Academy of Sciences of the Czech Republic
Institute of Physiology

Charles University in Prague
1st Faculty of Medicine

Control of white adipose tissue metabolism:

role of AMP-activated protein kinase

and

modulation by omega-3 polyunsaturated fatty acids

Mgr. Olga Horáková

Summary of Ph. D. Thesis

Supervisor: Jan Kopecký, MD, DSc.

Praha 2006

This thesis is based on the following articles, referred to in the text by their capital letter as indicated here:

A. **Matějková O**, Mustard KJ, Šponarová J, Flachs P, Rossmeisl M, Mikšík I, Thomason-Hughes M, Grahame Hardie D, Kopecký J. (2004). Possible involvement of AMP-activated protein kinase in obesity resistance induced by respiratory uncoupling in white fat. *FEBS Lett.* 569(1-3):245-8.

B. Šponarová J, Mustard KJ, **Horáková O**, Flachs P, Rossmeisl M, Brauner P, Bardová K, Thomason-Hughes M, Braunerová R, Janovská P, Grahame Hardie D, Kopecký J. (2005). Involvement of AMP-activated protein kinase in fat depot-specific metabolic changes during starvation. *FEBS Lett.* 579: 6105-6110.

C. Flachs P, **Horáková O**, Brauner P, Rossmeisl M, Pecina P, Franssen-van Hal N., Ružicková J, Šponarová J, Drahota Z, Vlček C, Keijer J, Houštek J, Kopecký J. (2005). Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce β-oxidation in white fat. *Diabetologia* 48: 2365-2375.

D. Flachs P, Mohamed-Ali V, **Horáková O**, Rossmeisl M, Hosseinzadeh-Attar MJ, Hensler M, Ružičková J, Kopecký J. (2006) Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet. *Diabetologia.* 49 :1-4

CONTENTS

Abbreviations:

α1 AMPK, α1 isoform of α subunit of AMPK ; ACC, acetyl-CoA carboxylase; ALA, α-linolenic acid; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; aP2, fatty acid binding protein 4; ATP, adenosine triphosphate; BAT, brown adipose tissue; COX IV, subunit IV of mitochondrial cytochrome oxidase; CPT-1, carnitine-palmitoyl transferase I; CR, caloric restriction; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAS, fatty acid synthase, G3P, glycerol-3-phosphate; HF, high fat; HSL, hormone-sensitive lipase; mRNA, messenger RNA; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; NEFA, non-esterified fatty acids; NRF, nuclear respiratory factor, OXPHOS, oxidative phosphorylation; PEPCK, phosphoenolpyruvate carboxykinase; PGC-1, PPAR γ coactivator 1; PPAR, peroxisome proliferator-activated receptor; PUFA; polyunsaturated fatty acids; SCD, stearoyl-CoA desaturase; TAG, triacylglycerol(s); TNF-α, tumor necrosis factor α; UCP, uncoupling protein; WAT, white adipose tissue.

1. INTRODUCTION

Regulating energy levels is fundamental process in every living organism. The endogenous systems regulating energy balance have developed during the course of evolution, when the conservation of energy reserves and the efficient replenishment of exhausted energy stores were essential for survival and reproduction [1]. According to 'energy balance equation theory' any increase in energy intake or decrease in energy expenditure, which imbalance the equation, will result in accumulation of fat in the body [2]. Obesity, an excessive accumulation of adipose tissue is associated with the risk of developing type 2 diabetes mellitus, coronary heart disease, hypertension, sleep apnoca, asthma, certain form of cancer and osteoarthritis of small and large joints [3].

Many pieces of evidence suggest that body fat content is controlled, at least partially, by the metabolism of adipose tissue itself. First, many candidate genes for obesity have important roles in adipocytes [4]. Second, mice that are prone or resistant to obesity were created by transgenic modification of adipose tissue (reviewed in [5]). And third, trought its secretory function, white adipose tissue lies at the heart of a complex network of factors capable of either improving (leptin, adiponectin) or reducing (TNF-α, resistin) insulin action in relevant tissues, including skeletal muscles and liver. This raises the possibility that the developement of drugs targeting adipose function represents a new terapeutic approach to senzitize peripheral tissues to insulin. This could be of particular therapeutic benefit in pathology associated with white adipose tissue mass dysregulation, such as lipodystrophy and obesity.

1.1. *White adipose tissue*

White adipose tissue (WAT), in addition to adipocytes, contains stromal-vascular cells. Unilocular adipocytes, characteristic of WAT, contain a single large lipid droplet which pushes the cell nucleus against the plasma membrane, giving the cell a signet-ring shape. Mitochondria are found predominately in the thicker portion of the cytoplasmic rim near the nucleus.

The largest depots are found subcutaneously and in the abdominal region. Visceral and subcutaneous adipose tissues depots display different metabolic properties, manifested by differences in the expression level of genes involved in fat cell metabolism, and in the secretion of adipose factors [6;7].

Adipogenesis is driven by transcription factors CCAAT/enhancer-binding protein (C/EBP) α, β and δ, peroxisome proliferator-activated receptor (PPAR) γ and adipocyte determination and differentiation factor-1/sterol regulatory element-binding protein-1c (ADD-1/SREBP-1), whose activation leads leads to the expression of many adipocyte specific proteins involved in glucose and lipid metabolism [8].

Fatty acids are either provided by the diet or can be synthesized *de novo* from glucose via acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). They are stored after esterification with G3P to form TAG droplets. G3P arises from glucose through glycolysis, a pathway occurring at a high rate in postprandrial situations. In contrast, during fasting, fatty acids are released from WAT into the blood via lipolysis of the TAG stores, mediated by homone-sensitive lipase, and used for fuel by other tissues. At the same time they reesterify part of these fatty acids back to TAG, leading to a recycling process. Mitochondrial β-oxidation of fatty acids is regulated by the entry of fatty acids into the mitochondria by malonyl-CoA, which is a potent inhibitor of CPT-1 [9].

Like in other tissues, mitochondria represent the main source of ATP even in white fat. Oxidative phosphorylation (OXPHOS) varies depending on cellular activities, and is regulated by both gene expression and the electrochemical potential difference of H^+. Mitochondrial proteins are coded by genes of both nuclear (nDNA) and maternal mitochondrial (mtDNA) DNA. Many conditions that lead to changes in bioenergetics result in mitochondrial proliferation. The expression of both mtDNA (by mtTFA) and nDNA for OXPHOS and uncoupling proteins (UCP) (by NRFs, etc.) is coordinated by a factor called PPAR γ coactivator 1 (PGC-1) [10].

