Charles University in Prague

Faculty of Science

Department of Molecular and Cellular Biology, Genetics and Virology

Academy of Sciences of the Czech Republic

Institute of Physiology, v.v.i

Molecular mechanisms affected by n-3 polyunsaturated fatty acids

Ph.D. Thesis

Michal Hensler

Supervisor: Pavel Flachs, RNDr, PhD

Prague 2011

Statement of authorship

I certify that the thesis represents valid work elaborated under the supervision of Pavel Flachs, RNDr, PhD, and that neither this manuscript nor one with substantially similar content under my authorship has been submitted in support of an application for any other academical degree. My participation in the published papers is specified at the end of the comments to each paper.

In Prague …….

Mgr. Michal Hensler

Statement of co-authors

I certify that Michal Hensler substantially contributed to the formation of the papers used as a basis of this thesis, and that his participation specified at the end of the comments to each paper is correct.

In Prague ……..

RNDr. Pavel Flachs PhD

Acknowledgements

I would like to express my sincere thanks to my supervisor Pavel Flachs and to Jan Kopecký, the head of the department, for the scientific and financial support through my PhD studies and to all collaborators and co-authors of our publications.

ÚVOD

Tuková tkáň a její hormony hrají nezastupitelnou roli ve fyziologii savců. Při nerovnováze mezi příjmem a výdejem energie dochází k růstu tukové tkáně, a ke změně jejího sekrečního profilu. S obezitou jsou spojována mnohá onemocnění, jako kardiovaskulární onemocnění, dyslipidémie, hypertenze a inzulínová rezistence.

n-3 polynenasycené mastné kyseliny s dlouhým řetězcem (LC n-3 PUFAs) z rybích olejů, hlavně eikosapentaenová (EPA) a dokosahexaenová (DHA) kyselina, zlepšují celkový lipidový metabolizmus a brání rozvoji obezity a inzulínové rezistence. Studie, prováděné na myších liniích náchylných k obezitě (C57BL/6), nám poskytují důležité poznatky, týkající se jejich vlivu na savčí tkáně a možného terapeutického využití.

Tato disertační práce je založena na pěti publikovaných článcích (A-E). Tři práce se věnovaly problematice bílé tukové tkáně. V těchto pracích jsme ukázali, že bílá tuková tkáň je flexibilní orgán a LC n-3 PUFAs hrají důležitou roli v její biologii. Naše výsledky ukazují, že LC n-3 PUFAs ovlivňují množství tukové tkáně mechanizmy závislými jak na potlačení proliferace, tak i diferenciace tukových buněk (publikace A). Anti-obezitní efekt EPA a DHA může být ještě více umocněn mírnou kalorickou restrikcí (10%). Výsledky z publikace B dokazují, že lipidový katabolizmus a tvorba proti-zánětlivých molekul v bílé tukové tkáni, může být umocněna právě zmíněnou kalorickou restrikcí. Jako první jsme prokázali, že LC n-3 PUFAs stimulují expresi a sekreci adiponektinu, hormonu zlepšujícího inzulínovou citlivost tkání (publikace C). V kombinované studii zaměřené na inzulínovou citlivost svalu, jsme popsali benefiční účinky LC n-3 PUFAs a rosiglitazonu, na syntézu glykogenu a celotělovou inzulínovou citlivost. Poslední práce byla zaměřena na játra a roli AMP-aktivované proteinové kinázy (AMPK). V této práci byly použity myši s celotělovým knock-out genem pro α2 katalytickou podjednotku. V experimentu bylo prokázáno, že benefiční účinky LC n-3 PUFAs na jaterní inzulínovou citlivost jsou závislé na funkční AMPK (publikace E).

Závěrem tato disertační práce ukázala, že EPA a DHA hrají důležitou roli v lipidové a glukózové homeostaze. Obohacení potravy o LC n-3 PUFAs, zvláště pak v kombinaci s rosiglitazonem nebo kalorickou restrikci, by se mohlo stát důležitou součástí prevence a léčby onemocnění doprovázených obezitou.

ABSTRACT

Adipose tissue and its hormones have an irreplaceable role in the physiology of mammals. The imbalance between energy intake and energy expenditure leads to the expansion of adipose tissue and changes in its secretion profile. With obesity are associated diseases including cardiovascular diseases, dyslipidemia, hypertension and insulin resistance, one of the major public health issues.

Long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs) from marine origin, mainly eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids exert numerous beneficial effects, such as improvements of lipid metabolism and prevention of obesity and diabetes. Studies with obesity-prone model mice (C57BL/6) provide us important knowledge regarding their effect on mammalian tissues and to test potential therapeutic interventions.

The thesis is based on five published studies (A-E). Three studies are focused on white adipose tissue. In these works we proved that adipose tissue is a flexible organ and LC n-3 PUFAs are potent regulators of adipose tissue biology. Our results document that LC n-3 PUFAs affect adipose tissue mass by a mechanism, which depends on counteraction of both, differentiation and proliferation of adipose cell (publication A). The anti-obesity effect of EPA and DHA could be magnified by mild calorie restriction (10%). Our results demonstrate activation of lipid catabolism and synergistic induction of anti-inflammatory lipid markers in white adipose tissue (publication B). LC n-3 PUFAs are also involved in improvement of whole-body insulin sensitivity. We showed for the first time that EPA and DHA stimulate expression and secretion of insulin-senziting hormone adiponectin from mature adipocytes (publication C). In the study focused on muscle insulin sensitivity we described the beneficial effect of combination treatment using EPA and DHA and the anti-diabetic drug rosiglitazone. EPA, DHA and rosiglitazone exerted an additive effect on muscle glycogen synthesis and its sensitivity to insulin (publication D). The last study was focused on the liver and the role of AMP–activated protein kinase (AMPK) in improvement of hepatic insulin sensitivity mediated by LC n-3 PUFAs. In this study mice were used with a whole-body deletion of the α2 catalytic subunit of AMPK. We demonstrated that LC n-3 PUFAs prevent hepatic insulin resistance in AMPKα2-dependent manner (publication E).

In conclusion, this PhD thesis shows that marine lipids, mainly EPA and DHA, play an important role in lipid and glucose homeostasis. Diet supplementation of LC n-3 PUFAs, especially in combination with rosiglitazone or calorie restriction, could become an important part in the prevention and in the treatment of metabolic disorders associated with obesity.

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

A. **Michal Hensler**, Kristina Bardova, Zuzana Macek Jilkova, Walter Wahli, Daniel Meztger, Pierre Chambon, Jan Kopecky and Pavel Flachs. *The inhibition of fat cell proliferation by n-3 fatty acids in dietary obese mice.* Lipids Health Dis. 2; 10:128, 2011, (IF = 2,247).

B. Flachs P, Rühl R, **Hensler M**, Janovska P, Zouhar P, Kus V, Macek Jilkova Z, Papp E, Kuda O, Svobodova M, Rossmeisl M, Tsenov G, Mohamed-Ali V, Kopecky J. *Synergistic induction of lipid catabolism and anti-inflammatory lipids in white fat of dietary obese mice in response to calorie restriction and n-3 fatty acids.* Diabetologia 54: 2626-38, 2011, (IF= 6,973).

C. Flachs P, Mohamed-Ali V, Horakova O, Rossmeisl M, Hosseinzadeh-Attar MJ, **Hensler M**, Ruzickova J, Kopecky J. *Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet*. Diabetologia 49: 394-7, 2006, (IF = 5,247).

D. Kuda O, Jelenik T, Jilkova Z, Flachs P, Rossmeisl M, **Hensler M**, Kazdova L, Ogston N, Baranowski M, Gorski J, Janovska P, Kus V, Polak J, Mohamed-Ali V, Burcelin R, Cinti S, Bryhn M, Kopecky J. *n-3 fatty acids and rosiglitazone improve insulin sensitivity through additive stimulatory effects on muscle glycogen synthesis in mice fed a high-fat diet*. Diabetologia 52: 941-951, 2009, (IF = 6,551).

E. Jeleník T, Rossmeisl M, Kuda O, Jilkova ZM, Medrikova D, Kus V, **Hensler M**, Janovska P, Miksik I, Baranowski M, Gorski J, Hébrard S, Jesen TE, Flachs P, Hawley S, Viollet B, Kopecky J. *AMP-activated protein kinase α2 subunit is required for the preservation of hepatic insulin sensitivity by n-3 polyunsaturated fatty acids*. Diabetes 59: 2737-2746, 2010, (IF = 8,505).

The above papers are included in full in this PhD thesis. For the complete list of my published articles, see List of my publications

LIST OF ABBREVIATIONS

15d-PGJ_2	15-deoxy-$\Delta^{12,14}$ - prostaglandin J_2
AA	arachidonic acid
AMPK	AMP–activated protein kinase
BAT	brown adipose tissue
cHF	corn oil based high-fat diet
cHF+CR	cHF diet with 10% of calorie restriction
cHF+F	corn oil based high-fat diet supplemented with fish oil
cHF+F+CR	cHF+F diet with 10% of calorie restriction
cHF+F+TZD	cHF+F diet supplemented with thiazolidinedione
cHF+TZD	cHF diet supplemented with thiazolidinedione
CLS	crown-like structures
COX3	cytochrome c oxidase subunit III
CR	calorie restriction
DAG	diacylglycerol
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FFA	free fatty acid
GLUT4	glucose transporter 4
HMW	high-molecular weight
IL	interleukin
imTG	intramuscular triglyceride
IRS	insulin receptor substrate
LC	long chain
NEFA	non-esterified fatty acids
NFκB	nuclear factor NF-kappa-B
NRF-1	nuclear respiratory factor 1
PGC-1α	peroxisome proliferative activated receptor, gamma, coactivator 1α

PI3K	phosphatidylinositol 3-kinase
PPAR	peroxisome proliferator-activated receptor
PUFA	polyunsaturated fatty acid
RQ	respiratory quotient
SCD-1	stearoyl-Coenzyme A desaturase 1
SREBP1	sterol regulatory element binding protein 1
TG	triacylglycerol
TNFα	tumor necrosis factor-alpha
TZD	thiazolidinediones
UCP1	uncoupling protein 1
VLDL	very low-density lipoprotein
WAT	white adipose tissue

CONTENTS

1 INTRODUCTION

In the last decades, obesity has become a worldwide problem. The health complications connected with being overweight, such as heart disease, type 2 diabetes and certain type of cancer, represent a social and economic problem. The global obesity epidemic has stimulated intense interest in the study of mechanisms leading to the balance between energy intake and energy expenditure.

The use of experimental mouse models of diet- induced obesity and mice with the deletion of genes involved in lipid and glucose metabolism provide invaluable knowledge and have been pushed to the forefront of this effort.

1.1 Long-chain polyunsaturated fatty acids

Long-chain polyunsaturated fatty acids (LC PUFAs) are fatty acids with two or more double bonds. LC PUFAs are classified as n-3 and n-6 on the basis of the location of the last double bond relative to the terminal methyl end of the molecule.

The LC n-6 and LC n-3 PUFAs are essential in human diet. The metabolically important arachidonic (20:4n-6; AA), eicosapentaenoic (20:5n-3; EPA) and docosahexaenoic (20:6n-3; DHA) acids are synthesized from linoleic (18:2n-6; LA) and α-linolenic (18:3n-3; ALA) acids, respectively. Western diet is consisting mainly of LC n-6 PUFAs, widely distributed in most vegetable oils (i.e. soybean, corn, sunflower). Instead, LC n-3 PUFAs are mainly abundant in fish oils. LC n-6 and LC n-3 PUFAs are competitively metabolized by the same pathway (Fig.1.), with an excess of one causing a significant decrease in the conversion of the other. Many studies have shown that more important is ratio between LC n-6 and LC n-3 PUFAs, rather than the absolute amount of each single molecule. Increased LC n-6/n-3 ratio is associated with pathogenesis of cardiovascular diseases, cancer, inflammation, obesity and insulin resistance states. Currently in the western diet the LC n-6/n-3 ratio ranges from 10/1 to 20/1, which markedly contrasts with the ratio 1/1 in the diet of our ancestors.

The LC n-6 and LC n-3 PUFAs have metabolically distinct and often opposing physiological functions. In very general terms, LC n-6 PUFAs compared with LC n-3

PUFAs are more obesogenic and promotes inflammation. With the increasing amount of EPA and DHA in diet, the body fat stores are reduced (1), lipid profiles are improved and the inflammatory process is inhibited (2; 3).

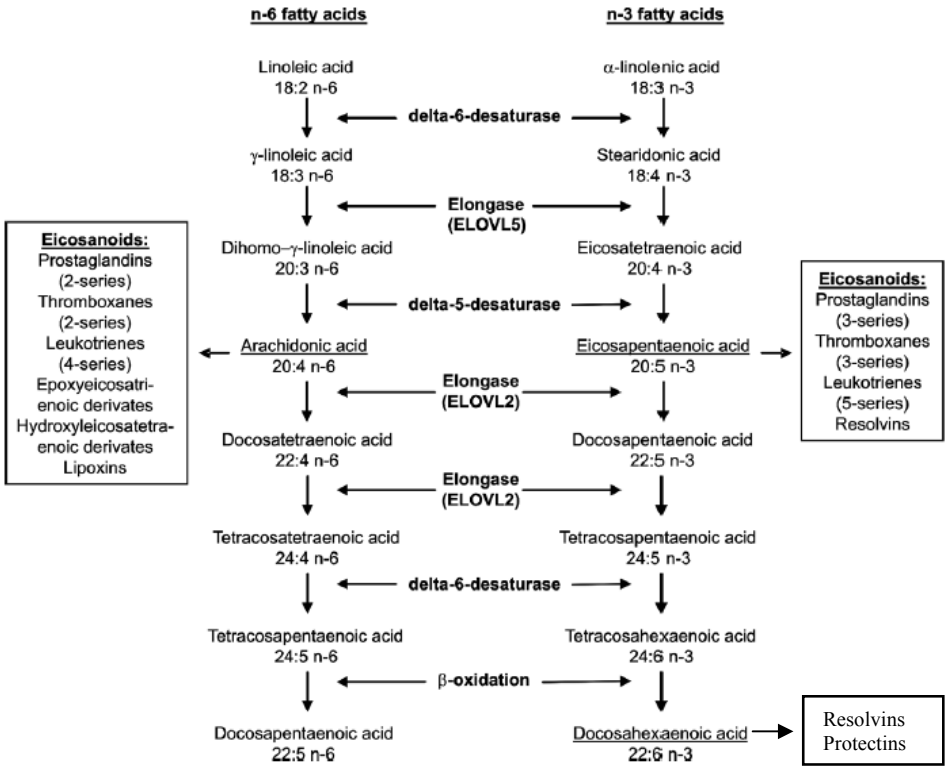

Figure 1. Transformation LA and ALA to their higher unsaturated derivates (AA, EPA, DHA) by consecutive desaturation and elongation reactions. The synthesis of metabolites from AA, EPA and DHA is also shown [adapted from (4)]. *ELOVL*, elongation of long chain fatty acids.

