Charles University in Prague

1st Faculty of Medicine

Summary of PhD Thesis

Analysis of the involvement of α2-AMPK in the beneficial effects of n–3 polyunsaturated fatty acids on obesity-associated metabolic derangements

Tomáš Jeleník

Prague
2010

Doctoral studies in Biomedicine

Charles University in Prague
and Academy of Sciences of the Czech Republic

Section: Biochemistry and Pathobiochemistry

Section chairman: Prof. MUDr. Jiří Kraml, DrSc.

Workplace: Department of Adipose Tissue Biology
Institute of Physiology, v.v.i
Academy of Sciences of the Czech Republic
Videnska 1083, 14220 Praha 4

Author: Ing. Tomáš Jeleník

Supervisor: MUDr. Martin Rossmeisl, PhD.
Department of Adipose Tissue Biology
Institute of Physiology, v.v.i
Academy of Sciences of the Czech Republic
Videnska 1083, 14220 Praha 4

Opponents:

Abstract of PhD thesis was sent:

Defence of PhD thesis

The full text of the PhD thesis is available at the Department of Science and Research and Inernational Relations of the First Faculty of Medicine, Charles University in Prague.

CONTENTS

SOUHRN

Je známo, že n-3 polynenasycené mastné kyseliny s dlouhým řetězcem (n-3 LC-PUFA) mají benefiční účinky na obezitou-indukovaná metabolická onemocnění, avšak jejich účinnost při snižování obezity a inzulínové rezistence u lidí je nízká. Cílem dizertační práce bylo otestovat různé přístupy, jak zvýšit efektivitu n-3 LC-PUFA a také zjistit, jaké je zapojení α2 podjednotky AMP-aktivované proteinové kinázy (α2-AMPK) v mechanizmech účinku n-3 LC-PUFA.

Nejdříve byly na myším modelu testovány chemické deriváty DHA. Substance-2, α-ethyl ester DHA, podávána v 10 % obvyklé dávky n-3 LC-PUFA, měla prevenční a částečně reverzní účinky v rozvoji obezity, akumulace tuku, glukózové intolerance, dyslipidémie a zánětu bílého tukového depa. Dále, kombinace n-3 LC-PUFA a nízké dávky antidiabetika rosiglitazonu, měla aditivní účinky v prevenci rozvoje dyslipidémie, inzulínové rezistence, akumulace tuku a hypertrofie adipocytů u myší krmených vysokotukou dietou, za současné indukce sekrece adiponektínu. Podávaní kombinační diety také léčilo již rozvinutou glukózovou intoleranci u obézních myší.

Hlavní část této práce byla zaměřena na studium zapojení α2-AMPK v mechanizech účinku n-3 LC-PUFA. Na myším modelu s delecí genu pro α2-AMPK jsme zjistili, že benefiční účinky n-3 LC-PUFA v prevenci vzniku inzulínové rezistence jsou zprostředkovány přes α2-AMPK. Dominantním místem účinku byla játra, kde n-3 LC-PUFA zvyšovaly aktivitu α2-AMPK a inzulínovou senzitivitu v závislosti na přítomnosti α2-AMPK. Zlepšení inzulínové senzitivity nebylo asociováno se změnami v jaterním obsahu triacylglycerolů ale se změnami v obsahu diacylglycerolů. Účinky n-3 LC-PUFA na obsah diacylglycerolů v játrech závisely, podobně jako v případě inzulínové senzitivity, na přítomnosti funkční α2-AMPK. Jaké jsou přesné mechanizmy aktivace α2-AMPK pomocí n-3 LC-PUFA a dráhy vedoucí od AMPK ke zlepšení inzulínové senzitivity, bude předmětem dalšího výzkumu.

SUMMARY

It is well established that n-3 polyunsaturated fatty acids with long chain (n-3 LC-PUFA) have beneficial effects on the obesity-induced metabolic disorders in mice. However, in obese humans, the potency of these fatty acids to positively affect obesity and insulin resistance has been shown to be lower. The aim of the studies described in this thesis was to verify various approaches aimed at increasing efficiency of n-3 LC-PUFA and to study the involvement of α2 subunit of AMP-activated protein kinase (α2-AMPK) in the mechanisms of action of these compounds.

Firstly, various chemical derivatives of DHA were tested in mice. Substance-2, the α-ethyl ester of DHA, completely prevented and even partially reversed the development of obesity, fat accumulation, impaired glucose tolerance, dyslipidemia and white adipose tissue inflammation, even though the dose was only 10 % of that normally used in mice for the treatment with n-3 LC-PUFA. Secondly, the combination of n-3 LC-PUFA and a low-dose of anti-diabetic rosiglitazone prevented, in additive manner, development of dyslipidemia and insulin resistance, reduced the accumulation of body fat and adipocyte hypertrophy, while inducing adiponectin in mice fed a high-fat diet. This treatment also reversed impaired glucose tolerance in obese mice.

The major part of this thesis was aimed to study the mechanism of n-3 LC-PUFA action and the possible involvement of α2-AMPK. We found, by using the model of α2-AMPK knockout mice, that beneficial effects of n-3 LC-PUFA on the prevention of insulin resistance were mediated by α2-AMPK. The liver appeared to be the site of a dominant effect of n-3 LC-PUFA, since their inclusion in the diet resulted in the activation of α2-AMPK primarily in the liver and improved hepatic insulin sensitivity in the α2-AMPK-dependent manner. The improvement of liver insulin sensitivity was not associated with the changes in liver triacylglycerol levels; however, it was closely related to the content of diacylglycerols that are known to affect insulin sensitivity. Similarly to hepatic insulin sensitivity, also the effect of n-3 LC-PUFA on diacylglycerol levels was α2-AMPK-dependent. The precise mechanisms of α2-AMPK activation by n-3 LC-PUFA as well as downstream effectors of α2-AMPK activation in this context remain to be elucidated.

1 INTRODUCTION

1.1 Mechanisms linking obesity to insulin resistance

Obesity, which is a major risk for insulin resistance, has reached epidemic proportions globally. Together with genetic predispositions, it can lead into a loss of metabolic fuel homeostasis and outbreak of type 2 diabetes (1).

Main role of insulin is to stimulate glucose uptake by peripheral tissues and suppress glucose production in the liver and lipolysis in the adipose tissue. Insulin resistance is characterized by an inadequate response of insulin target tissues. To maintain glucose and lipid homeostasis, body can, at least partially, compensate for decreased efficiency of insulin by increased insulin secretion. However, insufficient metabolic action of insulin leads to metabolic complications such as hyperglycemia and/or dyslipidemia, which further promote insulin resistance and cause serious tissue damage by subsequent mechanisms. Among the main mechanisms responsible for the development of insulin resistance in different organs could be overloading with toxic metabolites, induction of inflammation, disruption of secretory functions and activation of anti-stress mechanisms.

1.1.1 Central role of adipose tissue

Adipose tissue has a regulatory role in the development of obesity-associated insulin resistance. Metabolic overload of adipose tissue can result in dysregulation of its normal functions, which can negatively impact other organs in the body.

Positive energy balance causes adipose tissue to become hypertrophic and subsequently hyperplastic. Adipocytes can store excess energy up to the state of fullness and cannot expand beyond a “critical size” (2). Excess lipids are then ectopically stored in the liver, muscle or heart and provoke insulin resistance of these tissues (3). Moreover, hypertrophied adipocytes are resistant to the antilipolytic effect of insulin (4). Increased flux of non-esterified fatty acids (NEFA) may impair liver metabolism, leading to increased hepatic glucose production (hyperglycemia) and stimulation of very low-density lipoprotein triacylglycerol (VLDL-triacylglycerol) secretion (hyperlipidemia).

