in populations all over the world. Both energy excess (e.g. obesity) and deficit (e.g. cachexia, sarcopenia) are important worldwide challenges affecting more and more people.

Due to unprecedented food abundance and technological progress in developed countries, overconsumption of calorie-rich food is combined with sedentary lifestyle, therefore obesity reached epidemic proportions in the modern world. In 2014 1.9 billion adults were overweight and, among them, 600 million were obese all over the world [WHO 2016]. This prevalence is constantly growing, by 2030 1.35 billion and 573 million individuals are projected to be overweight and obese [Kelly et al. 2008]. It is well-known, that obesity increases risk of a number of medical conditions including type 2 diabetes mellitus, cardiovascular diseases, depression, certain cancers or respiratory disorders.

It is interesting, that having lower or even normal body mass index (BMI) seems to be more harmful in the elderly than being overweight [Wannamethee and Atkins 2015]. One of the possible explanations of this phenomenon is the muscle loss (sarcopenia) in the elderly. Aging societies and increasing expected lifespan contribute to the rising occurrence of aging anorexia leading to cachexia and/or sarcopenia [Cruz-Jentoft et al. 2014]. According to estimations more than 5-13% of people over 70 years of age are affected by sarcopenia [von Haehling et al. 2010]. This common clinical problem is the precursor of several severe health problems, such as frailty, fractures, functional/physical disabilities, poor quality of life and premature death [Thomas 2010].

The above mentioned abnormalities present considerable public health burdens with serious impact on health status and health cost. That makes it essential to achieve deeper understanding regarding the functions and mechanisms of healthy and pathological energy processes and it underlines the importance of research aimed at age-related alterations in energy metabolism.
1.1. Peptidergic regulation of the energy homeostasis

In an ideal situation, body weight (BW) of adults does not change significantly over a period of time, although energy intake and expenditure fluctuate every day. Thus, regulation of BW can be considered to be a homeostatic system. It is assumed, that an individual set-point is established for BW and energy storage, determined by environmental and genetical factors [Keesey and Hirvonen 1997], [Székely and Szelényi 2005], [Arora and Anubhuti 2006]. The control of BW appears to maintain this set-point and its function is based on a feedback system [Juhász et al. 2007]. The central nervous system receives various forms of afferent signals from the periphery, which modify the activity of central neurotransmitters, modulator substances, and consequently, efferent mechanisms are activated. This way, the system ensures nearly permanent BW and energy balance in the long run.

Some afferent signals represent the actual feeding state and influence the sensation of satiety and hunger. These afferent signals include mechanical signals indicating stretch of the stomach and other areas of the intestines, and hormonal signals originating from the gastrointestinal (GI) tract during a meal, such as cholecystokinin, glucagon-like peptide-1, ghrelin or peptide YY [Morley 1987], [Wilding 2002], [Székely and Szelényi 2005], [Woods 2005]. Satiety signals, through the vagus nerve act at the nucleus of the solitary tract (NTS), which projects amongst others, to the arcuate nucleus (ARC), to the ventromedial (VMN) and paraventricular (PVN) nuclei of hypothalamus; or the circulating substances act directly primarily at the ARC and area postrema [Székely and Szelényi 2005], [Valassi et al. 2008].

The other type of afferent signals indicate the nutritional state. These are proportional to the BW and to the amount of fat in the body, thus they are considered to be signals of fat mass [Baskin et al. 1999], [Porte et al. 2002]. The most important adiposity signals are leptin and insulin, which are transported from the circulation into the brain through the blood-brain barrier [Wilding 2002]. They bind primarily to the ARC and partly to some other structures, e.g. area postrema, NTS, VMN or dorsomedial nucleus of hypothalamus [Palkovits 2003], [Székely and Szelényi 2005].

The ARC is localised near the third ventricle, where the blood-brain barrier is specially modified to allow the entry of peripheral peptides and proteins (median eminence) [Ambach et al. 1976]. Due to its ideal position, the ARC is the main hypothalamic area involved in the control of BW, where various signals for energy
homeostasis are integrated, such as adiposity- and satiety signals or other hypothalamic and supra-hypothalamic inputs [Valassi et al. 2008].

ARC contains interconnected 'first-order' neuron groups: laterally located anorexigenic neurons [i.e. those inhibiting food intake (FI)] and medially located orexigenic neurons (i.e. those enhancing FI) [Palkovits 2003]. Elevated level of leptin (e.g. in fat accumulation) stimulates the expression of anorexigenic neuropeptides and inhibits the release of orexigenic ones from the ARC [Palkovits 2003]. In contrast low adiposity signal (e.g. in starvation) leads to orexigenic predominance [Wilding 2002], [Valassi 2008]. Orexigenic neurons in ARC express neuropeptide Y (NPY) and agouti-related protein (AgRP) [Bewick et al. 2005], [Hahn et al. 1998]. Anorexigenic neurons produce cocaine and amphetamine-regulated transcript (CART) and the alpha-melanocyte-stimulating hormone (alpha-MSH) precursor pro-opiomelanocortin (POMC) [Elias et al. 1998], [Konturek et al. 2005].

Both neuron groups project to 'second-order' neurons. These neurons are partly located in the lateral hypothalamic area (LHA) and perifornical area, where orexigenic neuropeptides are produced, such as melanin-concentrating hormone (MCH) and orexins [Sobrino Crespo et al. 2014]. They are inhibited by anorexigenic and stimulated by orexigenic peptides expressed in the ARC [Konturek et al. 2005]. The other main location of 'second-order' neurons is the PVN. These neurons are activated by anorexigenic neurotransmitters from the ARC (e.g. alpha-MSH derived from POMC), but they also receive important inputs from other hypothalamic nuclei [Palkovits 2003], [Konturek et al. 2005]. PVN is responsible for the secretion of catabolic (i.e. anorexigenic and hypermetabolic) thyrotropin-releasing hormone (TRH), corticotropin-releasing factor (CRF) and oxytocin [Lu et al. 2003], [Valassi et al. 2008]. These observations suggest that PVN plays a role in the integration of nutritional signals with the thyroid and the hypothalamic-pituitary axis and it has also an important role in reproductive processes [Neary et al. 2004].

For simplified schematic summary drawing see Fig.1.
Fig. 1: Schematic model of the peptidergic regulation of energy balance with special emphasis on the activation route of corticotropin-releasing factor. Abbreviations: alpha-MSH: alpha-melanocyte stimulating hormone; AgRP: agouti-related peptide; CART: cocaine and amphetamine-regulated transcript; FI: food intake; GI tract: gastrointestinal tract; LHA: lateral hypothalamic area; MCH: melanin-concentrating hormone; MR: metabolic rate; NPY: neuropeptide Y; POMC: pro-opiomelanocortin; PVN: paraventricular nucleus of the hypothalamus; TRH: thyrotropin-releasing hormone
1.2. Age-related changes of body weight

During the course of aging, long-term trends emerge in the regulation of energy balance resulting in middle-aged obesity and aging anorexia leading to loss of active tissues and cachexia and also to sarcopenia in old age [Scarpace et al. 2000], [Morley 2001a], [Morley 2001b], [Di Francesco et al. 2007], [Pétervári et al. 2011a], [Sertié et al. 2015], [Tay et al. 2015], [Wysokińska et al. 2011], [Jura and Kozak 2016], [Loenneke and Loprinzi 2016]. As most mammals also show similar trends in their long-term BW development (Fig. 2) [Székely et al. 2011] a dysregulation of energy homeostasis may also contribute to these phenomena.

![Body weight (BW) changes during aging in male Wistar rats.](image)

**Fig. 2:** Body weight (BW) changes during aging in male Wistar rats. [Székely et al. 2011]

Earlier studies demonstrated the potential role of age-related shifts in the responsiveness to such centrally administered catabolic mediators as leptin [Pétervári et al. 2014] or alpha-MSH [Pétervári et al. 2010], [Rostás et al. 2015] in the development of the above mentioned BW trends.

Leptin, as an adiposity signal is one of the most potent regulators of long-term energy balance with a complex catabolic activity involving FI suppression and hypermetabolism [Balaskó et al. 2014]. It has been reported, that its effects show special age-related pattern in rats. In studies applying intracerebroventricular (ICV) leptin infusion the hypermetabolic and anorexigenic effects changed in disparate ways during aging. Leptin-induced anorexia was maintained during the course of aging, however its strength varied across age-groups. It was strong in the youngest groups, weaker in aging
rats and became more pronounced in the oldest group. In contrast, hypermetabolism declined continuously with aging [Pétervári et al. 2014]. Moreover, responsiveness to acute central leptin injections showed a similar age-related pattern regarding FI and metabolic rate (MR) [Rostás et al. 2016].

Endogenous melanocortin agonist alpha-MSH is also an important member of the catabolic circuit. It has been proven, that central acute and chronic anorexigenic effects of alpha-MSH show similar pattern during aging to those of the leptin administration (strong in young adults, diminished in middle-aged and pronounced in old rats) [Pétervári et al. 2010], [Pétervári et al. 2011b]. Furthermore, this pattern was observed also in the hypermetabolic effects of central alpha-MSH injections, as opposed to leptin administration [Rostás et al. 2015]. In addition, ICV alpha-MSH infusion induced weak hypermetabolic response in the youngest group and strong hypermetabolic effects in middle-aged, which was maintained also in old animals [Pétervári et al. 2011b].

Based on these findings, the question arises, whether other potent members of the catabolic circuit, such as CRF may also contribute to the above mentioned age-associated obesity and/or to the development of aging anorexia and sarcopenia.

1.3. Urocortin family

The mammalian urocortin or corticotropin neuropeptide family includes four peptide, namely CRF, urocortin 1 (Ucn1), urocortin 2 (Ucn 2) and urocorin 3 (Ucn 3). These peptides are all encoded by separate genes well conserved across mammalian and non-mammalian species [Stengel and Taché 2014], which proves the physiological importance of this system.

CRF was named for its role in the hypothalamo-pituitary-adrenal axis (HPA axis), since it is the releasing factor of adrenocorticotropic hormone (ACTH) [Tsigos and Chrousos 2002]. This 41-aa peptide was isolated from ovine hypothalamus by Vale and coworkers in 1981 [Vale et al. 1981]. It is produced predominantly in the parvocellular neurons of the PVN of the hypothalamus [Morin et al. 1999], but it has been detected, among other sites, in the cerebral cortex, locus coeruleus, medial preoptic area, stria terminalis, amygdala and in the hippocampus [Morin et al. 1999], [Wang et al. 2011], [Janssen and Kozicz 2013].

Ucn1 is a 40 aa peptide characterized in 1995 [Vaughan et al. 1995]. It was named based on its similarity to CRF and urotensin1 (CRF-related peptide in fish) [Fekete and
More recently described urocortins, 39-aa Ucn2 and 38-aa Ucn3 were identified in 2001 [Reyes et al. 2001], [Lewis et al. 2001], [Hsu and Hsueh 2001]. Highest expression of Ucn1 is found in the Edinger–Westphal nucleus in mammalian brain and it is also detected in the lateral hypothalamus, supraoptic and motor nuclei of the brainstem [Bittencourt et al. 1999], [Fekete and Zorilla 2007], [Kozicz et al. 1998]. The main central localisation of Ucn2 is the PVN, the ARC, the locus coeruleus and the supraoptic nucleus [Reyes et al. 2001], [Stengel and Taché 2014], while Ucn3 expression is found among other sites in the amygdala, in the preoptic region, in the PVN, in the median preoptic nucleus and in the rostral prefrontral hypothalamus [Janssen and Kozicz 2013], [Richard et al. 2002], [Stengel and Taché 2014]. It should be noted, that CRF and other urocortins are not restricted to the brain, they have also been identified in several peripheral tissues, e.g. in the placenta, in the GI tract, in the pancreas, in the adrenal glands, in muscles and the liver [Stengel and Taché 2010], [Fekete and Zorilla 2007], [Aubry 2013].

The urocortin family has been demonstrated to participate in the regulation of the energy balance. All members of it reduce FI both in central and peripheral administration [Heinrichs and Richard 1999] and they also elicit matching hyperthermic/hypermetabolic effects [Fekete and Zorilla 2007] therefore they promote negative energy balance.

