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Determination of Desaturase and Elongase Activity in Plasma of Diabetics

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Abstract

The thesis deals with the relationship among enzymes of lipid metabolism, fatty acids and progression of type 2 *diabetes mellitus*. The progression of diabetes was determined by the level of glycosidic haemoglobin. The ultracentrifugation and two chromatographic methods were used to determine plasma concentration of fatty acids. The separation of total lipids into individual classes was achieved by thin layer chromatohraphy. The amount of fatty acids in these classes was determined after derivatization using gas chromatograph Agilent Technologies 7890 GC System with a flame ionization detector. Lipid enzyme activity was expressed as indices. The results were statistically evaluated in relation to diabetes compensation. Along with the lipid metabolism differences in the disease, also the difference in fatty acid profile of individual fractions was observed. The detailed description of these changes is described below. The importance of this work is underlined by the fact that there is nearly one million cases of type 2 diabetes in the Czech Republic. The main benefit of the research focused on effects of individual fatty acids and lipid metabolism enzymes ?? is more effective dietary recommendation and maintenance of diabetes compensation in treated patients.

Abstrakt

Předložená disertační práce se zabývá vztahy mezi enzymy lipidového metabolismu, mastnými kyselinami a progresí onemocnění diabetes mellitus 2. typu. Progrese diabetu byla určována úrovní hladiny glykovaného hemoglobinu. Pro zjištění koncentrací mastných kyselin v plazmě byla použita kombinace ultracentrifugace a dvou chromatografických metod. První byla tenkovrstevná chromatografie, pomocí které byly lipidy rozděleny do jednotlivých tříd. Podíl mastných kyselin v těchto třídách byl po derivatizaci zjišťován na plynovém chromatografu Agilent Technologies 7890A GC System s plamenově ionizačním detektorem. Aktivita lipidických enzymů byla stanovena výpočtem jako indexy. Statisticky zpracované výsledky byly vyhodnoceny ve vztahu ke kompenzaci diabetu. Se změněným lipidovým metabolismem u tohoto onemocnění byly pozorovány i diference v profilu mastných kyselin v jednotlivých frakcích. Podrobnějšímu popisu těchto změn se věnuje následující práce. Význam této práce podtrhuje fakt, že jen v České republice máme něco pod milion diabetiků typu 2. Přínos ve zkoumání vlivu jednotlivých mastných kyselin a enzymů lipidového metabolismu dle mého názoru leží hlavně v efektivnějších dietetických doporučeních a udržení kompenzace diabetu u léčených pacientů.

Keywords

diabetes, lipids, desaturase, elongase, gas chromatography, thin layer chromatography

Klíčová slova

diabetes, lipidy, desaturázy, elongáza, plynová chromatografie, tenkovrstvá chromatografie

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Introduction

Diabetes mellitus is one of the problems of modern world that grows significantly with the rise of civilization and the „western“ way of life. This disease interferes in many spheres of our being, whether medical, economical or social.

Type 2 diabetes mellitus, also known as non-insulin dependent diabetes mellitus, is a metabolic disease that usually develops in adulthood. It is related to obesity, decreased physical activity, unhealthy diets and western lifestyle in general. It is caused by a reduced sensitivity of the body's own tissues to insulin, pancreatic hormone, which is the main factor that affects carbohydrate metabolism. The insulin is produced in beta islet cells. If there is a higher glucose level in a blood, the pancreas secretes more and more insulin and eventually overburdened beta cells will atrophy.

Although the diabetes is identified as civilization disease, the first mention of it appeared more than three and a half thousand years ago in Ancient Egypt. The term diabetes was first used by Aretaeus of Cappadocia at around the begining of our era.

The main problem of the diabetes mellitus is its unsatisfactorily compensated form. Diabetes mellitus is the most commonn cause of kidney failure and blindness and it is very strong risk factor for atherosclerosis development (if it has not been already developed before). What is more, with the exception of atherosclerosis, diabetes mellitus is the most common cause of lower limbs amputation in the Czech Republic. An early diagnosis and disciplined patients can contribute in elimination, or at least significant mitigation of all these adverse effects.

In diabetic patients, not only increased glucose levels or increased glycosidic haemoglobin can be found, but also changes in concentration of other different substances (like lipids, amino acids and enzymes) is notable The main interest of this work is the concentration of fatty acids and activity of lipidic enzymes (desaturases and elongases). Their activity plays a key role in the biosynthesis of unsaturated higher fatty acids.

The relationship among fatty acids, glycosidic haemoglobin and enzyme activities was investigated.

Although a mass significant improvement of the population lifestyle is not expected, the results of this research could be used in the prevention, diagnosis and treatment of civilization diseases, diabetes mellitus including.