Hypertrophy of adipocytes also negatively affects the secretion profile of adipokines (e.g. adiponectin), resulting in systemic decrease of insulin sensitivity. Finally, the larger is the adipocyte, the more fragile it becomes when submitted to a common physical stress (5). Therefore, adipocyte size is an important determinant of cell death. The removal of dead adipocytes results in a low-grade inflammation and shift towards pro-inflammatory cytokines secretion, which have been shown to impair insulin sensitivity (6).

1.1.2 Hepatic insulin resistance

The sensitivity of liver to insulin is important to maintain glucose and lipid homeostasis. Impairments of pathways regulated by insulin may result in a development of hyperglycemia and liver steatosis (7). It has been shown, that liver steatosis is associated with insulin resistance (8), and lowering liver triacylglycerol pools correlates with improved insulin sensitivity in several rodent models as well as in humans [(9); (10)]. However, whether the impaired insulin sensitivity is a consequence of hepatic steatosis or vice versa remains still unresolved (11). Furthermore, dissociations of hepatic steatosis and insulin resistance have been found (12). For instance, increased flux of fatty acids into neutral triacylglycerols protects liver from the cytotoxic effects of fatty acid side products (13). There are several lines of evidence, that diacylglycerol rather than triacylglycerol accumulation affects the development of insulin resistance. It has been shown that excess diacylglycerols activate protein kinase C-ε (PKCε), which in turn interferes with ability of insulin to phosphorylate IRS-2 on tyrosine residues, thus inhibiting insulin signaling (14) (see Figure 1.1-1). On the other hand, inhibition of PKCε prevents hepatic insulin resistance in fatty liver disease (14).

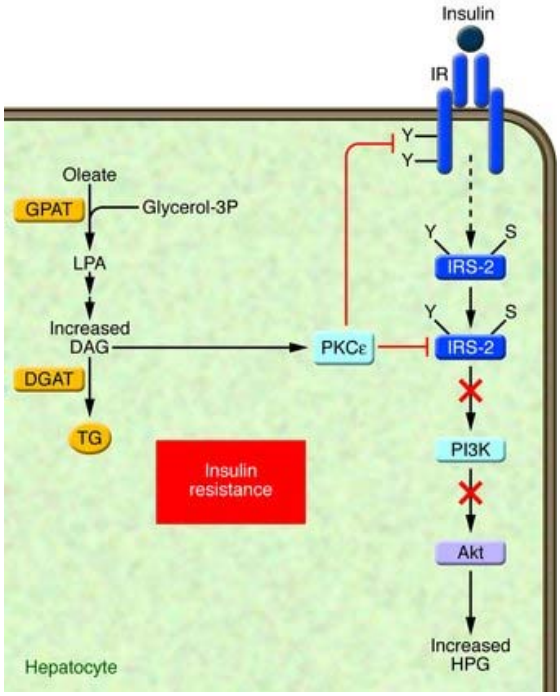

Figure 1.1-1 Mechanism by which diacylglycerols affect insulin sensitivity in the liver. HPG – hepatic production of glucose. Adapted from (7)

1.1.3 Skeletal muscle insulin resistance

Fatty acids and glucose are metabolized in the skeletal muscle, depending on the metabolic state of the tissue. Oversupply of fatty acids, for example from increased lipolysis of triacylglycerol stores in insulin-resistant adipocytes, can lead to accumulation of various lipid metabolites, which negatively correlates with insulin sensitivity. Ceramide and diacylglycerol levels seem to be the most important players (15). Insulin-resistant muscle is metabolically inflexible, i.e. the ability to switch from lipid to carbohydrate metabolism is low (16). Glucose uptake and glycogen synthesis of insulin-resistant muscle is decreased and this can be observed in obese and diabetic patients (17).

1.2 Role of AMPK in regulating whole body metabolism

Obesity-related insulin resistance and type 2 diabetes are characterised by defective energy metabolism. Preservation of intracellular ATP concentrations within an appropriate range is needed to sustain unimpaired metabolism. Adenosine

monophosphate-activated protein kinase (AMPK) is considered to be in the centre of the mechanisms that regulate metabolism on the cellular and whole-body level. Activation of AMPK by various stimuli leads to induction and inhibition of energy-producing and energy-consuming metabolic pathways, respectively.

1.2.1 AMPK structure and tissue distribution

AMPK is a heterotrimeric protein comprising one catalytic α subunit and two regulatory subunits (β and γ) (18). In mammals, two isoforms for α (α1, α2) and β (β1, β2) and three for γ-subunit (γ1, γ2, γ3) are known, while all 12 isoform combinations are possible. α1- and α2-AMPK catalytic subunits account each for about 50 % of total AMPK activity in the liver (19), while α1 predominates in adipose tissue (20) and α2 in muscle tissue (21).

1.2.2 Upstream targets of AMPK

AMPK can be activated both allosterically by AMP binding on the γ subunit and by reversible phosphorylation on Thr-172. Two upstream kinases have been identified to phosphorylate AMPK: LKB1 kinase mediating the regulation of the glucose and lipid metabolism (22) and Ca^{2+}/calmodulin-dependent protein kinase β (CaMKKβ) involved in response to increased Ca^{2+} concentrations during contraction in skeletal muscle (23). Also adiponectin is known to activate AMPK (24).

1.2.3 Downstream targets of AMPK

A number of downstream targets and processes that are regulated by AMPK have been described. Generally, activation of AMPK downregulates biosynthetic pathways such as fatty acid and cholesterol synthesis and switches on catabolic pathways that generate ATP, such as fatty acid oxidation, glucose uptake and glycolysis. Mechanisms, by which AMPK mediates its action, are based on the change of enzymes activity by phosphorylation and/or the regulation of gene expression. For example, mRNA levels of lipogenic genes such as sterol regulatory element binding protein-1c (SREBP-1c) (25) and carbohydrate responsive element-binding protein (ChREBP) (26) were decreased after activation of AMPK by the AMP analogue AICAR (5-aminoimidazole-4-carboxyamide ribonucleoside) or by anti-diabetic drug metformin. AMPK directly phosporylates acetyl CoA carboxylase (ACC) and thus inhibits fatty acid synthesis and

induces fatty acid oxidation through malonyl-CoA (27). The AMPK-dependent decrease in cholesterol biosynthesis is mediated by phosphorylation and inhibition of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase. AICAR treatment in rodents has hypoglycemic effects but along with the liver, muscle AMPK participates in this effect by increasing glucose uptake into muscle cells. The down-regulation of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) gene expression and enzyme activity by AMPK is responsible for its lowering effects on gluconeogenesis [(26); (28)]. In adipose tissue, AMPK inhibits lipolysis via the phosphorylation and consequent inactivation of hormone-sensitive lipase (29). AMPK could have the role in mediating insulin-independent glucose transport in adipocytes (30). In the skeletal muscle, AMPK activity promotes glucose metabolism by stimulating glucose uptake via glucose transporters (GLUT4) and glycogen storage [(31); (32)].

Various treatments, which led to the activation of AMPK and the subsequent change of metabolic processes, were associated with beneficial effects on metabolic parameters in the models of insulin resistance and dyslipidemia. For example, AICAR lowered plasma glucose levels, improved insulin sensitivity, adiposity and plasma triacylglycerol levels in obese and insulin resistant rat model (33). Furthermore, over-expression of AMPK in the liver led to the similar effects in the obese mice (26). Finally, AICAR infusion in type 2 diabetic patients decreased hepatic glucose production and plasma glucose levels (34). Thus, targeting AMPK by therapeutic treatments could be useful in the prevention or reversal of metabolic disorders such as obesity, insulin resistance and metabolic syndrome.

1.3 Long-chain polyunsaturated fatty acids of n-3 family

1.3.1 Nomenclature

Long-chain polyunsaturated fatty acids of the n-3 series (n-3 LC-PUFA), for example eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, are important essential fatty acids (Table 1.3-1). Humans or rodents can not synthesize n–3 LC-PUFA *de novo*. Therefore, the supplementation of these substances from the diet is needed. Main pathways of n–3 LC-PUFA utilization in the body are: 1) incorporation into plasma

phospholipids; 2) precursors for the formation of eicosanoid and docosanoid signaling molecules; and 3) regulation of gene transcription by acting as ligands of transcription factors.

