Univerzita Karlova

1. lékařská fakulta

Autoreferát disertační práce

UNIVERZITA KARLOVA
1. lékařská fakulta

Účinky stabilních analogů anorexigenních neuropeptidů v modelech metabolického syndromu

Effects of stable analogs of anorexigenic neuropeptides in models of metabolic syndrome

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Disertační práce bude nejméně pět pracovních dnů před konáním obhajoby zveřejněna k nahlížení veřejnosti v tištěné podobě na Oddělení pro vědeckou činnost a zahraniční styky Děkanátu 1. lékařské fakulty.

ABSTRAKT

Obezita je celosvětový zdravotní problém, ale účinná léčba je nedostatečná. Anorexigenní neuropeptidy, jako je peptid uvolňující prolaktin (PrRP), mají potenciál pro léčbu obezity a s ní spojených komplikací. Ve své přirozené formě mají tyto látky určité nevýhody, například: špatnou biologickou dostupnost, nízkou stabilitu a neschopnost procházet hematoencefalickou bariérou po periferním podání. V naší laboratoři byly navrženy lipidizované analogy PrRP. Lipidizace činí tento peptid stabilnějším a schopným působit centrálně po periferním podání.

Cílem této disertační práce bylo prozkoumat dlouhodobý antiobezitní účinek PrRP palmitoylovaného na pozici 11 (palm^{11}-PrRP31) a objasnit tak mechanismy účinku tohoto peptidu. Ke studiu obezity a metabolických parametrů souvisejících s obezitou byly použity tři modely: Wistar Kyoto (WKY) potkani s obezitou navozenou vysokotukovou dietou (DIO) s intaktním leptinem a leptinovým receptorem, a experimentální modely s narušenou funkcí leptinu: *ob/ob* myši s deficitem leptinu a fa/fa potkani s narušenou signalizací leptinu.

Příjem vysokotukové diety zvýšil u WKY potkanů tělesnou hmotnost. Tito potkani vykazovali silnou glukózovou intoleranci a zvýšenou mRNA expresi enzymů regulujících *de novo* lipogenezi. Léčba palm^{11}-PrRP31 vedla k signifikantnímu snížení kumulativního příjmu potravy, tělesné hmotnosti a plazmatické hladiny leptinu, snížení glukózové intolerance a exprese lipogenetických enzymů. U *ob/ob* myší s deficitem leptinu kombinace palm^{11}-PrRP31 a leptinu v chronickém podání vykazovala synergistický účinek. V mladším věku kombinovaná léčba snížila glykémii a hmotnost jater, ve starším věku vedla ke snížení tělesné hmotnosti a hladiny cholesterolu a zvýšení tělesné teploty. Naopak léčba palm^{11}-PrRP31 nesnížila tělesnou hmotnost ani z ní vyplývající poruchy u potkanů *fa/fa* s poruchou signalizace leptinu.

Palm^{11}-PrRP31 prokázal antiobezitní a antidiabetické účinky u potkanů s obezitou indukovanou vysokotukovou dietou, kteří měli intaktní leptin a leptinový receptor. V modelech s porušenou leptinovou signalizací jsme zjistili, že pro anorexigenní účinky palm^{11}-PrRP31 je nezbytná funkční leptinová signalizace. Palmitoylované analogy PrRP jsou tedy atraktivními kandidáty pro léčbu obezity indukované vysokoenergetickou dietou a z ní vyplývajících poruch.

ABSTRACT

Obesity is a worldwide health problem and an effective treatment is still scarce. Anorexigenic neuropeptides, such as prolactin-releasing peptide (PrRP), have a potential for the treatment of obesity and its complications, but in their natural form they have several limitations such as poor bioavailability, low stability and inability to cross the blood-brain barrier after peripheral administration. Recently we have designed lipidized analogs of PrRP. Lipidization makes this peptide more stable and able to act centrally after peripheral administration.

The aim of this study was to investigate the chronic effect of PrRP palmitoylated at position 11 (palm^{11}-PrRP31) on obesity and obesity-related metabolic parameters and to clarify mechanisms of its action. We used three rodent models of obesity: Wistar Kyoto (WKY) rats with high-fat diet-induced obesity (DIO) having intact leptin and leptin receptor as well as rodents with disrupted leptin function: leptin deficient *ob/ob* mice and *fa/fa* rats with a disturbed leptin signaling.

Consumption of a high-fat diet in DIO WKY rats increased their body weight, caused strong glucose intolerance and increased liver mRNA expression of enzymes of *de nov*o lipogenesis. Palm^{11}-PrRP31 treatment significantly decreased cumulative food intake, body weight, plasma leptin level, attenuated glucose intolerance as well as expression of liver lipogenesis enzymes. In leptin deficient *ob/ob* mice, palm^{11}-PrRP31 and leptin showed a synergistic effect in chronic treatment at a younger age on attenuating hyperglycemia and liver weight. At an older age it showed a decrease in body weight, cholesterol level and an increase in body temperature. On the other hand, there was a beneficial effect on obesity and related disturbances occurred in *fa/fa* rats with leptin signaling disruption after palm^{11}-PrRP31 treatment.

Our data suggest a good efficacy of palm^{11}-PrRP31 with diet-induced obesity with intact leptin and leptin receptor. Through the rodents with disturbed leptin signaling, we showed that leptin signaling is necessary for palm^{11}-PrRP31 anorexigenic and related effects. Thus, palmitoylated PrRP analogs are attractive candidates for treatment of humans with high energy diet-induced obesity and derived disturbances.

CONTENT

1. INTRODUCTION

Obesity is becoming a major public problem worldwide. Abdominal obesity in combination with dyslipidemia and/or hyperglycemia (impaired glucose tolerance), as well as insulin resistance and hypertension, are the most observed components of metabolic syndrome, which are risk factors in various chronic diseases including type 2 diabetes mellitus (T2DM), cardiovascular diseases, psychological deficits or fatty liver disease (Guo, 2014;Pan et al., 2014;Engin, 2017;Tune et al., 2017;Blüher, 2019).

There were only four drugs for short-term pharmacotherapy in the world that acted as appetite suppressants and recently several long-term pharmacotherapies have been established as chronic anti-obesity treatments. On the other hand, anorexigenic peptides have a minimal side effect during long-term anti-obesity treatment (Arch, 2015) and are promising tools for the treatment of obesity. These neuropeptides are usually of brain origin with an anorexigenic effect in animal models e.g., prolactin-releasing peptide (PrRP), cocaine- and amphetamine-regulated transcript (CART) peptide, and α-melanocyte-stimulating hormone (α-MSH) (Patel, 2015;Meneguetti et al., 2019;Gao et al., 2020;Williams et al., 2020).