1.1.1 Biological actions and mechanisms of LC n-3 PUFAs

One of the major impacts of LC n-3 PUFAs on mammalian organisms is a hypolipidemic and anti-obesity effect. Many studies have shown that EPA and DHA prevent development of obesity (1), improve insulin sensitivity, ameliorate low-grade inflammation in adipose tissue and lowering the risk of cardiovascular diseases (5; 6). LC n-3 PUFAs directly influence lipid metabolism by promoting fatty acid oxidation and repressing lipid synthesis. EPA and DHA rapidly regulate transcriptional factors including PPARs, SREBP1, LXR, HNF4α, NFκB (7; 8). Most importantly, LC n-3 PUFAs down regulate expression of lipogenic enzymes, including stearoyl CoA

desaturase (SCD-1). SCD-1 is a key regulatory enzyme in the conversion of saturated fatty acid to monounsaturated fatty acids and in assembly of VLDL particles.

The activation of fatty acid oxidation is mainly due to direct interaction of EPA and DHA or their metabolites with PPARα. The type of dietary fat affects the lipogenic activity not only in the liver but also in the adipose tissue. Our previous results demonstrated that diet enriched with 15% of EPA and DHA concentrate (EPAX 1050TG), resulted in the up regulation of genes for mitochondrial biogenesis and oxidative metabolism, namely PGC-1α and NRF-1. Mitochondrial carnitine palmitoyl transferase 1, the main control point for β-oxidation was increased after EPA and DHA administration (9).

EPA and DHA could reduce accumulation of body fat by limiting both hypertrophy and hyperplasia of fat cells. Many studies have shown that LC n-3 PUFAs have anti-proliferative properties in various cell types (10; 11). In vitro studies have shown that LC n-3 PUFAs and mainly DHA induce apoptosis in post confluent preadipocytes (12). Experiments on mice showed that dietary EPA and DHA reduces cellularity of adipose tissue (1).

Many effects of LC n-3 PUFAs depend on the formation of their active metabolites, eicosanoids and other lipid mediators (13). LC n-3 PUFAs can form several potent anti-inflammatory lipid mediators, i.e. resolvins and protectins (14; 15). These bioactive substances control inflammation via stimulating resolution (Fig. 2.). EPA, DHA are converted to the E-series and D-series of resolvins, respectively. DHA is also converted via lipoxygenase to another new family of mediators named protectins. Protectin D1 has anti-inflammatory properties (16). Resolvins and protectins attenuates the activity of nuclear transcription factors, such as NFκB, and reduce the production of pro-inflammatory enzymes and cytokines, including cyclo-oxygenase 2 (COX2), tumor necrosis factor alpha (TNFα), and interleukin beta (IL-1β). Eicosanoids derived from LC n-3 PUFAs have also anti-arrhythmic and anti-thrombotic functions (4).

It has been demonstrated that LC n-3 PUFAs enhanced AMP-activated protein kinase (AMPK) activity in the liver and adipose tissue (17; 18). Activation of AMPK in the liver causes suppression of transcription factors (SREBP-1c, ChREBP) and consequent reduction of hepatic lipogenic genes (19).

LC n-3 PUFAs intake prevented development of insulin resistance, however EPA and DHA could not revert insulin resistance in type 2 diabetes patients (20). LC n-3 PUFAs also stimulate secretion of insulin-senziting hormone adiponectin (21).

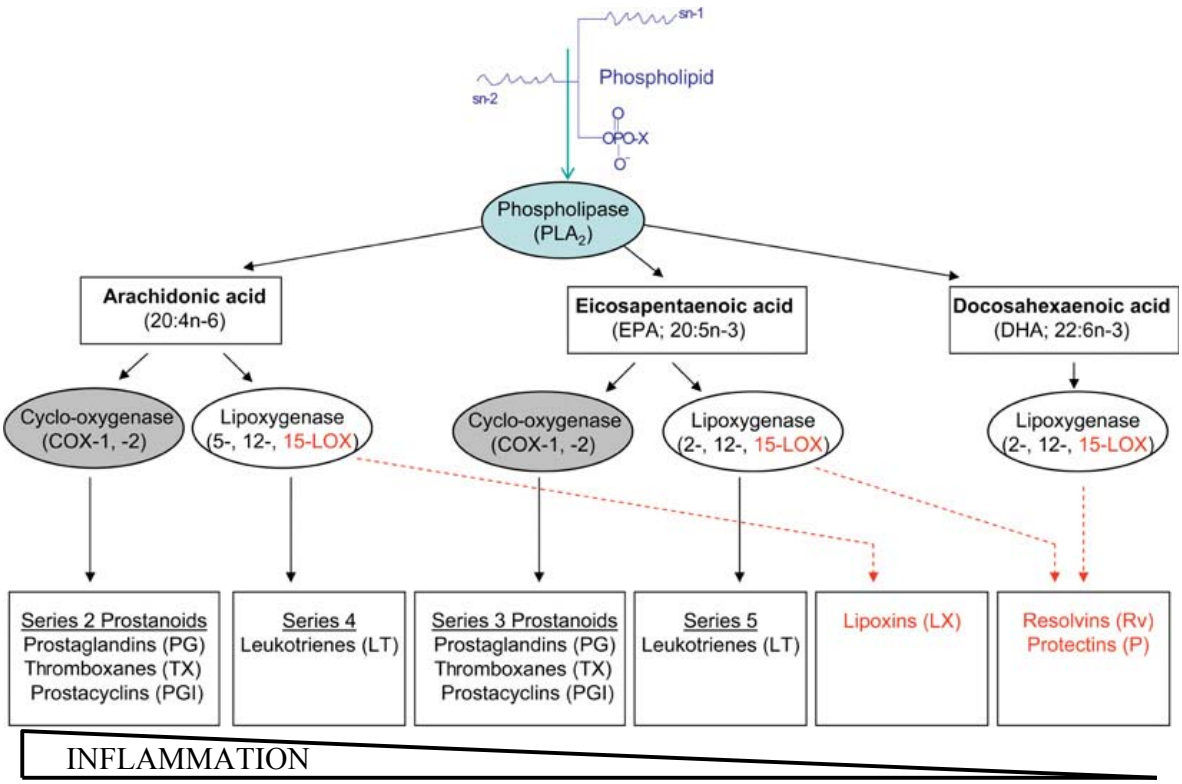

Figure 2. Formation of active metabolites from LC-PUFAs
LC-PUFAs bound to membrane phospholipids at the sn-2 position are released by PLA2 in response to various physiological and pathophysiological stimuli. The released LC-PUFAs are used as substrates for the production of active lipid metabolites [adapted from (22)].

1.1.1.1 Peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptors (PPARs) α, β/δ, γ, are ligand-activated transcription factors that are members of the nuclear-hormone receptor superfamily. PPARs mediate adaptive metabolic responses to increased systemic lipid availability following activation by binding of naturally occurring endogenous or dietary lipids or theirs derivatives (23).

PPARs form heterodimers with retinoid X receptor α (RXRα) and bind to PPREs (peroxisome proliferator response elements), which are present in promoters of

PPAR– regulated genes. PPARs, like other nuclear receptors interact with coactivators or repressors (24; 25).

Three types of PPARs have been identified. **(i)** PPARα is mainly expressed in the tissues with a high capacity for fatty acid (FA) oxidation, such as liver, heart, skeletal muscle, brown adipose tissue and others. Genes activated by PPARα are involved in FA uptake, activation and oxidation **(ii)** PPARβ/δ is expressed in many tissues. It has been shown, that PPARβ/δ increase mitochondrial content and enhance mitochondrial capacity in muscle (26) **(iii)** PPARγ is mainly expressed in adipose tissue and activation promotes lipid storage, as well as preadipocyte differentiation to mature adipocytes.

PPARα and lipid metabolism

Peroxisome proliferator-activated receptor α activates a set of genes encoding proteins involved in fatty acid catabolism. Moreover, PPARα has been shown to be involved in the regulation of obesity (27; 28). Similar expression of PPARα has been found in rodents and humans (29). In rodents, PPARα is regulated by various physiological conditions such as starvation, glucocorticoids, insulin and leptin (30-32). Activation of PPARα in the liver reduces plasma triacylglycerols, VLDL production and enhances the catabolism of LDL particles. PPARα stimulate lipid oxidation and increase HDL-cholesterol levels (33). PPARα can be activated through different ligands including synthetic or naturally ligands. Both saturated and unsaturated fatty acids act as direct natural ligands for PPARα. DHA is a more potent PPARα activator compared with other fatty acids (34).

PPARγ in adipogenesis

The cellular and molecular events during the transition from undifferentiated fibroblast-like preadipocytes into mature adipocytes are very extensively studied. The adipogenic transcription factor PPARγ plays a pivotal role in the transcriptional cascade that occurs during adipogenesis (35). PPARγ is essential for adipocyte survival and *in vivo* inducible knockout of PPARγ in differentiated adipocytes leads to adipocyte death followed by generation of new adipocytes (36).

Two isoforms of PPARγ are generated by alternative splicing. PPARγ2 is mostly expressed in adipogenic cells, while PPARγ1 is found in a wide range of tissues, including the liver, skeletal muscle, adipose tissue and bone.
PPARγ directly controls the expression of many genes involved in the key functions of adipocytes, like fatty acid uptake, lipogenesis insulin signalling and adipokine production (37-40).

Two coactivators of PPARs are known, PPARγ coactivator-1 and -2 (PGC-1,-2). PGC-1 promotes mitochondrial biogenesis and induces genes associated with components of the electron transport chain (41).
The most potent natural ligand of PPARγ is 15-deoxy-$\Delta^{12,14}$- prostaglandin J_2 (15d-PGJ_2) formed through the action of cyclo-oxygenase and of prostaglandin synthase.

1.1.1.2 AMP-activated protein kinase

LC n-3 PUFAs activate the AMP-activated protein kinase (AMPK) (17) that plays an important role in the regulation of both lipid and glucose metabolism. AMPK is a cellular energy sensor, activated by an increase in the intracellular AMP/ATP ratio. AMPK functions as a heterotrimeric complex consisting of a catalytic (α) and regulatory (β and γ) subunits (42).

The activation of AMPK leads to the production of ATP and switch off anabolic pathway. Glucose uptake as well as fatty acid oxidation is stimulated and simultaneously lipogenesis, cholesterol synthesis and gluconeogenesis are inhibited by AMPK. Anti-diabetic drugs, i.e. thiazolidinediones and metformin (43) are able to activate AMPK. Adipokines such as leptin and adiponectin are also potent activators of AMPK (44).

1.2 Insulin resistance and ectopic accumulation of lipids

Insulin resistance is the major risk factor for developing type 2 diabetes caused by the inability of insulin-target tissues to respond properly to insulin. Insulin signalling pathway is initiated by insulin binding to its receptor, phosphorylation and activation of

insulin-receptor substrates (IRSs). Phosphorylation of IRSs leads to activation of phosphatidylinositol 3-kinase (PI3K). This enzyme, through signal intermediates, activates Akt2 which promotes the docking and fusion of glucose transporter 4 (GLUT4)-containing vesicles to the plasma membrane.

Hypertrophic adipocytes lose their capacity to store lipids and lipids from the blood stream are stored in the non-adipose organs. Ectopic lipids and mainly theirs metabolites can interfere with insulin signalling pathway and causes insulin resistance in liver or skeletal muscle (lipotoxic effect) (for detail see Fig.3.).

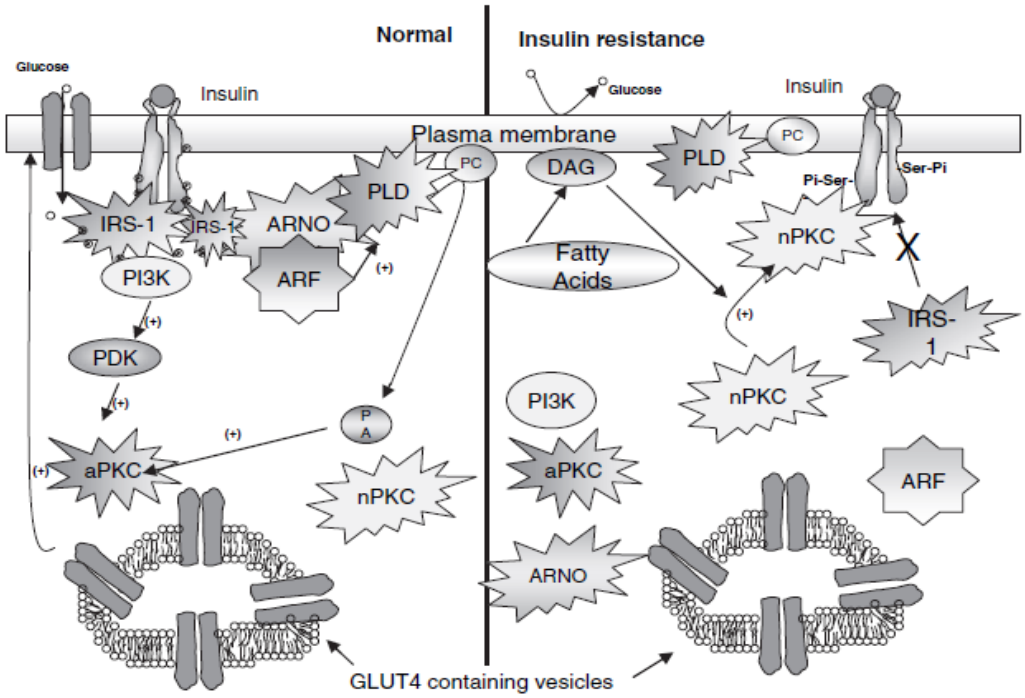

Figure 3. Protein kinase C (PKC)–mediated effects on insulin signalling. The left panel symbolizes normal insulin-mediated GLUT4 transport via activation of atypical PKC (aPKC). The right panel symbolizes insulin resistance due to aberrant activation of novel PKCs (nPKCs), which serine-phosphorylate the insulin receptor, thereby inhibiting the tyrosine autophosphorylation required for insulin receptor substrate (IRS) docking. Incidentally, IRSs are also serine phosphorylated by nPKCs (not shown). ARNO, cytohesin/ARF nucleotide-binding site opener; PLD, phospholipase D; PC, phosphatidylcholine; PA, phosphatidic acid; ARF, ADP ribosylation factor; PI3K, phosphoinositide 3 kinase; DAG, diacylglycerol; PDK, 3-phosphoinositide-dependent protein kinase [adapted from (45)].

1.2.1 White adipose tissue

The main role of white adipose tissue is to store free energy in the form of triacylglycerols and during fasting state, releasing free fatty acids (FFAs) into the blood stream as a source of energy for others organs. Adipose tissue is extremely sensitive to insulin concentration, more than muscle or liver.

In insulin resistant individuals, insulin does not suppress adipose tissue lipolysis and the ability of adipose tissue to take up lipids from the circulation in the post-prandial state is impaired. Plasma FFA and TG concentration is typically elevated, which leads to lipid accumulation in non-adipose organs (46). Ectopic lipid accumulation in non-adipocyte cells causes metabolic complications due to lipotoxic effects.