Table 1.3-1 Nomenclature of selected n–3 LC-PUFA

Trivial name	Abbreviation	Systematic name	Carboxyl reference
Eicosapentaenoic acid	EPA	(5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoic acid	C 20:5 (Δ□5,8,11,14,17)
Docosahexaenoic acid	DHA	(4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid	C 22:6 (Δ□4,8,12,15,19)

1.3.2 Biological effects and mechanisms

Naturally occurring n–3 LC-PUFA are obtained mainly from oils of marine fish. n-3 LC-PUFA act as hypolipidemics, suppressors of VLDL-triacylglycerol production (35), while they also reduce cardiac events and decrease progression of atherosclerosis (36). Several studies in obese humans even demonstrated reduction of adiposity after n–3 LC-PUFA supplementation [(37); (38)]. However, in diabetic patients n–3 LC-PUFA appear to have little effect on glycaemic control [(37); (39); (40)]. In rodents fed a high-fat diet, n–3 LC-PUFA efficiently prevented development of obesity [(41); (42); (43)], as well as impaired glucose tolerance [(44); (45)]. Low–grade inflammation of white adipose tissue could be also prevented (46).

n-3 LC-PUFA have been shown to decrease the levels of main glycolytic and lipogenic transcription factors including SREBP-1c (47) , ChREBP (11) as well as other genes that are regulated by these transcription factors (ACC, fatty acid synthase (FAS), stearoyl-coenzyme A desaturase-1 (SCD1)). In addition, n-3 LC-PUFA diminish malonyl-CoA concentration in the liver (48), which acutely regulates the rates of fatty acid synthesis and oxidation. The effects of n–3 LC-PUFA are furthermore largely mediated by peroxisome proliferator-activated receptors PPAR–α and PPAR–β/δ. n-3 LC-PUFA from fish oil are capable of inducing adiponectin (49), while influencing the secretion of other adipokines [(50); (6)]. The beneficial effects of n-3 LC-PUFA on the prevention of diet-

induced insulin resistance were associated with increased glucose uptake and GLUT4 mRNA and protein levels in adipocytes (51).

Besides acting directly as regulatory ligands, n–3 LC-PUFA act also through their active metabolites, eicosanoids, and other lipid molecules (52). Another mechanism of n-3 LC-PUFA action is mediated by the change in the biological membranes properties. In this way, the mobility of membrane-associated proteins, enzymes, and hormones can be affected.

1.4 Involvement of AMPK in the mechanisms of n-3 LC-PUFA action

As described above, n-3 LC-PUFA ingestion has beneficial effects on the lipid and glucose metabolism, in particular on the suppression of hepatic lipogenesis and gluconeogenesis, induction of fatty acid oxidation, and glucose uptake. All of these events have been shown to be affected by AMPK activation. Furthermore, it has been demonstrated that feeding rodents with n-3 LC PUFA enhanced AMPK activity in the liver (53), intestine (54), and adipose tissue [(55); (56)]. Therefore, it can be suggested, that n-3 LC-PUFA could mediate their effects, at least on the metabolic events mentioned above, by modulating the AMPK activity. Possible mechanisms of AMPK activation by n-3 LC-PUFA could involve: 1) changes in energy status of the cells, reflected by the change in AMP/ATP ratio; 2) modulation of upstream kinases or phostphatase activity; 3) induction of adiponectin secretion from adipose tissue or 4) other indirect mechanism. However, the precise mechanism of AMPK activation by n-3 LC-PUFA is not known.

2 SPECIFIC AIMS OF WORK

The main objective of this thesis was to analyze mechanisms involved in the beneficial effect of n-3 LC-PUFA on the development of obesity and associated impairments of insulin sensitivity and other metabolic disturbances.

The specific aims of this thesis were:

1. to study the involvement of α2-AMPK in the beneficial effects of n-3 LC-PUFA by using knock-out mice with a targeted deletion of α2 catalytic subunit of AMPK (study 1),

2. to investigate the efficacy of n-3 LC-PUFA chemical derivatives to prevent and reverse the impairment of lipid and glucose metabolism by high-fat feeding (study 2),

3. to evaluate possible additive improvements of insulin sensitivity induced by the combination treatments of n-3 LC-PUFA and anti-diabetic drug rosiglitazone in the model of high-fat diet-induced obesity in mice (study 3).

3 METHODS

3.1 Animals and diets

We used the following mouse strains in our studies:

1. male and female whole-body α2-AMPK knock-out mice (α2-AMPK KO) and WT littermate controls (WT),
2. male C57BL/6N mice (Charles River Laboratories, Sulzfeld, Germany);
3. male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME, USA).

Mice were bred in the animal facility of the Department of Adipose Tissue Biology, Institute of Physiology, Academy of the Sciences of the Czech Republic, v.v.i. At 4 weeks of age, mice were weaned onto a standard laboratory chow (Chow; lipid content ~3.4 % wt/wt; extruded R/MH diet; Ssniff Spezialdiäten, Soest, Germany) and maintained at 22 °C on a 12h light–dark cycle (light on from 06:00 a.m.) with a free access to food and water until the initiation of the experiment.

Table **3.1-1** briefly summarizes the names and lipid composition of the experimental diets used in the studies described in this thesis.

Table 3.1-1 High-fat diets used in the experiments

Name	**Lipid composition**
cHF	lipids ~35% wt/wt (~60 energy %), mostly corn oil
Substances 1-4	based on cHF; 1,5 % of dietary lipids replaced by various DHA derivatives termed Substance 1-4
DHA	based on cHF; 15 % of dietary lipids replaced by DHA (~99%; ethyl ester; Pronova BioPharma AS, Lysaker, Norway)
cHF+F	based on cHF; 15 % of dietary lipids replaced by n-3 LC-PUFA concentrate (46% DHA, 14% EPA); (EPAX 1050TG of the EPAX AS, Lysaker, Norway)
cHF+TZD	based on cHF; supplemented with rosiglitazone (10 mg/kg diet) (Avandia, GlaxoSmithKline)
cHF+F+TZD	based on cHF; 15 % of dietary lipids replaced by n-3 LC-PUFA and supplemented with rosiglitazone (10 mg/kg diet)

All high-fat diets contained 210 mg α-tocopherol/kg diet. Protein content was ~20.5 % wt/wt, carbohydrate content was ~35 % wt/wt and energy density 22.8 kJ/g.

3.2 Experimental design

Study 1

Two separate experiments were performed. In both experiments, 4-month-old WT and α2-AMPK KO mice of both genders were randomly assigned (n = 13-15) to either chow, cHF or cHF+F diet (

Table **3.1-1**). During dietary treatments, which lasted for 9 weeks, fresh rations of food were distributed every 2 days. Food consumption and body weights were recorded once a week.

Study 2

At 3 months of age, male mice of either C57BL/6N or C57BL/6J genetic background were randomly assigned to a cHF diet, while some mice were maintained on STD diet. Two experimental approaches were used:

1) In the "*prevention study"*, various DHA-derivatives (57) were admixed to the cHF diet (Substances-1 to -4) and administered to the 3-month-old C57BL/6N mice for a period of 4 months.

2) In the "*reversal study"*, obesity and impaired glucose tolerance were induced in C57BL/6J mice by feeding cHF diet for a period of 4 months prior to the subsequent 2-month-long administration of Substance-2 admixed to the cHF diet.

Study 3

At 3 month of age, male C57BL/6N mice were randomly assigned to a cHF diet or to the treatments by cHF+F, cHF+TZD and cHF+F+TZD. Some mice were maintained on the chow diet. Various analyses were performed after 5 to 20 weeks after initiation of the treatment (58).

All experiments were conducted under the guidelines for the use and care of laboratory animals of the Institute of Physiology and followed the 'Principles of laboratory animal care' (NIH publication no. 85-23, revised 1985).