1 Definition of thesis objectives

The aim of the work was to extend the knowledge about fatty acids and enzymes of lipid metabolism in the clinical picture of patients with type 2 diabetes, to focus on the less studied areas of this problematics, like branched chain fatty acids and HDL lipoproteins, and to search for significant associations with the progression of diabetes, expressed as glycosidic haemoglobin level.

2 Theoretical part

2.1 Type 2 diabetes

A non-insulin dependent diabetes mellitus (NIDDM) is a metabolic disease, occurrence of which is still increasing in the Central European population. This disease is characterized not only by increased glucose and glycosidic haemoglobin (HbA1c) level but also by disruption of lipid metabolism with all its inevitable consequences (Flowers, 2009).
Dietary fatty acids (FA) are partly reflected by the FA profile of various biological media including adipose tissue (which reflects the intake of past months to years), erythrocyte membranes, and plasma or serum (which indicates the intake during several weeks). However, the FA profile in biological tissues is also strongly dependent on the endogenous metabolism of FAs. It has been shown that the suppression of the activity of the regulatory enzymes by experimental diabetes provoked an alteration in the FA composition of phospholipids in different tissues (Lands, 1995).
Dyslipidemia of NIDDM patients may be characterized by decreased content of plasma n-3 and n-6 polyunsaturated fatty acids (PUFA) and increased content of cytotoxic saturated fatty acids (SFA) that enhance insulin resistance, causing disappearance of pancreatic β-cells, and also have proinflammatory effects (Eitel et al., 2002; Laaksonen et al., 2002; Maedler et al., 2003; Němcová-Fürstová et al., 2011). N-3 PUFA helps to prevent glucose intolerance and have anti-inflammatory properties (Nanri et al., 2011; Wu et al., 2012).

2.2 Branched chain fatty acids

Information about intake, significance and metabolism of isomeric saturated fatty acids (branched chain fatty acids - BCFA) are inadequate, as well as details about their favorable or adverse influence on the human organism (Ran-Ressler et al., 2008). One of the few published studies, working with rats, suggests that the increased concentration of isomeric saturated fatty acids in the gastrointestinal tract of unborn offspring positively correlates with decreased incidence of necrotizing enterocolitis (a disease affecting premature babies) (Ran-Ressler et al., 20011a). There is increasing promotion of healthy lifestyle and consumption of dairy products that contain relatively high amount of BCFA. Ran-Resseler (Ran-Ressler et al., 20011b) describes the content of these isomeric fatty acids in milk, supplied by American supermarkets, reaches up to 2 %.
It is known that straight-chain fatty acids are degraded only in the mitochondrial matrix, while the isomeric saturated fatty acids and very long-chain fatty acids are firstly oxidized in peroxisomes and only then transported as acylcoenzyme A to mitochondria (Mukherji et al., 2003). This process is controlled by peroxisome proliferator-activated receptors (PPAR), which represent the nuclear factors regulating production of lipogenic enzymes, which are necessary for implementing the aforesaid both peroxisome and mitochondrial fatty acid oxidation. There are several kinds of PPAR receptors. In humans, PPARα are expressed especially in the liver, skeletal muscles, kidney and vascular endothelium, wherein regulate the expression of many

genes and these genes encode enzymes participating on the peroxisome proliferation in fatty acid oxidation in peroxisomes and mitochondria (Memon et al., 2001). PPARβ / δ were found in various tissues, but information about them is still limited; some authors (Michalík et al., 2003) suggests that they also interfere with lipid metabolism – these PPAR lower serum triglyceride concentration by increase of VLDL (very low-density lipoproteins) elimination. PPARγ is the best-studied group of receptors yet, the most prominent in adipose tissue, where occur both two isoforms - PPARγ1 and PPARγ2, in smaller quantities are expressed in the spleen, cells of the hematopoietic system and skeletal muscles, where it is represented mainly by PPARγ1 isoform (Kawaguchi et al., 2003; Spiegelman, 1998). Activation of PPAR is preferably implemented by polyunsaturated and essential fatty acids, while saturated fatty acids are less effective (Holeček, 2006).
Effect of isomeric saturated fatty acids on PPAR have not been studied yet.

2.3 HDL lipoproteins

HDL (high-density lipoproteins) particles allow the transfer of non-esterified cholesterol to the liver in two ways: directly via HDL_2 and indirectly via other lipoproteins like intermediate density lipoproteins and chylomicron residues to which cholesterol is transferred from HDL_2 by CETP (cholesterol ester transfer protein). High levels of oxidized fatty acids in HDL are associated with impaired HDL function especially in patients with rheumatoid arthritis (Charles-Schoeman et al., 2018; Holeček, 2006).
HDL are important especially for pre-diabetics and type 2 diabetics. Low HDL-cholesterol and high triglycerides might contribute to sustain the abnormalities in insulin secretion that characterize the pre-diabetic state (Natali et al., 2017).