In addition to their roles in energy homeostasis CRF and urocortins also take part in stress processes and in the development of anxiety and depressive disorders [Bale and Vale 2004], [Fekete and Zorilla 2007], [Janssen and Kozicz 2013], [Waters et al. 2015]. Urocortins have been proposed to be involved in the modulation of glucose homeostasis. For instance, Ucn2 produced in the skeletal muscles inhibits glucose uptake [Chen et al. 2006], [Kuperman and Chen 2008] or Ucn3 expressed in pancreas contributes to the release of insulin [Kuperman and Chen 2008]. They also inhibit gastric emptying. Moreover their participation is suggested in the development of normal hearing and in the protection of the cardiovascular system [Fekete and Zorilla 2007], [Kuperman and Chen 2008].
1.4. Corticotropin-releasing factor (CRF)

1.4.1. Effects of CRF

a. Effects of CRF on the energy homeostasis

CRF participates in a great variety of regulatory processes in humans and also in laboratory rodents. Although CRF is generally regarded as a peptide, which plays an important role in the HPA-axis, it has a not less significant role in the regulation of energy balance. There is a considerable body of evidence indicating that CRF is an endogenous catabolic agent, its anorexigenic and hypermetabolic effects result in weight loss [Krahn et al. 1988], [Richard 1999].

In the PVN CRF level is increased in states of energy excess (overfeeding) or poor glucose utilization [Seeley et al. 1996]. Whereas, states of negative energy balance like fasting or cold exposure reduce CRF and CRF receptor level [Fekete et al. 2000], [Hatalski et al. 1998]. In such cases, the expression of CRF-binding protein (CRF-inactivating protein) is enhanced in brain areas involved in the anorexigenic and thermogenic actions [Richard 1999].

During acute stress anorexigenic effects are elicited through stimulation of POMC/CART neurons in the ARC by increased CRF levels and via additional decrease of NPY secretion [Kyrou et al. 2006], [Chrousos 2000].

However, CRF is able to decrease FI even in the absence of stress [Crespi et al. 2004]. Acute central administration of CRF (injection into the PVN or into the ventricles) suppresses spontaneous food consumption and fasting-induced re-feeding [Cullen et al. 2001], [Morley, 1987], [Arora and Anubhuti 2006]. These anorexigenic effects are accompanied by hypermetabolism and increased brown-fat thermogenesis [Brown et al. 1982], [LeFeuvre et al. 1987], [Carlin et al. 2006].

Not only acute, but also chronic central administration of CRF has catabolic effects without the development of functional tolerance. ICV CRF infusion enhanced the weight of brown adipose tissue [Cullen et al. 2001] and caused a fall in BW partly by suppression of FI partly by enhancement of metabolism [Arase et al. 1988].

Besides central injection and infusion, peripheral administration of CRF also has an effect on energy homeostasis. Peripheral CRF infusion and injection decreased FI and increased energy expenditure and fat oxidation in humans and rodents [Smith et al. 2001], [Hope et al. 2000]. Thus, central and also peripheral administration of CRF evokes catabolic, i.e. anorexigenic and hypermetabolic effects, which appear to be coordinated.
Administration of CRF also stimulates colonic motility, decreases colonic transit time [Zorilla et al. 2003] and induces gastric stasis [Maillot et al. 2000], [Zorilla et al. 2003], which generates a satiety signal. Moreover large dose of CRF is proved to evoke conditioned taste aversion and visceral illness [Krahn et al. 1988], [Zorilla et al. 2004], which may also contribute to the anorexigenic effects.

b. Other CRF effects

The main functions of CRF are thought to be the activation of the HPA axis [Rivier and Vale. 1983], [Muglia et al. 1995], and coordination of the body’s responses to stress [Koob and Bloom 1985]. CRF induces ACTH release from the anterior pituitary leading to glucocorticoid expression in the adrenal cortex, which exerts a negative feedback effect on the hypothalamic CRF release/expression [Rivier et al. 1982], [Vale et al. 1983], [Tsigos and Chrousos 2002]. Prolonged glucocorticoid secretion enhances FI by inhibition of CRF and by stimulation of NPY expression [Chrousos 2000]. CRF has also been shown to induce fear and is known to be involved in mood, anxiety and in depressive disorders [Buwalda et al. 1997], [Bale and Vale 2004], [Wasserman et al. 2010], [Aubry 2013], [Kormos and Gaszner 2013], [Waters et al. 2015]. It also takes part in the activation of the sympathetic nervous system [Brown et al. 1982].

It has been proven, that CRF plays an important role in thermoregulation. It increases the thermogenesis in the brown adipose tissue through the activation of the sympathetic nervous system [Brown et al. 1982], [LeFeuvre et al 1987]. Apart from the phenomenon of stress-induced hyperthermia [Nakamori et al. 1993], both central CRF injection and infusion were shown to induce hyperthermia [Heinrichs et al. 2001], [Richard et al. 2002], [Buwalda et al. 1997], but the exact mechanisms still remain highly controversial. Some studies suggest that fever-like, coordinated hyperthermic effects of CRF develop in a prostaglandin-independent way [Figueiredo et al. 2010], while others consider prostaglandins to be important factors in it [Telegdy and Adamik 2008]. Moreover, according to other studies CRF is not only a febrigenic agent, but also acts as an endogenous antipyretic substance, i.e. it suppresses fever [Holdeman et al. 1985].

In addition, this peptide is known to influence the motor activity [Contarino et al. 2000], [Ohata and Shibasaki 2004], the immune- [De Souza 1995] and the reproductive systems [Heinrichs and Richard 1999], reward-[Koob 2013] and learning mechanisms [Hashimoto et al. 2001].
1.4.2. Receptors and CRF binding protein

Two receptor subtypes (CRF1 and CRF2) mediate the effects of corticotropins. Both receptors belong to class B of the family of seven-transmembrane G-protein coupled receptors, sharing 65–68% overall homology at the amino acid level [Vaughan et al. 1995], [Chatzaki et al. 2004]. CRF elicits its effects by binding predominantly to CRF1 and to a lesser extent to CRF2 receptors [Vaughan et al. 1995], [Liaw et al. 1997], [Perrin and Vale 1999], [Reul and Holsboer 2002], while Ucn1 shows similar affinity to both receptor types [Liaw et al. 1997], [Perrin and Vale 1999], [Vaughan et al. 1995]. Other members of the corticotropin peptide family, such as Ucn2 or Ucn3 show enhanced affinity to CRF2 receptors [Cullen et al. 2001], [Lewis et al. 2001], [Ohata and Shibasaki 2004], [Stengel and Taché 2014]. (Fig 3)

![Fig. 3: Members of the urocortin family, their receptors and corticotropin-releasing factor binding protein (CRF-BP). [Kuperman and Chen 2008]](image)

Widespread expression of CRF1 receptor was described in hypothalamic nuclei [Potter et al. 1994], [Reul and Holsboer 2002], [Van Pett et al. 2000] and also in the anterior pituitary [Van Pett et al. 2000]. Additionally, they are found in the forebrain, in the septal region, in the amygdala [Justice et al. 2008], in the cerebral cortex and in the cerebellum [Reul and Holsboer 2002], [Van Pett et al. 2000]. Anxiogenic actions, depressive behavior, increased locomotor activity and hyperthermic/hypermetabolic effects have been attributed to the activation of this receptor type (Fig. 4) [Figueiredo et al. 2010], [Reul and Holsboer 2002], [Van Pett et al. 2000]. However, the dominant receptor regarding the anorexigenic effects is the CRF2 receptor, although some data
suggest that CRF1 receptor is also able to suppress food intake. This receptor mediates rapid onset, short-term anorexia, independently of CRF2 receptor activation [Zorilla et al. 2003], [Zorilla et al. 2004] [Kuperman and Chen 2008], which has been mainly attributed to anxiety, fear and different kind of stress situations, especially emotional and restrain stress [Hotta et al. 1999], [Richard et al. 2002], [Zorilla et al. 2003].

![Schematic model of the most relevant roles of the main corticotropin-releasing factor receptor types.](image)

**Fig. 4:** *Schematic model of the most relevant roles of the main corticotropin-releasing factor receptor types.*

CRF2 receptor expression appears to be more restricted in the brain relative to that of CRF1 receptors. It was detected in the VMN, in the lateral septum, in the PVN and in the ARC [Van Pett et al. 2000], [Currie et al. 2001], [Fekete and Zorilla 2007], [Chen et al. 2013]. These are proved to be important brain sites involved in the regulatory effects of the corticotropin system regarding food intake and energy balance [Currie et al. 2001], [Richard et al. 2002], [Stengel and Taché 2014]. Moreover, CRF2 receptors are also found in the amygdala, in the hippocampus, in the NTS or in the area postrema [Bittencourt and Sawchenko 2000], [Van Pett et al. 2000]. This receptor type has three functional splice variants differing structurally in the N terminal domain: CRF2a, CRF2b and CRF2c [Hauger et al. 2003]. CRF2b is predominantly found in mammalian species, CRF2a is a better-conserved isoform, which is found even in more primitive species, while CRF2c is expressed only in human brain [Kostich et al. 1998], [Stengel and Taché 2014]. Various studies established the primary role of CRF2 receptor in mediating the anorexigenic actions of central CRF administration or that of other corticotropin agonists Ucn2 or Ucn3 showing enhanced affinity to CRF2 receptors (Fig. 4) [Smagin et al. 1998], [Cullen et al. 2001], [Lewis et al. 2001], [Ohata and Shibasaki 2004], [Stengel and Taché 2014].
contrast with that of CRF1 receptors, CRF2 receptor-mediated anorexia is known to be late-onset and eliciting prolonged effect [Zorilla et al 2003]. The great majority of the studies suggest that specific CRF2 receptor agonists play an important role in stress-adaptation and in the late phase of the stress response [Ryabinin et al. 2012]. Moreover, CRF2 receptors are known to mediate anxiolytic, antidepressive behaviour (Fig. 4) [Van Pett et al. 2000], [Reul and Holsboer 2002]. Ucn 2 and 3 decrease locomotor activity, blood pressure and heart rate (Fig. 4) [Richard et al. 2002], [Ohata and Shibasaki 2004], [Nakamura et al. 2009], which may also confirm the role of CRF2 receptors in anxiolytic mechanisms. However, other studies suggest that the two main CRF receptor types do not show a simple dualism regarding the stress response, anxiety or depression. The CRF system seems to be a more complex circuit, the effects of which depend on the brain site and the neuron type [Janssen and Kozicz 2013].

The CRF2 receptor has a soluble isoform: sCRF2a, which contains only the first, ligand-binding extracellular domain of the CRF2 receptor [Chen et al. 2005]. It binds CRF and Ucn1 with high affinity, while it shows low affinity for type 2 urocortins, Ucn2 and Ucn3 [Chen et al. 2005]. The localisation of this variant is similar to that of the CRF1 receptor, which suggests that it may inhibit the signaling of CRF1 receptor [Chen et al. 2005].

In addition to receptors, effects of CRF are influenced by a 37 kD glycoprotein, called CRF binding protein (CRF-BP). CRF-BP was first isolated from blood of pregnant women and female primates [Petraglia et al. 1993], but it is also known to be widely expressed in the mammalian brain, including the hypothalamus, cerebral cortex, and amygdala [Potter et al. 1991], [Potter et al. 1992]. According to in vitro experiments CRF-BP affinity for CRF and Ucn1 is similar or even stronger than those of CRF receptors, while it has low affinity for both Ucn2 and Ucn3 (Fig. 3) [Heinrichs and Richard 1999], [Fekete and Zorilla 2007]. The exact function of this glycoprotein is not entirely known. It has been hypothesized that CRF-BP limits CRF- and urocortin-mediated processes. For instance CRF-BP is supposed to prevent the inappropriate activity of HPA axis during pregnancy [Linton et al. 1993]. Expression sites of CRF-BP are partly identical with those of CRF and CRF receptors, supporting the above mentioned hypothesis [Potter et al. 1992]. On the other hand, CRF-BP is also found in brain regions, where CRF or CRF receptors are not expressed [Janssen and Kozicz 2013], suggesting other, CRF-independent functions.
1.4.3. CRF and aging

During the course of aging, characteristic changes of the corticotropin system have been reported in humans and mammals [Hatzinger et al. 1996]. Most studies on animals and humans have reported increased hypothalamic CRF expression in old age compensated by some CRF1 receptor downregulation, [Scaccianoce et al. 1990], [Tizabi et al. 1992], [Ceccatelli et al. 1996], [Bao and Swaab 2007], [Aguilera 2011]. Nevertheless, a few studies described unchanged or even reduced CRF expression in old age-groups [Cizza et al. 1994], [Kasckow et al. 1999]. Post mortem immunohistochemical and in situ hybridization studies in brain samples of men also suggest an overactivity of the CRF system, a finding which is more marked in specimens from depressed patients or those diagnosed with Alzheimer's disease [Raadsheer et al. 1994], [Bao and Swaab 2007]. Other investigators demonstrated that hyperactivity of the HPA axis contributes to deterioration of the central and peripheral nervous system associated with aging in humans and in experimental animals [Sapolsky 1999], [Swaab et al. 2005], [Ferrari and Magri 2008].