Besides free fatty acids WAT release many proteins - 'adipokines'. They have many physiological effects on different organs and are involved in obesity-associated complications, such as insulin resistance, endothelial dysfunction, arterial hypertension and atherosclerosis [11, 12]. Leptin is released by fat cells in amounts mirroring overall body fat stores. Circulating leptin levels give the brain a reading of energy storage for the purposes of regulating appetite and metabolism. The peripheral mechanism, which results in intramyocellular lipid depletion via promoting fatty acid oxidation and TAG synthesis by activation of AMPK, will enhance insulin sensitivity [13]. Adiponectin exhibits several actions on skeletal muscle, liver and vessels and appears to be involved in the regulation of energy balance and insulin action and also seems to have anti-inflammatory and anti-atherogenic properties [14, 15]. Low plasma adiponectin concentrations predicted a decrease in insulin sensitivity and an increase of type 2 diabetes [16]. Administration of adiponectin

led to an increase in glucose utilisation and fatty acid oxidation in cultured myocytes and in soleus muscle of mice *in vivo*. In hepatocytes AMPK was activated as well, leading to a reduction in gluconeogenesis [17].

1.2. *AMP-activated protein kinase*

AMPK, serving as a metabolic master switch in response to alterations in cellular energy charge, plays a key role in regulation of carbohydrate and fat metabolism. Phosphorylation by AMPK may result in increases or decreases in the rate of the metabolic pathway in which the protein target plays a regulatory role [18] and AMPK can also influence metabolism by regulating gene expression [19]. The kinase is activated in physiological situations when cellular production of AMP is increased - exercise, heat shock, glucose or oxygen deprivation.

Its known effects among others include: (i) inhibition of fatty acid synthesis and lipolysis in adipocytes due to phosphorylation of ACC-α and HSL [20, 21, 22], (ii) activation of fatty acid oxidation in muscle due to phosphorylation of ACC-β [23], (iii) induction of mitochondrial biogenesis possibly through activation of NRF-1 [24], and (iv) downregulation of lipogenic genes mediated by transcription factor SREBP-1 in liver [25], as well as in 3T3-L1 adipocytes, in the latter case by downregulating PPARγ [26].

1.3. *aP2-UCP1 transgenic mice*

The UCPs regulate discharge the proton gradient that is generated by the respiratory chain across the inner mitochondrial membrane. This energy-dissipatory mechanism can serve functions such as thermogenesis, maintenance of the redox balance, or reduction in the production of reactive oxygen species. The most well-known member of the family is UCP1, which is uniquely expressed in brown adipose tissue (BAT) and plays an important role in cold- and diet-induced thermogenesis.

Transgenic aP2-*Ucp1* mice, in which the UCP1 gene is driven by the fat-specific aP2 promoter to achieve enhanced expression in both brown and white fat, represent an excellent model to study the effects of respiratory uncoupling on fat accumulation *in vivo*. These animals are partially resistant to obesity induced by age, genetic background, and HF diet. The resistance to obesity reflects lower accumulation of TAG in all fat depots, except for gonadal fat, which becomes relatively large [27, 28].

The results of the morphometric analysis indicated induction of mitochondrial biogenesis by ectopic UCP1 in the unilocular adipocytes [29]. Expression of the genes for

ACC and FAS was significantly depressed by the transgene in both subcutaneous and epididymal white fat depot. Fatty acid synthesis was reduced up to fourfold by ectopic UCP1 in the white fat of transgenic aP2-*Ucp1* mice, reflecting the magnitude of UCP1 expression in different fat depots [30]. The transgenic UCP1 decreased norepinephrine-induced lipolysis and also down-regulated the expression of HSL and lowered its activity in adipocytes [31]. The phenotype of aP2-*Ucp1* mice suggest that respiratory uncoupling is involved in the modulation of white adipose tissue metabolism.

1.4. *Fasting*

In early post-absorptive state blood glucose and insulin levels fall back to normal, glucagon release triggered. Glucagon promotes glycogenlysis and gluconeogenesis in liver. Decreased insulin also promotes lipolysis by activation HSL in WAT and release of amino acids as alanine and glutamine, substrate for gluconeogenesis, from muscle. Several tissues use fatty acids in preference to glucose. If a fast becomes prolonged, mobilisation of fatty acids is increased by stimulation of adipose tissue by norepinehrine. Glucose is spared for brain.Under conditions extraordinarily prolonged fasting (starvation) ketone bodies are mobilized from liver.

Of the fatty acids released in lipolysis, some are reesterified. Because of reesterification glyceroneogenesis is induced by fasting. The expression and activity of PEPCK-C is increased. During fasting one can assume that the obvious glyceroneogenic precursors are lactate and certain amino acids like alanine [32].

The expression of lipogenic enzyme genes (ACC, FAS) is markedly reduced in adipocytes but not in stromal-vascular fraction cells isolated from subcutaneous depot of 48-h starved male rats [33]. During fasting increase also β-oxidation in adipocytes [34].

1.5. *Polyunsaturated fatty acids*

Every organism contains broad range of fatty acids. They are not only the main structural component of every cell, bounded in phospholipids, or an important source of energy, stored as TAG. But they also play an active role in the cell metabolism. The activities of fatty acids may be dependent on their having a higher or lower ability to interact with enzymes or receptors, as compared with other fatty acids.

Omega-3 and omega-6 fatty acids cannot be interconverted, and both are essential nutrients. Linoleic acid (LA; 18:2, ω-6) is a major fatty acid in plant lipids. α-Linolenic acid (ALA; 18:3, ω-3) is found in higher plants and algae. Eicosapentaenoic acid (EPA; 20:5, ω-3)

and docosahexaenoic acid (DHA; 22:6, ω-3) are major fatty acids of marine algae, fish and fish oils.

The consumption of omega-3 PUFA leads to a substantial decrease in plasma TAG concentrations and reduced adipose tissue mass. In addition to effects on plasma lipids, PUFA can prevent the developement of insulin resistance [35, 36]. Dietary PUFA and their metabolites are able to affect gene transcription, mRNA processing and modulate posttranslational modifications of proteins [37, 38]. PUFAs are known to suppress lipogenic gene transcription by downregulating the expression of the SREBPs [39, 40] and as activators/ligands for the PPARs [41]. Dietary PUFA downregulate the expression of SCD-1 in adipose tissue therefore contribute to the ability of PUFAs to reduce adipose tissue growth [42].