Food intake (FI) regulation is a complex system where peripheral and central signals are involved. Peripheral signals or peripheral hormones involved in FI regulation are e.g., leptin, insulin and ghrelin, while central signals involved in FI regulation are so-called neuropeptides. There are many anorexigenic or orexigenic neuropeptides involved in FI regulation e.g., α-MSH, CART peptide, PrRP, corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), oxytocin, neuropeptide Y (NPY), melanin-concentrating hormone (MCH), orexins or galanin. The hypothalamus is the main center of FI regulation and consists of several nuclei such as the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the lateral hypothalamic nucleus (LHA), the ventromedial nucleus (VMH) and the dorsomedial nucleus (DMN). The ARC is a key hypothalamic nucleus in the regulation of appetite where anorexigenic neuropeptides that reduce appetite are released: pro-opiomelanocortin (POMC) and CART, as well as orexigenic neuropeptides that increase appetite, AgRP and NPY (Figure 1).

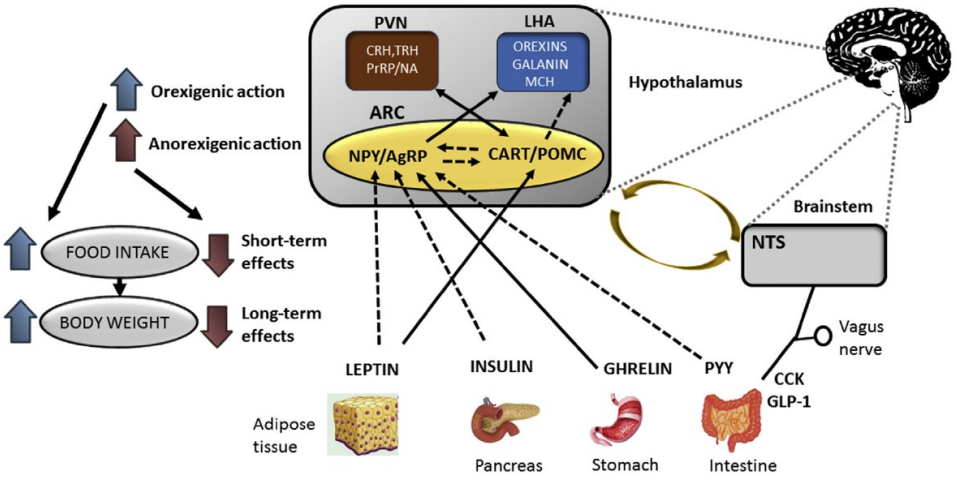

Figure 1: The scheme of central and peripheral factors regulating food intake (modified from (Mikulášková et al., 2016))

AgRP agouti-related peptide, ARC arcuate nucleus, CART cocaine- and amphetamine-regulated transcript peptide, CCK cholecystokinin, CRH corticotrophin-releasing hormone, GLP-1 glucagon-like peptide-1, LHA lateral hypothalamic area, MCH melanin-concentrating hormone, NPY neuropeptide Y, NTS nucleus tractus solitarius, POMC pro-opiomelanocortin, PrRP prolactin-releasing peptide, PVN paraventricular nucleus, TRH thyrotropin-releasing hormone.

PrRP was first isolated in 1998 by Hinuma (Hinuma et al., 1998) and this anorexigenic neuropeptide is involved mainly in FI regulation and energy expenditure, but also in stress regulating and in the cardiovascular system its potential neuroprotective properties were shown. (Samson et al., 1998;Maruyama et al., 2001;Zhang et al., 2001;Lawrence et al., 2002;Onaka et al., 2010;Holubová et al., 2019). PrRP belongs to so-called RF amide peptides and it binds with a high affinity to a GPR10 receptor but also with a lower affinity to an NPFF2 receptor. PrRP neurons are localized mostly in NTS and slightly in hypothalamic DMN, while immunoreactive cell bodies were detected in DMN, VMN, NTS and the ventrolateral medulla oblongata (ME), and immunoreactive fibers were found in high concentration in the posterior pituitary (Takayanagi and Onaka, 2010;Pražienková et al., 2019). PrRP cooperates with other anorexigenic peptides, mainly leptin or CCK.

Two isoforms, a 20 amino acid peptide named PrRP20 and a 31 amino acid peptide named PrRP31 are derived from the same preproprotein and they share an identical C-terminus (Figure 2) (Lin, 2008;Pražienková et al., 2019).

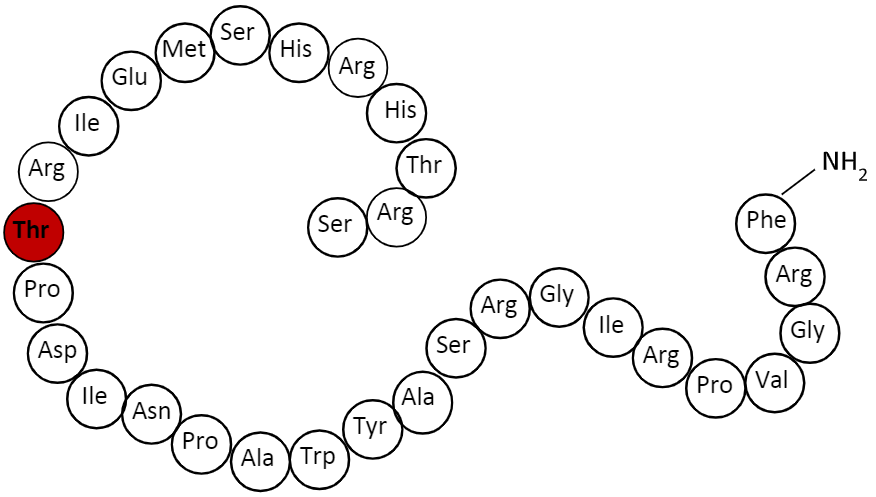

Figure 2: Structure of human prolactin-releasing peptide

Human PrRP31 peptide; threonine (in red) is the beginning of PrRP20.