Hypertrophic adipocytes, generated in obesity, become more fragile and apoptotic. Macrophages infiltrating adipose tissue (47) produce many inflammatory cytokines (i.e. IL-6, TNFα, IL-1) that are known to cause insulin resistance not only in adipocytes but also in other cells (48-51). Hypertrophic adipocytes also reduce adiponectin and increases leptin expression, inhibits mitochondrial respiration and biogenesis (52; 53).

1.2.2 Liver

Lipids overload could led to non-alcoholic fatty liver disease (NAFLD) (54). The intracellular accumulation of lipids, namely their derivates diacylglycerol (DAG) causes resistance to insulin in terms of inhibition of hepatic glucose production and stimulation of glycogen synthesis.

DAG activates novel protein kinase C (nPKC) (55), a known serine kinase that has been shown to reduce insulin-stimulated IRS-1 tyrosine phosphorylation. This inhibitory phosphorylation results in the reduction of PI3K activity (56) and decrease of GLUT4 translocation to the plasma membrane.

Hyperinsulinemia and hyperglycemia may also induced transcriptional factors SREBP-1c and ChREBP respectively, which promotes *de novo* lipogenesis (57; 58) and increase lipid content in the liver.

1.2.3 Skeletal muscle

Skeletal muscle is the primary site of glucose disposal in the insulin-stimulated state. Many studies measuring intramuscular triglyceride (imTG) content have shown a relationship between accumulation of imTG and insulin resistance (59). Muscle insulin resistance has been associated with reduction of tyrosine phosphorylation on IRS-1 and with increased IRS-1 serine phosphorylation, leading to diminished activity of PI3K (60; 61). PI3K and downstream kinase activate glycogen synthase and GLUT4 translocation in the muscle. Muscle glycogen synthase is the major pathway for glucose disposal. High-fed feeding was associated with activation of nPKC isoforms (-ε,-θ) (62; 63) and DAG and ceramides play primary roles in lipid-induced insulin resistance (64; 65).

Fatty acids compete with glucose for substrate oxidation. Increasing the plasma fatty acid concentration caused a reduction of muscle glycogen synthesis and whole-body glucose oxidation. Although results of some studies have indicated a reduction in expression of the PGC-1a in the muscles of patients with type 2 diabetes mellitus and decreases mitochondrial oxidative capacity and mitochondrial ATP synthesis (66; 67).

1.2.4 Modulation of insulin resistance by TZDs and LC n-3 PUFAs

Thiazolidinediones

The main role of the thiazolidinediones (TZDs, for details see chapter 1.4) to improve insulin resistance state is to redistribute fat from the liver and muscle into the adipocyte and reduce accumulation of muscle and hepatic triglycerides (68; 69). This clearance of lipids from non-adipose tissue organ suppresses activation of nPKC and augments insulin action. Activation of AMPK, promotion of secretion of adiponectin and reduction of inflammation also contributes to the improvement of insulin sensitivity by TZDs.

LC n-3 polyunsaturated fatty acids

Dietary intakes of LC n-3 PUFAs have a protective effect against a high fat diet that induces insulin resistance (70). The main effect of EPA and DHA on insulin action is due to activation of PPARα and down regulation of lipogenic enzymes (71). It leads to prevention of cell against lipotoxic effect. Anti-inflammatory effect of LC n-3 PUFAs are also involved in reduction of insulin resistance. EPA and DHA stimulate expression and production of adiponectin, insulin-senziter hormone, in mature adipocytes (21).

1.3 Adipose tissue

Adipose tissue has an important impact on the whole organism. It is not an inert cell mass contributing only to the storage of fat but also functions as an endocrine organ, contributing to inflammation and the innate immune response.
In mammals, we recognize two distinct types of adipocytes: white and brown adipocytes.

1.3.1 White adipose tissue

White adipose tissue is highly specialized to store energy in the form of triacylglycerols. During energy deprivation, lipids are released from adipose tissue as a source of energy for other organs. White adipocytes have triacylglycerols in one unique droplet filling the most of the intracellular space.

Adipose tissue mass can expand by increasing adipocyte size (hypertrophy) and/or by increasing the numbers of adipocytes (hyperplasia). It has been showed that the number of adipocytes is set and stays constant in adulthood in lean and obese individuals. It was discovered that approximately 10 % of fat cells are renewed annually and half of all the adipocytes are replaced every eight years (72).

Adipose tissue is not only composed of adipocytes but also preadipocytes, mesenchymal stem cells, macrophages, endothelial cells and fibroblasts are included (73). Adipocytes, beyond their role in lipid storage, can influence whole-body metabolism through modulation of systemic free fatty acid levels as well as secretion of

cell-specific proteins collectively known as adipokines. White adipose tissue plays a crucial role in lipid-mediated insulin resistance in liver and skeletal muscle (see above chapter 1.2).

1.3.2 Brown adipose tissue

Brown adipocytes have cytoplasmatic lipids arranged as numerous small droplets. Brown adipose tissue (BAT) contains large amount of mitochondria and its main role is an adaptive thermogenesis. This process depends on the expression of uncoupling protein-1 (UCP1), which is a unique marker for BAT. UCP1 is an inner mitochondrial membrane protein that uses mitochondrial proton gradient for heat production instead of ATP synthesis.

In humans, BAT develops in the fetus during gestation and in the newborn child is located mostly around the vasculature and organs (74; 75). BAT exists in humans during the whole life, but with a decline of BAT depots with increasing age. Additionally, a study in mice showed that under the specific conditions (i.e. thiazolidinediones, cold exposure) white adipocytes can be transdifferentiated into brown adipocytes (76; 77). This new population of white adipocyte containing a UCP1 is called “brite“ adipocytes.

1.3.3 Adipose tissue as an endocrine organ

Adipose tissue secretes a number of specific hormones (adipokines), which act as potent messengers to distant organs such as muscle, liver and brain. Adipokines play a role in food intake, regulation of energy balance, lipid and glucose metabolism, insulin action, angiogenesis and vascular remodelling (78; 79).
Leptin and adiponectin, the two most studied adipokines to date, may exert regulatory effects on the hypothalamus, liver, pancreatic cells and skeletal muscle.

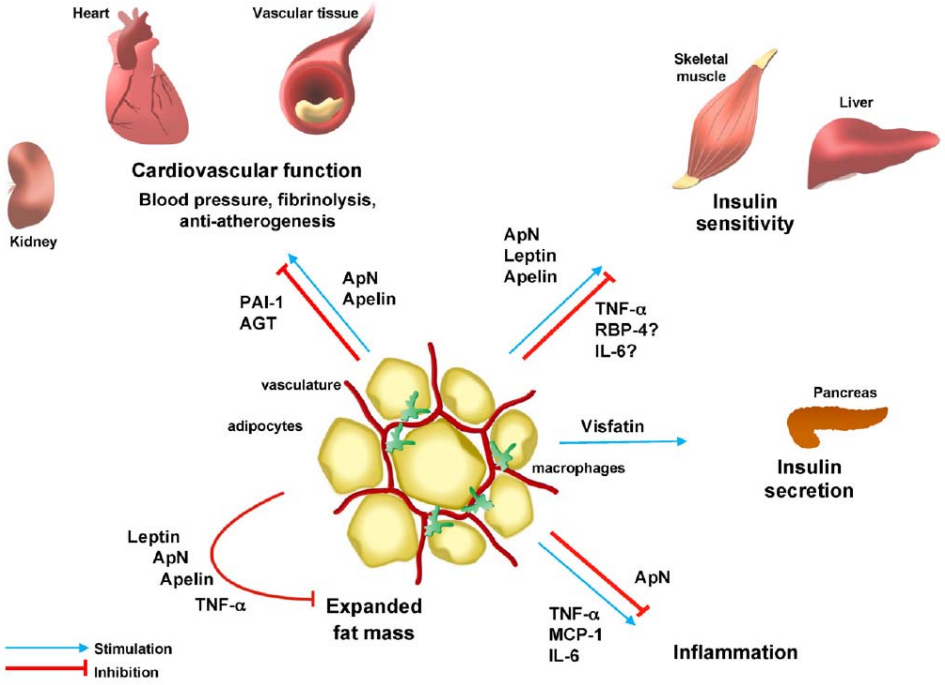

Figure 4. Adipokines secreted from adipose tissue and their involvement in pathophysiology. AGT, angiotensinogen; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor type 1; RBP-4, retinol binding protein-4; TNF-α, tumour necrosis factor-α; [adapted from (80)].

1.3.3.1 Leptin

The first characterised adipokine was leptin (81). Adipocytes are the most important source of leptin and leptin plasma levels directly correlate with adipose tissue mass (82). Leptin level falls when adipose tissue mass is low and during fasting conditions. Adipocytes also express leptin receptors, suggesting an autocrine and paracrine role of the hormone. The main site for leptin action is the hypothalamus (82). Local administration of leptin in hypothalamic regions reduces food intake and body weight in animal (83). Several studies found the leptin receptor in liver, skeletal muscle, heart, kidney, and pancreas, among others. Leptin promotes fatty acid oxidation, reduces ectopic fat accumulation on non-adipose tissues and increases insulin sensitivity (84). This effect is mediated by activation of the AMPK in skeletal muscle (85). In the liver,

leptin induces gene of lipid catabolism and inhibits lipogenesis. Obesity is often associated with hyperleptinemia, which led to the concept of leptin resistance (86).

1.3.3.2 Adiponectin

Adiponectin, which is secreted mainly from adipocytes, regulates lipid and glucose metabolism, stimulates fatty acid oxidation, suppresses hepatic gluconeogenesis, increases insulin sensitivity, protects against chronic inflammation and regulates food intake and body weight (87-89). In plasma, adiponectin circulates in three forms: an LMW (low-molecular weight form) trimer of aprox. 67kDa, MMW (middle-molecular weight form) a hexamer of 120kDa and the most biologically active HMW (high-molecular weight form) multimer of >300 kDa (90; 91). Trimeric and hexameric forms of adiponectin play an important role in the brain to regulate food intake (92). HMW adiponectin is more metabolically active and is associated with an improvement in insulin sensitivity in peripheral tissue (93). This effect is mediated through phosphorylation and activation of AMPK in skeletal muscle and liver (94). Adiponectin also increased fatty-acid combustion and energy consumption, via PPARα activation, in liver and skeletal muscle.

Two adiponectin receptors are identified (95). AdipoR1 is expressed ubiquitously, abundantly in muscle, while AdipoR2 is expressed mainly in the liver (93).

Anti-diabetic drugs from the class of TZD (see chapter 1.4) as well as LC n-3 PUFAs increased plasma adiponectin levels (21; 96). It has been shown that adiponectin concentration is decreased in obesity and also inflammation markers such as TNFα and IL-6 suppressed adiponectin gene transcription (97).

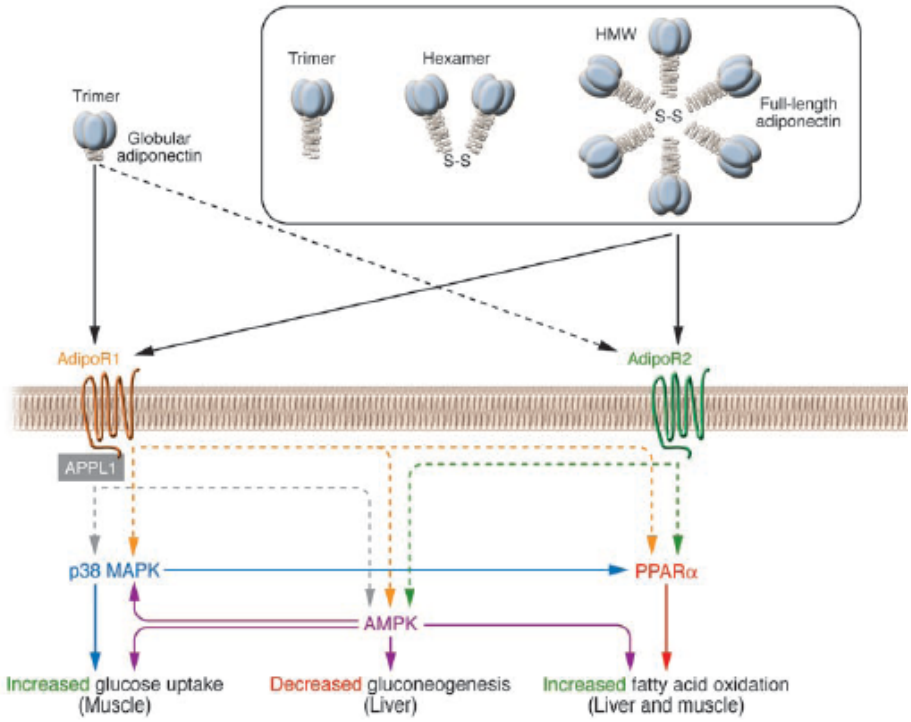

Figure 5. Signal transduction by adiponectin receptors [adapted from (93)]. APPL1, adaptor protein containing pleckstrin homology domain, phosphotyrosine-binding domain, and leucine zipper motif 1.

1.3.3.3 Interleukin-6

Interleukin-6 (IL-6) is a pro-inflammatory cytokine and is secreted from adipose tissue, liver and muscle. IL-6 is positively correlated with adiposity and increasing levels of IL-6 are observed in patient with type2 diabetes (98). It is estimated that approximately 30% of total circulating IL-6 is produced by adipose tissues. The primary sources of circulating IL-6 are macrophages that have infiltrated WAT (99). IL-6 has been shown to suppress insulin-stimulated metabolic actions in hepatocytes. This effect is mediated by the induction of SOCS-3 (suppressor of cytokine signaling-3) expression (100). Moreover, IL-6 suppresses LPL activity and inhibits adiponectin production in adipocytes (101). Plasma IL-6 concentrations predict the development of type 2 diabetes (102).

1.4 Thiazolidinediones

Thiazolidinediones, such as rosiglitazone, pioglitazone or troglitazone are a new class of medications used to improve glycemic control, lipid profile and insulin sensitivity (103). The molecular target of these compounds is PPARγ. Stimulatory effects on PPARγ induce mitochondrial biogenesis and modulate the transcription of essential genes involved in preadipocyte differentiation (104; 105). TZDs inhibit the expression of numerous inflammatory mediators (i.e. TNFα, IL-6) and macrophage infiltration into white adipose tissue (106). TZD treatment has been known to improve hepatic insulin action and cause a significant increase in HMW adiponectin levels (107).
TZDs have been shown to activate AMPK in many tissues and increase catabolism of fatty acids.

As most drugs, TZDs have side-effect on the organism. Due to a relatively high risk of adverse cardiac effects, the use of rosiglitazone was banned by the Europe in 2010 (108), whereas it is increasingly restricted by the US Food and Drug Administration. Rosiglitazone and pioglitazone therapy increase the risk of osteoporosis [reviewed in (109)] and enhance accumulation of body fat (110).

1.5 Calorie restriction

Calorie restriction (CR) delays the development of chronic diseases and prolongs lifespan in mice (111). Both, in rodents and primates, 30-40% reduction in calorie intake has been shown to reduce pathological phenotypes associated with obesity and type2 diabetes (112). Mice on CR increase expression of genes for fatty acid oxidation and decrease expression of genes for fatty acid synthesis compared with ad libitum fed controls. Calorie restriction increase mitochondrial respiration as well as mitochondrial number (113).