PUFAs enhance uncoupling of OXPHOS [43]. Rats fed with DHA and EPA have increased cytochrome c oxidase activity in brown adipose tissue and diet induced thermogenesis [44]. This is due to the stimulation of mitochondrial and peroxisomal fatty acid β-oxidation by omega-3 PUFA [45].

There is a high incorporation of EPA and DHA in membrane phospholipids. Altered fluidity may lead to changes of membrane protein functions and cell signalling. The eicosanoids produced from omega-3 fatty acids, particularly EPA and DHA, are less inflammatory, cause vasodilation, and inhibit platelet aggregation, compared with those produced from omega-6 fatty acids.

2. SPECIFIC AIMS OF WORK

General goal of this work was to learn whether changes in energy charge and metabolism of WAT may affect content of body fat and improve of obesity-associated disorders. Also site-specific response of subcutaneous and epididymal fat pads were studied. The specific aims of this thesis were:

A. to verify the hypothesis that respiratory uncoupling modulates activity of AMPK in white adipose tissue;

B. to investigate whether AMPK could be involved in the different responses of subcutaneous and epididymal fat depots to starvation;

C. to evaluate the effect of diets rich in omega-6 and omega-3 fatty acids on adipose tissue energy metabolism; and

D. to test the hypothesis that whole body effects of EPA and DHA involve induction of adiponectin.

3. METHODS

Note: Only those methods which I used personally in my work are in more detail described in this chapter. For detailed description of all methods used in the projects see enclosed publications.

Animals
Male C57BL/6J mice were used for all experiments. Animals were housed in a controlled environment (20°C; 12-h light-dark cycle; light from 6:00 a.m.) with free access to water and diet. Mice were sacrificed by cervical dislocation. Epididymal and subcutaneous white fat depots were dissected, flash frozen and stored in liquid nitrogen for biochemical and RNA analysis.
Publication A: Control mice and their transgenic littermates, hemizygous for the aP2-Ucp1 transgene, were sacrificed at 6 months of age
Publication B: Three- to four-month-old mice were caged singly for one week and than sacrificed between 9:00-10:00 a.m., while some of these mice were denied access to food for 6, 12, and 24 h before the sacrifice, respectively, and controls were allowed free access to food.
Publication C and D: At 4 months of age, singly caged animals were randomly assigned for 4 weeks to one of the high-fat diets described previously (see Tables 1–3 of reference [46] and the Electronic Supplementary Material [ESM] Tables 1 and 2); the sHFf diet, which contained 20% (wt/wt) flax-seed oil (rich in ALA) as the only lipid, or the sHFf-F2 diet, which had the same composition as the preceding diet except that 44% of lipids were replaced by n-3 PUFA concentrate containing 6% EPA and 51% DHA (EPAX 1050TG; Pronova Biocare, Lysaker, Norway; EPA/DHA concentrate). Some 4-month-old animals were also habituated for 2 weeks to the cHF diet, derived from standard chow and containing 35% (wt/wt) lipids of very low n-3 PUFA content. These were then assigned for 5 weeks to the cHF diet or to the cHF-F1 diet, which had the same composition as the cHF diet except that 15% (wt/wt) of lipids were replaced with EPAX 1050TG [46].

WAT explants
WAT(100 mg) was incubated in 1 0 ml of serum-free medium (Cellgro, Hyclone, USA) for 24h at 37oC/ 5% CO2. At the end of the incubation the culture supernatant was stored at -70oC.

Differentiation of 3T3-L1 adipocytes
Eleven days after induction of cell differentiation by dexamethasone-roziglitasone mix and 24 h before RNA isolation, a complete change of the medium was performed using serum-free Dulbecco's modified Eagle medium containing 0.5% fatty acid-free BSA, 830 nmol/l insulin and 0.5 mmol/l oleic acid in the form of BSA/fatty acid sodium salt. Medium was further supplemented with ethanolic solution of fatty acid: (1) 0.2 mmol/l oleic acid; (2) 0.2 mmol/l ALA; or (3) 0.2 mmol/l DHA, and incubated for 1 h before use.

RNA isolation
Total RNA was isolated and purified using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the protocol of the supplier with additional phenol/chloroform extraction.

RNA analysis
Gene expression was analyzed by reverse transcription followed by the real time quantitative PCR (LightCycler Instrument, Roche, Germany) with specific primers. Levels of b-actin were used to correct for inter-sample variations.

Oxidation of fatty acids
Oxidation of oleic acid was measured using a modified protocol of Wang et al. [47]. Adipose tissue (~35 mg) sliced up into 5–10 fragments was pre-labeled with 40μCi/mL [9,10(n)-3H]oleic acid in 300 μl of 2% FA-free BSA-KRB buffer containing 5 mM glucose for 75 min at 37°C and under 5% CO_2 (gentle shaking). Fragments were washed three times and re-suspended in 500 ul of the buffer and incubated for additional 4 h at 37°C. Oxidation was assessed by measuring the amount of ^3H_2O released into the medium. The content of free fatty acids in adipose tissue fragments was measured in 5% homogenate (w/v in H2O) using a NEFA C kit from Wako Chemicals (Richmond, VA) and results were calculated according to a published formula [47].

Quantification of AMPK and ACC
The total content of the α1 AMPK and the phosphorylated form of AMPK (pAMPK) was determined in tissue lysates by Western blotting using antibodies against total α1AMPK and phosphospecific antibodies against Thr-172. For the quantification of total ACC and the phosphorylated form of ACC (pACC), tissue lysates (10 μg protein) were subjected to SDS–PAGE using pre-cast 3–8% Tris-acetate gels (Invitrogen). Protein was transferred to nitrocellulose membranes (BioRad, Hercules, CA) and the membranes incubated in Odyssey Blocking buffer (Li-Cor Biosciences,Lincoln, NE). Phosphospecific sheep antibodies against the Ser-221 site on ACC-2, that also recognize the homologous site (Ser-79) on ACC-1 (1.46 lg/ml in blocking buffer containing 0.2% Tween-20), were used to quantify pACC (incubation for 1 h). The membranes were washed 5x5 min with TBS (10 mM Tris–HCl, pH 7.4, 0.5 M NaCl) containing Tween-20 (0.2%) and immersed in blocking buffer containing 0.2% Tween-20 and 1 μg/ml anti-sheep IgG conjugated to IR dye 680 (Molecular Probes, Leiden, The Netherlands) and 1 μg/ml streptavidin conjugated to IR Dye 800 (Rockland Inc., Philadelphia, PA) and left shaking for 1 h, protected from light. The membranes were then washed 5x5 min using TBS-Tween (0.2%) and 1x10 min in TBS and scanned using the Odyssey IR Imager (Li-Cor Biosciences). The results were quantified using Odyssey software.