PrRP has potential as an anti-obesity treatment, but in its natural form has low stability and is not able to cross the blood-brain barrier (BBB) after peripheral administration. Modification such as lipidization makes this peptide more stable and able to act centrally after peripheral administration. In our previous studies we demonstrated that analogs lipidized by 8-18 carbon chain fatty acids at N-terminus showed high binding affinities for both GPR10 and NPFF-2R similar to the analogs palmitoylated through linkers to Lys^{11} e.g., $palm^{11}$-PrRP31 (Maletínská et al., 2015;Pražienková et al., 2017;Karnošová et al., 2021). Nevertheless, natural PrRP20 or PrRP31 had no effect on FI after peripheral administration, as well as analogs lipidized with shorter carbon chains. But palm-PrRP31, myr-PrRP20 or $palm^{11}$-PrRP31 significantly and dose-dependently decreased FI in lean overnight-fasted or freely-fed mice after peripheral administration (Maletínská et al., 2015;Pražienková et al., 2017;Pirník et al., 2021).

Knowledge about animal models for metabolic studies is very important and rodent models are the most commonly used. However, there is no fully effective model to understand all disease mechanisms connected with metabolic syndrome. Nevertheless, diet induced obesity (DIO) models when a high-fat (HF) diet is employed allow us a better understanding of the promoting metabolic syndrome, which is a multifactorial disease, and are closer to human obesity and metabolic syndrome. On the other hand, genetic factors also play an important role in obesity development. Rodent models with genetically disrupted production or signaling of some specific factors of FI regulation develop more severe pathology, where long-feeding (diet)

programs are not needed to induce obesity. However, they are not so similar to human metabolic syndrome and obesity (Srinivasan and Ramarao, 2007;Nilsson et al., 2012;Fuchs et al., 2018;Mráziková et al., 2021).

The most used rodents for DIO are C57BL6J mice as well as Sprague Dawley (SD) rats. In these rodent models, most of the metabolic syndrome features were found e.g., obesity, prediabetes or diabetes, glucose intolerance and disrupted central leptin, and insulin signaling (Fuchs et al., 2018;Kwitek, 2019;Preguiça et al., 2020). Rodent models of leptin deficiency or leptin signaling disturbances are widely used as spontaneous genetic models of obesity and related complications. Leptin deficient *ob/ob* mice do not produce leptin. These mice develop severe early-onset obesity from four weeks of age and have a defect in the thermogenesis of brown adipose tissue (BAT) which could lead to an increased hepatic lipogenesis. Leptin deficiency in *ob/ob* mice also leads to a hyperinsulinemia and mild hyperglycemia, apparent from 8 to 12 weeks of age. These mice have severe liver steatosis but do not develop steatohepatitis (Kennedy et al., 2010;Fuchs et al., 2018;Kořínková et al., 2020). Leptin receptor-deficient *db/db* mice are obese, hyperinsulinemic and hyperglycemic (depending on the strain and age) and are widely used for the study of T2DM and its complications. The Obese Zucker *fa/fa* rats similarly as *db/db* mice develop obesity because of a defect in the leptin receptor and also could be hyperinsulinemic and hyperglycemic depending on the strain and age. These models are mainly used for pharmacological studies of anti-obesity drugs (Ramarao and Kaul, 1999;Kwitek, 2019). Zucker diabetic (ZDF) rats are lean and diabetic. These rats are mainly used to study potential antidiabetic compounds (Wang et al., 2014).

2. AIMS OF THE THESIS

Anorexigenic neuropeptide, PrRP, has a potential as a treatment for obesity but in its natural form has low stability and is not able to cross BBB after peripheral administration. Modification, such as lipidization makes this peptide more stable and able to act centrally after peripheral administration. Lipidized analogs of PrRP were previously tested in our laboratory at IOCB. The most potent analogs, palm-PrRP31 and palm^{11}-PrRP31, were then tested in rodent models for their potential anti-obesity and glucose-lowering properties. In my thesis, studies of rodent models with features of metabolic syndrome are described.

The aims of my thesis were:

Investigation of chronic effect of palm^{11}-PrRP31 and liraglutide in WKY rats fed an HF diet.

The first aim of my thesis was to evaluate an effect of palm^{11}-PrRP31 and liraglutide in WKY rats fed an HF diet for one year (as the most common type of human-like obesity) by monitoring the metabolic parameters related to obesity and glucose intolerance.

The study of chronic effect of palm^{11}-PrRP31, leptin and their combination in *ob/ob* leptin deficient mice.

The second aim was to determine the long-time effect of palm^{11}-PrRP31 alone or in combination with leptin in leptin deficient mice that develop early onset obesity. We have hypothesized that supplementation of leptin will influence the effect of palm^{11}-PrRP31, namly: metabolic parameters as well as signaling in the brain.

Research of chronic effect of palm^{11}-PrRP31 in *fa/fa* rats with leptin signaling disturbances.

The third aim was to study anorexigenic impact of palm^{11}-PrRP31 in *fa/fa* rats with non-functional leptin receptor in order to investigate a potential involvement of leptin signaling in the effect of palm^{11}-PrRP31.

3. METHODS

Analog of **human PrRP palmitoylated at position 11 (palm^{11}-PrRP31)** with the sequence SRTHRHSMEIK(N-γ-E(N-palmitoyl)) TPDINPAWYASRGIRPVGRF-NH2 was synthesized at the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences (IOCB CAS), Prague, Czech Republic, in the peptide synthesis laboratory by Miroslava Blechová, as previously described (Pražienková *et al*. 2017).

Three rodent models of obesity: **Wistar Kyoto (WKY) rats fed HF diet**, **leptin deficient *ob/ob* mice** (Kořínková et al., 2020) and **rats with leptin-signaling disturbance (*fa/fa* rats)** were used to study the effects of palm^{11}-PrRP31. At the end of the experiments, the mice and rats were fasted overnight, plasma samples were collected from the tail veins for determination of the biochemical parameters and an oral glucose tolerance test (OGTT) was performed. Tissue samples of all mice and rats were dissected and stored in -80°C until use.