3 Processing methodology

3.1 Chromatographic separation

Before lipid separation on the thin layer chromatography proteins had to be removed from samples. To the centrifuge tube with 500 µL of human plasma or serum (5 tubes for samples and one for standard) 2.5 mL of reagent (isopropyl alcohol, n-heptane and phosphoric acid in the ratio 40:20:1) was added. The denaturation of proteins in samples was caused in this way. The resulting mixture was stirred using a vortex mixer and was conditioned at room temperature for 10 minutes. To the content in the test tube 1 mL of methanol-toluene mixture (1:4) and 1.5 mL of distilled water was added. The resulting mixture was centrifuged 10 minutes at 3 000 rpm. The top organic layer was moved into a clean tube and evaporated under nitrogen atmosphere to a volume about 30 µL. Thus prepared samples were applied to glass chromatography plate covered with silica gel and divided into five fractions: phospholipids (PL), diglycerides (DG), free fatty acids (FFA), triglycerides (TG) and cholesterol esters (CE). Preparation of plate: A starting line was drawn 2.5 cm from the bottom of the plate. The other margins were 1 cm. Internal field of the board was split into 6 lines, each 3 cm wide. Mobile phase was prepared by mixing 160 mL of hexane, 40 mL of diethyl ether and 6 mL of acetic acid, 98 %. Chromatography took about one hour, untill the head reached the finish line at the top of the plate. On the dried plate individual fractions in „standard“ line were visualized using 2,7-dichlorofluorescein (0.2 g of 2,7-dichlorofluorescein in 100 mL of isopropyl alcohol). After drying individual fractions were visible under UV light (240 nm). Then all prepared fractions were moved to tubes with teflon sealed caps.

$$R_F = \frac{\text{migration distance of solvent front}}{\text{migration distance of substance}}$$

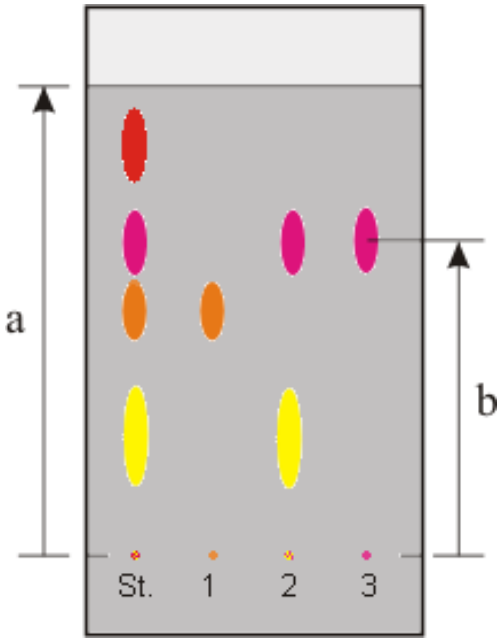

Figure 1: Thin layer chromatography – representation of retardation factor
Labels: a – migration distance of solvent front, b – migration distance of substance

3.2 Derivatization of fatty acids

All fractions were isolated and, after the addition of internal standard (cis-13,16,19-docosatrienoic acid, 10 μg/mL) and methanol-toluene mixture (1:4), thus prepared samples were hydrolyzed and converted to the corresponding methyl esters of fatty acids. This was carried out by adding 200 μL of acetyl chloride and heating to 100 °C for one hour. After cooling to laboratory temperature, the excess of acetyl chloride was neutralized by adding 5 mL of potassium carbonate solution, 6 %. After addition of carbonate solution, the tube was intensively stirred for two minutes and subsequently centrifuged at 3 000 rpm for ten minutes. The top organic layer was moved into a clean tube and evaporated under nitrogen atmosphere in a volume about 100 μL. The solution containing the preconcentrated lipid fractions was transferred to vials with chromatographic separation attachment, which were carefully sealed with a rubber septum cap.

3.3 Chromatographic analysis

Methyl esters of fatty acids were separated using HP-88 column. The volume of sample injected was 1 μL (split 1:10). Injector temperature was 250 °C, flame ionization detector temperature was 280 °C. Column temperature program was as follows: initial temperature of 130 °C for 1 min → gradient 2 °C/min to 176 °C hold for 2 min → gradient 1 °C/min to 186 °C hold for 1 min → gradient 0,1 °C/min to 190 °C hold for 1 min → gradient 1 °C/min to 220 °C hold for 4 min. Total analysis time took 92 minutes. The internal standard calibration curve was used for the quantification of individual free fatty acids. We were able to determine 39 fatty acids.