2 AIMS OF THE THESIS

The general goal of this thesis was to extend our knowledge of how polyunsaturated fatty acid series 3 affect the mammalian organism and improve lipid profile and glucose homeostasis in diet-induced obesity. Considerable effort was expended on finding new possible strategies for prevention and the treatment of obesity and obesity-associated disorders, i.e. insulin resistance. Special attention was focused on role of adipose tissue metabolism and secretory function in the effect of LC n-3 PUFAs.
The specific aims of the thesis correspond to individual publications:

1. Many experiments on mice have shown that reduction of adiposity by LC n-3 PUFAs were associated with both, a shift in adipose tissue metabolism and a decrease in tissue cellularity. The aim of the publication A was to further characterize the effects of LC n-3 PUFAs on fat cell proliferation and differentiation in obese mice.

2. Calorie restriction is an essential component in the treatment of obesity and associated diseases. LC n-3 PUFAs have a many beneficial effects on the mammalian organism and could prevent the development of obesity and insulin resistance. In the publication B we aimed to characterise the effectiveness and underlying mechanisms of the combination treatment with LC n-3 PUFAs and 10% calorie restriction in the prevention of obesity and associated disorders in mice.

3. Diets rich in LC n-3 PUFAs protect against insulin resistance and obesity in rodents and increase insulin sensitivity in healthy humans. In the publication C we tested whether the anti-diabetic effects of EPA and DHA involve enhanced production of insulin-senziting hormone adiponectin.

4. One of the major effects of DHA and EPA is decreasing lipids in the blood stream, but they do not improve glycemic control in obese and diabetic patients. On the other hand, thiazolidinediones like rosiglitazone improve whole-body

insulin sensitivity. In the publication D we tested the hypothesis that a combined treatment with DHA and EPA and low-dose of rosiglitazone would correct, by complementary additive mechanisms, impairments of lipid and glucose homeostasis in obesity.

5. The induction of obesity, dyslipidemia, and insulin resistance by high-fat diet in rodents can be prevented by LC n-3 PUFAs. We tested a hypothesis whether AMP-activated protein kinase has a role in the beneficial effects of LC n-3 PUFAs (publication E).

3 RESULTS:

3.1 Publication A:

The inhibition of fat cell proliferation by n-3 fatty acids in dietary obese mice.
Michal Hensler, Kristina Bardova, Zuzana Macek Jilkova, Walter Wahli, Daniel Meztger, Pierre Chambon, Jan Kopecky and Pavel Flachs.

The aim of this study was to further characterize the effects of LC n-3 PUFAs on fat cell proliferation and differentiation in obese mice.

A model of inducible and reversible lipoatrophy (aP2-Cre-ER^{T2} PPARγ$^{L2/L2}$ mice) was used. Selective ablation of PPARγ in mature adipocyte was achieved by injection of tamoxifen.

Obese male mice were randomly assigned (day 0) to the following groups: (i) mice injected by corn-oil-vehicle only, i.e. "control" mice, and fed cHF; (ii) mice injected by tamoxifen in corn oil, i.e. "mutant" mice, fed cHF; (iii) control mice fed cHF diet with 15% of dietary lipids replaced by LC n-3 PUFAs concentrate (cHF+F) ; (iv) mutant mice fed cHF+F. Blood and tissue samples were collected at days 14 and 42.

After tamoxifen injection, mutant mice achieved a maximum weight loss within 10 days, independent of diet. Afterward, all mice started to gain body weight with a different dynamics in various groups (Fig. 6A). cHF mice gained body weight much faster than the cHF+F mice and in the end of the study, the body weight of the cHF mice were bigger than compared with the cHF+F mice. The anti-obesity effect was more pronounced in mutant mice fed cHF+F diet. The brown and white adipose tissue was significantly reduced in mutant mice killed at day 14. At day 42, there were no differences between control and mutant mice within same diets, while cHF+F diet became the main force of reduction of adiposity.

At day 14, the mean size of adipocytes was smaller in mutant as compared with control mice and it was decreased further in response to dietary LC n-3 PUFAs. Importantly, at day 42, mean size of adipocytes was similar in all the groups.

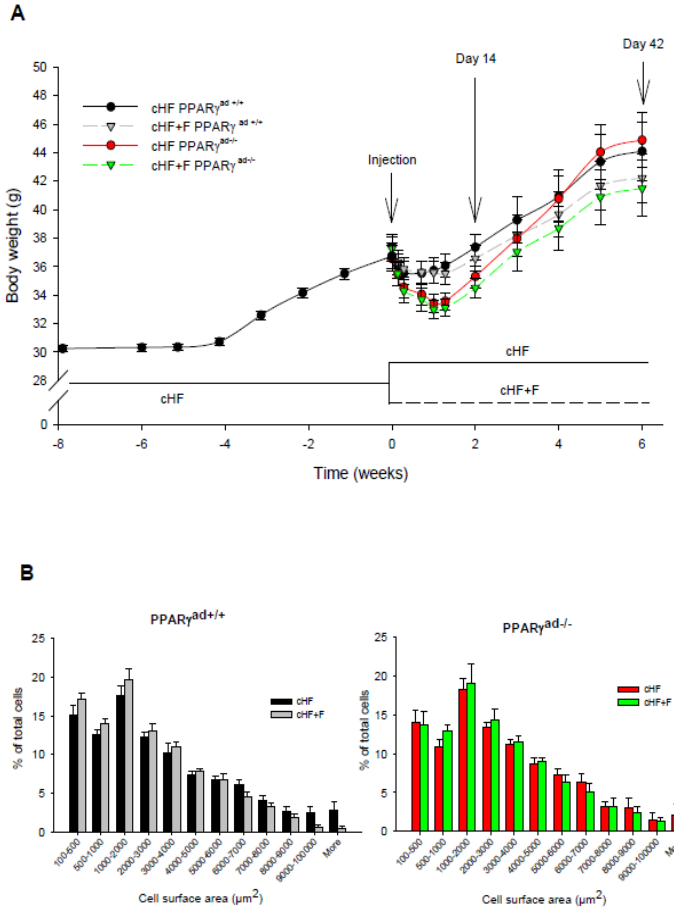

Figure 6. After 8 weeks of high-fat (cHF) feeding, mice were randomly assigned to one of the following groups: (i) control mice, fed cHF (cHF PPARγ$^{ad+/+}$); (ii) mutant mice, fed cHF (cHF PPARγ$^{ad-/-}$); (iii) control mice, fed cHF enriched by LC *n-3* PUFAs (cHF+F PPARγ$^{ad+/+}$); and (iv) mutant mice, fed cHF+F (cHF+F PPARγ$^{ad-/-}$). Part of mice were killed at day 14, while the remaining mice were killed at day 42. **A** Body weights; **B** Frequency distribution of adipocyte cell surface area in epididymal fat at day 42. Data are means ± SE; n=10.

Plasma levels of total adiponectin and HMW form were lower in mutant as compared with control mice at day 14. Later on, total and HMW adiponectin level were similar in both control and mutant mice, with a tendency to be higher in the mutant mice.

Expression of selected genes was quantified in WAT. At day 14, a marked down-regulation of SCD-1 was found, compared to cHF diet. At day 42, only dietary LC n-3 PUFAs diminished SCD-1 expression independently on genotype. Genes connected to lipid metabolism; PPARα, COX3 and CIDEC were transiently down regulated in the mutant as compared with control mice at day 14 only. Dietary LC n-3 PUFAs induced expression of these genes at both time points, with the strongest effects elicited in the mutant mice in response to the longer treatment.

In summary, mutant mice achieved a maximum weight loss within 10 days post-injection, followed by a compensatory body weight gain, which was significantly faster in the cHF as compared with the cHF+F mutant mice. Also in control mice, body weight gain was depressed in response to dietary LC n-3 PUFAs. At day 42, no significant differences in adipocyte size between groups was found, although body weight and adiposity was lower in the cHF+F as compared with the cHF mice, with a stronger effect in the mutant than in control mice. Gene expression documented depression of

adipocytes maturation during the reconstitution of adipose tissue in the cHF+F mutant mice.

My contributions to this work were management and coordination of the animal and tissue culture experiments and phenotypical and biochemical characterisation of the model. Histological analysis of adipose tissue was performed by KB and ZMJ. WW, DM and PC created transgenic murine model.

3.2 Publication B:

Synergistic induction of lipid catabolism and anti-inflammatory lipids in white fat of dietary obese mice in response to calorie restriction and n-3 fatty acids.
Flachs P, Rühl R, Hensler M, Janovska P, Zouhar P, Kus V, Macek Jilkova Z, Papp E, Kuda O, Svobodova M, Rossmeisl M, Tsenov G, Mohamed-Ali V, Kopecky J.

The aim of this study was to characterize the efficacy and underlying mechanisms of the combination treatment with LC n-3 PUFAs and 10% calorie restriction (CR) in the prevention of obesity and associated disorders in mice.

4 weeks old mice C57BL/6J weaned onto chow laboratory standard diet were habituated to a corn oil based high-fat diet (cHF; lipid content 35% wt/wt) for two weeks and then randomly assigned for 5 weeks or 15 weeks to various dietary treatments: (i) cHF, *ad libitum* (free access to food), (ii) cHF+F, *ad libitum*, which 15% of dietary lipids were replacing by LC n-3 PUFAs concentrate, (iii) cHF+CR, ratio of food was reduced by 10% compared with *ad libitum* fed mice on the same type of diet, (iv) cHF+F+CR, diet enriched by LC n-3 PUFAs and food reduced by 10%.

cHF feeding increased the body weight of the mice. Either cHF+F or cHF+CR treatment partially prevented the cHF-induced obesity, while combination treatment (cHF+F+CR) provided a full protection during the experiment. cHF diet induced accumulation of lipids in the liver and skeletal muscle. In the liver, all the treatments decreased contain of triacylglycerols at 5 weeks, with the strongest effect of cHF+F+CR diet in response to

the longer treatment. In the skeletal muscle, the cHF-induced triacylglycerols accumulation was significantly prevented only by the combination (cHF+F+CR) treatment lasting for 15 weeks. After 5 weeks, all treatments showed hypolipidemic effect, with the most pronounced effect in combination treatment. This effect was negatively correlated with the plasma level of β-hydroxybutyrate, the marker of lipid catabolism. Glucose levels in plasma were similar in all the groups, however, insulin levels were markedly decreased by cHF+CR treatment and even more by the cHF+F+CR treatment.

Combination treatment improved metabolic flexibility (which is associated with insulin sensitivity), based on both the response of plasma level of NEFA and glucose to FASTED to RE-FED transition and the glucose-induced increase in RQ (for data see publication B).

For better understanding, which way LC n-3 PUFAs and CR or both play role against development of obesity, genes involved in mitochondrial biogenesis and lipid catabolism in epididymal and subcutaneous adipose tissue, interscapular brown fat, liver and gastrocnemius muscle were measured (Fig.7).

Gene expression analysis in epididymal fat showed a strong induction of mitochondrial biogenesis and increasing oxidative capacity of mitochondria by the combination treatment (for more details see publication B). In contrast, no significant changes were observed in this cluster of genes in brown fat (Fig. 7c), liver (Fig. 7d) or skeletal muscle (Fig. 7e). Concerning the genes of β-oxidation such as very long chain, long chain and medium chain acyl-CoA dehydrogenases (*Vlcad, Lcad* and *Mcad*), additive induction of *Vlcad* and *Lcad* was noticed in the epididymal fat (Fig. 7a) and a slight increase was observed in response to cHF+F or cHF+F+CR treatments in the liver (Fig 7d).

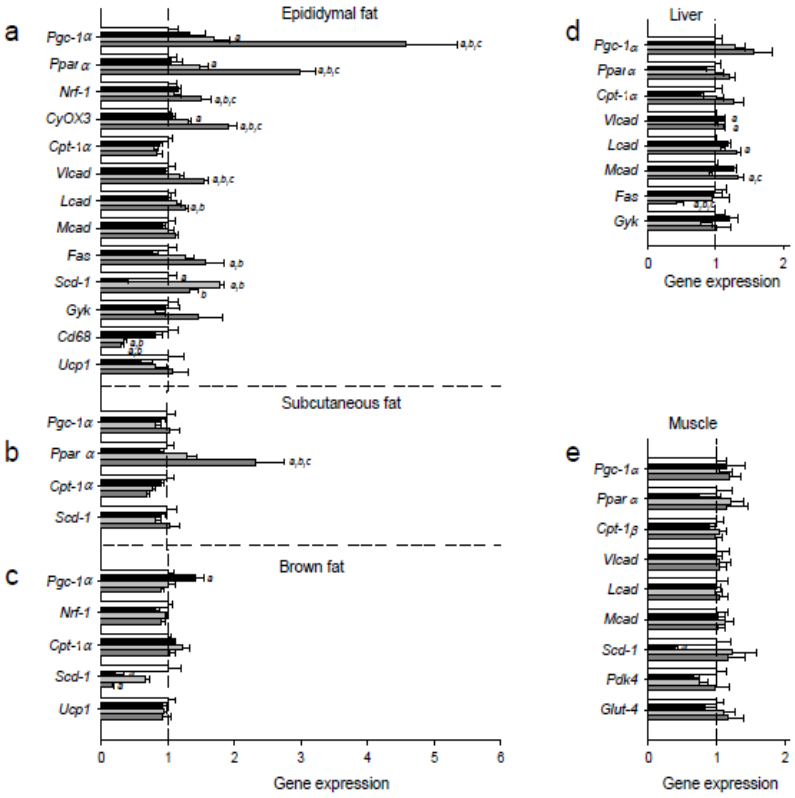

Figure 7. Specific induction of mitochondrial genes in epididymal fat. Quantitative real-time PCR data showing relative levels of gene expression (cHF= 1) in epididymal (a) and subcutaneous (b) WAT, interscapular brown fat (c), liver (d), and gastrocnemius muscle (e) after 5 weeks of the treatment. Data are means±SEM; n=10; white bars, cHF; black bars, cHF+F; light grey bars, cHF+CR; dark grey bars, cHF+F+CR; [a,b,c] Significant difference (ANOVA) compared with cHF, cHF+F and cHF+CR, respectively.

To confirm the synergistic induction of mitochondrial fatty acid oxidation by LC n-3 PUFAs and calorie restriction in epididymal fat, biochemical assessment was performed *ex-vivo* after 5 weeks of the dietary treatment. cHF diet stimulated radiolabeled palmitate oxidation in fragments of epididymal fat (~1.3-fold) compared with chow, and this activity was further significantly stimulated only by the combination treatment (~1.6-fold). Similar results have seen in isolated adipocytes from epididymal fat.

To assess changes in lipid catabolism in the other tissues, palmitate oxidation was measured in isolated hepatocytes and in skeletal muscles, after 5-weeks treatment. Only combination treatment increased the hepatic fatty acid oxidation compared to the cHF mice, but it had no effect on fatty acid oxidation in either gastrocnemius or soleus muscle (Tab 1).