Ten 8-week-old **WKY rats** were fed the Ssniff® diet (WKY LF) while 40 rats were fed an HF diet (WKY HF) for 52 weeks. Before the start of treatment, BW was monitored once a week. OGTT was performed and plasma samples were collected from tail vessels at 60 weeks of age for determination of the biochemical parameters. At the age of 60 weeks, 24 WKY rats on an HF diet with the highest BW were selected and divided into three experimental groups. The rats were treated from Monday to Friday with saline (WKY HF saline, n=8), palm^{11}-PrRP31 at a dose of 5 mg/kg (WKY HF palm^{11}-PrRP31, n=8) or with liraglutide at a dose of 0.2 mg/kg (WKY HF liraglutide, n=8). Rats fed with the Ssniff® diet formed the control group (WKY LF saline, n=8). During the six-week dosing period, BW and FI were measured twice per week.

Five-week-old male ***ob/ob* mice** and their wild-type (WT) controls fed with the Ssniff® diet were randomly divided into two experiments (experiment 1 and experiment 2) in groups of 8-10 animals. In this study, we wanted to explore the potential interaction between leptin and PrRP in younger mice treated for two weeks (experiment 1) and in older mice treated for eight weeks (experiment 2). Saline, leptin (5 or 10 μg/kg twice a day) and palm^{11}-PrRP31 (5 mg/kg twice a day) were used as a treatment. In experiment 1, mice were treated for two weeks from eight weeks of age. The following groups were used (n=8-10): WT saline, *ob/ob* saline, *ob/ob* leptin (5 μg/kg), *ob/ob* palm^{11}-PrRP31 (5 mg/kg) and *ob/ob* leptin + palm^{11}-PrRP31 (5 μg/kg + 5 mg/kg). In experiment 2, mice were treated for eight weeks from 16 weeks of age and the following groups of 10 animals were used: WT saline, *ob/ob* saline, *ob/ob* leptin (10 μg/kg),

ob/ob palm^{11}-PrRP31 (5 mg/kg) and *ob/ob* leptin + palm^{11}-PrRP31 (5 μg/kg + 5 mg/kg). During the dosing period, FI and BW were monitored daily. One week before the end of both experiments, rectal temperature was measured.

Homozygous **Zucker *fa/fa* male rats** (*fa/fa*) and their lean littermates, *fa*/+- (control) rats, were provided with a standard Ssniff® diet. Before the start of treatment, BW of *fa/fa* and control rats was monitored once per week. At the age of 32 weeks, fasted blood samples were collected from the tail veins to determine the basic biochemical parameters of the rat plasma. From 32 weeks of age, control rats were infused with saline (control saline group, n=8), while *fa/fa* rats were infused with saline (*fa/fa* saline group, n=7) or with palm^{11}-PrRP31 (5 mg/kg BW per day) (*fa/fa* palm^{11}-PrRP31 group, n=8) for two months. These three groups were infused using Alzet osmotic minipumps (Alzet, Cupertino, CA, USA), which are certified to infuse 6 μl of solution daily. Alzet osmotic minipumps were implanted IP under a short-term ether anesthesia and were replaced after four weeks with new ones. During the dosing period, BW and FI were measured twice per week.

Statistics: The data are presented as means ± S.E.M. Statistical analysis was performed using unpaired *t*-test or one-way, followed by Dunnett´s multiple comparisons test or two-way ANOVA followed by Bonferroni´s multiple comparisons test as indicated in **Figures legends and Tables** with Graph-Pad Prism software (Graph-Pad Software, San Diego, CA, USA). The differences were considered significant at $P<0.05$. The rate of insulin resistance was expressed with a homeostatic model assessment (HOMA) index calculated by (fasting glucose level, mmol/l) x (fasting insulin level, pmol/l) divided by 22.5 (Lansang et al., 2001).

4. RESULTS

4.1 Palm[11]-PrRP31 significantly decreased body weight and improved glucose intolerance in obese WKY rats

The results written in this chapter have not been published yet, but a manuscript is in preparation.

The consumption of an HF diet for 52 weeks resulted in a significantly higher BW in WKY HF compared to WKY LF. The average BW of the WKY LF group was 439 ± 7, while the average BW of the WKY HF group was 589 ± 7. During the six weeks of IP treatment with saline, palm[11]-PrRP31 or liraglutide, FI and BW change of WKY HF saline rats was similar to WKY LF saline rats, while both peptide treatments significantly lowered BW and FI compared to the saline-treated WKY HF group (Figure 3).

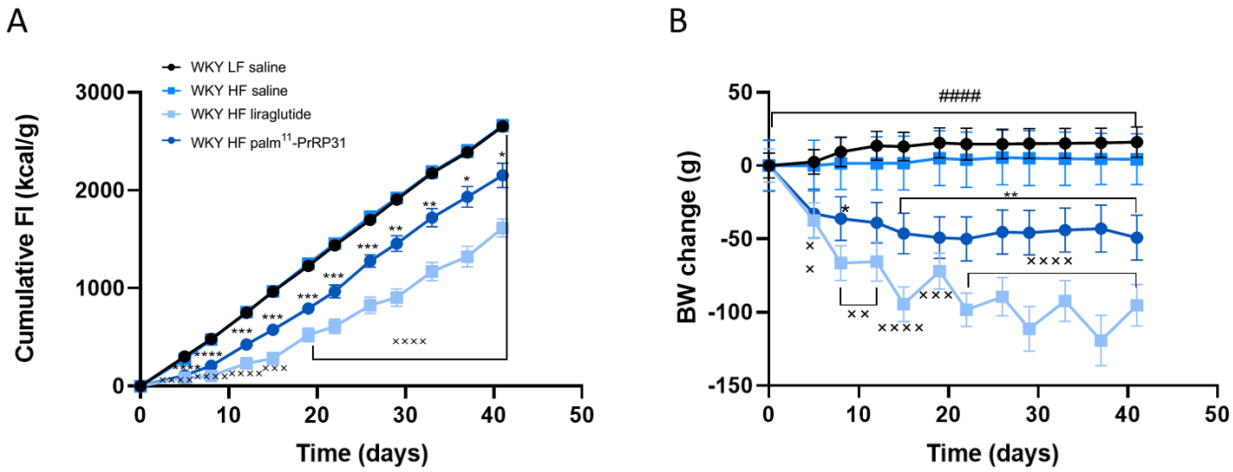

Figure 3: Chronic effect of palm[11]-PrRP31 and liraglutide on cumulative FI (A) and BW change (B) in DIO WKY rats during treatment (60-66 weeks).