3.3 List of methods

Methods accomplished by author are depicted in *italic*.

- *blood and plasma metabolites* (*glucose, NEFA, triacylglycerols, cholesterol,* insulin, adiponectin multimeric complexes*)*
- *tissue glycerolipid content*
- fatty acid composition of lipid fractions
- tissue ceramide content
- *RNA extraction and real-time quantitative analysis*
- activity of α1 and α2 AMPK isoforms
- determination of liver ATP, ADP and AMP
- light microscopy and immunohistochemical analysis
- *intraperitoneal glucose tolerance test*
- *hyperinsulinemic-euglycemic clamp technique*
- in vivo *VLDL-triacylglycerol synthesis: Tyloxapol test*
- indirect calorimetry measurement
- nor-epinephrine stimulated lipolysis

Statistics

Data were analyzed by two-way ANOVA using the SigmaStat software. All values are presented as means±SE. Comparisons were judged to be significant at $p \leq 0.05$.

4 RESULTS AND DISCUSSION

4.1 The involvement of α2-AMPK in the beneficial effects of n-3 LC-PUFA (Study 1)

9 weeks of cHF feeding induced obesity and adiposity in male WT mice characterized by the increased body weight gain, increased epididymal and dorsolumbar white adipose tissue mass and hypertrophy of adipocytes when compared to chow-fed controls, whereas the average food consumption did not significantly change. The weight of non-adipose tissues remained unaffected. Furthermore, cHF-feeding proved to have detrimental effects on the whole-body insulin sensitivity (Figure 4.1-1, A) as assessed by the hyperinsulinemic-euglycemic clamp. This was due to impaired whole-body glucose turnover rates and glycogen synthesis, and a lower potency of insulin to suppress hepatic glucose production (Figure 4.1-1, B). The blood glucose levels were unchanged by cHF-feeding, while insulin levels were increased. Normoglycemia together with hyperinsulinemia in cHF-fed mice indicated the presence of insulin resistance, which was confirmed by the clamp studies.

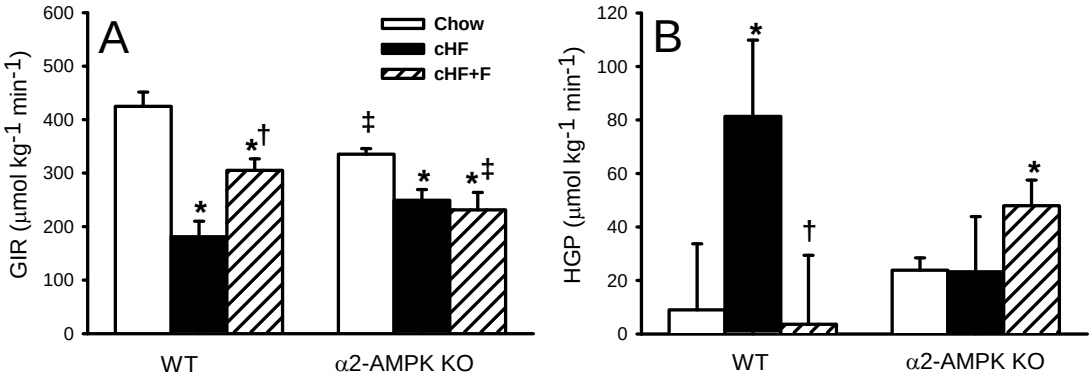

Figure 4.1-1Insulin sensitivity of male mice. Whole-body insulin sensitivity expressed by glucose infusion rate (GIR) (A) and hepatic glucose production (HGP) (B) assessed by hyperinsulinemic-euglycemic clamp in α2-AMPK KO and WT male mice fed for 9 weeks with control low-fat diet (Chow), control high-fat diet (cHF) or high-fat diet, in which 15% of lipids were replaced by n-3 LC-PUFA concentrate (cHF+F). Data are presented as means ± SE (n = 5-8). **P* < 0.05 vs. genotype chow; †*P* < 0.05 vs. genotype cHF; ‡*P* < 0.05 vs. WT on respective diet.

The next question was how the above mentioned parameters changed after replacing 15 % of fat in cHF diet with n-3 LC-PUFA concentrate and whether α2-AMPK was involved in these effects. Wild type mice treated with cHF+F diet for 9 weeks were protected against high-fat diet-induced obesity and adiposity, as documented by a significantly lower body weight gain and the weight of epididymal and dorsolumbar white adipose tissue mass, while the average food consumption was unaffected. Furthermore, the level of macrophage infiltration evaluated by the immunohistochemical detection of MAC-2 tended to be reduced and the secretion of HMW form of adiponectin, the adipokine with insulin sensitizing properties, was increased by cHF+F diet. All of these effects of cHF+F diet rich in n-3 LC-PUFA were primarily due to a reduction in the epididymal adipocyte size. The potency of cHF+F to decrease epididymal adipocyte size could be explained by the increased rates of basal lipolysis in epididymal adipose. Importantly, any of these effects of cHF+F were independent of the α2-AMPK, since a similar phenotype was observed in α2-AMPK KO mice

The insulin sensitivity measured by hyperinsulinemic-euglycemic clamp was improved by cHF+F diet rich in n-3 LC-PUFA (Figure 4.1-1). Mice on this diet had better suppression of hepatic glucose production by insulin. The insulin sensitivity of peripheral tissues, assessed by the rate of glucose turnover, was only slightly and non-significantly improved. On the other hand, whole-body glycogen synthesis was ameliorated by cHF+F. The beneficial effects of n-3 LC-PUFA in the prevention of obesity-induced insulin resistance in rodent models are known (44). However, it has been shown for the first time in this study, that n-3 LC-PUFA mediate their beneficial effects on the prevention of obesity-induced insulin resistance in α2-AMPK-dependent manner. First of all, insulin inefficiently suppressed hepatic glucose production in α2-AMPK KO mice fed the cHF+F, while whole-body glycogen synthesis was not improved. The question is what is the mechanism of AMPK action in the beneficial effects of n-3 LC-PUFA? The activation of AMPK in animals fed n-3 LC-PUFA rich diet was demonstrated before [(53); (54)], even though another work argued against the involvement of AMPK in n-3 LC-PUFA effects (59). In our study, the α2-AMPK activity in the liver measured *ex vivo* was increased by cHF+F diet supplemented with n-3 LC-PUFA. Activation of liver AMPK by various treatments, for example by AICAR

(the AMP mimetic) or metformin, led to a suppression of liver gluconeogenesis [(6); (25)]. Thus, the activation of liver AMPK by n-3 LC-PUFA could be responsible for the improved suppression of hepatic glucose production and whole-body insulin sensitivity.

It has been described that liver steatosis is associated with insulin resistance, and that lowering of liver triacylglycerols pools in several rodent models correlated with improved insulin sensitivity (9). Also in this study, improved liver insulin sensitivity in wild-type mice in response to cHF+F was accompanied by decreased liver triacylglycerol levels and by reduced plasma triacylglycerol, NEFA, and cholesterol levels. However, this was not the case in cHF+F-fed α2-AMPK KO mice, which exhibited decreased liver steatosis and plasma lipids but insulin resistance was still present. The question of causal relationship between liver triacylglycerol levels and insulin sensitivity still remains controversial (7). There are several lines of evidence, that diacylglycerols determine the development of insulin resistance (see chapter 1.1.2, (61)). Diacylglycerols activate protein kinase C-ε (PKCε), which in turn inhibits insulin signaling pathways (14). In our study, wild-type mice fed cHF+F diet had decreased levels of liver diacylglycerols, and this was accompanied by improved insulin sensitivity. The effects of cHF+F on both liver diacylglycerol content and liver insulin sensitivity were abolished in α2-AMPK KO mice, thus, n-3 LC-PUFA could improve insulin sensitivity by activating AMPK, which could in turn result in a decrease of liver diacylglycerol levels. How the stimulation of AMPK axis would affect diacylglycerol levels in the liver remains to be elucidated.