1.4.4. CRF and gender differences

An intriguing additional feature of the corticotropin system is that its functions show not only age, but also gender differences [Gordon et al. 2016], [Hiroshige et al. 1973], [Miśkowiak et al. 1988]. For instance, gender-dependent changes were demonstrated during a 14-day CRF infusion. CRF administration produced anorexia and weight loss in male Wistar rats, while it failed to induce any significant change in females [Gordon et al. 2016]. In addition, difference in the circadian rhythm of hypothalamic CRF was also reported in male and female Wistar rats [Hiroshige et al. 1973].

Moreover, other elements of the HPA axis are also proved to show age- and gender dependence [Veldhuis et al. 2013], [Handa and Weiser 2014]. Therefore, age-related alterations of the hypothalamic corticotropin system may also show different patterns in males and females. Such differences may also provide some explanation for the gender differences in the long-term BW development of male and female rats: in contrast with males showing middle-aged obesity and a decline in BW in old age-groups, females maintain a stable low BW throughout life [Ferreira et al. 2012], [Székely et al. 2011].
1.4.5. **CRF and other catabolic mediators**

As it was mentioned above, regulation of energy homeostasis is based on a complex circuit of anabolic and catabolic mediators. Thus, CRF and other peptides from the urocrin family are also interconnected with these systems.

Leptin, acting as an adiposity signal, has been reported to stimulate CRF and CRF2 receptor expression [Sobrino Crespo et al. 2014], [Richard 1999], while pretreatment with CRF antagonists decreased anorexigenic effects of leptin [Sobrino Crespo et al. 2014], [Székely and Szelényi 2005]. Moreover CRF-induced anorexia was maintained in leptin receptor deficient rats [Kochavi et al. 2001], which suggests that CRF anorexia is partly independent of leptin.

Catabolic melanocortins activated among others by leptin enhance the activity of the PVN and increase CRF expression [Lu et al. 2003] through melanocortin 4 receptors (MC4R). However, catabolic effects of melanocortins are only partly mediated by CRF [Tachibana et al. 2007], [Kawashima et al. 2008]. On the other hand both in MC4R-deficient mice and in specific MC4R antagonism catabolic effects of CRF remained intact [Marsh et al. 1999], [Vergoni et al. 1999]. In fasting rats, after the refeeding, the decreased MC4R level normalized, except in animals with adrenalectomy, when CRF level was continuously high [Germano et al. 2008]. These processes suggest a negative feedback relationship between CRF and the melanocortin system. Moreover, they also demonstrate that CRF acts downstream to melanocortins.

Based on all these previous findings, the question arises, whether corticotropins (downstream to melanocortins and leptin) may also contribute to the metabolic dysregulation characterizing aging.
2. PRIMARY HYPOTHESES AND AIMS

I. According to our first hypothesis acute catabolic (i.e. anorexigenic and hypermetabolic) CRF effects contribute to middle-aged obesity and aging anorexia. We also hypothesized, that catabolic (i.e. anorexigenic and hypermetabolic) CRF effects vary with aging similarly to those of melanocortins and leptin. Moreover, acute catabolic effects of CRF change in different, non-parallel, i.e. disparate ways in male and female Wistar rats during the course of aging.

Therefore we aimed
1. to assess anorexigenic and hypermetabolic effects of ICV CRF injection in different age-groups of male and female Wistar rats,
2. to analyse the potential involvement of acute central catabolic CRF effects in the development of age-related obesity and aging anorexia.

II. We also hypothesized, that age-related changes in central chronic CRF effects take part in the development of the special pattern of long-term BW regulation in male rats, similar to the catabolic activators of CRF.

Thus, we aimed
1. to investigate the anorexigenic and hypermetabolic responsiveness to a 7-day ICV CRF infusion in different age-groups of male Wistar rats,
2. to analyse whether age-related variations of CRF effects may also contribute to middle-aged obesity and aging anorexia leading to weight loss in old age-groups.

III. We proposed that, the age-related changes of endogenous CRF activity in the PVN contribute to the life-long BW development in male rats.

Thus, we aimed to analyze mRNS expression of CRF in the PVN in different age-groups of male Wistar rats. We also aimed to assess the potential contribution of the CRF expression to the above mentioned long-time BW development.
3. MATERIALS AND METHODS

3.1. Animals

Various age groups of male (M) and female (F) Wistar rats were used in the experiments of the present studies from the Colony of the Institute for Translational Medicine of the Medical School, University of Pécs, Hungary: young adult, younger and older middle-aged, aging and old (3-, 6- and 12-, 18- and 24-months old, respectively). Maximal life-span of our Colony reaches 30 months, about 50% of rats survive 26 months, but after the age of 24 months surgical interventions involve high mortality. Following experiments, animals were sacrificed; no repeated testing across age-groups was possible.

Mean BW of control vs. CRF- treated animals of these age- and gender-groups were as follows: 3-month M: 380.8 ± 14.9 g vs. 359.2 ± 11.1 g, 3-month F: 195.8 ± 8.4 g vs. 219.9 ± 5.6 g; 6-month M: 375.1 ± 7.1 g vs. 382.8 ± 10.1 g, 6-month F: 255.2 ± 4.1 g vs. 247.7 ± 2.7 g; 12-month M: 515.9 ± 22 g vs. 512.1 ± 20.15 g, 12-month F: 258.4 ± 16.8 g vs. 261.8 ± 18.2 g; 18-month M: 516.8 ± 13.8 g vs. 496.9 ± 19.4 g, 18-month F: 252.6 ± 8.8 g vs. 259.7 ± 15.2 g; 24-month M: 465.2 ± 12.8 g vs. 488.4 ±14.2 g, 24-month F: 273.7 ± 15.4 g vs. 281.1 ± 6.5 g. Initial BWs of treated and those of control animals did not differ within age-groups, as shown by one-way ANOVA (p< 0.05 in all cases).

In acute experiments ICV CRF or pyrogen- free saline (PFS) injections were administered to young adult (N_M = 14, N_F = 12), younger (N_M = 14, N_F = 16) and older middle-aged (N_M = 15, N_F = 13), aging (N_M = 16, N_F = 16) and old (N_M = 10, N_F = 18) male and female rats. However, in chronic experiments 7-day ICV CRF- or PFS infusions were applied to 3- (N_M= 16), 12- (N_M=12), 18- (N_M=12) and 24-months old (N_M=15) male rats. For CRF gene expression analysis different age- groups (3-month N_M= 6, 12-month N_M= 6, 18-month N_M= 6, 24-month N_M= 7) of intact male rats were used.

After they have reached the appropriate age, rats were housed in individual plastic home-cages (375 x 215 mm, height 149 mm) containing wood-chip bedding. Cages were equipped with a steel grid top with feeder and bottle container. The animals were kept under conditions of controlled illumination (with 12:12 hours dark-light regime, lights were on from 06:00 am) and at an ambient temperature of 24-25 °C, which provided a thermoneutral temperature in the nest. Standard rat chow (11 kJ/g; CRLT/N rodent chow, Szindbád Kft., Gödöllő, Hungary) and tap water were available ad libitum, except for the
24-h fasting period in acute experiments when only water was provided for the appropriate groups. Spontaneous daily FI and BW were measured every day at 09.00 h, consequently the animals were habituated to regular handling.

3.2. Substances applied

During acute and chronic experiments corticotropin-releasing factor (CRF-41, Bachem, AG Switzerland) dissolved in PFS and PFS alone (as control) were delivered into the right lateral cerebral ventricle.

3.2.1. Applied substances in acute experiments

In acute experiments CRF dissolved in PFS in a volume of 5 µl or 5 µl PFS was filled the proximal end of a 20-25 cm-long polyethylene tube attachment of the injection cannula. A small bubble separated the substance prepared for ICV injection from the PFS that filled the distal part of the tube. All injections were given remotely, without causing any acute discomfort to the rats and they were administered around 09.00 h, early in the inactive phase of the day.

During acute experiments two different doses of CRF injection were used for thermoregulatory analysis and for tests of FI.

When testing anorexigenic effects, the animals received 0.3 µg CRF dissolved in PFS or PFS after 24-h fasting. The dose was chosen based on previous reports of the literature [Semjonous et al. 2009], [Zorilla et al. 2004].

Initially three different doses of CRF (0.3, 1 and 3 µg) were tested in the thermoregulatory experiments of young adult male rats and all doses elicited similar, significant hyperthermic responses. However, the 1 µg dose elicited the largest increase in oxygen consumption (p < 0.05). Therefore, the 1 µg dose was applied in all subsequent thermoregulatory experiments. In these studies CRF was injected at a thermal steady state (usually 60-90 min following the start of the experiment).

3.2.2. Applied substances in chronic experiments

In chronic tests CRF solution or PFS alone were administered into the right lateral ventricle via Alzet osmotic minipumps at a flow rate of 1 µl/h for 7 days. The applied dose of CRF (0.2 µg/µl/h) was chosen according to earlier observations [Rivest et al. 1989].
3.3. Experimental protocols

For testing acute anorexigenic and thermoregulatory effects of CRF, an ICV guide cannula was implanted into the right lateral ventricle after an at least 7-day adaptation to the experimental systems and at least 7 days before the ICV injection. Several days before the first experiment, angiotensin II tests (see below) were performed in order to check the correct location of the cannula. During experiments, each animal in each age-group received CRF and its solvent in random order. For thermoregulatory analysis and for tests of FI separate groups of animals had to be used. For testing the anorexigenic effects of CRF injection, 24 h fasting was applied right before the injections, while in thermoregulatory experiments fasting was not required. After the experiments the position of the injection sites were checked again post mortem using brain coronal sections. (Fig. 5 a, b)

![Diagram](image)

**Fig. 5a:** Schedule for food intake measurements in acute experiments
Fig. 5b: Schedule for thermoregulatory measurements in acute experiments

In chronic experiments the measurements were performed in a biotelemetric system. After the adaptation and 5-7 days before the ICV cannula implantation an emitter was implanted intraperitoneally. Following the full recovery of the surgical procedure ICV cannula and osmotic minipump were simultaneously implanted into the right lateral ventricle and underneath the nape of the neck. After the 7-day infusion rats were euthanized, indicators of body composition were determined and injection sites were checked macroscopically. (Fig. 6)

Fig. 6: Schedule for chronic experiments
3.4. **Surgeries**

**3.4.1. Intracerebroventricular cannula implantation**

An ICV cannula (in acute experiments 22-gauge stainless-steel guide cannula (with O.D. 0.71 mm); in chronic experiments Alzet, Brain-kit) was implanted into the right lateral cerebral ventricle of rats using a stereotaxic apparatus for the purpose of ICV injections or infusion. The tip of the guide cannula or that of the Brain-kit (fixed to the skull by dental cement) was positioned at A: -1.0 mm (to bregma), L: 1.5 mm (right lateral to bregma), V: 3.5 mm (ventral to dura) (coordinates according to the Rat Brain Atlas [Paxinos and Watson 2006].

In acute experiments a stylet closed the lumen of the guide cannula which was replaced during the experiments by a 28-gauge injection cannula (with O.D. 0.36 mm) outreaching the guide cannula by about 0.5 mm.

In chronic experiments the implanted Brain-kit was attached to a simultaneously inserted osmotic minipump.