Data are presented as means ± S.E.M. Statistical analysis was performed by two-way ANOVA with Bonferroni´s *post hoc* test. BW body weight, FI food intake. Significance is $^{\#}p<0.05$, $^{\#\#\#\#}p<0.0001$ WKY LF saline *vs* WKY HF saline; $^{x}p<0.05$, $^{xx}p<0.01$, $^{xxx}p<0.001$, $^{xxxx}p<0.0001$ WKY liraglutide *vs* WKY HF saline; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 WKY palm[11]-PrRP31 *vs* WKY HF saline (n= 6-8).

At the end of the experiment, glycated hemoglobin (HbA1c) as well as HOMA index were significantly increased in WKY HF saline compared to the WKY LF saline group (data are not shown). WKY HF saline revealed strong glucose intolerance based on OGTT results compared to WKY LF saline (Figure 4). Treatment with both palm[11]-PrRP31 and liraglutide

significantly decreased HbA1c in WKY HF liraglutide compared to WKY HF saline. HOMA index was non-significantly decreased after both treatments (data are not shown), but palm^{11}-PrRP31 significantly improved glucose intolerance and lowered corresponding AUC in WKY on an HF diet (Figure 4).

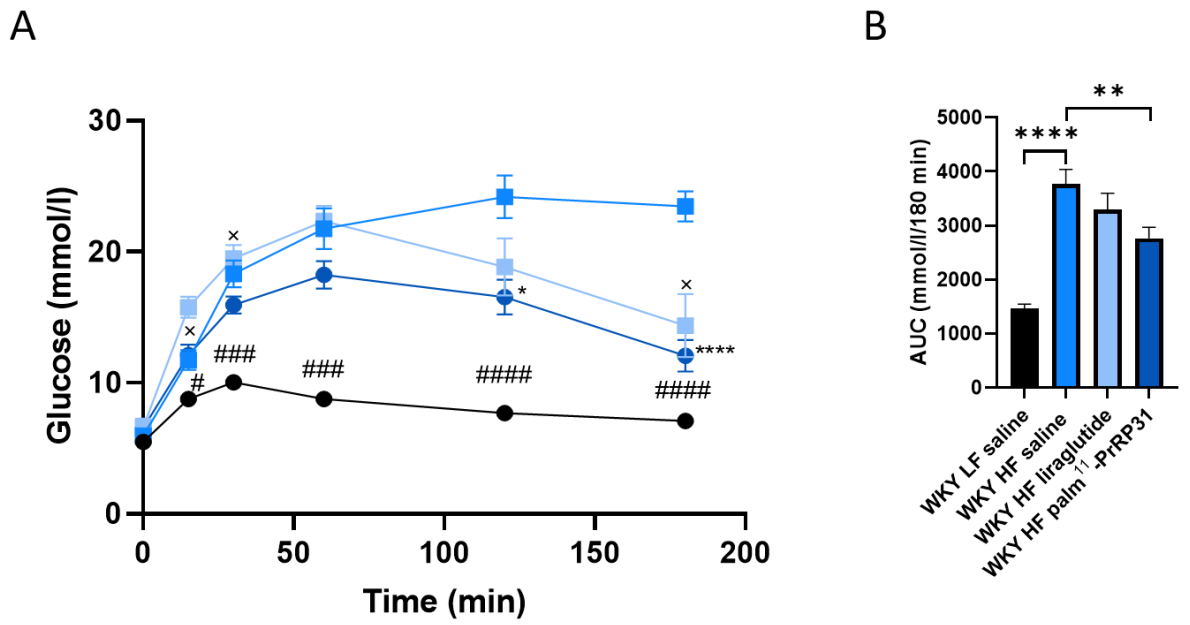

Figure 4: Chronic effect of palm^{11}-PrRP31 and liraglutide on OGTT (A), corresponding AUC (B) in DIO WKY rats at the end of the experiment (66 weeks).

Data are presented as means ± S.E.M. Statistical analysis was performed by one-way ANOVA with Dunnett´s *post hoc* test or two-way ANOVA with Bonferroni´s *post hoc* test. AUC area under the curve. Significance is $^{\#}p<0.05$, $^{\#\#\#}p<0.001$, $^{\#\#\#\#}p<0.0001$ WKY LF saline *vs* WKY HF saline; $^{x}p<0.05$ WKY liraglutide *vs* WKY HF saline; *p<0.05, ****p<0.0001 WKY palm^{11}-PrRP31 *vs* WKY HF saline (n= 6-8).

At the end of the experiment, liver lipogenesis was increased by an HF diet. mRNA expression of liver *SREBP-1*, as well as mRNA expression of lipogenetic enzymes *Acaca*, *Fasn*, and *Scd-1* were significantly increased in WKY HF saline in comparison to WKY LF saline (Figure 15C). Treatment with palm^{11}-PrRP31 significantly attenuated mRNA expression of *Acaca*, *Fasn* and *Scd-1*, while only *Scd-1* mRNA expression was significantly decreased by liraglutide treatment (data are not shown).

4.2 Synergistic effect of palm^{11}-PrRP31 and leptin in leptin deficient *ob/ob* mice

The results obtained in the experiments performed in *ob/ob* mice were published in the Journal of Molecular Endocrinology (Kořínková et al., 2020).

Five-week-old *ob/ob* mice and their wild-type littermates were randomly divided into two experiments (experiment 1 and experiment 2). In experiment 1, mice were treated with saline, palm^{11}-PrRP31, leptin and palm^{11}-PrRP31 + leptin for two weeks from eight weeks of age, while in experiment 2 mice were treated for eight weeks from 16 weeks of age, exactly as in experiment 1. FI was significantly decreased in experiment 1 (but not in experiment 2) after leptin + palm^{11}-PrRP31 treatment compared to the *ob/ob* saline group, but leptin and palm^{11}-PrRP31 alone did not significantly change FI (Figure 5). Analogously, treatment with the combination of palm^{11}-PrRP31 and leptin significantly decreased BW in *ob/ob* mice in both experiment 1 and experiment 2, while leptin or palm^{11}-PrRP31 alone did not significantly attenuate BW (Figure 5).