The gender differences in several metabolic parameters were observed. For instance, the potency of n-3 LC-PUFA to protect against high-fat diet-induced obesity was lower in female mice, compared to male mice. Interestingly, while the effects of n-3 LC-PUFA to decrease plasma triacylglycerol, NEFA and cholesterol levels in random-fed state were genotype-independent in male mice, in females, the lack of functional α2-AMPK led to a disappearance of these beneficial effects. This study was primarily aimed to study the male mice, and therefore more experiments would be needed to resolve the mechanisms responsible for these gender differences.

4.2 Beneficial effects of DHA derivatives (Study 2)

The efficiency of n-3 LC-PUFA to treat insulin resistance in obese humans is relatively low. We showed that replacement of 1.5% of dietary lipids by various chemical DHA-derivatives affected the development of diet-induced obesity and associated metabolic traits in C57BL/6 mice fed a high-fat diet (57). Substance-2 (α-ethyl DHA ethyl ester) completely prevented and even partially reversed the development of obesity, fat accumulation, impaired glucose tolerance, dyslipidemia and white adipose tissue inflammation.

The assessment of mRNA levels of various metabolic genes revealed some information on the mechanisms of action of these DHA-derivatives, namely Substance-2. The properties of epididymal white adipose tissue (tissue cellularity, the size of adipocytes, macrophage infiltration) were profoundly affected by Substance-2. The expression of CD68 and MCP-1, two factors that are closely linked to the function of macrophages, was decreased by Substance-2. In line with this observation, histological analysis of epididymal white adipose tissue revealed that Substance-2 completely prevented obesity-associated macrophage infiltration of adipose tissue. The reduction of macrophage infiltration should have beneficial systemic effects, since macrophages represent an additional source of pro-inflammatory cytokines, which induce insulin resistance and contribute to a state of chronic low-level inflammation in obesity (62). Similar to the effects of n-3 LC-PUFA, Substance-2 also partially prevented down-regulation of GLUT4 in white adipose tissue, otherwise induced by high-fat diet. In the liver, Substance-2 induced lipid oxidation as documented by the upregulation of PPARα and its target genes AOX1 and CPT1α. Moreover, lipogenic genes such as SCD1 and FDPS were also induced. This simultaneous stimulation of *in situ* lipogenesis and lipid oxidation by Substance 2 in the liver suggests induction of futile substrate cycling, which may be responsible for the reduced accumulation of triacylglycerols in the tissues.

4.3 Additive beneficial effects of combination treatments (Study 3)

Combination of life style changes with pharmacological interventions is required for treatment of diabetes and other metabolic diseases associated with obesity. We showed that long-term treatment combining partial replacement of dietary lipids by n-3 LC-PUFA and a low dose of thiazolidinedione rosiglitazone markedly and in additive manner prevented development of dyslipidemia and insulin resistance, reduced accumulation of body fat and adipocyte hypertrophy, while inducing adiponectin in mice fed a high-fat diet. Importantly, this treatment also reverted impaired glucose tolerance in obese mice.

Hyperinsulinemic-euglycemic clamps in mice showed synergistic induction of glycogen synthesis at the insulin-stimulated conditions by the combination treatment, indicating that skeletal muscle was the major organ responsible for the additive effects of n-3 LC-PUFA and rosiglitazone combination on whole-body glycemic control (Figure 4.3-1) .

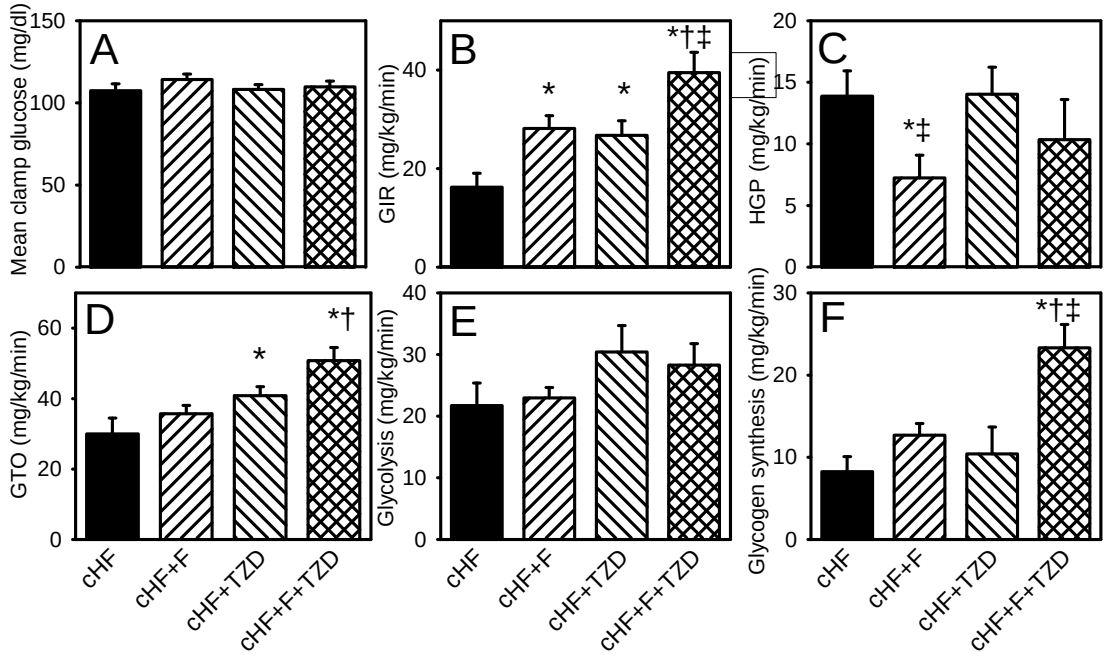

Figure 4.3-1Insulin sensitivity of male mice. Mean clamp glucose (A), glucose infusion rate (GIR) (B), hepatic glucose production (HGP) (C), glucose turn-over rates (GTO) (D), , whole-body glycolysis (E) and glycogen synthesis (F) assessed by hyperinsulinemic-euglycemic clamp in C57BL/6J male mice fed for 8 weeks with control high-fat diet (cHF), high-fat diet, in which 15% of lipids were replaced by n-3 LC-PUFA concentrate (cHF+F), or high-fat diet supplemented with 10 mg/kg diet of rosiglitazone (cHF+TZD) or both, n-3 LC-PUFA and rosiglitazone supplemented (cHF+F+TZD). Data are presented as means ± SE (n = 6-9). *$P < 0.05$ for difference from cHF; †$P < 0.05$ for difference from cHF+F; ‡$P < 0.05$ for difference from cHF+TZD (ANOVA).

Interestingly, and in accordance with the previous studies, neither the treatment by n-3 LC-PUFA [(44); (45)] nor rosiglitazone alone (at the relatively low dose used; (63)) significantly affected the rate of glycogen synthesis. n-3 LC-PUFA but not rosiglitazone were able to depress hepatic glucose production under hyperinsulinemic conditions, suggesting improvement of hepatic insulin sensitivity by the former treatment. Given the ability of n-3 LC-PUFA to prevent development of impaired glucose tolerance, the above data also suggest that the effect on hepatic glucose production may dominate in the effect of n-3 LC-PUFA. On the other hand, the reversal of impaired glucose tolerance and insulin resistance by thiazolidinedione may depend more on the enhancement of insulin action in skeletal muscle. This effect could be mediated either by TZD, or, according to our results, even more potently by the combination of n-3 LC-PUFA and TZD. A putative mechanism behind the synergistic effect of the combination treatment might involve reductions of muscle ceramide content.