Palmitate oxidation rates in liver and skeletal

	Chow	cHF	cHF+F	cHF+CR	cHF+F+CR
Hepatocytes					
FA oxidation (dpm/µg DNA)	17±3	31±2*	36±4	27±2	42±4[a]
Gastrocnemius muscle					
FA oxidation (dpm/mg tissue)	N.E.	42±5	N.E.	N.E.	44±4
Soleus muscle					
FA oxidation (dpm/mg tissue)	N.E.	123±12	N.E.	N.E.	140±10

Tab 1. After 5 weeks of the treatment, oxidation of [1-^{14}C] palmitate was measured either in isolated hepatocytes, or in whole soleus muscle (oxidative type), or in a fragment of gastrocnemius muscle (mixed type). Data are expressed as means±SEM; *n*= 8-12; *a*- significant difference (ANOVA) compared to cHF. *- significant difference (*t*-test) compared to Chow. N.E. not estimated.

Mitochondrial oxidative capacity was characterized in permeabilized adipocytes isolated from epididymal fat, using respirometry. Adipocytes from the cHF+F+CR mice exerted ~2-fold higher oxygen consumption compare to the cHF mice.
Obesity is associated with low-grade adipose tissue inflammation. Imunohistochemical analysis of epididymal fat after 5 weeks of various treatments revealed a reduction of content macrophages aggregated in crown-like structures (CLS) surrounding death adipocytes compare with cHF. mRNA marker of macrophages CD68 was also decreased in the same pattern as CLS in the epididymal fat. Dietary LC n-3 PUFAs supplementation, CR or both inhibited formation of various pro-inflammatory eicosanoids in the adipose tissue and liver as well as induced the anti-inflammatory molecules (for data see publication B and ESM).

In summary, dietary LC n-3 PUFAs augment the anti-obesity effects of mild calorie restriction in mice while improving lipid metabolism and glucose homeostasis. These effects probably reflect in large synergistic induction of mitochondrial fatty acid oxidation in white adipose tissue, linked to a suppression of low-grade inflammation of this tissue.

My main contributions to this work were performing of animal experiments, RNA isolation from all tissues, control of quality of isolated RNA, reverse transcription and quantification of gene expression using qRT-PCR. Ex-vivo analysis of fatty acids oxidation in fragments of epididymal fat, gastrocnemius and soleus muscle.

3.3 Publication C:

Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet. *Flachs P, Mohamed-Ali V, Horakova O, Rossmeisl M, Hosseinzadeh-Attar MJ, Hensler M, Ruzickova J, Kopecky J.*

We tested whether the anti-diabetic effects of EPA and DHA involve enhanced production of insulin-senziting hormone adiponectin.

The study was performed to the background of obesity-promoting high-fat diet (cHF), or cHF diet, in which 15 % of total lipids were replaced with LC n-3 PUFAs concentrate (cHF-F1). Adult male C57BL/6J mice that either had free access to food of had their food intake restricted by 30%. At the end of the treatment, systemic markers of lipid and glucose metabolism and full-length adiponectin and leptin were measured.

In mice with free access to food, plasma triacylglycerols, NEFA, and insulin levels were lower in the presence of EPA and DHA, while glucose and leptin levels were not significantly altered. Food restriction resulted in net loss of body weight and adipose tissue mass. Plasma levels of triacylglycerols, glucose, insulin and leptin, but not adiponectin were also decreased by food restriction (Tab 2). EPA and DHA increase plasma adiponectin levels, independent of food intake (Tab 2).

Effects of EPA/DHA and caloric restriction on body weight, markers of lipid and glucose metabolism, and adipokines in plasma

	Diet				
	Free access to food		Caloric restriction		
	cHF	cHF-F1	cHF	cHF-F1	*n*
Body weight (g)[a]	37.5±1.2	34.8±1.2[b]	25.1±0.4[c]	25.2±0.6[c]	10
Plasma levels of:	–	–	–	–	–
NEFA (mmol/l)	0.47±0.02	0.33±0.02[b]	0.44±0.05	0.38±0.05	10
Triacylglycerols (mmol/l)	1.46±0.11	0.79±0.07[b]	0.51±0.06[c]	0.52±0.05[c]	10
Glucose (mmol/l)	10.10±0.22	10.04±0.38	7.55±0.44[c]	7.27±0.33[c]	10
Insulin (ng/ml)	2.77±0.52	0.17±0.04[b]	1.68±0.22[c]	0.16±0.06[b]	7
Adiponectin (µg/ml)	9.0±0.3	12.1±0.5[b]	9.2±1.1	11.2±0.5[b]	10
Leptin (ng/ml)	42.5±7.9	40.1±0.9	2.4±0.4[c]	2.5±0.2[c]	10

Tab 2. Mice had either free access to cHF or cHF-F1diets or were subject to calorie restriction. Data are means ±SE. [a] mice were used in our previous study. [b] significant effect of diet. [c] significant effect of caloric restriction.

At the whole-tissue level, no significant effect of the diet to gene expression of adiponectin and leptin was detected. However, in adipocytes, isolated from dorsolumbar and epididymal fat, the expression level of adiponectin was stimulated by EPA and DHA (tab 3). The stimulation of adiponectin secretion was more pronounced in epididymal than in dorsolumbar fat (2.5- and 1.9-fold stimulation, respectively). Gene expression of leptin level in isolated adipocytes and secretion from adipose tissue explants was unchanged.

Gene expression and adipokine production in WAT depots of mice with free access to cHF or cHF-F1 diets

	Dorsolumbar fat		Epididymal fat		
	cHF	cHF-F1	cHF	cHF-F1	*n*
Gene expression	–	–	–	–	–
Tissue	–	–	–	–	–
Adipoq (AU)	0.77±0.08	0.94±0.07	0.82±0.03	0.88±0.06	10
Lep (AU)	0.51±0.05	0.48±0.03	0.61±0.04	0.47±0.07	10
Adipocytes	–	–	–	–	–
Adipoq (AU)	3.21±0.25	6.13±0.87[b]	2.83±0.23	7.18±0.82[b]	7–8
Lep (AU)	2.92±0.83	3.57±0.91	1.70±0.40	3.11±1.06	7–8
Adipokine production[a]	–	–	–	–	–
Adiponectin (µg/ml)	0.31±0.04	0.32±0.03	0.32±0.03	0.47±0.03[b]	9
Leptin (ng/ml)	36.1±3.1	34.7±3.6	33.6±6.4	20.9±3.3	9

Tab 3.Transcript levels were evaluated by qRT-PCR using RNA isolated from whole tissues or collagenase-liberated adipocytes. Tissue explants were used to measure adipokine production. Data are means±SEM. [a] Levels in medium after incubation of WAT. [b] Significant effect of diet. AU, arbitrary units

In conclusion, we show for the first time that EPA and DHA stimulate adiponectin expression and increase the levels of circulating adiponectin, largely independent of food intake or adiposity and explain, to some extent, their anti-diabetic effects.
My main contributions to this work were the RNA isolation, control of quality of isolated RNA, reverse transcription and quantification of gene expression using qRT-PCR.

3.4 Publication D:

n-3 fatty acids and rosiglitazone improve insulin sensitivity through additive stimulatory effects on muscle glycogen synthesis in mice fed a high-fat diet.
Kuda O, Jelenik T, Jilkova Z, Flachs P, Rossmeisl M, Hensler M, Kazdova L, Ogston N, Baranowski M, Gorski J, Janovska P, Kus V, Polak J, Mohamed-Ali V, Burcelin R, Cinti S, Bryhn M, Kopecky J.

The aim of this study was to determine mechanisms of action LC n-3 PUFAs and TZD could have additive or synergistic effects in combination treatment, to improve lipid and glucose homeostasis in obesity. In this work was studied the effects of (i) low-dose of rosiglitazone, (ii) partial replacement of dietary lipids by LC n-3 PUFAs, and (iii) a combination of both.

First, a 'prevention study' was performed to characterise the effects of LC n-3 PUFAs, rosiglitazone and their combination, on developing obesity and impaired glucose tolerance (IGT) in mice fed high-fat diet. 3 months old male mice (C57BL/6N) were randomly assigned to a corn oil-based high-fat diet (cHF; lipid content ~35.2% wt/wt, mainly corn oil) or to the following treatments: (i) cHF diet supplemented with EPA and DHA (cHF+F) as concentrate of LC n-3 PUFAs (46% DHA, 14% EPA; 1050TG; EPAX, Lysaker, Norway) replacing 15% of dietary lipids; (ii) cHF diet supplemented with rosiglitazone (cHF+TZD) (10 mg/kg diet); and (iii) cHF diet supplemented with EPA, DHA and rosiglitazone (cHF+F+TZD). Some mice were maintained on the standard chow diet. Various analyses were performed at 5 to 20 weeks after initiation of treatment.

Differences in body weight gain, due to cHF feeding, becoming apparent at 4 weeks (Fig. 8a). Treatment by cHF+F or by cHF+TZD diets tended to prevent body weight gain (Fig. 8a). cHF+ F+TZD treatment significantly lowered body weight gain compare to cHF diet. Changes of body weight correlated with the changes in adipose tissue mass, mainly with significant reduction of subcutaneous (data not shown) and epididymal fat (Fig. 8b). cHF diet induced hypertrophy of adipocytes in epididymal fat at 8 weeks, accompanied by increased content of macrophages (Fig. 8c), indicating induction of

low-grade inflammation of adipose tissue. Adipocytes hypertrophy and macrophage infiltration was significantly decreased by combination diets (cHF+F, cHF+TZD), especially by cHF+F+TZD.

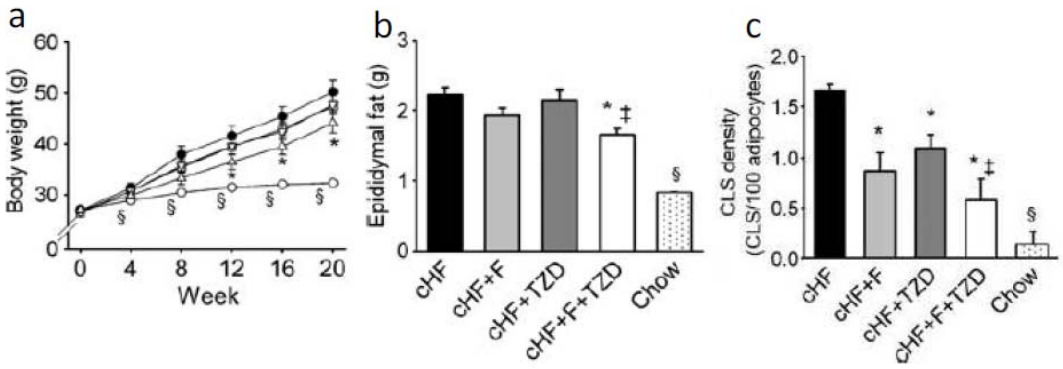

Figure 8. Body weight (a), weight of epididymal fat (b) and macrophage infiltration (c). **a** Three-month-old mice were placed on cHF diet or various cHF-based diets (cHF+F, cHF+TZD and cHF+F+TZD), or maintained on a chow diet; this treatment lasted for up to 20 weeks. **a** Body weights during 20-week treatment by cHF (black circles), cHF+F (white squares), cHF+TZD (white inverted triangles), cHF+F+TZD (white triangles) or chow (white circles) diet (n=16). **b** Epididymal fat at 8 weeks, weight of fat depot. **c** Relative count of crown-like structures (CLS), formed by MAC-2-positive macrophages surrounding adipocytes. The morphometry data are based on measurements of more than 1,000 cells taken randomly from six different areas per animal. Data are means±SE (n=7–8). *p≤0.05 for difference from cHF (ANOVA); †p≤0.05 for difference from cHF+F (ANOVA); ‡p≤0.05 for difference from cHF+TZD (ANOVA); §p≤0.05 for difference from cHF (t test)

Compared with standard chow, the cHF diet induced accumulation of triacylglycerols in liver and gastrocnemius, with a stronger effect observed at 20 weeks. Compared with cHF, none of the treatments significantly affected the triacylglycerol accumulation, except for cHF+TZD, which increased liver triacylglycerols at 8 weeks. The cHF+F and cHF+TZD increased levels of immunoreactive adiponectin in plasma. The strongest induction was observed with a combination of the two treatments (cHF+F+TZD). Multimeric adiponectin complexes in plasma were also analysed. The ratio between high molecular weight and total adiponectin was increased by the all treatments, with the highest effect observed in cHF+F+TZD-fed animals.

Analysis of gene expression from gastrocnemius revealed a trend (Fig. 9a) for induction by combination treatment of *Pdk4, Cpt-1α and Cpt-1β* genes, involved in switching from lipid to glucose catabolism. Interestingly, the cHF+TZD diet strongly induced expression of *Scd1*, while cHF+F and cHF+F+TZD treatments had an opposite effect.

Analysis of hepatic gene (Fig. 9b) revealed a down regulation of gluconeogenic genes *Pck2* and *G6pc* by cHF+F+TZD. This treatment also strongly induced expression of genes involved in fatty acid oxidation in liver. Both diets supplemented with TZD strongly induced expression of *Scd1*, while cHF+F had no effect. *De novo* lipogenesis was down regulated by diet enriched by LC n-3 PUFAs.

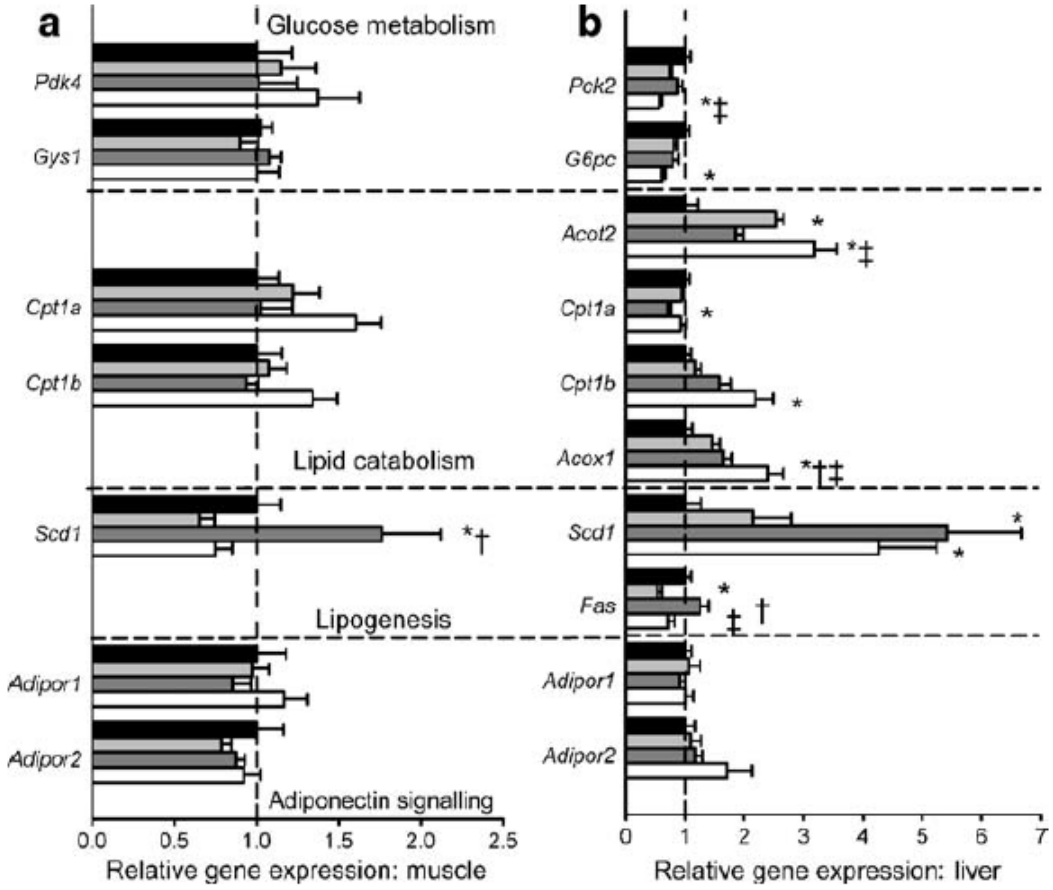

Figure 9. Quantitative RT-PCR data showing gene expression in mouse gastrocnemius muscle (a) and liver (b) after 8 weeks of dietary treatments. Black bars, cHF; light grey bars, cHF+F; dark grey bars, cHF+TZD; white bars, cHF+F+TZD. Data are means±SE (n=6–7). *p≤0.05 for difference from cHF (ANOVA); †p≤0.05 for difference from cHF+F (ANOVA); ‡p≤0.05 for difference from cHF+TZD (ANOVA).