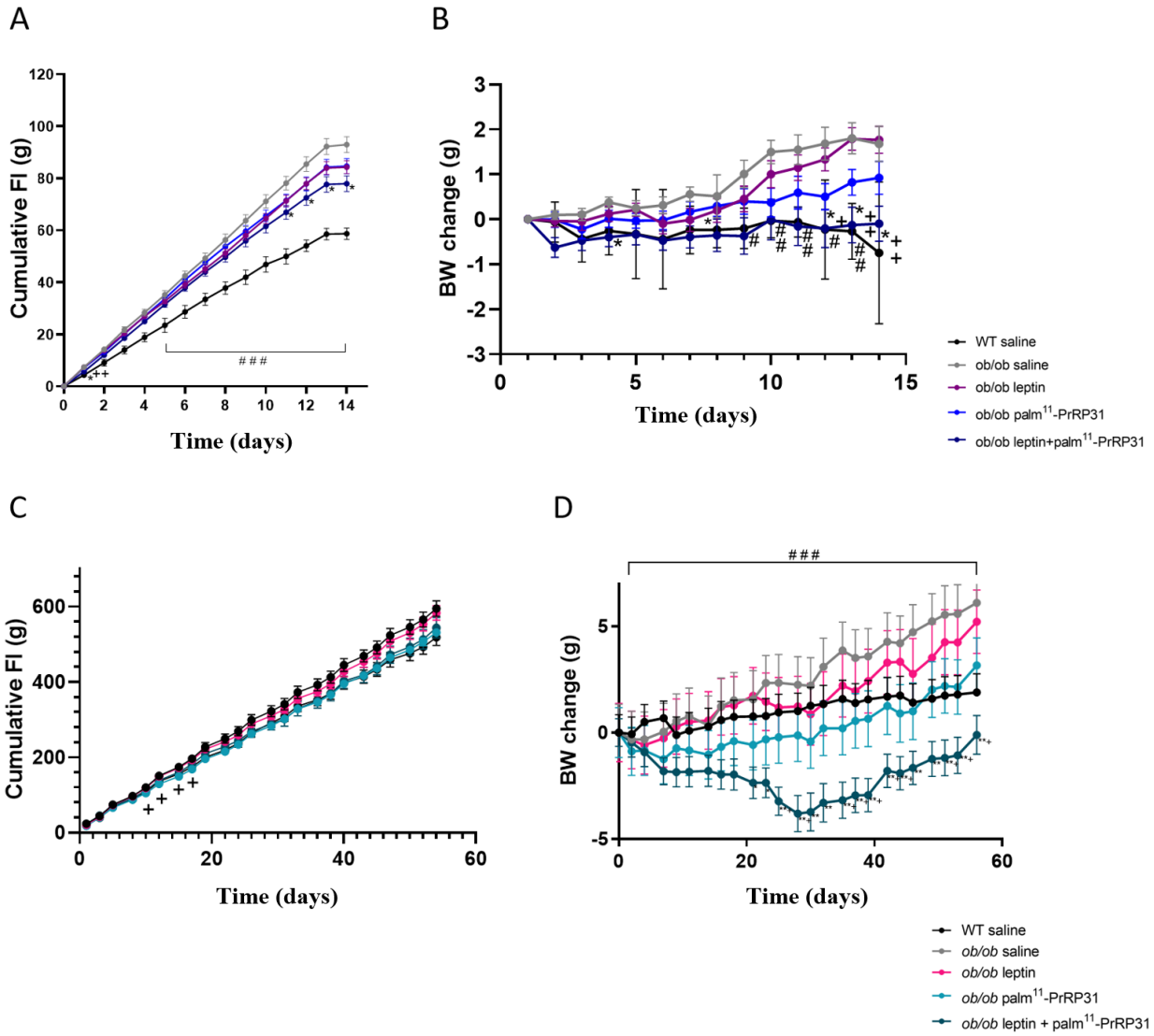

Figure 5: Chronic effect of palm^{11}-PrRP31, leptin and leptin + palm^{11}-PrRP31 on FI and BW in experiment 1 (A, B) and experiment 2 (C, D)

Data are presented as means ± S.E.M. Statistical analysis was performed by two-way ANOVA with Bonferroni´s *post hoc* test. FI food intake, BW body weight. Significance is *p<0.05, **p<0.01 WT saline *vs ob/ob* saline; $^{\#}$p<0.05, $^{\#\#}$p<0.01 $^{\#\#\#}$p<0.001 *ob/ob* leptin + palm^{11}-PrRP31 *vs ob/ob* saline; $^{+}$p<0.05, $^{++}$p<0.01. *ob/ob* leptin + palm^{11}-PrRP31 *vs ob/ob* leptin (n= 8-10).

Saline-treated *ob/ob* mice developed intolerance to glucose during OGTT in comparison with WT saline in experiment 2. Leptin, palm^{11}-PrRP31 and leptin + palm^{11}-PrRP31 did not improve glucose intolerance (Figure 6).

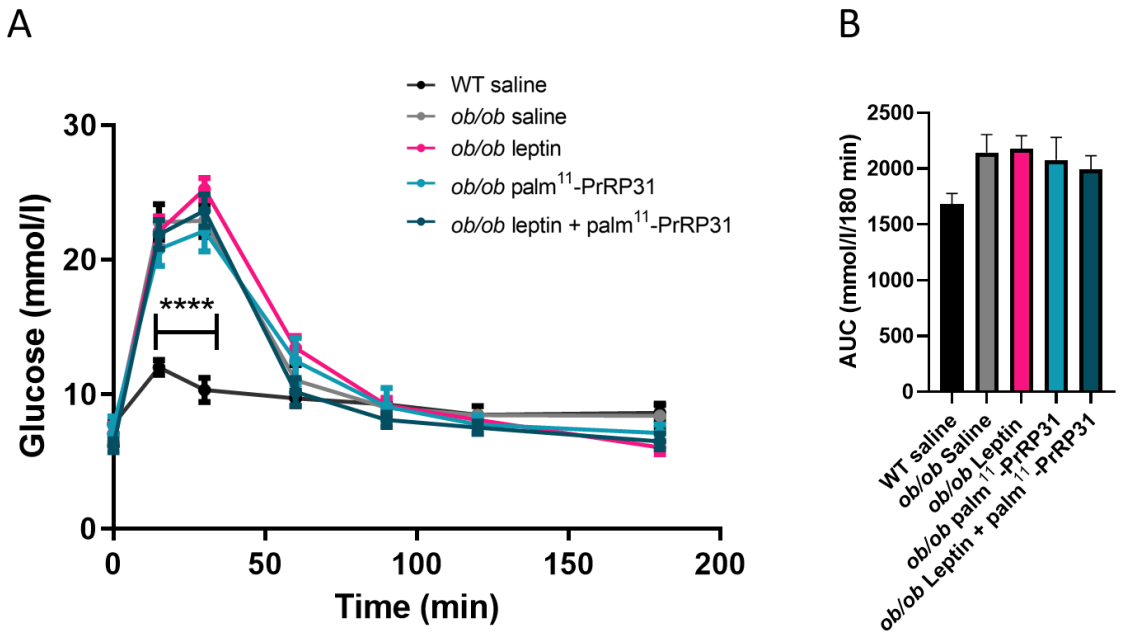

Figure 6: Chronic effect of palm^{11}-PrRP31, leptin and palm^{11}-PrRP31 + leptin on OGTT (A) AUC (B) in experiment 2