The additive improvements of insulin sensitivity correlated with the hypolipidemic effect of the treatments. The suppresion of plasma triacylglycerol levels possibly resulted either from an increased triacylglycerol uptake by muscle and other tissues induced by both TZD (64) and n-3 LC-PUFA (65), or from a decreased hepatic VLDL-triacylglycerol production. The former mechanism has not been studied in our experiments, however, we have demonstrated the inhibition of hepatic VLDL-triacylglycerol production by either n-3 LC-PUFA or rosiglitazone, while the strongest effect was observed in the combination treatment (Figure 4.3-2).

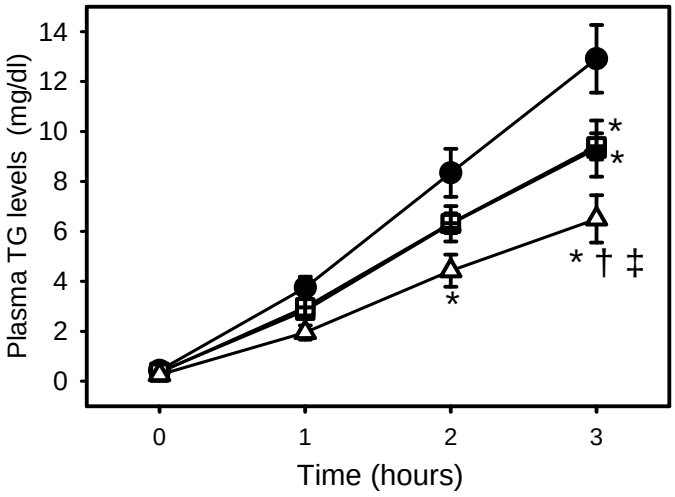

Figure 4.3-2Hepatic VLDL-triacylglycerol production. At 3 months of age, mice were randomly assigned to various diets, and after 8 weeks of treatment, mice were fasted for 6 h, anesthetized, injected with Triton WR1339, and plasma triacylglycerol levels were measured before (time 0), and at 1,2, and 3 hour after injection in the cHF (black circles), cHF+F (empty squares), cHF+TZD (crossed squares), and cHF+F+TZD (empty triangles)-fed mice. Data are presented as means ± SE (n=5-8). *Significantly different from cHF; †significantly different from cHF+F; ‡significantly different from cHF+TZD (ANOVA).

The decrease of hepatic triacylglycerol production by n-3 LC-PUFA may represent a functional outcome of the coordinated suppression of lipogenic genes by n-3 LC-PUFA (66). In addition, a stimulation of AMP-activated protein kinase by n-3 LC-PUFA, resulting in a metabolic switch from lipogenesis to lipid catabolism, may be also involved (53).

In contrast, the mechanism of suppression of VLDL-triacylglycerol formation by rosiglitazone must be different, since rosiglitazone increased both SCD1 expression and triacylglycerol content in the liver. The mechanism may reflect increased rate of fatty acid re-esterification induced by rosiglitazone (67) rather than suppresion of de novo lipogenesis in hepatocytes. These data, in accordance with the effects of the treatments on muscle SCD1, document differential modulation of the genes involved in de novo fatty acid synthesis (FAS) and desaturation (SCD1) by n-3 LC-PUFA and rosiglitazone. That rosiglitazone treatment induced expression and activity of SCD1 in association with insulin sensitization has been observed before (68), but the underlying mechanism remains unknown.

5 CONCLUSIONS

With respect to the specific aims of the thesis, the following conclusions could be drawn:

1. Among the beneficial effects of n-3 LC-PUFA, it is primarily the effect on hepatic insulin sensitivity, which shows a clear α2-AMPK dependency. Moreover, there is an association between the improvement in hepatic insulin sensitivity and hepatic levels of diacylglycerols, but not triacylglycerols. The precise mechanism of AMPK action in the beneficial effects of n-3 LC-PUFA remains to be elucidated.

2. Among the four DHA-derivatives tested, Substance-2 (α-ethyl DHA ethyl ester) appeared to exhibit a similar range of beneficial effects on obesity and associated metabolic disorders as naturally occurring n-3 LC-PUFA, but with a higher efficacy. Therefore, this compound could qualify as a novel drug for the treatment of obesity, dyslipidemia and insulin resistance.

3. Combined use of n-3 LC-PUFA and a low dose rosiglitazone generated additive effects in the prevention as well as reversal of adipose tissue hypertrophy, hyperlipidemia and impaired glycemic control in mice fed an obesogenic diet. Multiple mechanisms underlined the beneficial effects of the combination treatment with a prominent synergistic stimulation of muscle glycogen synthesis in response to insulin. The combined use of n-3 LC-PUFA and thiazolidinedions thus represents a prospective strategy in the treatment of type 2 diabetes and other obesity-associated metabolic disorders. The inclusion of n-3 LC-PUFA in the pharmacological treatment may reduce the dose requirements and the incidence of adverse side-effects associated with the thiazolidinedione-based therapy.

6 REFERENCES

1. Despres,JP, Lemieux,I: Abdominal obesity and metabolic syndrome. *Nature* 444:881-887, 2006
2. Farnier,C, Krief,S, Blache,M, Diot-Dupuy,F, Mory,G, Ferre,P, Bazin,R: Adipocyte functions are modulated by cell size change: potential involvement of an integrin/ERK signalling pathway. *Int.J.Obes.Relat Metab Disord.* 27:1178-1186, 2003
3. Yki-Jarvinen,H: Ectopic fat accumulation: an important cause of insulin resistance in humans. *Journal of the Royal Society of Medicine* 95:39-45, 2002
4. Campbell,PJ, Carlson,MG, Hill,JO, Nurjhan,N: Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification. *Am.J.Physiol* 263:E1063-E1069, 1992
5. Monteiro,R, de Castro,PMST, Calhau,C, Azevedo,I: Adipocyte size and liability to cell death. *Obesity Surgery* 16:804-806, 2006
6. Tilg,H, Moschen,AR: Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat.Rev.Immunol.* 6:772-783, 2006
7. Postic,C, Girard,J: Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J.Clin.Invest* 118:829-838, 2008
8. Sakurai,M, Takamura,T, Ota,T, Ando,H, Akahori,H, Kaji,K, Sasaki,M, Nakanuma,Y, Miura,K, Kaneko,S: Liver steatosis, but not fibrosis, is associated with insulin resistance in nonalcoholic fatty liver disease. *Journal of Gastroenterology* 42:312-317, 2007
9. Savage,DB, Choi,CS, Samuel,VT, Liu,ZX, Zhang,DY, Wang,A, Zhang,XM, Cline,GW, Yu,XX, Geisler,JG, Bhanot,S, Monia,BP, Shulman,GI: Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. *J.Clin.Invest.* 116:817-824, 2006
10. Petersen,KF, Dufour,S, Befroy,D, Lehrke,M, Hendler,RE, Shulman,GI: Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* 54:603-608, 2005
11. Dentin,R, Benhamed,F, Pegorier,JP, Foufelle,F, Viollet,B, Vaulont,S, Girard,J, Postic,C: Polyunsaturated fatty acids suppress glycolytic and lipogenic genes through the inhibition of ChREBP nuclear protein translocation. *J.Clin.Invest* 115:2843-2854, 2005
12. Monetti,M, Levin,MC, Watt,MJ, Sajan,MP, Marmor,S, Hubbard,BK, Stevens,RD, Bain,JR, Newgard,CB, Farese,RV, Hevener,AL, Farese,RV: Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver. *Cell Metabolism* 6:69-78, 2007
13. Listenberger,LL, Han,XL, Lewis,SE, Cases,S, Farese,RV, Ory,DS, Schaffer,JE: Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proceedings of the National Academy of Sciences of the United States of America* 100:3077-3082, 2003