Euglycemic-hyperinsulinemic clamps were used to characterize the changes in insulin sensitivity. In the hyperinsulinemic conditions, the amount of exogenous glucose required to maintain euglycemia, i.e. the glucose infusion rate, was higher in mice treated by cHF+F+TZD. Hepatic glucose production in the hyperinsulinemic conditions was decreased in the cHF+F mice, suggesting that EPA and DHA improve hepatic

insulin sensitivity. Whole-body glycogen synthesis was strongly stimulated by cHF+F+TZD, while cHF+F and cHF+TZD diets had no effect. These results were confirmed by ex *vivo* glycogen synthesis in diaphragm (Fig. 10).

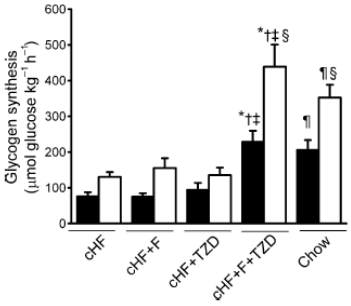

Figure 10. Glycogen synthesis in diaphragm muscle. At 5 weeks after initiation of treatment as described, left and right hemidiaphragms of mice were dissected and incubated with [U 14C]glucose in the absence (black bars) or presence (white bars) of 250 μU/ml insulin to measure the rate of glycogen synthesis. Data are means±SE (n=7–8). *p≤0.05 for difference from cHF; †p≤0.05 for difference from cHF+F; ‡p≤ 0.05 for difference from cHF+TZD (ANOVA); §p≤0.05 for difference from non-insulin-stimulated value within the diet (ANOVA); ¶p≤0.05 for difference from cHF (t test)

Metabolic changes described in ´prevention study´ were also analyzed in ´reversal study´, where mice were obese and insulin resistant. Compared with cHF-fed animals, body weight gain, triacylglycerols and NEFA levels were suppressed by cHF+F, while cHF+TZD decreased plasma triacylglycerols and insulin levels. Only cHF+F+TZD showing additive effects of EPA and DHA and rosiglitazone in the reversal of obesity. The cHF+F+TZD diet decreased plasma NEFA, cholesterol, triacylglycerols, insulin levels and improved glucose tolerance test.

In conclusion, combined treatment of LC n-3 PUFAs with low-dose rosiglitazone improved lipid and glucose homeostasis. This combination affects adipose hypertrophy, hyperlipidemia and stimulated muscle glycogen synthesis. The combined use of LC n-3 PUFAs and TZDs thus represents a potential strategy for treatment of type2 diabetes and other obesity-associated metabolic disorders.

My main contributions to this work were the RNA isolation, control of quality of isolated RNA, reverse transcription and quantification of gene expression using qRT-PCR. Immunophoretic analysis of multimeric forms of adiponectin.

3.5 Publication E:

AMP-activated protein kinase α2 subunit is required for the preservation of hepatic insulin sensitivity by n-3 polyunsaturated fatty acids.
Jeleník T, Rossmeisl M, Kuda O, Jilkova ZM, Medrikova D, Kus V, Hensler M, Janovska P, Miksik I, Baranowski M, Gorski J, Hébrard S, Jesen TE, Flachs P, Hawley S, Viollet B, Kopecky J.

The aim of this study was to tested a hypothesis whether AMP-activated protein kinase (AMPK) plays a role in the beneficial effects of LC n-3 PUFAs.

Four-month-old whole-body AMPK α2 knock-out (AMPKα2$^{-/-}$) mice and wild-type littermate controls were fed on either a Chow, high-fat diet (cHF) and high-fat diet in which 15% of the lipids were replaced by LC n-3 PUFAs concentrate (cHF+F) for nine weeks.

cHF-feeding induced greater body weight gain compared with the Chow-fed mice. However, this effect was less pronounced in AMPKα2$^{-/-}$ mice. Independently of genotypes, the cHF+F diet induced smaller body weight gain compare with the cHF diet. Diet enriched by LC n-3 PUFAs concentrate lowered plasma lipid levels independently of AMPKα2$^{-/-}$. Plasma levels of adiponectin (both total adiponectin as well as its biologically active HMW form), were increased only in wild-type animals in response to LC n-3 PUFAs.

Hyperinsulinemic-euglycemic clamps were performed to evaluate whole-body insulin sensitivity. LC n-3 PUFAs had protective effects from high-fat diet-induced insulin resistance in wild-type animals. In contrast, none of these beneficial effects of LC n-3 PUFAs were observed in AMPKα2$^{-/-}$ mice. Whole-body glycogen synthesis, which reflects insulin sensitivity of muscle glucose metabolism, was dependent on both diet and genotype. A similar pattern of changes in the glycogen synthesis in response to LC n-3 PUFAs were observed when measured directly in the skeletal muscle (Fig. 11).

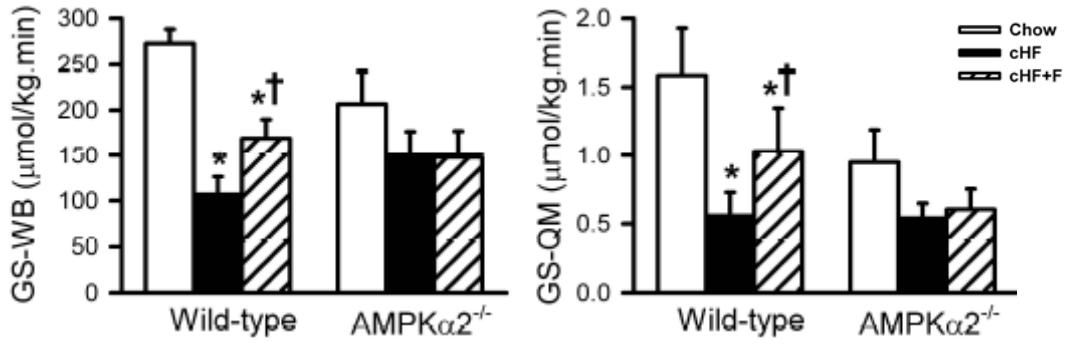

Figure 11. Insulin sensitivity assessed by hyperinsulinemic-euglycemic clamp. Whole-body glycogen synthesis (GS-WB); and glycogen synthesis in quadriceps muscle (GS-QM were measured in wild-type and AMPKα2$^{-/-}$ mice fed either a Chow diet, cHF, or cHF-F for 9 weeks. The data are the means _ SE (n _ 5–8). *P < 0.05 versus genotype Chow; †P < 0.05 versus genotype cHF; ‡P < 0.05 versus wild-type on respective diet.

cHF+F diet decreased the hepatic triglyceride content compared with cHF diet in both genotypes. Under hyperinsulinemic-euglycemic conditions, decreasing of liver triacylglycerols were in AMPKα2-dependent manner. *In vitro* experiments on isolated hepatocytes confirmed AMPK-dependent induction of fatty acid oxidation by LC n-3 PUFAs in the liver (Fig. 12).

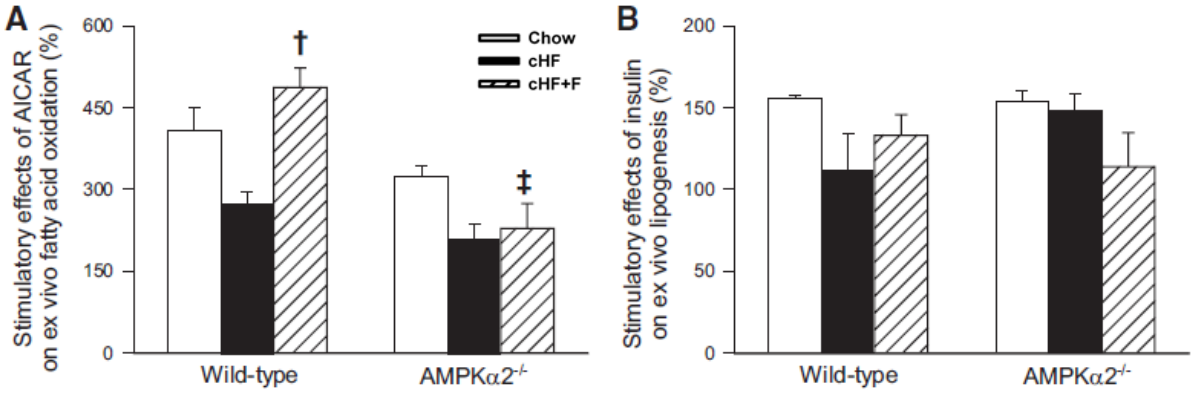

Figure 12. AICAR-stimulated fatty acid oxidation (*A*) and insulin-stimulated de novo fatty acid synthesis (*B*) in cultured hepatocytes isolated from wild-type and AMPKα2$^{-/-}$ mice fed for 9 weeks either a Chow diet, cHF, or cHF-F. The data are means ±SE (isolated hepatocytes, n = 3 in triplets). *P < 0.05 versus genotype Chow; †P < 0.05 versus genotype cHF; ‡P < 0.05 versus wild-type on respective diet.

Lipogenic genes, measured in the livers of mice after CLAMP, were down regulated by cHF feeding in all groups (except for SREPB-1c in AMPKα2$^{-/-}$ mice). This suppression was partially counteracted by cHF+F diet in wild-type but not AMPKα2$^{-/-}$ mice. Together with the *de novo* fatty acid synthesis data (tab 4), these results further support the AMPKα-dependent improvement of liver insulin sensitivity by LC n-3 PUFAs.

The basal rates of lipid metabolism in isolated hepatocytes of wild-type and AMPKα2$^{-/-}$ mice.

	Wild-type			AMPKα2$^{-/-}$		
	Chow	cHF	cHF+F	Chow	cHF	cHF+F
Fatty acid oxidation (pmol/h/mg protein)	13 ± 2	8 ± 1*	9 ± 1*	15 ± 2	12 ± 1‡	6 ± 1*†
Lipogenesis (pmol/h/mg protein)	108 ± 9	74 ± 24	43 ± 10*†	79 ± 10	51 ± 6	34 ± 6*

Tab 4. Wild-type and AMPKα2$^{-/-}$ mice were fed either a Chow diet or corn oil-based high-fat diets without (cHF) or with 15 % of the lipids in the form of n-3 PUFAs concentrate (cHF+F) for 9 weeks. *P < 0.05 vs. genotype Chow; †P < 0.05 vs. genotype cHF; ‡P < 0.05 vs. wild-type on respective diet. The data are presented as means ± SE (n = 3 analyzed in triplets).

To summarise, the preservation of hepatic insulin sensitivity by LC n-3 PUFAs in mice fed a high-fat diet depends on AMPKα2. The AMPKα2 subunit is an important in lipid metabolism and hepatic triglyceride accumulation, which are pronounced under insulin-stimulated condition.

My main contributions to this work were the RNA isolation, control of quality of isolated RNA, reverse transcription and quantification of gene expression using qRT-PCR.

4 DISCUSSION

LC n-3 PUFAs from marine origin, mainly EPA and DHA, have a large impact on the mammalian organism. These essential fatty acids influence lipid and glucose homeostasis by affecting gene expression, enzymes activity and the last but not least by producing of active metabolites (adipokines).

In this series of publications, we tried to expand our knowledge about the mechanism of EPA and DHA action (publications A, C, E) or using a combination treatment enhances their beneficial effect (publications B, D).

One of the most pronounced effects of LC n-3 PUFAs is protection against diet induced obesity in rodents. This protective effect is due to both reducing cellularity of adipose tissue and activation of fat burning.

For better understanding how LC n-3 PUFAs influence fat cell proliferation and differentiation in obese mice, we used a murine model of inducible and reversible lipoatrophy (publication A). Using this model showed that transgenic mice fed diet enriched by LC n-3 PUFAs have lower body weight gain compare with high-fed feeding animals. Reduction adiposity depends on counteraction of both, differentiation and proliferation of adipose cells. Anti-obesity effect is not only depending on suppression of proliferation activity of fat cells but also reduction of adipocyte hypertrophy is important too. In our arrangement of study, polyunsaturated fatty acids reduce adipocyte size and combination with mild calorie restriction (publication B) is more effective than each of the treatments applied separately. Our results demonstrate that this could be due to induction of fatty acid oxidation and mitochondrial biogenesis in adipocytes. Reducing the size of adipocytes resulted in prevention of macrophage infiltration into adipose tissue. In accordance with the previous findings (114), dietary LC n-3 PUFAs supplementation resulted in the inhibition of formation pro-inflammatory lipid mediators and promotes the anti-inflammatory molecules. Unexpectedly, 15-deoxy-$\Delta^{12,14}$- prostaglandin J_2 (15d-PGJ_2), an anti-inflammatory mediator and potent PPARγ agonist derived from AA, was synergistically up regulated by the combination treatment. Recent work showed that activation of PPARγ causes increasing of UCP-1 expression in white adipose tissue and transdifferentiation of white adipocytes to more active brite adipocytes (76). It is possible that 15d-PGJ_2 prostaglandin could play a similar role in transdifferentiation.

Obesity is often associated with insulin resistance and EPA and DHA are able to improve insulin sensitivity. We showed for the first time that EPA and DHA increase the

levels of circulating adiponectin (publication C) which is associated with improvement of insulin sensitivity in peripheral tissues.

Thiazolidinediones are currently often prescribed medicaments for curing diabetes. In the study focused on skeletal muscle we hypothesised that partial replacement of dietary lipids by LC n-3 PUFAs with combination of a low non obesogenic dose of rosiglitazone could lead to an improvement of insulin resistant state (publication D).