Statistics
Statistical significance was evaluated using unpaired t-tests or two-way ANOVA. Logarithmic or square root transformation was used to stabilise variance in cells when necessary. The Spearman correlation coefficient was used to evaluate the relationship between transcript levels. The level of significance for all tests was set at p=0.05.

4. RESULTS AND DISCUSSION

4.1. *Possible involvement of AMP-activated protein kinase in obesity resistance induced by respiratory uncoupling in white fat*

Since UCP1-mediated respiratory uncoupling in brown fat is involved in thermogenesis and in the control of energy balance [48], it may be hypothesised that uncoupling of OXPHOS in WAT would also increase energy expenditure and thermogenesis. It could be expect an effect of respiratory uncoupling on lipid metabolism in adipocytes and therefore reduction of body fat content. Consequences of the expression of transgenic UCP1 in white fat in our aP2-*Ucp1* transgenic mice, which induces the obesity resistance, has been studied in great detail *(for details see chapter 2.3.)*.

The presence of transgenic UCP1 resulted in a significant, 2-fold increase in the activity of the α1 isoform of AMPK (α1 AMPK) in subcutaneous fat. Our results are supported by finding that norepinephrine (NE) increased the activity of AMPK in BAT of wild type but not UCP1-KO mice [49]. Quantification of total ACC and pACC content in adipose tissue lysates also showed a modest but significant 1.3-fold increase of the pACC/ACC ratio in subcutaneous fat due to the transgenic modification. Increased phosphorylation of ACC could, in addition to decreasing of *de novo* fatty acid synthesis, raise a rate of fatty acid oxidation. Measurement of oleate oxidation in adipose tissue fragments showed 2.7-fold increase in the oleate oxidation in subcutaneous fat, but not epididymal fat of transgenic mice.

A significant diminution of PPARγ and aP2 mRNA level was found in subcutaneous but not in epididymal fat of aP2-*Ucp1* mice. In adipocytes, AMPK is known to inhibit both lipolysis and lipogenesis by regulating directly the enzymes engaged in lipid metabolism [20], as well as by downregulating PPARγ expression [26]. All the effects of transgenic UCP1 on the biochemical properties of white fat in the aP2-*Ucp1* mice seem to be in agreement with the activation of AMPK. Thus, activation of AMPK by decreased intracellular energy charge in the white fat of aP2-*Ucp1* mice explains the complexity of changes in adipose tissue metabolism observed in this obesity-resistant transgenic model.

4.2. *Involvement of AMP-activated protein kinase in fat depot-specific metabolic changes during starvation*

The time- and depot-dependent effects of starvation were studied during 24 hours food deprivation. Significant change was detected only in weight of subcutaneous fat after 12 and 24 hours of fasting compared with fed mice. The reduction of weight was accompanied by a decrease in the content of tissue lipids in subcutaneous but not in epididymal fat after 24 h. Starvation increased plasma levels of NEFA and it reached a peak after 12 hours of fasting

The expression of SREBP-1, a transcription factor controlling lipogenesis, declined only transiently in subcutaneous fat, while the expression was progressively abolished during 24h of starvation in epididymal fat. Since decrease in the rate of lipogenesis was observed due to starvation in WAT, also cellular level of malonyl-CoA, a potent inhibitor of fatty acid oxidation, could be affected. Previously, stimulation of fatty acid oxidation in adipocytes isolated from epididymal and perirenal fat of starved rats has been shown [47]. Our measurement of oleate oxidation in WAT fragments from fed mice and mice starved for 12 h showed significant stimulation of fatty acid oxidation by starvation in epididymal fat

Previously was shown that activation of AMPK in liver do the following: (i) to phosphorylate and inactivate ACC [18]; (ii) to suppress the transcriptional regulator SREBP-1, an effect that decreases the expression of ACC, FAS [25], and the first cominitted enzyme in the pathway of glycerolipid synthesis GPAT [50]; and (iii) to increase the activity [51] and expression [52] of CPT-1.

The separate regulation of lipid metabolism in epididymal and subcutaneous fats in response to starvation was in agreement with a differential activation of the AMPK. The activity of α1 AMPK in epididymal fat peaked after 12h of starvation.

The phosphorylation state of Ser79 of ACC, the specific site of inhibitory phosphorylation by AMPK, was raised in accord with the differential activation of α1 AMPK activity.

In order to understand the mechanism of the differential effect of fasting on lipid metabolism and activation of AMPK in subcutaneous and epididymal fat, we examined whether either PEPCK or UCP2 could be involved. Expression of both PEPCK [53] and UCP2 [54] genes in adipose tissue is known to be stimulated by food deprivation. In both cases, the stimulation may result in depression of the energy charge in adipocytes, because PEPCK govens ATP-consuming fatty acid re-esterification in the adipocyte during fasting

[53], while UCP2, which is abundant in white fat [55], may decrease the rate of ATP formation due to respiratory uncoupling in mitochondria [56, 57]

In different response of both fad pads to starvation could be involved fatty acid reesterification driven by PEPCK. Up to 30% of the fatty acid released by lipolysis in epididymal fat of the fasted rat are reesterified back to TAG. This futile cycle could decrease intracellular ATP levels. A strong induction of PEPCK in epididymal fat during fasting correlates with the activation of AMPK, similar to the situation in fasted liver [53].

The induction of UCP2 expression observed during fasting, which was higher in epididymal than subcutaneous fat, might increase the proton leak in mitochondria [56, 57], inhibit synthesis of ATP during oxidative phosphorylation and contribute to the differential activation of AMPK cascade in fat depots. In support of this, respiratory uncoupling in muscle cells *in vitro* [58] and in white adipose tissue in vivo in aP2-*Ucp1* mice, both stimulated AMPK.

Several studies have proposed another explanations for the site-specific differences in response: (i) differences in nerve control [59], (ii) differences in the balance in α_2- and β-adrenoceptors [60], (iii) differences in the sensitivity of adipocytes to adenosine-mediated suppression and (iv) differences in the adenylate-cyclase activity [61].