Before the first ICV injection in order to check the appropriate location of the guide cannula angiotensin II (Sigma, A9525, 20 ng/5 μl) was injected through a PE10 polyethylene tube attachment around 3 days after the implantation. Appropriate location was assumed if at least 5 ml water was consumed within 30 min [Pétervári et al. 2010]. Experiments started 7 days after the cannula implantation. (Fig. 5a, b) Rats with inappropriately located cannula were excluded. No such test was possible in case of the chronic experiments, the location of the Brain-kit cannula was checked only post mortem.

After acute and chronic experiments rats were sacrificed by intraperitoneal (IP) overdose of urethane (3-5 g/kg, Reanal). *Post mortem* check of the injection sites were performed by observing macroscopically the coronal sections of the removed brains that were fixed in 4% paraformaldehyde for 48 hours. Only rats with appropriate cannula location were included in the analysis.
3.4.2. E-mitter implantation

In chronic experiments after at least one week of adaptation to the biotelemetric system and 5-7 days prior to the start of the infusion, an e-mitter (HR E-Mitter, Sunriver, OR) was inserted intraperitoneally into the rats (Fig. 6). The implantation was performed according to our previous studies [Soós et al. 2010], [Pétervári et al. 2011b]. Such an e-mitter contains temperature-, activity measuring capsule and two heart rate (HR) sensors extensions (Fig. 7). During the operation the capsule was inserted into the abdominal cavity and sutured to the abdominal wall, while HR sensors were fixed subcutaneously to the chest at 45-60 degree angle to transverse plane of the heart (Fig. 7).

Fig. 7: E-mitter implantation
1.: Core temperature and activity measuring capsule
2., 3.: Heart rate sensors
3.4.3. Osmotic minipump implantation

After full recovery from the e-mitter surgery, an Alzet osmotic minipump was implanted simultaneously with the ICV cannula (Fig. 6). It was inserted subcutaneously underneath the nape of the neck which was attached to the Brain-kit. The minipump was filled with CRF dissolved in PFS or PFS and provided the delivery of these substances at controlled rates (1 μl/h) for 7 days.

The Alzet osmotic minipump consisted of a semipemalable membrane, an osmotic layer, a flexible impermeable reservoir containing the test solution and a flow moderator (Fig. 8). Interstitial fluid fluxed into the minipump due to the osmotic pressure difference between the osmotic layer and the tissue environment in which the pump was implanted. The influx of water compressed the flexible reservoir, removing the tested agent from the pump at a controlled, predetermined rate.

![Fig. 8: Structure of Alzet osmotic minipumps [Alzet website]](image)

3.4.4. Anesthesia

All surgical interventions were performed under IP ketamine + xylazine [78 mg/kg (Calyspol, Richter) + 13 mg/kg (Sedaxylan, Eurovet)] anesthesia, which provided 1-1.5 hours of deep narcotic state. Gentamycin injection (2 mg IP) was also applied to avoid infections.
3.5. Experimental methods

3.5.1. Assessment of central acute CRF effects on food intake

At least 14 days before the experiments, rats were transferred to the automated FeedScale system (Columbus, OH, Fig. 9) to get habituated to the environment and to the powdered form of rat chow (Fig 5a). The system allowed continuous recording of the amount of consumed food and prevented food hoarding. Data were registered every 10 minutes. On day 1 at 09.00 h food was removed for 24-h (Fig 5a). Five minutes before the re-feeding started (on day 2 at 09.00) assigned rat groups received 0.3 μg ICV CRF or 5 μl PFS injection to test the inhibitory effect of the peptide on 3-h cumulative FI.

Fig. 9: Structure of the automated food intake measuring (FeedScale) system.
1.Scale 2.Feeding apparatus with powdered rat chow
3. Drinking apparatus with tap water
3.5.2. Assessment of central acute CRF effects on thermoregulation and metabolic rate

During thermoregulatory analysis oxygen consumption (VO\textsubscript{2}, representing MR), core temperature (Tc) and tail skin temperature (Ts, indicating heat loss) were recorded in an indirect calorimeter. The tests were performed between 09.00 h and 15.00 h on semi-restrained rats, singly enclosed in cylindrical wire-mesh confiners (Fig. 10a) in separate tightly sealed plexiglass metabolic chambers continuously ventilated with room air (size: 20 x 30 x 18.5 cm; Fig. 10b). Four chambers were used simultaneously that were immersed into a thermostatically controlled water-bath (Fig. 10b). For thermoregulatory analysis of CRF a slightly subthermoneutral environment [ambient temperature (Ta) was 25 °C] was maintained. As the animals were previously accustomed to the semi-restraining cages for at least a week (Fig. 5b; starting with a 120-min, followed by a 180-, a 240- and two 300-min sessions), we could minimize the stress during the experiments, as shown by the normal initial Tc values between 37.2 and 37.6 °C. Nevertheless, the gradual moderate decline in the Tc and VO\textsubscript{2} values of control male (and to a lesser extent of female) PFS-treated rats indicates that some initial stress must have induced a slight increase in these values that declined later in the familiar environment. During tests the animals could not eat or drink.

Fig. 10a: *Semi-restrained rat in a cylindrical wire-mesh confiner.*
Fig. 10b: Schematic model of the indirect calorimeter system.
1.: Air supply pump; 2.: Metabolic chamber; 3.: Water bath;
4.: Extension of the intracerebroventricular cannula;
5.: Thermocouple recording ambient temperature;
6.: Thermocouple recording the tail skin temperature;
7.: Thermocouple recording the core temperature in the colon;
8.: Benchtop thermometer; 9.: Gas sensors; 10.: Computer

Oxygen consumption (ml/kg/min) and carbon-dioxide production (ml/kg/min) were determined by indirect calorimetry (Oxymax, Equal Flow, Columbus, OH) from the air flowing through the chambers (Fig. 10b). Data was registered in 10-min intervals for 3 hours.

For the recording of Tc and Ts copper-constantan thermocouples were applied and temperature data were collected by a Digi-Sense Benchtop Thermometer (Cole-Parmer) for electronic processing and evaluation. The colon thermocouple was inserted 10 cm beyond the anal sphincter and it was fixed by tape to the tail (Fig. 10b). The Ts thermocouple was fixed on the dorsal skin of the distal part of the tail (Fig. 10b). One thermocouple recorded Ta of the chamber (Fig. 10b). The rate of heat loss (heat loss index, HLI) was calculated from the relationship of the monitored temperatures: HLI = (Ts-Ta) / (Tc-Ta) [Romanovsky and Blatteis 1996]. HLI near 0 (when the Ts value approaches Ta) suggests vasoconstriction as a heat conserving mechanism, HLI near 1 (when the Ts value approaches Tc) indicates vasodilation as indication of heat loss. In the semi-restraining cages the animals could not turn around or reach the thermocouples. The thermocouples and the extensions of the ICV cannula were pulled through a tightly sealed port of the metabolic chamber allowing the remote administration of substances (Fig. 10b).
3.5.3. Biotelemetric measurements in central chronic CRF administration

The biotelemetric system consisted of intraperitoneally implanted e-mitters, MiniMitter chambers equipped with feeding and drinking apparatus, receivers (MiniMitter-VMFH series 4000, Sunriver, OR), placed under the MiniMitter chambers and a computer, that processed and stored the measured data (Fig. 11). E-mitters detected Tc and HR (representing MR, [Butler 1993]) and sent signals about these parameters to the receiver. Any change in the signal strength from the implanted e-mitter was interpreted as an indication that the transmitter has horizontally moved. In this way the receiver was capable of registering spontaneous horizontal locomotor activity (Act), which was recorded as an activity count. The receiver generated electrical fields, which powered the e-mitter. Thus, the transmitter did not require batteries and allowed long-term monitoring.

The biotelemetric system registered data every 5 minutes that were integrated into 12-h periods (two mean values per day), equivalent to the daily inactive and to the nighttime active phase. For primary data analysis, the VitalView software provided by the manufacturer (MiniMitter) was used. Data recorded on the day of the implantation of the osmotic minipump and ICV cannula and also on the following night, were excluded from the analysis because of the inflammatory response induced by the surgical procedures [Buwalda et al. 1997]. In this system FI and BW were measured manually daily.

Fig. 11: Structure of the biotelemetric system.
3.5.4. Sampling, RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

Intact normally fed male Wistar rats of each age-group were decapitated. The brains were quickly dissected and frozen in liquid nitrogen. PVN samples were punched from 1 mm thick slices (-2 to -3 mm from the Bregma, [Paxinos and Watson 2006]) of the brains cut on a brain matrix (Ted Pella, CA, USA) by two razor blades. Sections were placed on a chilled mat and the hypothalamic area containing the PVN was microdissected by a 1mm diameter Harris punching needle (Sigma-Aldrich Budapest, Hungary), samples were stored at -70°C until further processing.

The total RNA was isolated with the Pure LinkTM RNA Mini Kit (Life Sciences, Carlsbad CA, USA) according to the protocol suggested by the manufacturer. Samples were homogenized, RNA was purified by ethanol treatment, and eluted from the membrane. The total amount of RNA was determined by NanoDrop (Thermo Scientific). High-capacity cDNA kit was applied (Applied Biosystems, Foster City, CA, USA) to perform cDNA synthesis, using 1 µg of total RNA sample according to the official protocol.

For CRF gene expression analysis, qRT-PCR was performed using SensiFast SYBR Green reagent (BioLine). Amplifications were run on ABI StepOnePlus system. StepOne software was used to analyze gene expression, which was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene. Based on the quality of previous PCR reference curves in male Wistar rats and other rodents, the GAPDH was chosen as a reference gene [Pibiri et al. 2015], [Füredi et al. 2016]. The primer sequences are shown in Table 1. PCR conditions were also set according to previous studies [Füredi et al., 2016]: one cycle 95°C for 2 minutes, 40 cycles at 95°C for 5 seconds and 60°C for 30 seconds. The amplification of PCR products were calculated according to the 2-ΔΔCt method.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRF</td>
<td>5’-CCGGGCAGAGCAGTTAGC</td>
<td>5’-CAACATTTTCATTTCCCAGTAATCT</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5’-TGCCATCACTGCCACTCAGA</td>
<td>5’-GTCAGATCCACAACGGATACATTG</td>
</tr>
</tbody>
</table>

Table 1: Primer sequences of the tested corticotropin-releasing factor (CRF) gene and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene.
3.5.5. Other post mortem examinations

After the experiments rats were euthanized and the injection sites were checked macroscopically by coronal sections of the removed and fixed brains in all cases. Moreover, epididymal and retroperitoneal fat pads of the animals that received ICV infusion were removed and weighed, along with the tibialis anterior muscle, as indicators of body composition.

3.6. Statistical analysis

Each animal group contained at least 6 rats. SPSS 11.0 for Windows was used for the statistical analysis of the data with application of one-way, two-way (univariate or repeated-measures analysis) or repeated-measures ANOVA complemented by Tukey’s post hoc test, when more than two groups were compared. All results are presented as mean ± S.E.M. The significance was set at the level of p < 0.05.

3.7. Ethical issues

All experimental interventions and procedures were undertaken according to the general rules and following the special permission of the University of Pécs Ethical Committee for the Protection of Animals in Research (BA 02/2000-11/2011, valid for 5 years). These rules are in good accord with the main directives of the National Ethical Council for Animal Research and those of the European Communities Council (86/609/EEC, Directive 2010/63/EU of the European Parliament and of the Council).
4. RESULTS

4.1. Results of acute intracerebroventricular administration of CRF in different age-groups of male and female Wistar rats

Table 2 shows BW values of different male and female age-groups. Values of male Wistar rats (Table 2) were in accord with those observed in our previous studies [Balaskó et al. 2013], [Pétervári et al. 2014]: up to 18 months of age BW showed a rising tendency, then it declined in the oldest animals. Body weights (Table 2) of male rats exceeded those of corresponding females of the same age-group.

Following 24-h fasting, weight loss of rats ranged from 7% to 9% of initial BW in males, and from 3% to 10% in females.