Combination treatment decreased both, fasted glycemia and insulin levels in comparison with high-fat feeding mice. Measurement in diaphragm muscle showed synergistic induction of glycogen synthesis at the basal and insulin-stimulated conditions by the combination treatment. The improvement of insulin resistance state correlated with the hypolipidemic effect and with reduction of muscle ceramides concentration. Combination treatment also reduced low-grade adipose tissue inflammation and together with induction of adiponectin contributed to the beneficial effects on glucose homeostasis.

All the treatments significantly decreased the rate of VLDL synthesis, with the strongest reduction by combination treatment. The reduction of hepatic VLDL synthesis by EPA and DHA concentrate may result from suppression of lipogenic genes and activation of AMPK.

To confirm the hypothesis that AMPK in the liver is responsible for preservation of insulin sensitivity by LC n-3 PUFAs, we used knock-out mouse with genetically disrupted α2 catalytic subunit of AMPK (publication E).

Nine weeks of high-fed feeding induce insulin resistance in both genotypes but LC n-3 PUFAs significantly reduced fasted insulin levels only in wild-type animals. In accordance with our previous studies, adiponectin level was significantly increased by LC n-3 PUFAs but no significant increased of plasma adiponectin in AMPKα2$^{-/-}$ mice.

Under insulin-stimulated condition we showed that whole-body glycogen synthesis as well as muscle glycogen synthesis is dependent on LC n-3 PUFAs and AMPK. Moreover, AICAR-stimulated fatty acid oxidation in hepatocytes isolated from LC n-3 PUFAs treated mice were strongly reduced in knock-out mice. LC n-3 PUFAs have been shown to reduce hepatic content of triacylglycerols (71). In our hyperinsulinemic-euglycemic conditions only wild-type mice fed by diet enriched by EPA and DHA concentrate protected liver against the accumulation of lipids. This effect was absent in AMPKα2$^{-/-}$ mice. Our results showed that LC n-3 PUFAs completely prevented cHF diet-induced increase hepatic diacylglycerols in wild-type mice and this action is AMPKα2-dependent.

In summary, this PhD thesis shows that LC n-3 PUFAs from marine origin influence the mammalian organism in many ways. EPA and DHA and/or their active metabolites affect white adipose tissue by (i) reduction of cellularity, (ii) activation of mitochondrial biogenesis and β-oxidation and (iii) probably transdifferentiation of white adipocytes into brite adipocytes. LC n-3 PUFAs also play an important role in glucose homeostasis. EPA and DHA induce adiponectin secretion and improve insulin sensitivity in the liver and skeletal muscle, which is AMPK-dependent. Despite to the adverse side effects of the TZD-based therapy (see chapter 1.4) our results from combination treatment suggest that even lower dose of rosiglitazone in combination with EPA and DHA retains its effectiveness in improvement of glycemic control. Results from combination studies (calorie restriction and rosiglitazone) document the possibility of a new direction for the treatment of obesity-associated diseases.

5 CONCLUSIONS

1. Our results on the model of inducible and reversible lipoatrophy showed that LC n-3 PUFAs could decrease adiposity in obese mice by a mechanism, which depends on counteraction of both, differentiation and proliferation of adipose cells.
2. We demonstrated that combination treatment of LC n-3 PUFAs and mild calorie restriction is targeted to WAT. Combination treatment promotes mitochondrial fatty acid oxidation and reduces low-grade inflammation in this tissue.
3. We were one of the first of which proved that EPA and DHA is a potent modulator of adiponectin expression and secretion from mature adipocytes.
4. Combination treatment with low dose of rosiglitazone and EPA and DHA concentrate reduce adipocyte hypertrophy and prevented development of dyslipidemia and insulin resistance. Improvement of insulin action in muscle was established by increases of glycogen synthesis.
5. Using AMPKα2 knock-out mice showed that for the effect of LC n-3 PUFAs to preserve whole-body and especially hepatic insulin sensitivity is α2 subunit of AMPK is essential.

6 REFERENCES

1. **Ruzickova J, Rossmeisl M, Prazak T, Flachs P, Sponarova J, Vecka M, Tvrzicka E, Bryhn M and Kopecky J**. Omega-3 PUFA of marine origin limit diet-induced obesity in mice by reducing cellularity of adipose tissue. *Lipids* 39: 1177-1185, 2004.

2. **Wall R, Ross RP, Fitzgerald GF and Stanton C**. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr Rev* 68: 280-289, 2010.

3. **Harbige LS**. Fatty acids, the immune response, and autoimmunity: A question of n-6 essentiality and the balance between n-6 and n-3. *Lipids* 38: 323-341, 2003.

4. **Schmitz G and Ecker J**. The opposing effects of n-3 and n-6 fatty acids. *Progress in Lipid Research* 47: 147-155, 2008.

5. **Kris-Etherton PM, Harris WS and Appel LJ**. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 106: 2747-2757, 2002.

6. **Connor WE**. Importance of n-3 fatty acids in health and disease. *American Journal of Clinical Nutrition* 71: 171S-175S, 2000.

7. **Jump DB**. Dietary polyunsaturated fatty acids and regulation of gene transcription. *Current Opinion in Lipidology* 13: 155-164, 2002.

8. **Neschen S, Moore I, Regittnig W, Yu CL, Wang Y, Pypaert M, Petersen KF and Shulman GI**. Contrasting effects of fish oil and safflower oil on hepatic peroxisomal and tissue lipid content. *Am J Physiol Endocrinol Metab* 282: E395-E401, 2002.

9. **Flachs P, Horakova O, Brauner P, Rossmeisl M, Pecina P, Franssen-van Hal NL, Ruzickova J, Sponarova J, Drahota Z, Vlcek C, Keijer J, Houstek J and Kopecky J**. Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce beta-oxidation in white fat. *Diabetologia* 48: 2365-2375, 2005.

10. **Dommels YEM, Haring MMG, Keestra NGM, Alink GM, van Bladeren PJ and van Ommen B**. The role of cyclooxygenase in n-6 and n-3 polyunsaturated fatty acid mediated effects on cell proliferation, PGE(2) synthesis and cytotoxicity in human colorectal carcinoma cell lines. *Carcinogenesis* 24: 385-392, 2003.

11. **Calviello G, Palozza P, Maggiano N, Piccioni E, Franceschelli P, Frattucci A, Di Nicuolo F and Bartoli GM**. Cell proliferation, differentiation, and apoptosis are modified by n-3 polyunsaturated fatty acids in normal colonic mucosa. *Lipids* 34: 599-604, 1999.

12. **Kim HK, Della-Fera M, Lin J and Baile CA**. Docosahexaenoic acid inhibits adipocyte differentiation and induces apoptosis in 3T3-L1 preadipocytes. *Journal of Nutrition* 136: 2965-2969, 2006.

13. **Serhan CN**. Novel omega -- 3-derived local mediators in anti-inflammation and resolution. *Pharmacol Ther* 105: 7-21, 2005.

14. **Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N and Gronert K**. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. *Journal of Experimental Medicine* 192: 1197-1204, 2000.

15. **Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G and Moussignac RL**. Resolvins: A family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *Journal of Experimental Medicine* 196: 1025-1037, 2002.

16. **Serhan CN, Gotlinger K, Hong S, Lu Y, Siegelman J, Baer T, Yang R, Colgan SP and Petasis NA**. Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: Assignments of dihydroxy-containing docosatrienes. *Journal of Immunology* 176: 1848-1859, 2006.

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

18. **Kopecky J, Rossmeisl M, Flachs P, Kuda O, Brauner P, Jilkova Z, Stankova B, Tvrzicka E and Bryhn M**. n-3 PUFA: bioavailability and modulation of adipose tissue function. *Proc Nutr Soc* 68: 361-369, 2009.

19. **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**. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 108: 1167-1174, 2001.

20. **Nettleton JA and Katz R**. n-3 long-chain polyunsaturated fatty acids in type 2 diabetes: a review. *J Am Diet Assoc* 105: 428-440, 2005.

21. **Neschen S, Morino K, Rossbacher JC, Pongratz RL, Cline GW, Sono S, Gillum M and Shulman GI**. Fish Oil Regulates Adiponectin Secretion by a Peroxisome Proliferator-Activated Receptor-gamma-Dependent Mechanism in Mice. *Diabetes* 55: 924-928, 2006.

22. **Flachs P, Rossmeisl M, Bryhn M and Kopecky J**. Cellular and molecular effects of n-3 polyunsaturated fatty acids on adipose tissue biology and metabolism. *Clinical Sciences* 116: 1-16, 2009.

23. **Grimaldi PA**. Peroxisome Proliferator-Activated Receptors as sensors of fatty acids and derivatives. *Cellular and Molecular Life Sciences* 64: 2459-2464, 2007.

24. **Smith SA**. Peroxisome proliferator-activated receptors and the regulation of mammalian lipid metabolism. *Biochemical Society Transactions* 30: 1086-1090, 2002.

25. **Zieleniak A, Wojcik M and Wozniak LA**. Structure and physiological functions of the human peroxisome proliferator-activated receptor gamma. *Archivum Immunologiae et Therapiae Experimentalis* 56: 331-345, 2008.

26. **Gaudel C and Grimaldi PA**. Metabolic Functions of Peroxisome Proliferator-Activated Receptor beta/delta in Skeletal Muscle. *Ppar Research* 2007.

27. **Zhang F, Lavan B and Gregoire FM**. Peroxisome proliferator-activated receptors as attractive antiobesity targets. *Drug News & Perspectives* 17: 661-669, 2004.

28. **Chen XL, Matthews J, Zhou LB, Pelton P, Liang Y, Xu J, Yang M, Cryan E, Rybczynski P and Demarest K**. Improvement of dyslipidemia, insulin sensitivity, and energy balance by a peroxisome proliferator-activated receptor alpha agonist. *Metabolism-Clinical and Experimental* 57: 1516-1525, 2008.

29. **Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM and Mangelsdorf DJ**. Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. *Cell* 126: 789-799, 2006.

30. **Inoue J, Satoh SI, Kita M, Nakahara M, Hachimura S, Miyata M, Nishimaki-Mogami T and Sato R**. PPAR alpha gene expression is up-regulated by LXR and PXR activators in the small intestine. *Biochemical and Biophysical Research Communications* 371: 675-678, 2008.

31. **Lee Y, Yu X, Gonzales F, Mangelsdorf DJ, Wang MY, Richardson C, Witters LA and Unger RH**. PPAR alpha is necessary for the lipopenic action of hyperleptinemia on white adipose and liver tissue. *Proceedings of the National Academy of Sciences of the United States of America* 99: 11848-11853, 2002.

32. **Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B and Wrahli W**. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *Journal of Clinical Investigation* 103: 1489-1498, 1999.

33. **Carpentier YA, Portois L and Malaisse WJ**. n-3 fatty acids and the metabolic syndrome. *American Journal of Clinical Nutrition* 83: 1499S-1504S, 2006.

34. **Forman BM, Chen J and Evans RM**. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proceedings of the National Academy of Sciences of the United States of America* 94: 4312-4317, 1997.

35. **Tontonoz P, Hu E, Graves RA, Budavari AI and Spiegelman BM**. Mppar-Gamma-2 - Tissue-Specific Regulator of An Adipocyte Enhancer. *Genes & Development* 8: 1224-1234, 1994.

36. **Imai T, Takakuwa R, Marchand S, Dentz E, Bornert JM, Messaddeq N, Wendling O, Mark M, Desvergne B, Wahli W, Chambon P and Metzger D**. Peroxisome proliferator-activated receptor gamma is required in mature white and brown adipocytes for their survival in the mouse. *Proceedings of the National Academy of Sciences of the United States of America* 101: 4543-4547, 2004.

37. **Rosen ED and Spiegelman BM**. Molecular regulation of adipogenesis. *Annual Review of Cell and Developmental Biology* 16: 145-171, 2000.

38. **Lehrke M and Lazar MA**. The many faces of PPAR gamma. *Cell* 123: 993-999, 2005.

39. **Rangwala SM and Lazar MA**. Transcriptional control of adipogenesis. *Annual Review of Nutrition* 20: 535-559, 2000.

40. **Frohnert BI, Hui TY and Bernlohr DA**. Identification of a functional peroxisome proliferator-responsive element in the murine fatty acid transport protein gene. *Journal of Biological Chemistry* 274: 3970-3977, 1999.

41. **Wu ZD, Puigserver P, Andersson U, Zhang CY, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC and Spiegelman BM**. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 98: 115-124, 1999.

42. **Hardie DG**. Minireview: The AMP-activated protein kinase cascade: The key sensor of cellular energy status. *Endocrinology* 144: 5179-5183, 2003.

43. **Fryer LG, Parbu-Patel A and Carling D**. The Anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. *J Biol Chem* 277: 25226-25232, 2002.

44. **Hardie DG**. AMPK: a key regulator of energy balance in the single cell and the whole organism. *International Journal of Obesity* 32: S7-S12, 2008.

45. **Corcoran MP, Lamon-Fava S and Fielding RA**. Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise. *American Journal of Clinical Nutrition* 85: 662-677, 2007.

46. **Frayn KN**. Adipose tissue as a buffer for daily lipid flux. *Diabetologia* 45: 1201-1210, 2002.
47. **Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL and Ferrante AW**. Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation* 112: 1796-1808, 2003.

48. **Rui LY, Yuan MS, Frantz D, Shoelson S and White MF**. SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. *Journal of Biological Chemistry* 277: 42394-42398, 2002.

49. **Ueki K, Kondo T, Tseng YH and Kahn CR**. Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. *Proceedings of the National Academy of Sciences of the United States of America* 101: 10422-10427, 2004.

50. **Ueki K, Kondo T and Kahn CR**. Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Mol Cell Biol* 24: 5434-5446, 2004.

51. **Suganami T, Nishida J and Ogawa Y**. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes - Role of free fatty acids and tumor necrosis factor alpha. *Arteriosclerosis Thrombosis and Vascular Biology* 25: 2062-2068, 2005.

52. **Rutkowski JM, Davis KE and Scherer PE**. Mechanisms of obesity and related pathologies: The macro- and microcirculation of adipose tissue. *Febs Journal* 276: 5738-5746, 2009.

53. **Ye J**. Emerging role of adipose tissue hypoxia in obesity and insulin resistance. *Int J Obes* 33: 54-66, 2009.

54. **Browning JD and Horton JD**. Molecular mediators of hepatic steatosis and liver injury. *Journal of Clinical Investigation* 114: 147-152, 2004.

55. **Dries DR, Gallegos LL and Newton AC**. A single residue in the C1 domain sensitizes novel protein kinase C isoforms to cellular diacylglycerol production. *Journal of Biological Chemistry* 282: 826-830, 2007.

56. **Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF and Shulman GI**. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 103: 253-259, 1999.

57. **Shimomura I, Bashmakov Y and Horton JD**. Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. *Journal of Biological Chemistry* 274: 30028-30032, 1999.

58. **Iizuka K, Bruick RK, Liang G, Horton JD and Uyeda K**. Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. *Proceedings of the National Academy of Sciences of the United States of America* 101: 7281-7286, 2004.