The differencial response to starvation exhibited by epidydimal and subcutaneous fat show a different biological role played by these tissues in organism. The control of lipid and glucose metabolism in WAT by AMPK may represent a basic biological mechanism that contributes to regional differences in the metabolic properties of adipose tissue depots

4.3. *Polyunsaturated fatty acids of marine origin up-regulate mitochondrial biogenesis and induce β -oxidation in white fat*

Omega-3 PUFA of marine origin reduce adiposity in animals fed high-fat diet. The effects of EPA/DHA concentrate (6 % EPA, 51 % DHA) (sHFf-F2) admixed to high-fat diet (sHFf), rich in ALA, were studied in male C57BL/6J mice.

After 4 weeks of feeding sHFf-F2 diet decreased the body weight of mice and weight of epididymal fat compared with control mice feeding sHFf diet. Changes in gene expression in epididymal and subcutaneous fat from mice fed either sHFf or sHFf-F2 was analyzed. The most of detected upregulated genes in both depots belong to mitochondrial coded gene for components of the mitochondrial oxidative phosphorylation system (complex I , COX and subunit 6 of ATPase). Also gene for adipsin, fatty acid transport protein, was upregulated by EPA/DHA dietary uptake. On the other side was observed downregulation in expression gene

for SCD-1 in both epididymal and subcutaneous fat and acyl-CoA:diacylglycerol acyltransferase in epididymal depot only.

Induction of mRNA for transcription factors, coordinating mitochondrial biogenesis, PGC-1 and NRF-1 was caused in epididymal fat by the sHFf-F2 diet . The upregulation of the gene for CPT-1, downstream target of PGC-1, suggests stimulation of fatty acid oxidation in the epididymal fat of the sHFf-F2 mice. Indeed, oxidation of oleate was 1.5- to 1.8-fold higher in epididymal fat of the sHFf-F2 compared with the sHFf mice while fatty acid synthesis was decreased.

Our results indicate that omega-3 PUFA of marine origin (EPA and DHA) are more potent then omega-3 PUFA of plant (ALA).

In rodents, numerous studies have shown that PUFA from fish oils suppressed the expression of lipogenic genes in the liver and also upregulated the expression of genes involved in lipid oxidation in the liver and muscle tissues [62, 63]. The PUFA, particularly omega-3 series, may play a role in partitioning intracellular fuel to reduce lipid accumulation [64]. Transcriptional control of gene expression is a common mechanism by which lipids as well as other nutrients affect metabolism.

Also prostaglandins, reactive intermediates of PUFA, could be involved. Prostaglandins play a critical role in the adipocyte differentiation process [65]. Omega-3 PUFA exert an anti-adipogenic effect in adipose tissue in a site-specific manner by down-regulating series 2 prostaglandin synthesis. They prevent the development of visceral adipose tissue by down-regulating adipocyte differentiation [66].

In order to further address the molecular basis by which PUFA might direct influence adipose tissue gene expression were quantified PGC-1 and NRF-1 mRNA levels in 3T3-L1 adipocytes incubated for 24 h with various fatty acids. Compared with oleate, both ALA and DHA significantly increased the levels of both transcripts. This finding indicates that PUFA-mediated regulation of adipose tissue gene expression would not necessarily need extra-adipose factors. And the activation of PGC-1 transcription factor is acute and direct effect of omega-3 PUFA on WAT.

PUFAs act as potent hypolipidemic agents due to their ability to increase expression of genes encoding enzymes of lipid oxidation while concurrently decreasing those encoding lipogenic enzymes. EPA/DHA seems to be more potential then other PUFA. The mechanism of EPA/DHA mediated metabolic switch from lipid storage to lipid oxidation includes (i) attenuation of the malonyl-CoA inhibitory effect on CPT-1, (ii) induction of CPT-1 gene expression and (iii) stimulation of mitochondrial biogenesis via up-regulation of NRF-1. This

metabolic switch in adipocytes is induced independently of ALA intake and could reduce the development of obesity. Relatively minor changes in the composition of lipids in an obesity-promoting diet exert surprisingly pronounced effects.

4.4. *Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed high-fat diet*

In previous study was shown effect of dietary intake of fish oil omega-3 PUFA on hyperinsulinemia caused by HF diet. It also protected against down-regulation of GLUT4, an insulin-sensitive glucose transporter, in WAT by the high-fat diet. The expression of glucose transporter GLUT4 partially restored in adipose tissue of mice treated with EPA/DHA suggests that improved insulin sensitivity of adipose tissue could be responsible, at least partly, for the enhancement in whole body insulin sensitivity. We hypothesized that one of the adipose tissue secreted factors could be involved. Both leptin and adiponectin are responsible for increased fatty acid oxidation in response to the activation of AMPK in muscle [13, 14].

Adult male mice fed cHF or cHF-F1 (9 % of dietary fat substituted by EPA/DHA) diets *ad libitum* or 70% *ad libitum* (caloric restriction (CR)) for 5 weeks were compared. Decrease in plasma levels of NEFA was detected only in mice fed cHF-F1 diet *ad libitum*. TAG levels were lower in both groups animals with CR and was also affected by cHF-F1 diet. Levels of glucose and leptin declined due to CR only. On the other side levels of insulin and adiponectin were affected by cHF-F1 only. Levels of adiponectin were increased by cHF-F1 diet to a similar extent in both *ad libitum* fed and in the CR mice.

In adipocytes isolated from both depots expression of adiponectin was stimulated by EPA/DHA. The stimulative effect was stronger in adipocytes from epididymal fat. Levels of secreted adiponectin, measured in tissue explants, were increased by EPA/DHA in epididymal fat only.

Although both caloric restriction and dietary intake of EPA/DHA resulted in decrease in body weight and reduction of adipose tissue mass, effect on lipid and glucose metabolism markers and secretion of adipokines is different. By dietary EPA/DHA were regulated predominantly NEFA, insulin and adiponectin. Interestingly, EPA/DHA significantly reduced circulating insulin levels in a dose-dependent manner, suggesting improved whole-body insulin sensitivity and/or direct effect on pancreatic β-cells [46, 67].

The mRNA expression of adiponectin and its plasma level are significantly reduced in obese and diabetic mice and humans [15, 68]. The induction of adiponectin by EPA/DHA

could not result from reduction of adiposity, since the strong decrease of fat content due to CR did not influence adiponectin levels.

Adiponectin influences glucose and fatty acid metabolism through an insulin-sensitizing effect that appears to be mediated through activation of AMPK in liver, muscle and adipocytes [17, 69]. In parallel with its activation of AMPK, adiponectin stimulates phosphorylation of ACC, fatty acid oxidation, glucose uptake and lactate production in myocytes, phosphorylation of ACC and reduction of molecules involved in gluconeogenesis in the liver, and reduction of glucose levels *in vivo* [17]. Our results suggest that the protection against HF diet-induced insulin resistance is at least partially mediated by adiponectin, but not leptin.