3.4 Calculation of enzyme activity indices

The 16:1n7/16:0 ratio was calculated as indice of stearoyl-CoA desaturase 1, i.e. 9-desaturase (9D) activity. Furthermore, the 20:4n6/20:3n6 and 20:3n6/18:2n6 ratios were calculated as indices of 5-desaturase (5D) and 6-desaturase (6D) activities, respectively. The ratio of 18:0 to 16:0 was calculated as an elongase activity indice.

3.5 Statistical analysis

Obtained chromatograms were subsequently integrated using GC ChemStation B04.03 software and gained data were statistically processed using MS Excel 2007, SigmaStat 3.5 and Statistica 12.0. Differences in variables between the groups were evaluated using Mann Whitney test. P value less than 0.05 was considered statistically significant.

4 Results

4.1 Relationships between glycosidic haemoglobin and fatty acid profile

4.1.1 Polyunsaturated fatty acids

Total PUFA were markedly decreased both in PL ($p = 0.033$) and CE ($p = 0.038$) fractions of type 2 diabetics (T2D) group compared to the control group, predominantly due to down-regulation of n-6 PUFA content.
Significantly lower sum of n-6 PUFA was found in T2D patients in comparison with the control group in PL ($p = 0.006$) and CE ($p = 0.030$) fractions. The difference was caused mainly by linoleic acid, which was significantly lower in PL ($p = 0.016$) and CE ($p = 0.029$) fractions in diabetic group. Index n6/n3 was lower in PL fraction ($p = 0.022$) of T2D group. No correlations were found between PUFA and HbA1c.

4.1.2 Saturated fatty acids

Lower value of SFA sum was found in FFA fraction in T2D patients ($p < 0.001$) compared to the controls. The most significant decrease was observed in case of stearic acid ($p < 0.001$) and lignoceric acid ($p < 0.001$).
The HbA1c positively correlated with palmitic acid ($r = 0.68$, $p< 0.001$) in TG and ($r = 0.40$, $p = 0.040$) in PL.

4.1.3 Monounsaturated fatty acids

Sum of monounsaturated fatty acids (MUFA) was markedly higher in diabetic group in FFA fraction ($p < 0.001$), mainly due to oleic acid ($p < 0.001$) and vaccenic acid ($p = 0.010$).
In case of MUFA (Fig. 2 F), our results revealed a negative correlation of oleic acid ($r = -0.46$, $p = 0.020$) with HbA1c in TG fraction.

4.1.4 Desaturases and elongases indices

Activity of 9 desaturation (9D) index, characterized by the ratio of 16:1 n7 to 16:0, was markedly lower in T2D patients ($p = 0.005$) in comparison with the control group in CE fraction; 6 desaturation (6D) index, characterized by the ratio of 20:3 n6 to 18:2 n6, was markedly higher in T2D patients ($p = 0.014$) compared to the control subjects. Elongation index (characterized by the ratio of 18:0 to 16:0) was in FFA fraction significantly lower in T2D patients ($p < 0.001$) compared to the control group. In PL fraction, desaturation and elongation indices were higher in T2D patients, but the difference did not reach the statistical significance.
Further we focused on relationships between enzyme activities and glycosidic haemoglobin as marker of compensation of diabetes. Negative correlation was found in case of 9D index in PL fraction ($r = -0.45$, $p = 0.020$) as well as in TG fraction ($r = 0.54$, $p = 0.004$).