59. **Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB and Storlien LH**. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* 46: 983-988, 1997.

60. **Belfort R, Mandarino L, Kashyap S, Wirfel K, Pratipanawatr T, Berria R, DeFronzo RA and Cusi K**. Dose-response effect of elevated plasma free fatty acid on insulin signaling. *Diabetes* 54: 1640-1648, 2005.

61. **White MF**. IRS proteins and the common path to diabetes. *American Journal of Physiology-Endocrinology and Metabolism* 283: E413-E422, 2002.

62. **Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, Goodyear LJ, Kraegen EW, White MF and Shulman GI**. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. *Diabetes* 48: 1270-1274, 1999.

63. **SchmitzPeiffer C, Browne CL, Oakes ND, Watkinson A, Chisholm DJ, Kraegen EW and Biden TJ**. Alterations in the expression and cellular localization of protein kinase C isozymes epsilon and theta are associated with insulin resistance in skeletal muscle of the high-fat-fed rat. *Diabetes* 46: 169-178, 1997.

64. **Montell E, Turini M, Marotta M, Roberts M, Noe V, Ciudad CJ, Mace K and Gomez-Foix AM**. DAG accumulation from saturated fatty acids desensitizes insulin stimulation of glucose uptake in muscle cells. *American Journal of Physiology-Endocrinology and Metabolism* 280: E229-E237, 2001.

65. **Schmitz-Peiffer C, Craig DL and Biden TJ**. Ceramide generation is sufficient to account for the inhibition of the insulin-stimulated PKB pathway in C2C12 skeletal muscle cells pretreated with palmitate. *Journal of Biological Chemistry* 274: 24202-24210, 1999.

66. **Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D and Groop LC**. PGC-1 alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nature Genetics* 34: 267-273, 2003.

67. **Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, Landaker EJ, Goldfine AB, Mun E, DeFronzo R, Finlayson J, Kahn CR and Mandarino LJ**. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. *Proceedings of the National Academy of Sciences of the United States of America* 100: 8466-8471, 2003.

68. **SchmitzPeiffer C, Oakes ND, Browne CL, Kraegen EW and Biden TJ**. Reversal of chronic alterations of skeletal muscle protein kinase C from fat-fed rats by BRL-49653. *American Journal of Physiology-Endocrinology and Metabolism* 273: E915-E921, 1997.

69. **Mayerson AB, Hundal RS, Dufour S, Lebon V, Befroy D, Cline GW, Enocksson S, Inzucchi SE, Shulman GI and Petersen KF**. The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. *Diabetes* 51: 797-802, 2002.

70. **Jucker BM, Cline GW, Barucci N and Shulman GI**. Differential effects of safflower oil versus fish oil feeding on insulin-stimulated glycogen synthesis, glycolysis, and pyruvate dehydrogenase flux in skeletal muscle: a 13C nuclear magnetic resonance study. *Diabetes* 48: 134-140, 1999.

71. **Neschen S, Morino K, Dong J, Wang-Fischer Y, Cline GW, Romanelli AJ, Rossbacher JC, Moore IK, Regittnig W, Munoz DS, Kim JH and Shulman GI**. N-3 Fatty Acids Preserve Insulin Sensitivity In Vivo in a PPAR alpha-dependent manner. *Diabetes* 56: 1034-1041, 2007.

72. **Arner P and Spalding KL**. Fat cell turnover in humans. *Biochem Biophys Res Commun* 396: 101-104, 2010.

73. **Cinti S**. *The adipose organ*. Milano, Italy: Editrice Kurtis, 1999.

74. **Aherne W and Hull D**. Brown Adipose Tissue and Heat Production in Newborn Infant. *Journal of Pathology and Bacteriology* 91: 223-&, 1966.

75. **Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S and Nuutila P**. Functional brown adipose tissue in healthy adults. *N Engl J Med* 360: 1518-1525, 2009.

76. **Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B and Nedergaard J**. Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J Biol Chem* 285: 7153-7164, 2010.

77. **Barbatelli G, Murano I, Madsen L, Hao Q, Jimenez M, Kristiansen K, Giacobino JP, De Matteis R and Cinti S**. The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. *American Journal of Physiology-Endocrinology and Metabolism* 298: E1244-E1253, 2010.

78. **Mohamed-Ali V, Pinkney JH and Coppack SW**. Adipose tissue as an endocrine and paracrine organ. *Int J Obes* 22: 1145-1158, 1998.

79. **Lau DCW, Shillabeer G, Li ZH, Wong KL, Varzaneh FE and Tough SC**. Paracrine interactions in adipose tissue development and growth. *International Journal of Obesity* 20: S16-S25, 1996.

80. **Maury E and Brichard SM**. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol* 314: 1-16, 2010.

81. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L and Friedman JM**. Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425-432, 1994.

82. **Maffei M, Fei H, Lee GH, Dani C, Leroy P, Zhang Y, Proenca R, Negrel R, Ailhaud G and Friedman JM**. Increased expression in adipocytes of ob RNA in mice with lesions of the hypothalamus and with mutations at the db locus. *Proc Natl Acad Sci USA* 92: 6957-6960, 1995.

83. **Campfield LA, Smith FJ, Guisez Y, Devos R and Burn P**. Recombinant Mouse Ob Protein - Evidence for A Peripheral Signal Linking Adiposity and Central Neural Networks. *Science* 269: 546-549, 1995.

84. **Toyoshima Y, Gavrilova O, Yakar S, Jou W, Pack S, Asghar Z, Wheeler MB and LeRoith D**. Leptin improves insulin resistance and hyperglycemia in a mouse model of type 2 diabetes. *Endocrinology* 146: 4024-4035, 2005.

85. **Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D and Kahn BB**. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 415: 339-343, 2002.
86. **Unger RH**. Lipid overload and overflow: metabolic trauma and the metabolic syndrome. *Trends in Endocrinology and Metabolism* 14: 398-403, 2003.

87. **Gil-Campos M, Canete R and Gil A**. Adiponectin, the missing link in insulin resistance and obesity. *Clinical Nutrition* 23: 963-974, 2004.

88. **Haluzik M, Parizkova J and Haluzik MM**. Adiponectin and its role in the obesity-induced insulin resistance and related complications. *Physiol Res* 53: 123-129, 2004.

89. **Hoffstedt J, Arvidsson E, Sjolin E, Wahlen K and Arner P**. Adipose tissue adiponectin production and adiponectin serum concentration in human obesity and insulin resistance. *Journal of Clinical Endocrinology & Metabolism* 89: 1391-1396, 2004.

90. **Pajvani UB, Du XL, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M and Scherer PE**. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin - Implications for metabolic regulation and bioactivity. *J Biol Chem* 278: 9073-9085, 2003.

91. **Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R and Kadowaki T**. Impaired multimerization of human adiponectin mutants associated with diabetes - Molecular structure and multimer formation of adiponectin. *J Biol Chem* 278: 40352-40363, 2003.

92. **Kubota N, Yano W, Kubota T, Ueki K, Yamauchi T, Terauchi Y, Ezaki O, Tobe K, Minokoshi Y and Kadowaki T**. Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Diabetes* 56: A7, 2007.

93. **Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K and Tobe K**. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* 116: 1784-1792, 2006.

94. **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 BB and Kadowaki T**. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Medicine* 8: 1288-1295, 2002.

95. **Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R and Kadowaki T**. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 423: 762-769, 2003.

96. **Banga A, Unal R, Tripathi P, Pokrovskaya I, Owens RJ, Kern PA and Ranganathan G**. Adiponectin translation is increased by the PPAR gamma agonists pioglitazone and omega-3 fatty acids. *American Journal of Physiology-Endocrinology and Metabolism* 296: E480-E489, 2009.

97. **Simons PJ, van den Pangaart PS, Aerts JMFG and Boon L**. Pro-inflammatory delipidizing cytokines reduce adiponectin secretion from human adipocytes without affecting adiponectin oligomerization. *J Endocr* 192: 289-299, 2007.

98. **Pradhan AD, Manson JE, Rifai N, Buring JE and Ridker PM**. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Jama-Journal of the American Medical Association* 286: 327-334, 2001.

99. **Eder K, Baffy N, Falus A and Fulop AK**. The major inflammatory mediator interleukin-6 and obesity. *Inflammation Research* 58: 727-736, 2009.

100. **Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW and Mooney RA**. Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J Biol Chem* 278: 13740-13746, 2003.

101. **Fasshauer M, Kralisch S, Klier M, Lossner U, Bluher M, Klein J and Paschke R**. Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* 301: 1045-1050, 2003.

102. **Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C and Pratley RE**. Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obesity Research* 9: 414-417, 2001.

103. **Goldberg RB, Kendall DM, Deeg MA, Buse JB, Zagar AJ, Pinaire JA, Tan MH, Khan MA, Perez AT, Jacober SJ and for the GLAI Study Investigators**. A Comparison of Lipid and Glycemic Effects of Pioglitazone and Rosiglitazone in Patients With Type 2 Diabetes and Dyslipidemia. *Diabetes Care* 28: 1547-1554, 2005.

104. **Tontonoz P, Hu E, Graves RA, Budavari AI and Spiegelman BM**. mPPARgamma2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev* 8: 1224-1234, 1994.

105. **Wilson-Fritch L, Nicoloro S, Chouinard M, Lazar MA, Chui PC, Leszyk J, Straubhaar J, Czech MP and Corvera S**. Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone. *J Clin Invest* 114: 1281-1289, 2004.

106. **Ricote M, Li AC, Willson TM, Kelly CJ and Glass CK**. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 391: 79-82, 1998.

107. **Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, Utzschneider KM, Kahn SE, Olefsky JM, Buchanan TA and Scherer PE**. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem* 279: 12152-12162, 2004.

108. **Nissen SE and Wolski K**. Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes. *N Engl J Med* 356: 2457-2471, 2007.

109. **Loke YK, Kwok CS and Singh S**. Comparative cardiovascular effects of thiazolidinediones: systematic review and meta-analysis of observational studies. *BMJ* 342: d1309, 2011.

110. **Yang X and Smith U**. Adipose tissue distribution and risk of metabolic disease: does thiazolidinedione-induced adipose tissue redistribution provide a clue to the answer? *Diabetologia* 50: 1127-1139, 2007.

111. **Sinclair DA**. Toward a unified theory of caloric restriction and longevity regulation. *Mechanisms of Ageing and Development* 126: 987-1002, 2005.

112. **Wheatley KE, Nogueira LM, Perkins SN and Hursting SD**. Differential effects of calorie restriction and exercise on the adipose transcriptome in diet-induced obese mice. *J Obes* 2011: 265417, 2011.

113. **Nisoli E, Tonello C, Cardile A, Cozzi V, Bracale R, Tedesco L, Falcone S, Valerio A, Cantoni O, Clementi E, Moncada S and Carruba MO**. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science* 310: 314-317, 2005.

114. **Todoric J, Loffler M, Huber J, Bilban M, Reimers M, Kadl A, Zeyda M, Waldhausl W and Stulnig TM**. Adipose tissue inflammation induced by high-fat diet in obese diabetic mice is prevented by n-3 polyunsaturated fatty acids. *Diabetologia* 49: 2109-2119, 2006.

7 LIST OF MY PUBLICATIONS

1) **Hensler M**, Bardova K, Jilkova ZM, Wahli W, Meztger D, Chambon P, Kopecky J, Flachs P. The inhibition of fat cell proliferation by n-3 fatty acids in dietary obese mice. *Lipids Health Dis.* 10:128. 2011 (IF = 2,240).

2) Flachs P, Rühl R, **Hensler M**, Janovska P, Zouhar P, Kus V, Macek Jilkova Z, Papp E, Kuda O, Svobodova M, Rossmeisl M, Tsenov G, Mohamed-Ali V, Kopecky J. Synergistic induction of lipid catabolism and anti-inflammatory lipids in white fat of dietary obese mice in response to calorie restriction and n-3 fatty acids. *Diabetologia* 54: 2626-38. 2011 (IF= 6,973).

3) Jelenik T, Rossmeisl M, Kuda O, Jilkova ZM, Medrikova D, Kus V, **Hensler M**, Janovska P, Miksik I, Baranowski M, Gorski J, Hébrard S, Jensen TE, Flachs P, Hawley S, Viollet B, Kopecky J. AMP-activated protein kinase α2 subunit is required for the preservation of hepatic insulin sensitivity by n-3 polyunsaturated fatty acids. *Diabetes.* 59:2737-46. 2010 (IF = 8,505).

4) Macek Jílková Z, Pavelka S, Flachs P, **Hensler M**, Kůs V, Kopecký J. Modulation of type I iodothyronine 5'-deiodinase activity in white adipose tissue by nutrition: possible involvement of leptin. *Physiol Res.* 59:561-9. 2010 (IF = 1,739).

5) Kuda O, Stankova B, Tvrzicka E, **Hensler M**, Jelenik T, Rossmeisl M, Flachs P, Kopecky J. Prominent role of liver in elevated plasma palmitoleate levels in response to rosiglitazone in mice fed high-fat diet. *J Physiol Pharmacol.* 60:135-40. 2009 (IF = 4,466).

6) Kuda O, Jelenik T, Jilkova Z, Flachs P, Rossmeisl M, **Hensler M**, Kazdova L, Ogston N, Baranowski M, Gorski J, Janovska P, Kus V, Polak J, Mohamed-Ali V, Burcelin R, Cinti S, Bryhn M, Kopecky J. n-3 fatty acids and rosiglitazone improve insulin sensitivity through additive stimulatory effects on muscle glycogen synthesis in mice fed a high-fat diet. *Diabetologia.* 52:941-51. 2009 (IF = 6,551).

7) Rossmeisl M, Jelenik T, Jilkova Z, Slamova K, Kus V, **Hensler M**, Medrikova D, Povysil C, Flachs P, Mohamed-Ali V, Bryhn M, Berge K, Holmeide AK, Kopecky J. Prevention and reversal of obesity and glucose intolerance in mice by DHA derivatives. *Obesity. 17:1023-31.* 2009 (IF = 2,798).

8) Ogston NC, Karastergiou K, Hosseinzadeh-Attar MJ, Bhome R, Madani R, Stables M, Gilroy D, Flachs P, **Hensler M**, Kopecky J, Mohamed-Ali V. Low-dose acetylsalicylic acid inhibits the secretion of interleukin-6 from white adipose tissue. *Int J Obes*. 32:1807-15. 2008 (IF = 3,640).

9) Kus V, Prazak T, Brauner P, **Hensler M**, Kuda O, Flachs P, Janovska P, Medrikova D, Rossmeisl M, Jilkova Z, Stefl B, Pastalkova E, Drahota Z, Houstek J, Kopecky J. Induction of muscle thermogenesis by high-fat diet in mice: association with obesity-resistance. *Am J Physiol Endocrinol Metab.* 295:E356-67. 2008 (IF = 4,129).

10) Flachs P, Mohamed-Ali V, Horakova O, Rossmeisl M, Hosseinzadeh-Attar MJ, **Hensler M**, Ruzickova J, Kopecky J. Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet. *Diabetologia*. 49:394-7. 2006 (IF = 5,247).

8 SELECTED PUBLICATIONS (A-E)