14. Samuel,VT, Liu,ZX, Wang,A, Beddow,SA, Geisler,JG, Kahn,M, Zhang,XM, Monia,BP, Bhanot,S, Shulman,GI: Inhibition of protein kinase C epsilon prevents hepatic insulin resistance in nonalcoholic fatty liver disease. *J.Clin.Invest.* 117:739-745, 2007
15. Koves,TR, Ussher,JR, Noland,RC, Slentz,D, Mosedale,M, Ilkayeva,O, Bain,J, Stevens,R, Dyck,JR, Newgard,CB, Lopaschuk,GD, Muoio,DM: Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. *Cell Metab* 7:45-56, 2008
16. Kelley,DE: Skeletal muscle fat oxidation: timing and flexibility are everything. *J Clin.Invest* 115:1699-1702, 2005
17. Golay,A, Munger,R, Assimacopoulos-Jeannet,F, Bobbioni-Harsch,E, Habicht,F, Felber,JP: Progressive defect of insulin action on glycogen synthase in obesity and diabetes. *Metabolism-Clinical and Experimental* 51:549-553, 2002
18. Woods,A, Cheung,PCF, Smith,FC, Davison,MD, Scott,J, Beri,RK, Carling,D: Characterization of AMP-activated protein kinase beta and gamma subunits - Assembly of the heterotrimeric complex in vitro. *J.Biol.Chem.* 271:10282-10290, 1996
19. Cheung,PC, Salt,IP, Davies,SP, Hardie,DG, Carling,D: Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. *Biochem.J.* 346 Pt 3:659-669, 2000
20. Lihn,AS, Jessen,N, Pedersen,SB, Lund,S, Richelsen,B: AICAR stimulates adiponectin and inhibits cytokines in adipose tissue. *Biochem.Biophys.Res.Commun.* 316:853-858, 2004
21. Stapleton,D, Mitchelhill,KI, Gao,G, Widmer,J, Michell,BJ, Teh,T, House,CM, Fernandez,CS, Cox,T, Witters,LA, Kemp,BE: Mammalian AMP-activated protein kinase subfamily. *J.Biol.Chem.* 271:611-614, 1996
22. Shaw,RJ, Lamia,KA, Vasquez,D, Koo,SH, Bardeesy,N, DePinho,RA, Montminy,M, Cantley,LC: The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science* 310:1642-1646, 2005
23. Hawley,SA, Pan,DA, Mustard,KJ, Ross,L, Bain,J, Edelman,AM, Frenguelli,BG, Hardie,DG: Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab* 2:9-19, 2005
24. 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, Kadowaki,T: Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat.Med.* 8:1288-1295, 2002
25. Zhou,G, Myers,R, Li,Y, Chen,Y, Shen,X, Fenyk-Melody,J, Wu,M, Ventre,J, Doebber,T, Fujii,N, Musi,N, Hirshman,MF, Goodyear,LJ, Moller,DE: Role of AMP-activated protein kinase in mechanism of metformin action. *J.Clin.Invest* 108:1167-1174, 2001
26. Foretz,M, Ancellin,N, Andreelli,F, Saintillan,Y, Grondin,P, Kahn,A, Thorens,B, Vaulont,S, Viollet,B: Short-term overexpression of a constitutively active form of AMP-activated protein kinase in the liver leads to mild hypoglycemia and fatty liver. *Diabetes* 54:1331-1339, 2005

27. Velasco,G, Geelen,MJH, Guzman,M: Control of hepatic fatty acid oxidation by 5'-AMP-activated protein kinase involves a malonyl-CoA-dependent and a malonyl-CoA-independent mechanism. *Arch.Biochem.Biophys.* 337:169-175, 1997
28. Andreelli,F, Foretz,M, Knauf,C, Cani,PD, Perrin,C, Iglesias,MA, Pillot,B, Bado,A, Tronche,F, Mithieux,G, Vaulont,S, Burcelin,R, Viollet,B: Liver AMPKalpha2 catalytic subunit is a key target for the control of hepatic glucose production by adiponectin and leptin but not by insulin. *Endocrinology* 147:2432-2441, 2006
29. Daval,M, Diot-Dupuy,F, Bazin,R, Hainault,I, Viollet,B, Vaulont,S, Hajduch,E, Ferre,P, Foufelle,F: Anti-lipolytic action of AMP-activated protein kinase in rodent adipocytes. *J Biol.Chem.* 280:25250-25257, 2005
30. Wu,X, Motoshima,H, Mahadev,K, Stalker,TJ, Scalia,R, Goldstein,BJ: Involvement of AMP-Activated Protein Kinase in Glucose Uptake Stimulated by the Globular Domain of Adiponectin in Primary Rat Adipocytes. *Diabetes* 52:1355-1363, 2003
31. Merrill,GF, Kurth,EJ, Hardie,DG, Winder,WW: AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. *American Journal of Physiology-Endocrinology and Metabolism* 36:E1107-E1112, 1997
32. Breen,DM, Sanli,T, Giacca,A, Tsiani,E: Stimulation of muscle cell glucose uptake by resveratrol through sirtuins and AMPK. *Biochem.Biophys.Res.Commun.* 374:117-122, 2008
33. Bergeron,R, Previs,SF, Cline,GW, Perret,P, Russell,RR, Young,LH, Shulman,GI: Effect of 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside infusion on in vivo glucose and lipid metabolism in lean and obese Zucker rats. *Diabetes* 50:1076-1082, 2001
34. Boon,H, Bosselaar,M, Praet,SF, Blaak,EE, Saris,WH, Wagenmakers,AJ, McGee,SL, Tack,CJ, Smits,P, Hargreaves,M, van Loon,LJ: Intravenous AICAR administration reduces hepatic glucose output and inhibits whole body lipolysis in type 2 diabetic patients. *Diabetologia* 51:1893-1900, 2008
35. Nestel,PJ, Connor,WE, Reardon,MF, Connor,S, Wong,S, Boston,R: Suppression by Diets Rich in Fish Oil of Very Low-Density Lipoprotein Production in Man. *J.Clin.Invest.* 74:82-89, 1984
36. Ruxton,CH, Reed,SC, Simpson Double Dagger,MJ, Millington,KJ: The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *J Hum.Nutr.Diet.* 17:449-459, 2004
37. Mori,TA, Bao,DQ, Burke,V, Puddey,IB, Watts,GF, Beilin,LJ: Dietary fish as a major component of a weight-loss diet: effect on serum lipids, glucose, and insulin metabolism in overweight hypertensive subjects. *Am.J.Clin.Nutr.* 70:817-825, 1999
38. Couet,C, Delarue,J, Ritz,P, Antoine,J-M, Lamisse,F: Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. *Int.J Obes.* 21:637-643, 1997