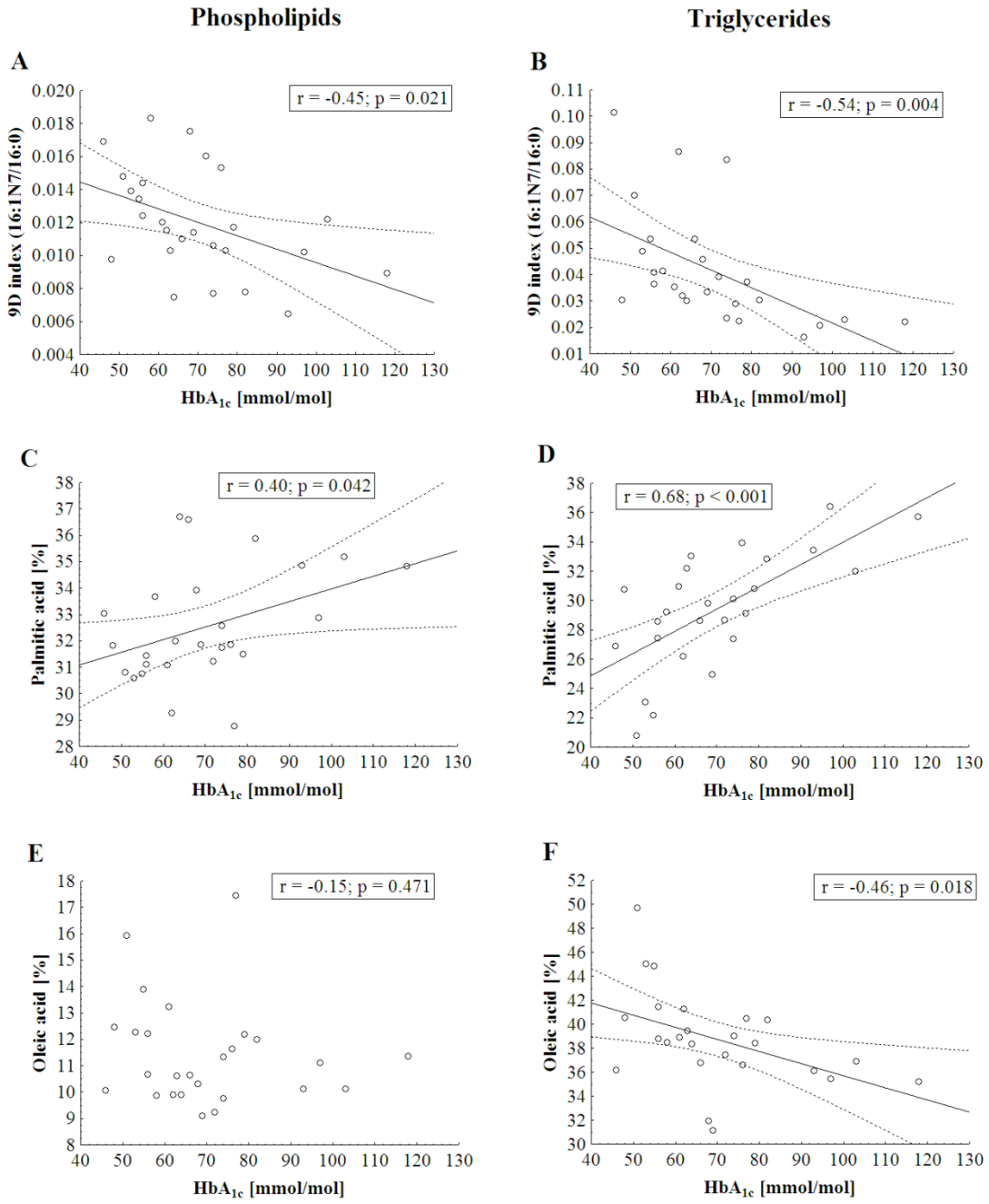

Figure 2: Cross-sectional relationships of glycosidic haemoglobin values with A) 9D index in PL fraction, B) 9D index in TG fraction, C) percentage of palmitic acid in PL fraction, D) percentage of palmitic acid in TG fraction, E) percentage of oleic acid in PL fraction, F) percentage of oleic acid in TG fraction in T2D