Data are presented as means ± S.E.M. Statistical analysis was performed by one-way ANOVA with Dunnett´s *post hoc* test or two-way ANOVA with Bonferroni´s *post hoc* test. AUC area under the curve. Significance is ****p<0.0001 WT saline *vs ob/ob* saline (n= 8-10).

In experiment 1, liver mRNA expression of *SREBP-1*, which controls the expression of *Acaca* and *Fasn*, did not differ between *ob/ob* saline and WT saline groups and no treatment affected it significantly. mRNA expression of *Acaca* and *Fasn* were significantly increased in *ob/ob* saline compared to WT saline, and leptin treatment significantly attenuated *Fasn* mRNA liver expression. Moreover, treatment with a leptin + palm^{11}-PrRP31 combination significantly lowered both *Acaca* and *Fasn* mRNA expression. The mRNA expression levels of enzymes regulating fatty acid oxidation, such as *Cpt-1a*, *Pparα* and *Pparγ*, were significantly increased in *ob/ob* saline compared to WT saline, but no treatment affected the expression (data are not shown).

4.3 Palm^{11}-PrRP31 treatment neither lowered body weight nor ameliorated glucose tolerance in *fa/fa* rats

The results obtained in this chapter are after major revision in Nutrition and Diabetes.

Before treatment, 32-week-old *fa/fa* rats had significantly increased BW. Average BW of the control group was 441 ± 9, while the average BW of the WKY HF group was 572 ± 11. During the two month treatment with palm^{11}-PrRP31, there were no significant differences in cumulative FI and BW between *fa/fa* saline rats and control saline rats. Moreover, there were no significant changes in BW and FI between *fa/fa* palm^{11}-PrRP31 and *fa/fa* saline rats (Figure 7).

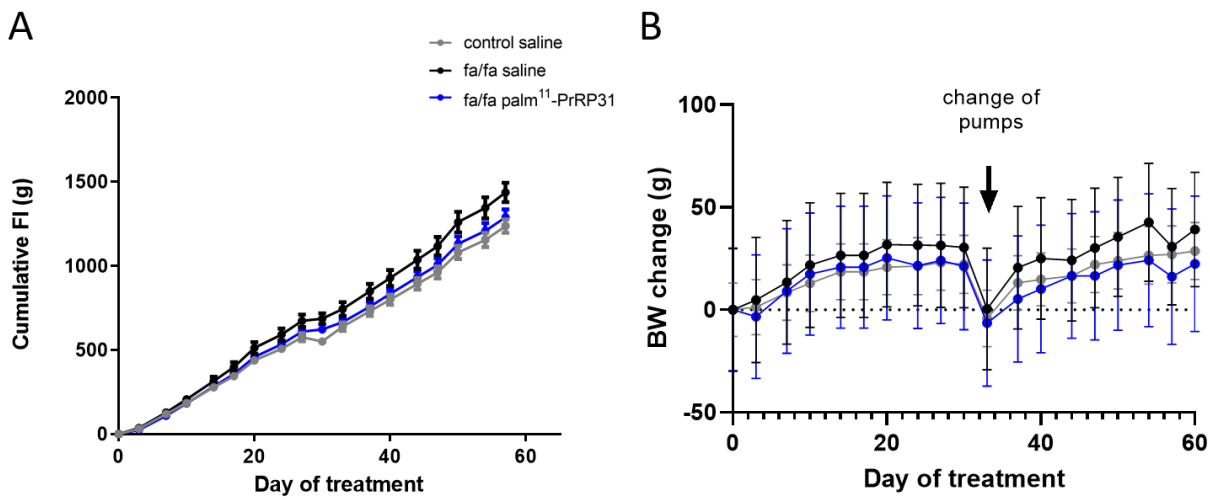

Figure 7: Chronic effect of palm^{11}-PrRP31 on Cumulative FI (A) and BW change (B) in *fa/fa* rats during treatment (32 - 40 weeks)

Data are presented as means ± S.E.M. Statistical analysis was performed by two-way ANOVA with Bonferroni´s *post hoc* test. FI food intake, BW body weight. Significance is *vs fa/fa* saline (n= 7, 8).

All saline and palm[11]-PrRP31 treated *fa/fa* rats were normoglycemic, however, *fa/fa* saline rats were glucose-intolerant based on OGTT results and treatment with palm[11]-PrRP31 did not improve their tolerance to glucose (Figure 8).

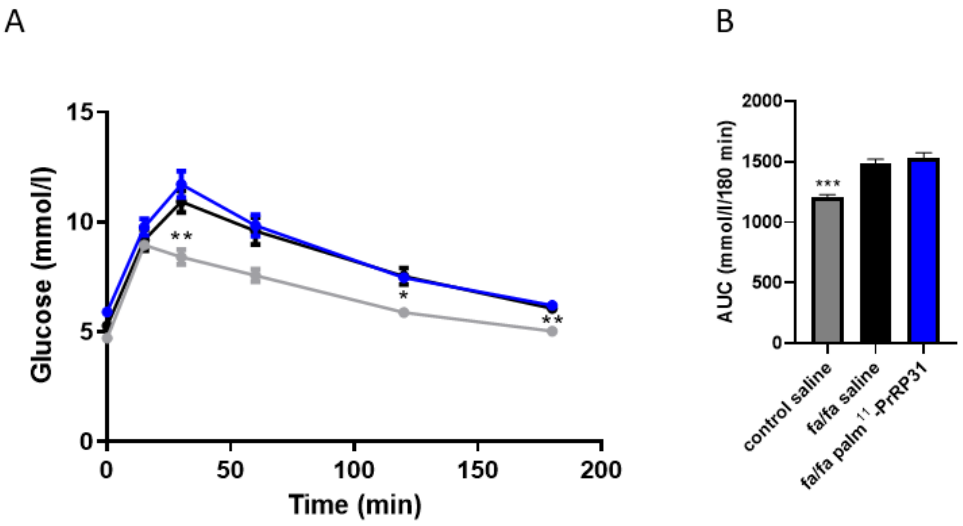

Figure 8: Chronic effect of palm[11]-PrRP3 on OGTT (A), AUC (B) in *fa/fa* rats at the end of the experiment (40 weeks)

Data are presented as means ± S.E.M. Statistical analysis was performed by one-way ANOVA with Dunnett´s *post hoc* test or two-way ANOVA with Bonferroni´s *post hoc* test. AUC area under the curve. Significance is **p<0.01, ***p<0.001, ****p<0.0001 control saline *vs fa/fa* saline (n= 7, 8).