<table>
<thead>
<tr>
<th>group (age and gender)</th>
<th>initial BW (g)</th>
<th>group (gender, age, treatment)</th>
<th>BW (g) before fasting</th>
<th>BW (g) before re-feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>M3</td>
<td>384.0±11.8</td>
<td>M3 PFS</td>
<td>387.4 ± 15.8</td>
<td>359.3 ± 13.4</td>
</tr>
<tr>
<td>M3</td>
<td></td>
<td>M3 CRF</td>
<td>360.5 ± 11.5</td>
<td>346.5 ± 18.8</td>
</tr>
<tr>
<td>M6</td>
<td>411.1±5.7</td>
<td>M6 PFS</td>
<td>416.5 ± 9.6</td>
<td>382.8 ± 10.1</td>
</tr>
<tr>
<td>M6</td>
<td></td>
<td>M6 CRF</td>
<td>405.7 ± 6.3</td>
<td>375.1 ± 7.1</td>
</tr>
<tr>
<td>M12</td>
<td>506.5±8.5&lt;sup&gt;a&lt;/sup&gt;&lt;b&gt; &lt;/sup&gt;</td>
<td>M12 PFS</td>
<td>507.3 ± 9.2</td>
<td>477.0 ± 8.9</td>
</tr>
<tr>
<td>M12</td>
<td></td>
<td>M12 CRF</td>
<td>505.8 ± 15.0</td>
<td>474.8 ± 15.6</td>
</tr>
<tr>
<td>M18</td>
<td>543.4±15.4&lt;sup&gt;a&lt;/sup&gt;&lt;b&gt; &lt;/sup&gt;</td>
<td>M18 PFS</td>
<td>532.8 ± 9.4</td>
<td>493.6 ± 17.7</td>
</tr>
<tr>
<td>M18</td>
<td></td>
<td>M18 CRF</td>
<td>554.0 ± 5.1</td>
<td>511.8 ± 24.0</td>
</tr>
<tr>
<td>M24</td>
<td>514.0±14.1&lt;sup&gt;a&lt;/sup&gt;&lt;b&gt; &lt;/sup&gt;</td>
<td>M24 PFS</td>
<td>526.3 ± 18.5</td>
<td>494.8 ± 18.0</td>
</tr>
<tr>
<td>M24</td>
<td></td>
<td>M24 CRF</td>
<td>484.4 ± 11.2</td>
<td>477.6 ± 11.8</td>
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<tr>
<td>F3</td>
<td>220.3±6.9</td>
<td>F3 PFS</td>
<td>233.2 ± 23.1</td>
<td>219.9 ± 5.6</td>
</tr>
<tr>
<td>F3</td>
<td></td>
<td>F3 CRF</td>
<td>207.4 ± 10.2</td>
<td>195.8 ± 8.4</td>
</tr>
<tr>
<td>F6</td>
<td>262.2±2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>F6 PFS</td>
<td>260.5 ± 3.1</td>
<td>247.7 ± 2.7</td>
</tr>
<tr>
<td>F6</td>
<td></td>
<td>F6 CRF</td>
<td>263.9 ± 4.3</td>
<td>255.2 ± 4.1</td>
</tr>
<tr>
<td>F12</td>
<td>275.0±12.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>F12 PFS</td>
<td>278.0 ± 17.5</td>
<td>261.8 ± 18.2</td>
</tr>
<tr>
<td>F12</td>
<td></td>
<td>F12 CRF</td>
<td>272.0 ± 18.8</td>
<td>258.4 ± 16.8</td>
</tr>
<tr>
<td>F18</td>
<td>278.0±9.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>F18 PFS</td>
<td>276.5 ± 17.2</td>
<td>259.7 ± 15.2</td>
</tr>
<tr>
<td>F18</td>
<td></td>
<td>F18 CRF</td>
<td>279.5 ± 10.8</td>
<td>252.6 ± 8.8</td>
</tr>
<tr>
<td>F24</td>
<td>298.2±10.2&lt;sup&gt;a&lt;/sup&gt;&lt;b&gt; &lt;/sup&gt;</td>
<td>F24 PFS</td>
<td>304.4 ± 10.4</td>
<td>281.1 ± 6.5</td>
</tr>
<tr>
<td>F24</td>
<td></td>
<td>F24 CRF</td>
<td>293.9 ± 16.3</td>
<td>273.7 ± 15.4</td>
</tr>
</tbody>
</table>

Table 2: Body weight values (BW) of different age-groups of male (M) and female (F) Wistar rats. Groups are listed according to age and gender, male and female rats were divided into five-five age-groups. In addition to the initial BW values characterizing male and female age-groups, pre-fasting and pre-re-feeding BW-s of corticotropin-releasing factor (CRF)- or pyrogen-free saline (PFS)-treated animals are also shown. Values are expressed as mean ± S.E.M. for a minimum of six-eight rats in each group. Regarding initial BW-s, the following statistically significant differences were denoted:
<sup>a</sup> 3-month male or female rats vs. other gender-matched age-groups (p<0.005),
<sup>b</sup> 6-month male or female rats vs. other gender-matched age-groups (p<0.05).
4.1.1. Acute anorexigenic CRF effects

In young adult (M3) and younger and older middle-aged (M6 and M12, respectively) male rats the ICV administered acute CRF injection caused a strong suppression of 3-h cumulative FI during re-feeding following 24-h fasting [repeated-measures ANOVA for the 3-h re-feeding period from 10 to 180 min for M3: F(1,14) = 10.280, p = 0.006, for M6: F(1,12) = 17.339, p = 0.001 and for M12: F(1,14) = 16.700, p = 0.001, Fig. 12]. The peptide failed to induce a significant anorexigenic response in aging (M18) and old (M24) rats (Fig. 12). These results suggest that the anorexigenic effects of an acute central injection of CRF show a gradual decline with aging [F(4,63) = 3.410, p= 0.014, as indicated by two-way ANOVA repeated-measures analysis].

Cumulative 3-h re-feeding of control female rats reached similar values as those of corresponding age-groups of males (Figs 12, 13). In female rats, anorexigenic effects of an acute ICV CRF injection (0.3 μg) were maintained at a significant level in all age-groups from F3 to F24 [repeated-measures ANOVA for the 3-h re-feeding period from 10 to 180 min for F3: F(1,10) = 21.365, p = 0.002, for F6: F(1,14) = 67.986, p < 0.001, for F18: F(1,10) = 10.210, p = 0.010, for F24: F(1,10) = 5.550, p = 0.040 and from 40 to 180 min for F12: F(1,10) = 5.749, p = 0.043, Fig. 13]. Therefore, in females no age-dependence of acute central CRF-anorexia was found within the observed age-groups [F(4,54) = 1.287, p = 0.287, as indicated by two-way ANOVA repeated-measures analysis].
Fig. 12: Cumulative food intake (FI) values during 3-h re-feeding following 24-h fasting upon an intracerebroventricular (ICV) corticotropin-releasing factor (CRF) or pyrogen-free saline (PFS) injection in different age-groups of male Wistar rats. M3, M6, M12, M18 and M24 indicate 3-month, 6-month, 12-month, 18-month and 24-month male Wistar rats. The groups consisted of minimum 6-8 animals. Asterisks (*) indicate significant (p<0.05) differences between 3-h re-feeding FI of CRF- vs. PFS-treated male rats (repeated-measures ANOVA).
Fig. 13: Cumulative food intake (FI) values during 3-h re-feeding following 24-h fasting upon an intracerebroventricular (ICV) corticotropin-releasing factor (CRF) or pyrogen-free saline (PFS) injection in different age-groups of female Wistar rats. F3, F6, F12, F18 and F24 indicate 3-month, 6-month, 12-month, 18-month and 24-month female Wistar rats. The groups consisted of minimum 6-8 animals. Asterisks (*) indicate significant differences (p<0.05) between FI of CRF- vs. PFS-treated female rats [repeated-measures ANOVA for the whole 3-h re-feeding period (from 10 to 180 min)]. Hashmark (#) indicates significant difference (p<0.05) between FI of CRF- vs. PFS-treated female rats [repeated-measures ANOVA (from 40 to 180 min)].
4.1.2. Acute hypermetabolic/hyperthermic CRF effects

The young adult male age-group showed a CRF-induced increase in MR [represented by the change in VO$_2$ ($\Delta$VO$_2$), at 25 °C Ta], with a consequent significant rise in Tc (shown as $\Delta$Tc in Fig. 14). The rise in VO$_2$ and Tc started directly upon the ICV injection. Fig. 14 demonstrates CRF-induced hypermetabolic/hyperthermic effects (1 µg dose) at 25 °C in young adult rats [ANOVA repeated measures for $\Delta$VO$_2$: F(1,12) = 21.335, p = 0.001; $\Delta$Tc: F(1,12) = 104.208, p <0.001]. No compensatory rise in heat loss was detectable, as indicated by HLI. Based on these data, CRF-induced hyperthermia appears to be coordinated, in which case both heat production and heat conservation (lack of vasodilation) promote the rise in Tc.

![Fig. 14: Changes of core temperature ($\Delta$Tc), oxygen consumption ($\Delta$VO$_2$) and heat loss index (HLI) induced by an intracerebroventricular (ICV) corticotropin-releasing factor (CRF) or pyrogen-free saline (PFS) injection in young adult male Wistar rats (M3). The groups consisted of minimum 6-8 animals. Asterisks (*) indicate significant differences (p<0.05) between $\Delta$Tc and $\Delta$VO$_2$ of CRF- vs. PFS-treated male rats (repeated-measures ANOVA).](image)
Regarding age-related variations of these reactions (Fig. 15), in addition to the strong response of young adult rats to CRF concerning maximal Tc (p < 0.001, one-way ANOVA) and VO$_2$ changes (p < 0.001, one-way ANOVA as compared with their respective controls), similar CRF-induced hyperthermic and hypermetabolic reactions were observed in older rats, as well (for $\Delta$Tcmax M6: p = 0.003, M12: p < 0.001, M18: p < 0.001 and M24: p = 0.004; for $\Delta$VO$_2$max M6: p = 0.005, M12: p = 0.007, M18: p = 0.023 and M24: p = 0.040).

**Fig. 15:** Maximal increases in core temperature ($\Delta$Tcmax) and oxygen consumption ($\Delta$VO$_2$max) induced by intracerebroventricular corticotropin-releasing factor (CRF) or pyrogen-free saline (PFS) injection in different age-groups of male Wistar rats. M3, M6, M12, M18 and M24 indicate 3-month, 6-month, 12-month, 18-month and 24-month male Wistar rats. The groups consisted of minimum 6-8 animals. Asterisks (*) indicate significant differences (p<0.05) between $\Delta$Tc and $\Delta$VO$_2$ of CRF- vs. corresponding PFS-treated male rats (one-way ANOVA).
Although these responses proved to be significant in all age-groups, the maximal increase in Tc and VO$_2$ (Fig. 15) declined with aging (two-way ANOVA univariate analysis for maximal rises in Tc or VO$_2$: F(9,55) = 3.929, p = 0.007 or F(9,55) = 2.67, p = 0.041, respectively). Heat loss mechanisms did not show any age-related alteration.

Young adult female rats showed a weaker CRF-induced increase in MR and Tc (at 25 °C Ta) than the corresponding male age-group. The rise in VO$_2$ and Tc started directly upon the ICV injection. Fig. 16 demonstrates CRF-induced hypermetabolic/hyperthermic effects (1 μg dose) in young adult female rats [ANOVA repeated-measures for ΔVO$_2$ from 10 to 180 min: F(1,12) = 8.254, p = 0.014; for ΔTc from 10 to 180 min.: F(1,12) = 13.342, p = 0.003]. No vasodilation, indicated by HLI occurred in these female rats (Fig. 16).

**Fig. 16:** Changes of core temperature (ΔTc), oxygen consumption (ΔVO$_2$) and heat loss index (HLI) induced by an intracerebroventricular (ICV) corticotropin-releasing factor (CRF) or pyrogen-free saline (PFS) injection in young adult female Wistar rats (F3). The groups consisted of minimum 6-8 animals. Asterisks (*) indicate significant differences (p<0.05) between ΔTc and ΔVO$_2$ of CRF- vs. PFS-treated female rats (repeated-measures ANOVA).
The maximal increase in Tc and VO$_2$ (Fig. 17) showed some variations with aging in female rats. The peptide induced hyperthermia (for ΔTcmax: F3: p < 0.001; F6: p=0.017; one-way ANOVA) and hypermetabolism (for ΔVO$_2$max: F3: p = 0.004; F6 p= 0.047; one-way ANOVA) in the young adult and younger middle-aged females. However, this response was weaker than in the corresponding male groups. Older female age-groups failed to show any significant change in Tc or VO$_2$. Thus, the hyperthermic/hypermetabolic effects of CRF showed a gradual age-dependent decline in female rats [two-way ANOVA univariate analysis, F(9,59) = 3.309, p = 0.037 for ΔTcmax and F(9,59) = 2.731, p = 0.016 for ΔVO$_2$max].