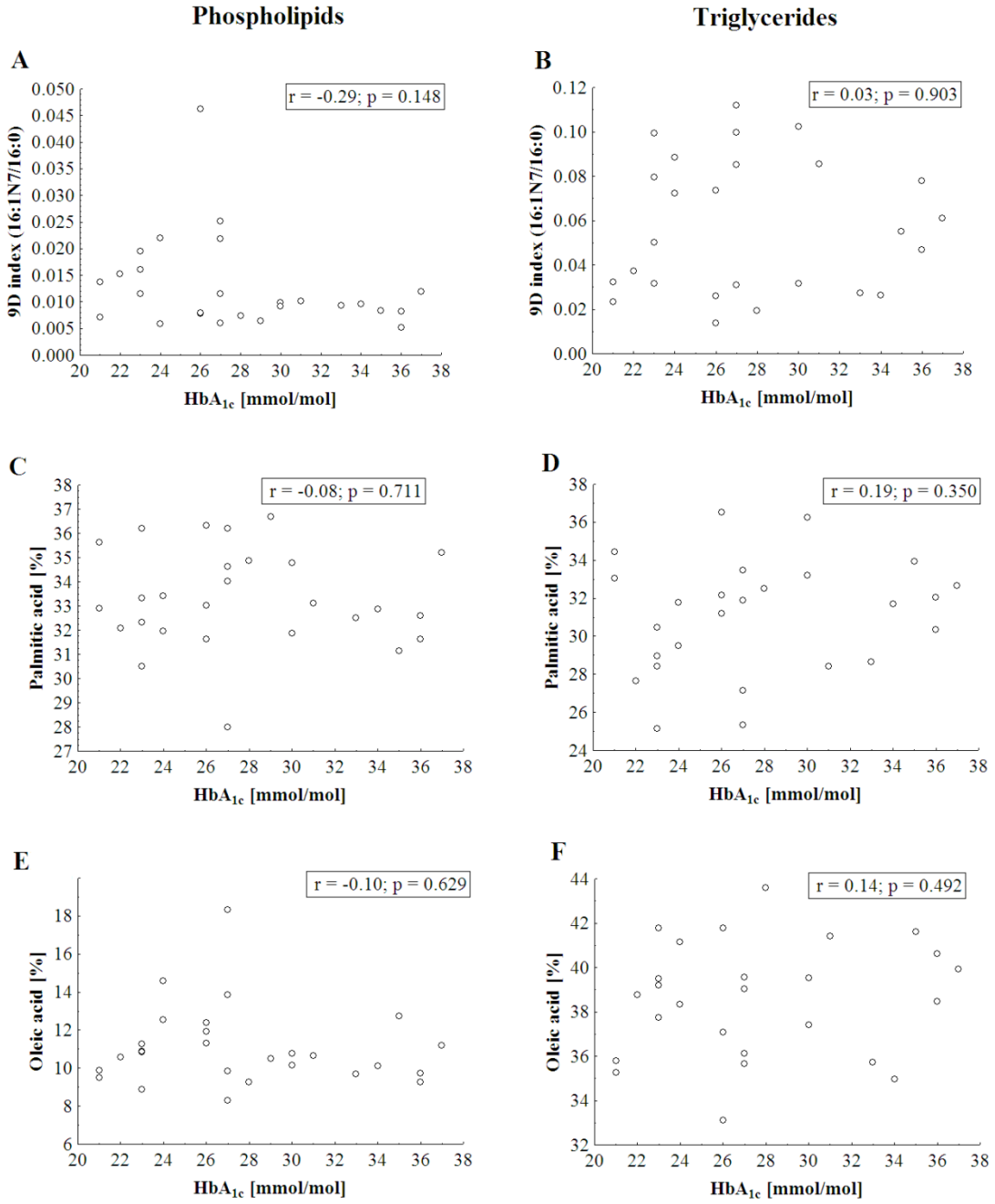

Figure 3: Cross-sectional relationships of glycosidic haemoglobin values with A) 9D index in PL fraction, B) 9D index in TG fraction, C) percentage of palmitic acid in PL fraction, D) percentage of palmitic acid in TG fraction, E) percentage of oleic acid in PL fraction, F) percentage of oleic acid in TG fraction in control group

4.2 Branched chain fatty acids

The following results are expressed as absolute concentrations of the relevant fatty acids in µmol/L. The most represented BCFA is 14-methylhexadecanoic acid in all measured lipidic fractions. Therefore we can consider it as a representative of total BCFA level. Statisticallly significant increase in this acid was found in DG and FFA fraction (see Fig. 4). Median of BCFA sum in these fractions are in DG 3.245 µmol/L

in controls and in FFA 4.53 μmol/L in controls. The richest fractions in the incidence of BCFA were TAG (median 19.02 μmol/L in controls) and CE (median 14.86 μmol/L in controls). There were found no significant differences in PL fraction.