5. CONCLUSIONS

A. The activation of AMPK can explain the complexity of metabolic changes in adipose tissue of aP2-*Ucp1* mice and suggests a new link between mitochondrial energy conversion and regulation of body weight.

B. The activation of AMPK is involved in differential response of epidydimal and subcutaneous fat to starvation. The control of lipid and glucose metabolism in WAT by AMPK may represent a basic biological mechanism that contributes to regional differences in metabolic properties of adipose tissue depots.

C. Dietary intake of EPA/DHA induces a metabolic switch from lipid storage to lipid oxidation in WAT by increasing expression and activity of CPT-1 and inducing of mitochondrial biogenesis via up-regulation of PGC-1 and NRF-1.

D. The intake of EPA/DHA stimulates adiponectin expression and increases the levels of circulating adiponectin, relatively independent of food intake and body fat mass.

6. REFERENCES

1. Lev-Ran, A. (1999) Thrifty fenotype: how applicable is it to obesity and type 2 diabetes? Diabetes Rev. 7, 1-22
2. Spiegelman, B.M., and Flier, J.S. (2001) Obesity and the regulation of energy balance. Cell 104(4). 531-43
3. Bray, G.A. (2000) Overweight, mortality and morbidity. In: Bouchard C. ed. Physical activity and obesity. Champain, IL: Human Kinetics, 31-53
4. Arner, P. (2000) Obesity is genetic disease of adipose tissue? Br. J. Nut.r 83 (Suppl 1), S9-S16
5. Kopecky, J., Rossmeisl, M., Flachs, P., Bardova, K., and Brauner P. (2001) Mitochondrial uncoupling and lipid metabolism in adipocytes. Biochem. Soc. Trans. 29, 791-79.
6. Arner, P. (2001) Regional differences in protein production by human adipose tissue. Biochem. Soc Trans. 29, 72-75
7. Wajchenberg, B.L. (2000) Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr. Rev. 21, 697-738
8. Gregoire, F.M., Smas, C.M., Sul, H.S. (1998) Understanding adipocyte differentiation. Physiol Rev 78(3),783-809
9. McGarry, J. D. and Brown, N. F. (1997) Mouse white adipocytes and 3T3-L1 cells display an anomalous pattern of carnitine palmitoyltransferase (CPT) I isoform expression during differentiation. Inter-tissue and inter-species expression of CPT I and CPT II enzymes. Eur J Biochem. 244, 1-14
10. Wallace, D.C. (1999) Mitochondrial diseases in man and mouse. Science. 283, 1482-8
11. Rajala, M.W., and Scherer, P.E. (2003) Minireview: the adipocyte—at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology 144, 3765- 3773
12. Trayhurn, P., and Beattie, J.H: (2001) Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. Proc Nutr Soc 60, 329-339
13. Minokoshi, Y., Kim, Y. B., Peroni, O. D., Fryer, L. G., Muller, C., Carling, D., and Kahn, B.B. (2002) Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 415, 339-343
14. Scherer, P.E., Williams, S., Fogliano, M., Baldini, G., and Lodish, H.F. (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 270, 26746-9.
15. Hu, E., Liang, P., and Spiegelman, B.M. (1996) AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 271, 10697-703
16. Stefan, N., Vozarova, B., Funahashi, T., et al. (2002) Plasma adiponectin concentration is associated with skeletal muscle insulin receptor tyrosine phosphorylation, and low plasma concentration precedes a decrease in whole-body insulin sensitivity in humans. Diabetes 51, 1884-8.
17. Yamauchi, T., Kamon, J., Minokoshi, Y., Ito, Y., Waki, H., Uchida, S., Yamashita, S., Noda, M., Kita, S., Ueki, K., Eto, K., Akanuma, Y., Froguel, P., Foufelle, F., Ferre, P., Carling, D., Kimura, S., Nagai, R., Kahn, B. B., and Kadowaki, T. (2002) Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 8, 1288-95.
18. Hardie, D. G., and Carling, D. (1997) The AMP-activated protein kinase. Fuel gauge of the mammalian cell? Eur. J. Biochem. 246, 259–273
19. Leclerc I, Kahn A, Doiron B. (1998) The 5'-AMP-activated protein kinase inhibits the transcriptional stimulation by glucose in liver cells, acting through the glucose response complex. FEBS Lett 431, 180-4

20. Sullivan, JE, Brocklehurst, KJ, Marley, AE, Carey, F, Carling, D, Beri, RK. (1994) Inhibition of lipolysis and lipogenesis in isolated rat adipocytes with AICAR, a cell-permeable activator of AMPactivated protein kinase. FEBS Lett 353, 33–36.

21. Garton, A.J., Campbell, D.G., Carling, D., Hardie, D.G., Colbran R.J., and Yeaman, S.J. (1989) Phosphorylation of bovine hormone-sensitive lipase by the AMP-activated protein kinase. A possible antilipolytic mechanism. Eur. J Biochem 179, 249–254

22. Corton, J.M., Gillespie, J.G., Hawley, S.A., and Hardie, D.G. (1995) 5-aminoimidazole-4-carboxamide ribonucleoside. A specific method for activating AMP-activated protein kinase in intact cells? Eur. J. Biochem 229, 558–565.

23. Merrill, G.F., Kurth, E.J., Hardie, D.G., and Winder, W.W. (1997) AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. Am. J. Physiol 36, E1107-E1112.

24. Bergeron, R., Ren, J.M., Cadman, K.S., Moore, I.K., Pen-et, P., Pypaert, M., Young, L.H., Semenkovich, C.F., and Shulman, G.I. (2001) Chronic activation of AMP kinase results in NRF-1 activation and mitochondrial biogenesis. Am J Physiol Endocrinol Metab 281, E1340-6.

25. Zhou, G, Myers, R, Li, Y, Chen, Y, Shen, X, Fenyk-Melody, J, Wu, M, Ventre, J, Doebber, T, Fujii, N, Musi, N, Hirshman, MF, Goodyear, LJ, and Moller, DE. (2001) Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 108, 1167-1174

26. Habinowski, SA, Witters, LA. (2001) The effects of AICAR on adipocyte differentiation of 3T3-L1 cells. Biochem Biophys Res Commun 286, 852–856.

27. Kopecky, J., Rossmeisl, M., Hodny, Z., Syrovy, I., Horakova, M., and Kolarova, P. (1996) Reduction of dietary obesity in the aP2-Ucp transgenic mice: mechanism and adipose tissue morphology. Am.J.Physiol 270, E776-E786.