**Fig. 17:** Maximal increases in core temperature (ΔTcmax) and oxygen consumption (ΔVO$_2$max) induced by intracerebroventricular corticotropin-releasing factor (CRF) or pyrogen-free saline (PFS) injection in different age-groups of female Wistar rats. F3, F6, F12, F18 and F24 indicate 3-month, 6-month, 12-month, 18-month and 24-month female Wistar rats. The groups consisted of minimum 6-8 animals. Asterisks (*) indicate significant differences (p<0.05) between ΔTc and ΔVO$_2$ of CRF- vs. corresponding PFS-treated female rats (one-way ANOVA).
4.2. Results of chronic intracerebroventricular administration of CRF in different age-groups of male Wistar rats

4.2.1. Effects of chronic CRF administration on body weight and body composition

Body composition values (calculated for 100 g BW) (Tables 3, 4, 5) and mean BW-s of different age-groups of control animals were in accord with those observed in our previous studies [Pétervári et al. 2010], [Balaskó et al. 2013]: BW of young adult rats was significantly lower than that of older animals (p < 0.05). Concerning body composition indicators, epididymal fat values were found to be different between young adult (3-month) rats vs. 18- or 12-month groups and between the 12- or 18-month vs. 24-month group (p<0.05, Table 3), whereas retroperitoneal fat pad of young adult rats differed significantly from values of 18-month, aging animals (p < 0.05; Table 4). No difference in muscle mass was detected in any group, except for the oldest (24-month) sarcopenic animals (p < 0.05 for rats 24- vs. 12 months of age; Table 5).

Regarding the age-related effects of a 7-day ICV CRF infusion on BW values, CRF treatment suppressed BW throughout the infusion period (as shown by repeated-measures ANOVA comparing the curves of treated vs. control rats) in the 3-month, 18- and 24-month age-groups, but not in middle-aged (12-month) animals [3-month: F(1,14) = 10.548, p = 0.006; 18-month: F(1,10) = 23.436, p = 0.001; 24-month: F(1,14) = 44.239, p < 0.001] (Fig. 18). By the end of the CRF infusion, significant reduction of retroperitoneal fat developed in the oldest groups [18-month: p = 0.009; 24-month: 0.036, one-way ANOVA; Table 4], while no change occurred in epididymal fat or muscle mass in any group (Table 3).
<table>
<thead>
<tr>
<th>Group (age)</th>
<th>Epididymal fat (g/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PFS</td>
</tr>
<tr>
<td>3-month</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td>12-month</td>
<td>0.53 ± 0.05*,#</td>
</tr>
<tr>
<td>18-month</td>
<td>0.55 ± 0.01*,#</td>
</tr>
<tr>
<td>24-month</td>
<td>0.39 ± 0.03</td>
</tr>
</tbody>
</table>

**Table 3:** Epididymal fat values at the end of a 7-day intracerebroventricular corticotropin-releasing factor (CRF) or pyrogen-free saline (PFS) infusion in different age-groups of male Wistar rats. Asterisks (*) indicate the significant difference (p<0.05) from 3-month old PFS-treated animals. Hashmarks (#) refer to significant differences (p<0.05) from 24-month old PFS-treated animals.

<table>
<thead>
<tr>
<th>Group (age)</th>
<th>Retroperitoneal fat (g/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PFS</td>
</tr>
<tr>
<td>3-month</td>
<td>0.29 ± 0.04</td>
</tr>
<tr>
<td>12-month</td>
<td>0.47 ± 0.07</td>
</tr>
<tr>
<td>18-month</td>
<td>0.60 ± 0.13*</td>
</tr>
<tr>
<td>24-month</td>
<td>0.44 ± 0.05</td>
</tr>
</tbody>
</table>

**Table 4:** Retroperitoneal fat values at the end of a 7-day intracerebroventricular corticotropin-releasing factor (CRF) or pyrogen-free saline (PFS) infusion in different age-groups of male Wistar rats. Asterisk (*) indicates the significant difference (p<0.05) from 3-month old PFS-treated animals. Hashmarks (#) refer to significant differences (p<0.05) between corresponding retroperitoneal fat values of CRF- vs. PFS-treated 18- or 24-month rat-groups.

<table>
<thead>
<tr>
<th>Group (age)</th>
<th>Tibialis anterior muscle (g/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PFS</td>
</tr>
<tr>
<td>3-month</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>12-month</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>18-month</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>24-month</td>
<td>0.15 ± 0.02*</td>
</tr>
</tbody>
</table>

**Table 5:** Values of tibialis anterior muscle at the end of a 7-day intracerebroventricular corticotropin-releasing factor (CRF) or pyrogen-free saline (PFS) infusion in different age-groups of male Wistar rats. Asterisk (*) indicates the significant difference (p<0.05) from 12-month old PFS-treated animals.
Fig. 18: Changes of body weight (ΔBW) prior to and during the course of a 7-day intracerebroventricular infusion of pyrogen-free saline (PFS, open symbols) or corticotropin-releasing factor (CRF, closed symbols) in different age-groups of male Wistar rats. Asterisks (*) indicate significant differences (p < 0.05) determined by repeated-measures ANOVA.
4.2.2. Chronic anorexigenic CRF effects

Concerning the anorexigenic effects, the CRF infusion elicited the strongest suppression during the first two days in all rats. Significant anorexia was detected for 2 days in the 3-month, for 7 days in the 18- and 24-month animals [3-month: F(1,14) = 7.430, p = 0.016; 18-month: F(1,10) = 6.803, p = 0.028; 24-month: F(1,14) = 19.771, p = 0.001, repeated-measures ANOVA] (Fig. 19). Fig. 20 describes 7-day cumulative energy intake in CRF-treated vs. respective control groups demonstrating the age-dependence and short-term feature of CRF-induced anorexia (18-month: p = 0.035; 24-month: p < 0.001, one-way ANOVA). Accordingly, anorexigenic effects of the CRF infusion were strongest in the oldest rats.

Fig. 19: Daily food intake (FI) values prior to and during the course of intracerebroventricular infusion of pyrogen-free saline (PFS, open bars) or corticotropin-releasing factor (CRF, closed bars) in different age-groups of male Wistar rats. The fall at day-0 was due to the surgical intervention. Asterisks (*) indicate significant differences (p < 0.05) in the variables during the indicated period between the CRF-treated versus control groups, as determined by repeated-measures ANOVA.
Fig. 20: Cumulative food intake (FI in kJ) values from day 1 to day 7 in the course of intracerebroventricular infusion of pyrogen-free saline (PFS, open bars) or corticotropin-releasing factor (CRF, closed bars) in different ag-gorupps of male Wistar rats. Asterisks (*) indicate significant differences (p < 0.05) between CRF- vs. PFS-treated rats as determined by one-way ANOVA. Above the bars the rates of CRF-induced suppression (the difference between values of corresponding control and CRF-treated groups expressed as percentage of the control energy intake) are also indicated.
4.2.3. Results of biotelemetric measurements in chronic CRF administration

Our present data demonstrate that mean nighttime control HR values of young adult rats differed from those of older age-groups [3- versus 12-, 18-, 24-month: F(1, 23) = 7.808, p = 0.001, repeated-measures ANOVA, p values of post hoc analysis: 3- vs. 12-month – 0.002, 3- vs. 18-month – 0.025, 3- vs. 24-month – 0.002] (Fig. 21). These results are in accord with our previous findings that demonstrated a decline in the control nighttime HR values of rats in the course of aging [Pétervári et al. 2014]. During the ICV CRF infusion mean daytime HR (inactive period, nadir of the circadian rhythm) failed to show significant increase during the infusion. The slight rise in HR in middle-aged and old rats on day 1 of the infusion is likely to be attributable to the surgery.

Fig. 21: Heart rate (HR) values averaged for 12-h periods before and during the course of an intracerebroventricular infusion of pyrogen-free saline (PFS, grey lines) or corticotropin-releasing factor (CRF, black lines) in different age-groups of male Wistar rats. The mean daytime (inactive phase) values are represented by open symbols, the mean nighttime HR values by closed symbols. The white-black bars underneath the abscissa refer to the day-night periods, respectively.
Mean basal (pre-infusion) day- and nighttime body temperature values did not change across our age-groups. Regarding hyperthermic effects of CRF, the infusion induced a 2-day elevation of mean daytime temperatures: [3-month: F(1,12) =7.836, p = 0.016, repeated-measures ANOVA] (Fig. 22) in young adult rats. The slight rise in the mean daytime Tc value of day 1 in aging animals is probably due to the surgery, while differences in other groups did not reach statistical significance.

Fig. 22: Core temperature (Tc) values averaged for 12-h periods before and during the course of an intracerebroventricular infusion of pyrogen-free saline (PFS, grey lines) or corticotropin-releasing factor (CRF, black lines) in different age-groups of male Wistar rats. The mean daytime (inactive phase) values are represented by open symbols, the mean nighttime Tc values by closed symbols. The white-black bars underneath the abscissa refer to the day-night periods, respectively. The deep fall in Tc at day-0 in all age-groups was due to the surgical intervention. Asterisk (*) indicates the significant difference (p < 0.05) for mean day-time body temperature values of 3-month PFS-treated versus age-matched CRF-treated rats for days 2 and 3 determined by repeated-measures ANOVA.
Act of young adult rats exhibited some diminishment on day 2 of the infusion that did not reach statistical significance. Otherwise no CRF-related alteration of Act was detectable (Fig. 23).

Fig. 23: Spontaneous horizontal locomotor activity (Act) values averaged for 12-h periods before and during the course of an intracerebroventricular infusion of pyrogen-free saline (PFS, grey lines) or corticotropin-releasing factor (CRF, black lines) in different age-groups of male Wistar rats. The mean daytime (inactive phase) values are represented by open symbols, the mean nighttime Act values by closed symbols. The white-black bars underneath the abscissa refer to the day-night periods, respectively. The falls at day-0 (both for the daytime and nighttime values) were due to the surgical intervention.
4.3. CRF gene expression in the paraventricular nucleus in different age-groups of male Wistar rats

In our study qRT-PCR measurements revealed that in the PVN of different age-groups of rats, CRF mRNA expression showed significant changes with aging (p = 0.036, one-way ANOVA). CRF mRNA expression appeared to increase until 18 months with a subsequent slight decline in the 24-month animals (Fig. 24). Post hoc analysis showed significant difference between the young and aging groups (3- versus 18-month rats: p =0.006). In addition, a rising tendency was observed between the middle-aged and aging groups (12- versus 18-month animals: p = 0.051) (Fig. 24).

![Relative expression of CRF mRNA in the PVN](attachment:image)

**Fig. 24**: Relative mRNA expression of corticotropin-releasing factor (CRF) gene by quantitative RT PCR in the paraventricular nucleus (PVN) in different age-groups of male Wistar rats. Data were evaluated by the delta delta Ct method for qRT-PCR analysis. CRF mRNA expression was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression. Asterisk (*) indicates significant difference (p<0.05) between 3-month and 18-month animals as determined by one-way ANOVA.
5. DISCUSSION OF FINDINGS

5.1. Acute anorexigenic CRF effects in male and female rats in the course of aging

In males, acute ICV CRF administration-induced suppression of re-feeding showed age-dependence, as it was significant in the young adult and also in the younger and older middle-aged groups, whereas this effect failed to develop in older animals (Fig. 12). This age-related pattern of acute central CRF-anorexia distinctly differs from that of acute central alpha-MSH- and also leptin-induced suppression of re-feeding reported in male Wistar rats [Pétervári et al. 2010] [Rostás et al. 2016]. According to those studies, male rats showed a strong decline of melanocortin- and leptin-induced anorexia in the middle-aged group (as compared with that seen in young adult animals) but pronounced effects were observed again in the old rats [Pétervári et al. 2010] [Rostás et al. 2016]. Such an age-related pattern potentially contributes to the explanation of middle-aged obesity and aging anorexia. In contrast, the age-related pattern derived from our experiments, characterizing acute central CRF-anorexia is unlikely to promote the development of either the middle-aged obesity or the aging anorexia in male Wistar rats.