39. Fasching,P, Ratheiser,K, Waldhausl,W, Rohac,M, Osterrode,W, Nowotny,P, Vierhapper,H: Metabolic effects of fish-oil supplementation in patients with impaired glucose tolerance. *Diabetes* 40:583-589, 1991
40. Pelikanova,T, Kohout,M, Valek,J, Kazdova,L, Base,J: Metabolic effects of omega-3 fatty acids in type 2 (non-insulin-dependent) diabetic patients. *Ann.N.Y.Acad.Sci.* 683:272-278, 1993
41. Ikemoto,S, Takahashi,M, Tsunoda,N, Maruyama,K, Itakura,H, Ezaki,O: High-fat diet-induced hyperglycemia and obesity in mice: Differential effects of dietary oils. *Metabolism* 45:1539-1546, 1996
42. Ruzickova,J, Rossmeisl,M, Prazak,T, Flachs,P, Sponarova,J, Vecka,M, Tvrzicka,E, Bryhn,M, 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
43. 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, Kopecky,J: Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce beta-oxidation in white fat. *Diabetologia* 48:2365-2375, 2005
44. Storlien,LH, Kraegen,EW, Chisholm,DJ, Ford,GL, Bruce,DG, Pascoe,WS: Fish oil prevents insulin resistance induced by high-fat feeding in rats. *Science* 237:885-888, 1987
45. Neschen,S, Morino,K, Dong,J, Wang-Fischer,Y, Cline,GW, Romanelli,AJ, Rossbacher,JC, Moore,IK, Regittnig,W, Munoz,DS, Kim,JH, Shulman,GI: N-3 Fatty Acids Preserve Insulin Sensitivity In Vivo in a PPAR{alpha}-Dependent Manner. *Diabetes* 56:1034-1041, 2007
46. Tsuchida,A, Yamauchi,T, Takekawa,S, Hada,Y, Ito,Y, Maki,T, Kadowaki,T: Peroxisome proliferator-activated receptor (PPAR)alpha activation increases adiponectin receptors and reduces obesity-related inflammation in adipose tissue: comparison of activation of PPARalpha, PPARgamma, and their combination. *Diabetes* 54:3358-3370, 2005
47. Xu,J, Teran-Garcia,M, Park,JHY, Nakamura,MT, Clarke,SD: Polyunsaturated fatty acids suppress hepatic sterol regulatory element-binding protein-1 expression by accelerating transcript decay. *J.Biol.Chem.* 276:9800-9807, 2001
48. Wilson,MD, Blake,WL, Salati,LM, Clarke,SD: Potency of Polyunsaturated and Saturated Fats As Short-Term Inhibitors of Hepatic Lipogenesis in Rats. *Journal of Nutrition* 120:544-552, 1990
49. 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 high-fat diet. *Diabetologia* 49:394-397, 2006
50. Toruner,F, Akbay,E, Cakir,N, Sancak,B, Elbeg,S, Taneri,F, Akturk,M, Karakoc,A, Ayvaz,G, Arslan,M: Effects of PPARgamma and PPARalpha agonists on serum leptin levels in diet-induced obese rats. *Horm.Metab Res.* 36:226-230, 2004
51. Peyron-Caso,E, Fluteau-Nadler,S, Kabir,M, Guerre-Millo,M, Quignard-Boulange,A, Slama,G, Rizkalla,SW: Regulation of glucose transport and transporter 4 (GLUT-4) in muscle and adipocytes of sucrose-fed rats: effects of N-3 poly- and monounsaturated fatty acids. *Horm.Metab Res.* 34:360-366, 2002

52. Serhan,CN: Novel omega -- 3-derived local mediators in anti-inflammation and resolution. *Pharmacol.Ther.* 105:7-21, 2005
53. Suchankova,G, Tekle,M, Saha,AK, Ruderman,NB, Clarke,SD, Gettys,TW: Dietary polyunsaturated fatty acids enhance hepatic AMP-activated protein kinase activity in rats. *Biochem.Biophys.Res.Commun.* 326:851-858, 2005
54. Gabler,NK, Radcliffe,JS, Spencer,JD, Webel,DM, Spurlock,ME: Feeding long-chain n-3 polyunsaturated fatty acids during gestation increases intestinal glucose absorption potentially via the acute activation of AMPK. *J.Nutr.Biochem.* 20:17-25, 2009
55. Gonzalez-Periz,A, Horrillo,R, Ferre,N, Gronert,K, Dong,B, Moran-Salvador,E, Titos,E, Martinez-Clemente,M, Lopez-Parra,M, Arroyo,V, Claria,J: Obesity-induced insulin resistance and hepatic steatosis are alleviated by {omega}-3 fatty acids: a role for resolvins and protectins. *FASEB J.* 2009
56. Kopecky,J, Rossmeisl,M, Flachs,P, Kuda,O, Brauner,P, Jilkova,Z, Stankova,B, Tvrzicka,E, Bryhn,M: n-3 PUFA: bioavailability and modulation of adipose tissue function. *Proc.Nutr.Soc.*1-9, 2009
57. 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 (Silver.Spring)* 2009
58. 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* 2009
59. Yokota,T, Oritani,K, Takahashi,I, Ishikawa,J, Matsuyama,A, Ouchi,N, Kihara,S, Funahashi,T, Tenner,AJ, Tomiyama,Y, Matsuzawa,Y: Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 96:1723-1732, 2000
60. Lochhead,PA, Salt,IP, Walker,KS, Hardie,DG, Sutherland,C: 5-aminoimidazole-4-carboxamide riboside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes PEPCK and glucose-6- phosphatase. *Diabetes* 49:896-903, 2000
61. Neschen,S, Morino,K, Hammond,LE, Zhang,DY, Liu,ZX, Romanelli,AJ, Cline,GW, Pongratz,RL, Zhang,XM, Choi,CS, Coleman,RA, Shulman,GI: Prevention of hepatic steatosis and hepatic insulin resistance in mitochondrial acyl-CoA : glycerol-sn-3-phosphate acyltransferase 1 knockout mice. *Cell Metabolism* 2:55-65, 2005
62. Wellen,KE, Hotamisligil,GS: Inflammation, stress, and diabetes. *J Clin Invest* 115:1111-1119, 2005
63. Kim,JK, Fillmore,JJ, Gavrilova,O, Chao,L, Higashimori,T, Choi,H, Kim,HJ, Yu,C, Chen,Y, Qu,X, Haluzik,M, Reitman,ML, Shulman,GI: Differential effects of rosiglitazone on skeletal muscle and liver insulin resistance in A-ZIP/F-1 fatless mice. *Diabetes* 52:1311-1318, 2003

64. Laplante,M, Festuccia,WT, Soucy,G, Blanchard,PG, Renaud,A, Berger,JP, Olivecrona,G, Deshaies,Y: Tissue-specific postprandial clearance is the major determinant of PPAR{gamma}-induced triglyceride lowering in the rat. *Am.J.Physiol Regul.Integr.Comp Physiol* 2008
65. Ton,MN, Chang,C, Carpentier,YA, Deckelbaum,RJ: In vivo and in vitro properties of an intravenous lipid emulsion containing only medium chain and fish oil triglycerides. *Clin.Nutr.* 24:492-501, 2005
66. Teran-Garcia,M, Adamson,AW, Yu,G, Rufo,C, Suchankova,G, Dreesen,TD, Tekle,M, Clarke,SD, Gettys,TW: Polyunsaturated fatty acid suppression of fatty acid synthase (FASN): evidence for dietary modulation of NF-Y binding to the Fasn promoter by SREBP-1c. *Biochem.J* 402:591-600, 2007
67. Gibbons,GF, Wiggins,D, Brown,AM, Hebbachi,AM: Synthesis and function of hepatic very-low-density lipoprotein. *Biochem.Soc.Trans.* 32:59-64, 2004
68. Riserus,U, Tan,GD, Fielding,BA, Neville,MJ, Currie,J, Savage,DB, Chatterjee,VK, Frayn,KN, O'Rahilly,S, Karpe,F: Rosiglitazone increases indexes of stearoyl-CoA desaturase activity in humans: link to insulin sensitization and the role of dominant-negative mutation in peroxisome proliferator-activated receptor-gamma. *Diabetes* 54:1379-1384, 2005

7 LIST OF PUBLICATIONS

7.1 Publications related to the thesis

Jelenik T, Rossmeisl M, Kuda O, Macek Jilkova Z, 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 (accepted in July 2010), (IF = 8,26)

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.
Journal of physiology and pharmacology 60(4): 135-40, 2009, (IF = 4,47)

Kuda O, **Jelenik T,** Jilkova Z, Flachs Z, Rossmeisl M, Hensler M, Kazdova L, Ogston N, Baranowski M, Gorski J, Janovska P, 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 high-fat diet.
Diabetologia 52: 941-951, 2009, (IF = 6,328)

Rossmeisl M, **Jelenik T,** Jilkova Z, Slamova K, Kus V, Hensler M, Medrikova D, Povysil C, Flachs P, Mohamed-Ali V, Bryhn M, Berge Kristin Holmeide K, Kopecky J.
DHA-derivatives in the prevention and reversal of obesity and glucose intolerance in mice.
Obesity 17: 1023–1031, 2009, (IF = 3,24)

7.2 Publications unrelated to the thesis

Jelenik T, Sabatkova Z, Demnerova K, Pazlarova J.
Two rapid diagnostic procedures for the identification of Campylobacter jejuni/coli in food matrix.
Czech Journal of Food Sciences 23:121-125, 2005, (IF = 0,472)