28. Kopecky,J., Hodny,Z., Rossmeisl,M., Syrovy,I., and Kozak,L.P. (1996) Reduction of dietary obesity in the aP2-Ucp transgenic mice: physiology and adipose tissue distribution. Am.J.Physiol. 270, E768-E775.

29. Rossmeisl, M., Barbatelli, G., Flachs, P., Brauner, P., Zingaretti, M.C., Marelli, M., Janovská, P., Horaková, M., Syrový, I., Cinti, S., and Kopecký, J. (2002) Expression of the uncoupling protein 1 from the aP2 gene promoter stimulates mitochondrial biogenesis in unilocular adipocytes in vivo. Eur J Biochem 269, 19-28

30. Rossmeisl, M., Syrový, I., Baumruk, F., Flachs, P., Janovská, P., and Kopecký, J. (2000) Decreased fatty acid synthesis due to respiratory uncoupling in adipose tissue. FASEB J 14, 1793-1799

31. Flachs, P., Novotný, J., Baumruk, F., Bardová, K., Bourová, L., Mikšík, I., Šponarová, J., Svoboda, P., and Kopecký, J. (2002) Impaired noradrenaline-induced lipolysis in white fat of aP2-Ucp1 transgenic mice is associated with changes in G-protein levels. Biochem J 364, 396-376

32. Forest, C., Franckhauser, S., Glorian, M., Antras-Ferry, J., Robin, D. and Robin, P. (1997) Regulation of gene transcription by fatty acids, fibrates and prostaglandins: the phosphoenolpyruvate carboxykinase gene as a model.Prostaglandins Leukotrienes Essential Fatty Acids 57, 47–56

33. Thumelin, S, Kohl, C, Girard, J, Pegorier, JP. (1999) Atypical expression of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase in subcutaneous adipose tissue of male rats. J Lipid Res 40, 1071-7

34. Commerford, SR, Pagliassotti, MJ, Melby, CL, Wei, Y, Gayles, EC, Hill, JO. (2000) Fat oxidation, lipolysis, and free fatty acid cycling in obesity-prone and obesity-resistant rats. Am J Physiol Endocrinol Metab 279, E875-85

35. Storlien, LH, Kraegen, EW, Chisholm, DJ, Ford, GL, Bruce, DG, Pascoe, WS. (1987) Fish oil prevents insulin resistance induced by high-fat feeding in rats. Science 237, 885-8.

36. Belzung, F, Raclot, T, Groscolas, R. (1993) Fish oil n-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets. Am J Physiol 264, R1111-8.

37. Clarke, S.D. (2004) The multi-dimensional regulation of gene expression by fatty acids: polyunsaturated fats as nutrient sensors. Curr Opin Lipidol 15, 13–18

38. Wahle, K., Rotondo, D., and Heys, SD. (2003) Polyunsaturated fatty acids and gene expression in mammalian systems. Proc Nutr Soc 62, 349–360

39. Apfel, R, Benbrook, D, Lernhardt, E, Ortiz, MA, Salbert, G, Pfahl, M. (1994) A novel orphan receptor specific for a subset of thyroid hormone-responsive elements and its interaction with the retinoid/thyroid hormone receptor subfamily. Mol Cell Biol 14, 7025-7035.

40. Schultz, JR, Tu, H, Luk, A, et al. (2000) Role of LXRs in control of lipogenesis. Genes Dev 14, 2831-2838

41. Forman, B.M., Chen, J., and. Evans, R.M (1997) Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. Proc Natl Acad Sci 94, 4312–4317.

42. Jones, B., Maher, M., Banz, W., Zemel, M., Whelan, J., Smith, P., Moustaid, N., (1996) Adipose tissue stearoyl-CoA desaturase mRNA is increased by obesity and decreased by polyunsaturated fatty acids, Am. J. Physiol 271, E44–E99.

43. Stillwell, W., Jenski, L. J., Crump, F. T., and Ehringer, W. (1997) Effect of docosahexaenoic acid on mouse mitochondrial membrane properties. Lipids 32, 497-506

44. Oudart, H., Groscolas, R., Calgaari, C., Nibbelink, M., Leray, C., Le Maho, Y., and Malan, A. (1997) Brown fat thermogenesis in rats fed high-fat diets enriched with n-3 polyunsaturated fatty acids. Int J Obesity 21, 955- 962

45. Ide, T., Murata, M., and Sugano, M. (1996) Stimulation of the activities of hepatic fatty acid oxidation enzymes by dietary fat rich in alpha-linolenic acid in rats. J. Lipid Res 37, 148-463.

46. Ruzickova, J, Rossmeisl, M, Prazak, T, Flachs, P, Sponarova, J, Vecka, M, Tvrzicka, E, Bryhn, M, Kopecky, J. (2004) Omega-3 PUFA of marine origin limit diet-induced obesity in mice by reducing cellularity of adipose tissue. Lipids 39, 1177-85.

47. Wang, T, Zang, Y, Ling, W, Corkey, BE, Guo, W. (2003) Metabolic partitioning of endogenous fatty acid in adipocytes. Obes Res 11, 880-7

48. Ricquier D., Boullaud F. (2000) The uncoupling protein homologes. Biochem J 345, 161-79

49. Inokuma, K, Ogura-Okamatsu, Y., Toda, Ch., Kimura, K., Yamashita, H. and Saito, M. (2005) Uncoupling Protein 1 Is Necessary for Norepinephrine-Induced Glucose Utilization in Brown Adipose Tissue. Diabetes 54, 1385–1391

50. Muoio, D.M, Seefeld, K., Witters, L.A., and Coleman, R.A. (1999) AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol-3-phosphate acyltransferase is a novel target. Biochem J 338, 783-791

51. Velasco, G, Gomez del Pulgar, T, Carling, D, and Guzman, M. (1998) Evidence that the AMP-activated protein kinase stimulates rat liver carnitine palmitoyltransferase I by phosphorylating cytoskeletal components. FEBS Lett 439, 317-320

52. Suchankova, G, Tekle, M, Saha, AK, Ruderman, NB, Clarke, SD, and Gettys, TW. (2005) Dietary polyunsaturated fatty acids enhance hepatic AMP- activated protein kinase activity in rats. Biochem Biophys Res Commun 326, 851-858

53. Gorin, E., Tal-Or, Z. and Shafrir, E. (1969) Glyceroneogenesis in adipose tissue of fasted, diabetic and triamcinolone treated rats. Eur J Biochem 8, 370–375

54. Millet, L, Vidal, H, Andreelli, F, Larrouy, D, Riou, JP, Ricquier, D, Laville, M, Langin, D. (1997) Increased uncoupling protein-2 and -3 mRNA expression during fasting in obese and lean humans. J Clin Invest 100, 2665-70.