Regarding the potential involvement of the major CRF receptor subtypes in the above described age-related pattern, CRF2 receptors (rather than CRF1 receptors) may play a decisive role in it. Although CRF shows much higher affinity to CRF1 receptors, their contribution to CRF-induced anorexia is mainly attributed to emotional stress [Hotta et al. 1999]. Moreover, these receptors activate the HPA axis [Van Pett et al. 2000] and therefore they are responsible for the dramatic increase in peripheral plasma corticosterone level upon an acute ICV CRF injection, described in previous studies [Cullen et al. 2001]. A rise in peripheral corticosterone level or even an ICV administration of the hormone failed to change FI in mice [Debons et al. 1986], whereas a CRF-induced increase in peripheral cortisol has been shown to be coupled with increased FI in healthy humans [George et al. 2010].

In addition, the vast majority of the related literature also attributes anorexigenic CRF effects to CRF2 receptor mediation [Cullen et al. 2001], [Lewis et al. 2001], [Ohata and Shibasaki 2004], [Stengel and Taché 2014]. Thus, our present findings may have implications for the potential lack of involvement of CRF2 receptors in aging-induced variations of BW in male rats.
In females, CRF-induced anorexia did not exhibit age-dependence within the studied age-groups, as it was maintained in all female animals (Fig. 13). This continuous efficacy of acute ICV anorexigenic actions of CRF may have helped to prevent rapid weight gain in middle-aged and older female animals, but apparently failed to induce weight loss up to 24 months of age. However, it cannot be excluded that even older age-groups of female rats (e.g. 30-month), not studied in the present work, would show anorexia and weight loss as a result of this maintained efficacy. In summary, a gender difference emerges in the age-related patterns of CRF-induced anorexia and of CRF2 receptor responsiveness in Wistar rats: these CRF effects decline in males, but they are maintained in females during aging (until 24 months of age).

5.2. Acute hyperthermic/ hypermetabolic CRF effects in male and female rats in the course of aging

Concerning hypermetabolic/hyperthermic effects in young adult males, ICV CRF administration elicited a prompt rise in VO\textsubscript{2} inducing a steady rise in Tc. This hyperthermia was accompanied by continuous heat conservation as indicated by the HLI (Fig. 14). This thermoregulatory response appears to be coordinated, i.e. similar to fever-like coordinated hyperthermias, in which increased heat production is accompanied by suppressed heat loss coordinated by thermoregulatory centers of the hypothalamus [Balaskó et al. 2013], [Steiner et al. 2002], [Székely and Szelényi 1979]. The role of CRF in thermoregulation is still controversial. Some studies suggested a role of CRF in the development of fever [Holdeman et al. 1985], [Rothwell 1990]. However, other findings contradict the hypothesis of the participation of CRF in febrigenesis. Neither non-selective cyclooxygenase (COX) inhibitors, nor COX-2 antagonists suppressed CRF-induced hyperthermia [Figueiredo et al. 2010], [Rothwell 1990], although prostaglandin E\textsubscript{2}, a product of COX, is a key mediator of fever (for review see [Blatteis 2007]). Antipyretic effects of centrally applied CRF were also demonstrated previously [Bernardini et al. 1984]. However, the present test also failed to detect compensatory vasodilation during the course of CRF-hyperthermia arguing for a coordinated hyperthermic response. The mechanisms of this seemingly coordinated hyperthermia still remain unknown.

Regarding the age-related pattern of hypermetabolic/hyperthermic acute central CRF actions in males, these effects are maintained at a significant level across all age-
groups, although a decline begins in the early middle-aged group continuing in the course of aging (Fig. 15). This pattern is similar to those of leptin injection in male Wistar rats [Rostás et al. 2016] and may contribute to the development of middle-aged obesity. In addition, the low hypermetabolic responsiveness of old rats to CRF may contribute to the diminished capacity of old populations to develop fever. These findings may also have implications for the potential involvement of CRF1 receptors in aging-induced variations of BW, since acute central hyperthermic CRF effects were shown to be mediated predominantly by CRF1 receptors [Figueiredo et al. 2010].

Young female rats exhibited a much weaker hypermetabolic/hyperthermic response upon acute central CRF administration (Fig. 16) as compared with that of males. Even an elevated dose of CRF (3 µg) failed to elicit an equivalent CRF-hyperthermia in females. This phenomenon cannot be explained by a diminished thermogenic capacity of females, as febrile responses of male and female rats to toxic agents did not differ in previous studies [Gordon et al. 1997], [Gordon and Mack 2003]. Moreover, heat production capacity of the brown adipose tissue was even enhanced in female rats [Justo et al. 2005]. It may be concluded that the hypermetabolic responsiveness of female Wistar rats to acute central CRF administration is weaker than that of the males. The decline of the response during the course of aging was faster than in males: from 12 months of age no significant rise of VO₂, or Tc was observed in older female animals (Fig. 17).

5.3. Chronic CRF effects on body weight and body composition in male rats in the course of aging

Concerning responsiveness to prolonged administration of exogenous CRF, our results regarding the effects of the CRF infusion on parameters of energy balance in young adult rats were in accord with previous observations [Rivest et al. 1989], [Buwalda et al. 1997]. Compared with the weak, but significant CRF-induced BW reduction in young adult rats, the oldest animals (18-, 24-month) showed strong weight loss during the course of the infusion, while middle-aged animals failed to lose weight (Fig. 18). This pattern was similar to those of alpha- MSH and leptin infusions [Pétervári et al. 2011b], [Pétervári et al. 2014] and it suggests the potential contribution of CRF in age-related body weight changes.

Accordingly, at the end of the infusion, post mortem body composition indicators demonstrated the biggest changes in the two oldest age-groups: retroperitoneal fat mass
was found to be reduced in CRF-treated animals (Table 4), whereas epididymal fat did not show any decline (Table 3). Although CRF-infusion–induced decreases in fat mass were reported previously by other researchers in young age-groups of different rat strains [Arase et al. 1988], [Cullen et al. 2001], in our study no change in fat mass indicators was detected either in young adult, or in middle-aged groups (Tables 3 and 4). In addition, failure of fat accumulation in rats of our study by the end of the CRF infusion supports the dominance of CRF2 receptor activation. In previous experiments chronic CRF1 receptor activation (using a 13-day ICV CRF-infusion with simultaneous administration of CRF2 receptor antagonist) was shown to increase fat mass by the end of the infusion [Cullen et al. 2001]. In contrast, in our study aging and old rats showed a loss of fat tissue by the end of the 7-day infusion.

Muscle mass indicators did not show any CRF- or PFS-induced change in any group (Table 5), indicating a lack of sarcopenic effects of our 7-day CRF infusion. This lack of sarcopenia (a typical consequence of overactivity of glucocorticoids) [Millward et al. 1976], [Kayali et al. 1987] in all our CRF-treated age-groups also indicates that effects of CRF-induced HPA axis activation [Rivest et al. 1989], [Cullen et al. 2001] and those of the inevitable rise in peripheral corticosterone level [Rivest et al. 1989], [Cullen et al. 2001] did not influence our results significantly. On the other hand, enhancement of CRF-induced weight loss in the oldest age-groups in our study may be, at least in part, ascribed to the relative diminishment of peripheral glucocorticoid release in old rats, as demonstrated by previous studies [Rebuffat et al. 1992], [Zambrano et al. 2015].

5.4. Chronic anorexigenic CRF effects in male rats in the course of aging

With regard to CRF infusion-induced anorexia, the FI suppression was of short duration in the young, but strong and persistent throughout the infusion in the two oldest age-groups (Figs. 19, 20). The above described findings strongly support the contribution of age-associated alterations in anorexigenic responsiveness to CRF in aging anorexia and cachexia and they do not contradict a potential role of these changes in middle-aged obesity. This age-related pattern was similar to those of activators of CRF such as melanocortin agonist alpha-MSH or adipose tissue derived leptin [Pétervári et al. 2011a], [Pétervári et al. 2014]. In addition age-related decline in the activity of antagonistic
orexigenic/anabolic mediator systems such as that of neuropeptide Y [Sahu et al. 1988] may be also found in the background.

As CRF also activates the HPA axis, the question arises, as to what extent would the inevitable rise in peripheral corticosterone level [Rivest et al. 1989], [Cullen et al. 2001] contribute to the CRF infusion-induced changes in energy balance. Previous reports demonstrated that a 13-day ICV CRF-infusion (applying a dose of 44 μg or 1 nmol/day using an Alzet osmotic minipump, similar to that in our study) increased the serum level of corticosterone substantially from the 4.3-7.5 μg/dl measured in controls to 16-20.5 μg/dl [Rivest et al. 1989], [Cullen et al. 2001]. These data suggest that effects of the high peripheral corticosterone level would contribute to the outcome of a CRF infusion. However, central injections of CRF proved to be efficient in suppressing FI even in hypophysectomized rats [Morley and Levine 1982]. This finding argues against a crucial role of peripheral corticosterone in CRF effects. Moreover corticosteron is reported to increase FI by inhibition of CRF and stimulation of NPY expression [Chrousos 2000]. Thus, age-related diminishment of glucocorticoid release (antagonizing central anorexigenic CRF effects) from the adrenal cortex of old rats upon ACTH activation [Rebuffat et al. 1992] may take part in the enhanced anorexigenic effects of CRF in the older age-groups.

Potential contribution of other factors, such as divergent age-related alterations in the activity of different subpopulations of CRF receptors may be also considered. In the present study, an important role of the CRF2 receptor emerges. Central CRF infusion-induced anorexia in young rats and especially the long-term suppression of FI in the aging and old age-groups suggest that the infusion must have activated CRF2 receptors. Our results are in accord with previous observations of Contarino and coworkers [Contarino et al. 2000], who found that CRF decreased FI equally in wild type and CRF1 receptor-deficient mice, demonstrating that CRF is able to induce anorexia via CRF2 receptors. Other studies also support the role of CRF2 receptor in anorexigenic CRF-effects [Cullen et al. 2001; Pelleysmounther et al. 2000].
5.5. Chronic CRF effects on metabolic rate and locomotor activity in male rats in the course of aging

A transient moderate CRF-induced hyperthermia was observed in the young adult group, but no change of Tc (day- or nighttime) developed in middle-aged or older rats (Fig. 22). In addition, increase of HR failed to reach a significant extent in any age-group (Fig. 21).

Hyperthermia/hypermetabolism induced by ICV infusions of leptin or alpha-MSH differed from those induced by the CRF infusion [Pétervári et al. 2011b], [Pétervári et al. 2014]. In case of middle-aged (12-month) rats, CRF infusion failed to elicit hyperthermia. However our previous reports demonstrate that in similar middle-aged male Wistar rats a similar 7-day ICV infusion of a melanocortin agonist alpha-MSH successfully induced hyperthermia for 4-5 days. The duration of the alpha-MSH-induced increase in HR also reached 5 days [Pétervári et al. 2011a]. A 7-day ICV leptin infusion was also capable of increasing Tc and HR of 12-month male Wistar rats [Pétervári et al., 2014]. Thus, hypermetabolic effects of the 7-day CRF infusion, at a dose appropriate for induction of anorexia in young adult rats, appear to be much weaker than those of corresponding doses of melanocortins or leptin [Pétervári et al. 2011a], [Pétervári et al. 2014] and did not show any remarkable age-related variations (Fig. 22).

The lack of increase in HR during the CRF infusion supports the potential contribution of CRF2R activation to the observed effects. Previous studies reported acute elevations of HR upon acute central CRF injections based on the activation of CRF1 receptor [Nijsen et al. 2000], whereas Ucn3 possessing especially high affinity to CRF2 receptor, injected into the nucleus tractus solitarii elicited bradycardia [Nakamura et al. 2009]. Here again, we hypothesize the mutual quenching of effects of CRF1 and CRF2 receptors concerning HR.

Apart from some surgical procedure-induced suppression Act failed to change in CRF-, alpha-MSH- or leptin-treated rats, alike in all age-groups (Fig. 23) [Pétervári et al. 2011a], [Pétervári et al. 2014]. Previous studies reported activity-enhancing effects of CRF1 receptor and also those of acute central CRF injection [Contarino et al. 2000], while others demonstrated the inhibitory influence of CRF2 receptor on motor activity [Ohata and Shibasaki 2004]. So, the lack of rise in nighttime Act in any of our animal groups may indicate CRF infusion-induced additional activation of CRF2 receptors,
counteracting the locomotor activity–inducing effects of the inevitable CRF1 receptor activation.