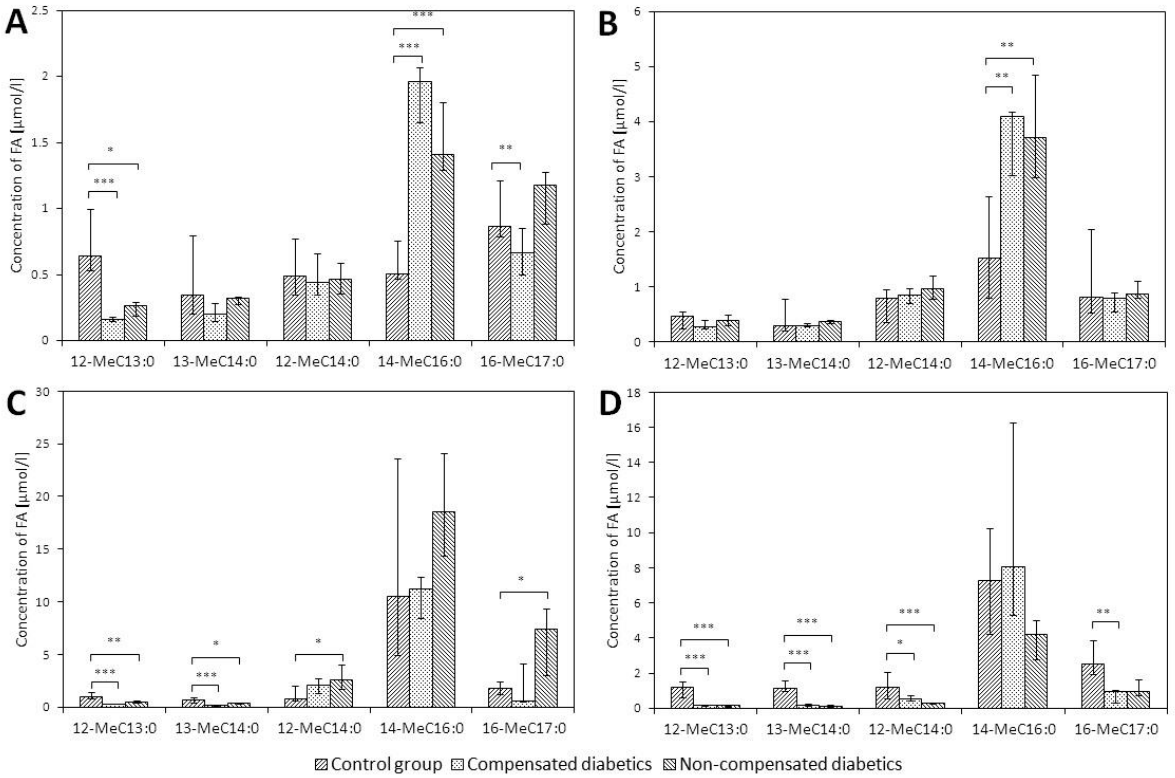

Figure 4: Branched chain fatty acid content in lipid fractions A) DG fraction, B) FFA fraction, C) TG fraction and D) CE fraction. Control group, compensated diabetics, decompensated diabetics. Data are presented as median ± IQR. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Our results show that in T2D compared to controls the content of isomeric saturated fatty acids increases in three plasma lipid fractions (DG - Figure 4A, FFA - Figure 4B and TG - Figure 4C), in phospholipid fraction we found no significant differences and in cholesterol esters fraction (Figure 4D) there was a noticeable decrease in the concentration of these acids.

This finding points to the fact that in type 2 diabetics reduced activation of PPAR receptors occurs and thus accumulation of both saturated and isomeric fatty acids takes place. We assume that the overall increase in isomeric SFA content relates to insulin resistance of adipose tissue in T2D, caused by reduced activation of PPAR receptors. This results in increased deposition of TG in the liver and formation of hepatic steatosis. This fact is in conformity with our finding of increased content of isomeric SFA in TG fraction which is formed in the liver. These isomeric SFA are not oxidized in liver peroxisomes in diabetics, but they are incorporated as undesirable component into triglycerides and secreted by the liver in form of VLDL, which are primarily designed as a source of energy for muscle and other organ cells (Holeček, 2006). The fate of these isomeric SFA has not been studied in detail so far. The question therefore arises - which organ removes these isomeric fatty acids from the circulation?

We have found reduced content of isomeric SFA in CE fraction, which may indicate their oxidation in peroxisomes of muscle cells. CE fraction is included in LDL

(low-density lipoproteins) and HDL. In type 2 diabetics LDL concentration is increased and HDL is decreased (Vijayaraghavan, 2010). From our results it is obvious that the content of CE is generally diminishing and these esters are not enriched with BCFA, these isomeric SFA are oxidized in extrahepatic organs because they do not return in the form of cholesterol esters to liver and their content is even lower than in the healthy control group.
Muscle peroxisomes (containing mainly PPARα and γ), are not so likely regulated via PPAR such as liver ones, they are intended to a simple oxidation of present FA and their function is apparently controlled only by a lack of energy. It is known that diabetics have an abundance of energy sources, but the cells process these sources insufficiently and by different metabolic pathways compared to the healthy population. Activation of PPARα receptors increases catabolism of FA and reduces the formation of Apo C-III, and thereby decreases the secretion of FFA, TG and consequently VLDL into the blood (Auwerx et al., 1996; Hřebíček, 2004; Schoonjans et al., 1996; Staels et al., 1995). Further activation of these receptors enhances capture of FFA in cells by the increase of activity of the transport protein - fatty acid translocase (FATP) and Acyl-CoA synthetase at the transcriptional level (Hřebíček, 2004; Mascaró et al., 1998, Motojima et al., 1998). Main target organ for FATP and Acyl-CoA synthetase is the liver where they reduce the amount of FA used in the production and secretion of TG and VLDL (Hřebíček, 2004). The situation is different for PPARγ. Our assumption that the isomeric SFA are oxidized predominantly in muscle peroxisomes is confirmed by the analysis of biopsy samples of human muscle and adipose tissue, which reflect the different tissue distribution of PPARγ (Loviscach et al., 2000).