55. Pecqueur, C, Alves-Guerra, MC, Gelly, C, Levi-Meyrueis, C, Couplan, E, Collins, S, Ricquier, D, Bouillaud, F, Miroux, B (2001) Uncoupling protein 2, in vivo distribution, induction upon oxidative stress, and evidence for translational regulation. J Biol Chem 276, 8705-12.

56. Echtay, KS, Winkler, E, Frischmuth, K, Klingenberg, M. (2001) Uncoupling proteins 2 and 3 are highly active H(+) transporters and highly nucleotide sensitive when activated by coenzyme Q (ubiquinone). Proc Natl Acad Sci U S A. 98, 1416-21.

57. Fink, BD, Hong, YS, Mathahs, MM, Scholz, TD, Dillon, JS, Sivitz, WI. (2002) UCP2-dependent proton leak in isolated mammalian mitochondria. J Biol Chem 277, 3918-25.

58. Fryer, L. G., Parbu-Patel, A., and Carling, D. (2002) Protein kinase inhibitors block the stimulation of the AMP-activated protein kinase by 5-amino-4-imidazolecarboxamide riboside. J Biol Chem 277, 25226-25232

59. Bray, GA, Inoue, S, and Nishizawa, Y. (1981) Hypothalamic obesity. The autonomic hypothesis and the lateral hypothalamus. Diabetologia 20 Suppl, 366-77

60. Arner, P, Hellstrom, L, Wahrenberg, H, Bronnegard, M. (1990) Beta-adrenoceptor expression in human fat cells from different regions. J Clin Invest 86, 1595-600.

61. Dieudonne, MN, Pecquery, R, and Giudicelli, Y. (1992) Characteristics of the alpha 2/beta-adrenoceptor-coupled adenylate cyclase system and their relationship with adrenergic responsiveness in hamster fat cells from different anatomical sites. Eur J Biochem 205, 867-73.

62. Baillie, RA, Takada, R, Nakamura, M, Clarke, SD. (1999) Coordinate induction of peroxisomal acyl-CoA oxidase and UCP-3 by dietary fish oil: a mechanism for decreased body fat deposition. Prostaglandins Leukot Essent Fatty Acids. 60, 351-6.

63. Yahagi, N, Shimano, H, Hasty, AH, Amemiya-Kudo, M, Okazaki, H, Tamura, Y, Iizuka, Y, Shionoiri, F, Ohashi, K, Osuga, J, Harada, K, Gotoda, T, Nagai, R, Ishibashi, S, and Yamada, N. (1999) A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. J Biol Chem 274, 35840-4.

64. Clarke, SD. (2001) Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome. J Nutr 131, 1129-32.

65. Forman, BM, Tontonoz, P, Chen, J, Brun, RP, Spiegelman, BM, and Evans, RM. (1995) 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ. Cell 83, 803-812.

66. Okuno, M, Kajiwara, K, Imai, S, Kobayashi, T, Honma, N, Maki, T, Sumga, K, Goda, T, Takase, S, Muto, Y, and Moriwaki, H. (1997) Perilla oil prevents the excessive growth of visceral adipose tissue in rats by down-regulating adipocyte differentiation. J Nutrition 127, 1752-1757.

67. Ikemoto, S, Thompson, KS, Itakura, H, Lane, MD, and Ezaki, O. (1995) Expression of an insulin-responsive glucose transporter (GLUT4) minigene in transgenic mice: effect of exercise and role in glucose homeostasis. Proc Natl Acad Sci U S A 92, 865-9.

68. Arita, Y., Kihara, S., Ouchi, N., et al. (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 257, 79-83.

69. Wu, X.D., Motoshima, H., Mahadev, K., Stalker, T.J., Scalia, R., Goldstein, B.J. (2003) Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. Diabetes 52, 1355- 1363.

7. LIST OF PUBLISHED PAPERS

Kopecký, J., Rossmeisl, M., Flachs, P., Kmoch, S., Bardová, K., Brauner, P., **Matějkova, O.**, Sponarova, J., Pražák, T., Thomason-Hughes, M., Hardie, DG. (2003). UCP1 expression in white adipose tissue and lipid metabolism. *Progress in Obesity Research* 9. Chpt. 45, pp 214-219.

Kopecky J, Rossmeisl M, Flachs P, Brauner P, Sponarova J, **Matejkova O**, Prazak T, Ruzickova J, Bardova K, Kuda O. (2004). Energy metabolism of adipose tissue--physiological aspects and target in obesity treatment. *Physiol Res.* 53 Suppl 1:S225-32.

Matejkova O, Mustard KJ, Sponarova J, Flachs P, Rossmeisl M, Miksik I, Thomason-Hughes M, Grahame Hardie D, Kopecky J (2004). Possible involvement of AMP-activated protein kinase in obesity resistance induced by respiratory uncoupling in white fat. *FEBS Lett.* 569(1-3):245-8.

Rossmeisl M, Flachs P, Brauner P, Sponarova J, **Matejkova O**, Prazak T, Ruzickova J, Bardova K, Kuda O, Kopecky J. (2004) Role of energy charge and AMP-activated protein kinase in adipocytes in the control of body fat stores. *Int. J. Obes. Relat. Metab. Disord.* 28 Suppl 4:S38-44.

Flachs P, **Horakova O**, Brauner P, Rossmeisl M, Pecina P, Franssen-van Hal N, Ruzickova J, Sponarova J, Drahota Z, Vlcek C, Keijer J, Houstek J, Kopecky J. (2005) Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce beta-oxidation in white fat. *Diabetologia* 48(11):2365-75.

Sponarova J, Mustard KJ, **Horakova O**, Flachs P, Rossmeisl M, Brauner P, Bardova K, Thomason-Hughes M, Braunerova R, Janovska P, Hardie DG, Kopecky J. (2005). Involvement of AMP-activated protein kinase in fat depot-specific metabolic changes during starvation. *FEBS Lett.* 579(27):6105-10.

Flachs P, Mohamed-Ali V, **Horáková O**, Rossmeisl M, Hosseinzadeh-Attar MJ, Hensler M, Ružičková J, Kopecký J. (2006) Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet. *Diabetologia.* 49 :1-4