5.6. CRF gene expression in the paraventricular nucleus in male rats in the course of aging

In the PVN, CRF gene expression increased with aging until 18 months with a subsequent slight decline in the 24-month group (Fig. 24). These results suggest an age-related rise in the endogenous gene expression of CRF in rats. Our findings are in accord with several previous studies demonstrating maintained or even increased CRF expression in old age [Scaccianoce et al. 1990], [Tizabi et al. 1992], [Bao and Swaab 2007], [Aguilera 2011]. These findings support the potential contribution of endogenous CRF effects to aging anorexia but not to middle-aged obesity.
6. SUMMARY OF NOVEL FINDINGS

I. Novel findings of acute experiments:
   • Anorexigenic effects of ICV CRF injections declined with aging in both male and female Wistar rats, although CRF-induced anorexia remained significant in all female groups.
   • Regarding hypermetabolic effects, female and male rats appear to share the tendency for age-related decline in the responsiveness to acute central CRF administration, nevertheless all age-groups of male animals showed pronounced hypermetabolic responsiveness.
   • Only age-related decline in the hypermetabolic responsiveness to central CRF injection was proved to be similar to the effects of central leptin injections. Other CRF- induced changes differed from the effects of central alpha- MSH or leptin injections.
   • The maintained anorexigenic efficacy of CRF in females may contribute to their lack of middle-aged obesity and may possibly promote later weight loss in older female animals. In males, no such association may be observed.
   • However, hypermetabolic effects declined with aging in both males and females, the high remaining level of CRF-induced hypermetabolism of old male rats, may contribute to the age-related weight loss.

II. Novel findings of chronic experiments
   • Chronic ICV CRF administration induced pronounced anorexia and BW loss in young adult, aging and old male rats in contrast with middle-aged animals.
   • Central CRF infusion did not show any remarkable age-related changes in hypermetabolic/hyperthermic effects.
   • Unlike hyperthermia/hypermetabolism, CRF-induced anorexia and consequent weight loss, appears to show similar age-related patterns as those previously described in case of chronic central melanocortin and leptin administrations.
   • With regard to our hypothesis, our results confirm the potential contribution of age-related changes in the anorexigenic responsiveness to a CRF infusion to aging anorexia and consequent weight loss in the old age-groups.
III. Novel findings of the investigation of CRF gene expression in the PVN:

- In the PVN, CRF gene expression increased with aging until 18 months with a subsequent slight decline in the 24-month group.
- Age-related changes in CRF gene expression in the PVN may contribute to the phenomena of aging anorexia.
7. LIMITATIONS AND PERSPECTIVES

Despite our positive results, our studies had numerous limitations. There is a lack of internal positive controls such as alpha-MSH in both acute and chronic studies, however the effects of alpha-MSH injection and infusion were investigated earlier separately. Acute and chronic effects were not possible to be examined in the same experimental model due to technical difficulties. In addition, acute effects of the CRF infusion could not be assessed in our experimental model, since the infusion started already 10-12 hours following the implantation of the osmotic minipump. Therefore, effects of surgery influenced data recorded during the first day of the infusion.

Gender differences raise the possibility of the involvement of sex hormones in long-term BW regulation and in CRF effects. Although a previous report [Rivest et al. 1989] described similar baseline serum levels of plasma corticosterone in male and female Wistar rats and a similar increase in their respective serum values by the end of a 13-day CRF infusion, to date the effects of estrogen or testosterone on CRF activity (from hypothalamic immunoreactivity to serum levels of the peptide) remain controversial [Haas and George 1989], [Handa and Weiser 2014]. Ovariectomy in different age-groups of rats (carried out a month before the experiments) may help identify the special contribution of estrogen to these gender differences. However, the contribution of sex hormones would decrease during the course of aging, as their levels decline following menopause or by old age in males.

Another important question that could not be addressed by the present work (due to technical obstacles) involves age-related changes in CRF1 receptor and CRF2 receptor systems in the hypothalamus. The role of CRF2 receptors promises to be more important from the point of view of this topic, as anorexigenic, but not hypermetabolic responsiveness to CRF seems to be involved in age-related body weight changes. Unfortunately, as yet, no specific CRF2 receptor antibody is available (probably due to the high rate of homology of CRF receptor subtypes, [Chatzaki et al. 2004], [Lukkes et al. 2011].

In the future differential involvement of CRF1 and CRF2 receptors in central CRF effects should be further investigated by the application of specific agonists (e.g. Ucn2 for CRF2 receptors) and antagonists (e.g. antalarmin for CRF1 receptors [Stengel and Taché 2014] and antisauvagine-30 for CRF2 receptors [Stengel and Taché 2014] of the
two major receptor types. In addition, differential age-related variations in the signal transduction pathways may also be assumed and investigated.

Our results suggest that the CRF system plays an important role in the development of aging anorexia and cachexia. However, corticotropin receptors show widespread presence in the brain with diverse effects that are not explored in all details yet. Therefore in order to identify clinically relevant, specific and safe new targets in the prevention or therapy of aging anorexia, further investigations are necessary especially regarding specific receptor subpopulations and signal transduction pathways.
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9. LIST OF PUBLICATIONS AND PRESENTATIONS

9.1. Articles related to the thesis


9.2. Articles not related to the thesis


Cumulative impact factor (without citable abstracts): 32.837
Citations: 27
Independent citations: 15
9.3. Presentations related to the thesis

1. **Tenk J.** A CRF hatása energetikai folyamatokban. IV. Grastyán Endre Szakkollégium Intézményi Konferencia 2010, Pécs

2. **Tenk J.** A corticotropin- releasing factor életkorfüggő hatásai az energia háztartás egyes paramétereire PTE ÁOK Házi Tudományos Diákköri Konferencia 2011, Pécs

3. **Tenk J.** A corticotropin- releasing factor anyagcsere folyamatokban betöltött szerepe az életkor függvényében. III. Nemzetközi IX. Országos Interdiszciplinális Grastyán Konferencia 2011, Pécs

4. **Tenk J.** A corticotropin- releasing factor életkorfüggő hatásai az energia háztartás egyes paramétereire. XVI. Korányi Frigyes Tudományos Fórum 2011, Budapest


8. **Tenk J, Mikó A, Füredi N, Lőrincz O:** Age- associated alterations in corticotropin effects on energy homeostasis are disparate. CROSS, 8th International Biomedical Croatian Student Summit 2012, Zágráb


11. **Tenk J**, Füredi N. Disparate age-associated alterations in corticotropin effects on energy homeostasis. 5th HMAA, Balatonfüred Amerikai Magyar Orvosszövetség Konferenciája 2012, Balatonfüred

12. **Tenk J**, Rostás I, Füredi N. Age-related shifts in the responsiveness to corticotropin- releasing factor (CRF) affecting energy homeostasis János Szentágothai Memorial Conference and Student Competition 2012, Pécs


14. **Tenk J**, Füredi N. Disparate age- associated alterations in acute corticotropin effects on energy homeostasis. CROSS, 9th International Biomedical Croatian Student Summit 2013, Zágráb

16. **Tenk J.** A corticotropin-releasing factor (CRF) akut életkorfüggő hatásai az energia háztartásban. V. Nemzetközi XI. Országos Interdisciplinális Grastyán Konferencia 2013, Pécs


19. **Tenk J.** Age-related shifts in acute central corticotropin effects on energy homeostasis. Amerikai Magyar Orvosszövetség (HMAA) Hungary Chapter Conference 2013, Balatonfüred

20. **Tenk J.** Age-associated alterations in acute corticotropin effects on energy homeostasis. 2nd International Doctoral Workshop on Natural Sciences 2013, Pécs


25. **Tenk J**, Szakács Zs, Rostás I, Soós Sz, Pétervári E, Balaskó M. Age-related alterations in acute central corticotropin effects regarding the parameters of energy balance. YES Meeting- 9th Young European Scientist Meeting 2014, Porto


27. **Tenk J.** Szakács Zs, Pilisi R, Rostás I, Balaskó M. Corticotropin releasing factor (CRF) in age-related metabolic dysregulation. Third International Symposium on Hypertension 2014, Eszék

28. **Tenk J.** Pagáts R, Rostás I, Soós Sz, Pétervári E, Székely M, Balaskó M. Age related shifts in the responsiveness to corticotropin-releasing factor concerning energy homeostasis. 5th Central European Congress on Obesity 2015, Budapest
9.4. Other presentations


2. Lőrincz O, Tenk J. A testösszetétel és életkor hatásai az energia-háztartás szabályozására: a leptin szerepe. PTE Mínősítő Konferencia - TÁMOP 4.2.3. „Nyitott Egyetem- a PTE tudásbázisának disszeminációja” 2011, Pécs


5. Füredi N, Tenk J, Mikó A. Age-associated alterations in cholecystokinin effects concerning energy balance are disparate. CROSS, 8th International Biomedical Croatian Student Summit 2012, Zágráb

6. Mikó A, Füredi N, Tenk J. Complex changes in the energy homeostasis of spontenously hypertensive rats (SHR). CROSS, 8th International Biomedical Croatian Student Summit 2012, Zágráb

7. Füredi N, Tenk J. Disparate age-related alterations in cholecystokinin (CCK) effects on energy homeostasis. 5th HMAA, Balatonfüred Amerikai Magyar Orvosszövetség Konferenciája 2012, Balatonfüred

9. Füredi N, Tenk J, Rostás I. Central and peripheral effects of cholecystokinin (CCK) on food intake during the course of aging. János Szentágothai Memorial Conference and Student Competition 2012, Pécs


15. Mikó A, Rostás I, Tenk J, Füredi N. Spontaneously hypertensive rats (SHR) show complex changes in their energy homeostasis. LIMSC- Leiden International Medical Student Conference 2013, Leiden

16. Rostás I, Mikó A, Füredi N, Tenk J. Complex effects of neuropeptide alpha-melanocyta- stimulating hormone on energy homeostasis during the course of aging. LIMSC- Leiden International Medical Student Conference 2013, Leiden
24. Füredi N, Rostás I, Tenk J, Mikó A, Pétervári E, Balaskó M. Cholecystokinin effects on energy balance depend on age and body composition. 9th János Szentágothai Memorial Conference and Student Competition 2013, Pécs
25. Rostás I, Tenk J, Mikó A, Füredi A. Complex effects of alpha-melanocyta-stimulating-hormone during the course of aging. Periodicum Biologorum- 3rd Congress of Croatian Physiological Society and 1st Regional Congress of the Phisiological Societies 2013, Rijeka


27. Rostás I, Tenk J, Mikó A, Füredi N. Complex effects of alpha-MSH during the course of aging. 2nd International Doctoral Workshop on Natural Sciences 2013, Pécs

28. Rostás I, Novinszky P, Tenk J, Soós Sz, Székely M, Pétervári E, Balaskó M. Central catabolic effects of leptin during the course of aging. IBRO Workshop 2014, Debrecen


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32. Rostás I, Rimai T, Varga E, Tenk J, Soós Sz, Székely M, Pétervári E, Balaskó M. Age- and nutritional state-related catabolic effects of a central leptin infusion. 2014. 08. 27.-30. Joint Meeting of the Federation of European Physiological Societies (FEPS) and the Hungarian Physiological Society, Budapest

33. Rostás I, Varga E, Rimai T, Tenk J, Soós Sz, Pétervári E, Balaskó M. Catabolic effects of central leptin infusion during aging. 9th Young European Scientist Meeting 2014, Porto


36. Nagy P, Novinszky P, Tenk J, Mikó A, Füredi N, Rostás I, Pétervári E, Balaskó M, Székely M, Soós S. Changes of efficacy of the centrally applied alpha-melanocyte-stimulating hormone (alpha-MSH) or neuropeptide Y (NPY) and peripherally administered cholecystokinin (CCK) in male and female rats of various ages. 15th Meeting of the Hungarian Neuroscience Society 2015, Budapest


39. Rostás I, Csernela Zs, **Tenk J**, Soós Sz, Pétervári P, Székely M, Balaskó M. Leptin in metabolic dysregulation: the influence of age and nutritional state. 5th Central European Congress on Obesity 2015, Budapest


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