In the liver, *Scd-1, SREBP-1* and *FABP4* mRNA expression was significantly increased and *PPARγ* expression was lowered in the *fa/fa* saline group compared to the control saline group (data are not shown).

5. DISCUSSION

A fully effective preclinical model to understand pathophysiological mechanisms connected to obesity and related metabolic complications is still missing. DIO rodent models are close to human obesity and metabolic syndrome but genetic factors also play an important role in obesity development. Rodent models with disrupted leptin system function develop more severe pathology, where long-term feeding with specific obesogenic diets is not needed, but they are not as close to human obesity (Srinivasan and Ramarao, 2007;Fuchs et al., 2018) as DIO models.

Anorexigenic peptides, such as PrRP are promising tools for the treatment of obesity. Lipidization, e.g., palmitoylation of PrRP leads to increased stability and half-life in organism and allows its central anorexigenic effect after peripheral administration. Thus, palmitoylated PrRP analogs are good candidates for obesity treatment (Maletínská et al., 2015;Špolcová et al., 2015;Holubová et al., 2016;Pražienková et al., 2017;Čermáková et al., 2019).

In my Ph.D. thesis, the analog of PrRP palmitoylated at position 11 (palm^{11}-PrRP31) was used for metabolic studies in different rodent models, such as DIO WKY rats with intact leptin and leptin receptor and in rodents with disrupted leptin signaling; in leptin deficient *ob/ob* mice and in *fa/fa* rats with leptin signaling disturbances.

We investigated the chronic effect of palm^{11}-PrRP31 in WKY rats fed HF diet. WKY fed with HF diet for 52 weeks developed obesity with worsened metabolic parameters and strong glucose intolerance. WKY HF rats had also increased *de nov*o liver lipogenesis. Palm^{11}-PrRP31 showed strong anorexigenic effect by significantly decreased cumulative FI, BW and plasma leptin level. Glucose lowering effect of palm^{11}-PrRP31 was also found after OGTT test, as well as decreased *de novo* liver lipogenesis in these rats fed HF diet.

Chronic effects of palm^{11}-PrRP31, leptin and their combination in *ob/ob* leptin deficient mice were studied in two experiments (Kořínková et al., 2020). *Ob/ob* mice develop early onset obesity with worsened related metabolic parameters and are hypothermic. Leptin and palm^{11}-PrRP31 synergistically decreased BW, cholesterol level and SOCS3 production and increased body temperature in older mice with a longer treatment. The synergistic effect of leptin and palm^{11}-PrRP31 was also demonstrated by lower liver weight and glucose levels after a shorter treatment in younger mice.

Research of chronic effect of palm^{11}-PrRP31 was studied in *fa/fa* rats with a disturbed leptin signaling. Palm^{11}-PrRP31 treatment neither lowered BW nor attenuated glucose tolerance and did not improve metabolic parameters related to obesity, probably due to leptin signaling disturbances.

6. CONCLUSIONS

Action of palm^{11}-PrRP31, a lipidized analog of anorexigenic neuropeptide PrRP, on obesity and related metabolic disorders was followed in three rodent models of obesity: WKY rats with DIO resulting from HF diet having intact leptin and leptin receptor and two rodent models with obesity resulting from disrupted leptin function - leptin deficient *ob/ob* mice, and *fa/fa* rats with a disturbed leptin signaling.

Based on the results from this PhD thesis, we would like to conclude that the full effect of palm^{11}-PrRP31 on food intake and BW decrease depends on undisturbed leptin signaling. Palm^{11}-PrRP31 showed strong anorexigenic, body weight-reducing, glucose tolerance-improving and lipogenesis-attenuating effects in WKY rats fed with an HF diet for 52 weeks, where leptin resistance resulted from excessive adipose tissue but leptin signaling apparatus was functional. The fact that a BW-lowering effect of palm^{11}-PrRP31 was not accomplished in *fa/fa* rats with leptin signaling disruption supports this idea. Palm^{11}-PrRP31 and leptin showed synergistic effect in leptin-deficient *ob/ob* mice, both at a younger and older age where palm^{11}-PrRP31 could manifest its full anti-obesity effect only when leptin was supplemented simultaneously. Leptin and palm^{11}-PrRP31 synergistically lowered liver weight and glucose levels in younger mice, decreased body and subcutaneous fat weight, decreased cholesterol levels and increased body temperature in older mice. Production of SOCS3, an inhibitor of leptin signaling, was attenuated by action of the combined substances.

In conclusion, our data suggest a good efficacy of palmitoylated PrRP analogs in rodent models of diet-induced obesity with intact leptin and leptin receptor revealing an anti-obesity and antidiabetic effect together with decreased lipogenesis, increased lipolysis and hypothalamic insulin signaling. Thus, palmitoylated PrRP analogs are attractive candidates for anti-obesity and glucose-lowering treatment of the most common human – high energy diet-induced obesity.

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Seznam publikací doktoranda v tomto uspořádání:

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Publications related to Ph.D. thesis:

1. **Kořínková L**, Holubová M, Neprašová B, Hrubá L, Pražienková V, Bencze M, Haluzík M, Kuneš J, Maletínská L, Železná B. Synergistic effect of leptin and lipidized PrRP on metabolic pathways in ob/ob mice. J Mol Endocrinol. 2020 Feb;64(2):77-90. doi: 10.1530/JME-19-0188. PMID: 31855558. **IF = 5.098**

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Commercial activities:

The research collaboration and license agreement between IOCB AS CR and IP AS CR and Novo Nordisk A/S was signed in August 2017, and I participated in this project.