4.3 HDL lipoproteins

Figures 5 and 6 show negative correlation of elongase index with HbA1c in diabetics in PL ($r = -0.47$; $p = 0.032$) and FFA ($r = -0.48$; $p = 0.029$). This decreasing trend means that palmitic acid (the main substrate for elongase) remains in HDL lipoproteins. This affects function of HDL in lipid metabolism concretely in lower efficiency of reverse cholesterol transport. In addition low elongase activity causes disruption in docosahexaenoic and arachidonic acid metabolism which has an influence on the fluidity of membranes and thus on the activity of transport proteins thereunder.
Negative relationship with HbA1c was found in case of arachidic acid ($r = -0.47$; $p = 0.029$) in PL (Fig. 7), lower concentration of arachidic acid occurred in poorly compensated diabetics compared to healthy donors (0.28 % vs. 0.20 %; $p = 0.019$). This foundation was never described in literature before. We can presume its influence on diabetes compensation from influence of SFA on fluidity and rigidity of membranes. We can assume that lower concentration of higher SFA leads to impairment of membrane function.

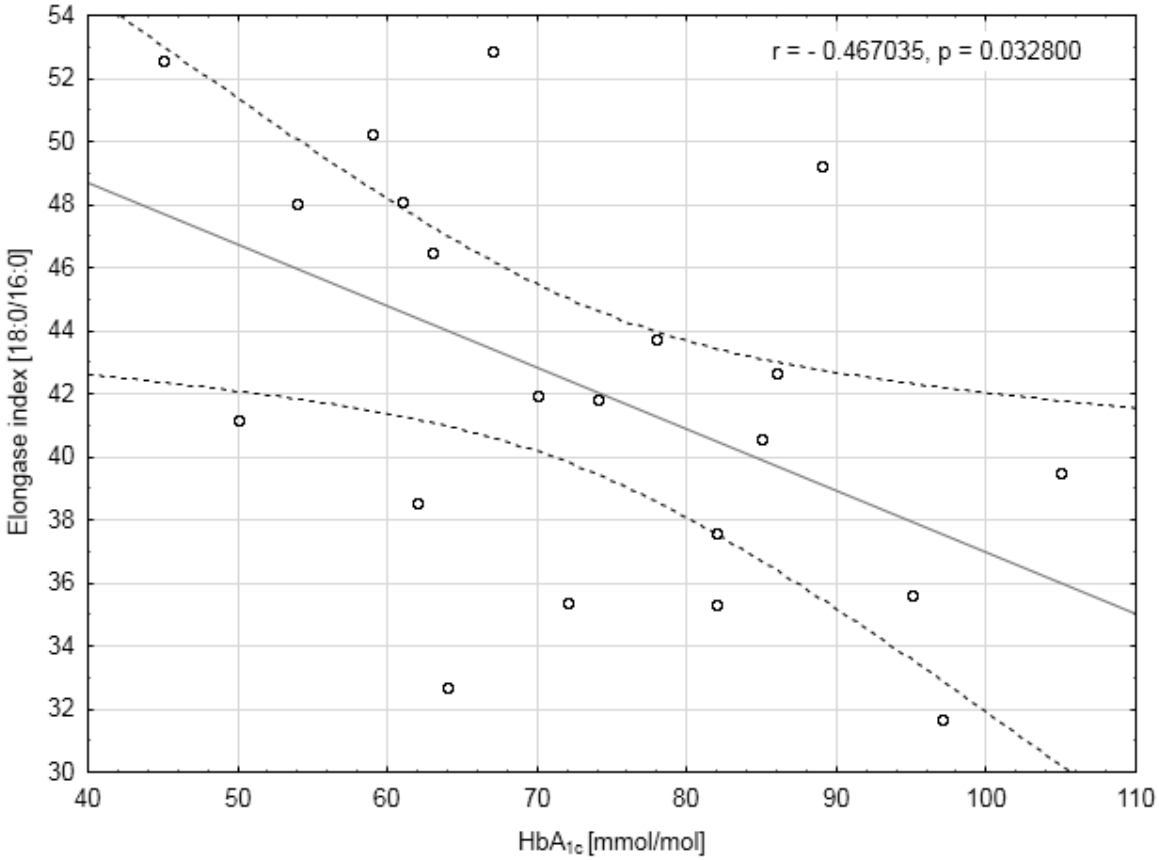

Figure 5: Cross-sectional relationship of HbA1c values with elongase index in PL

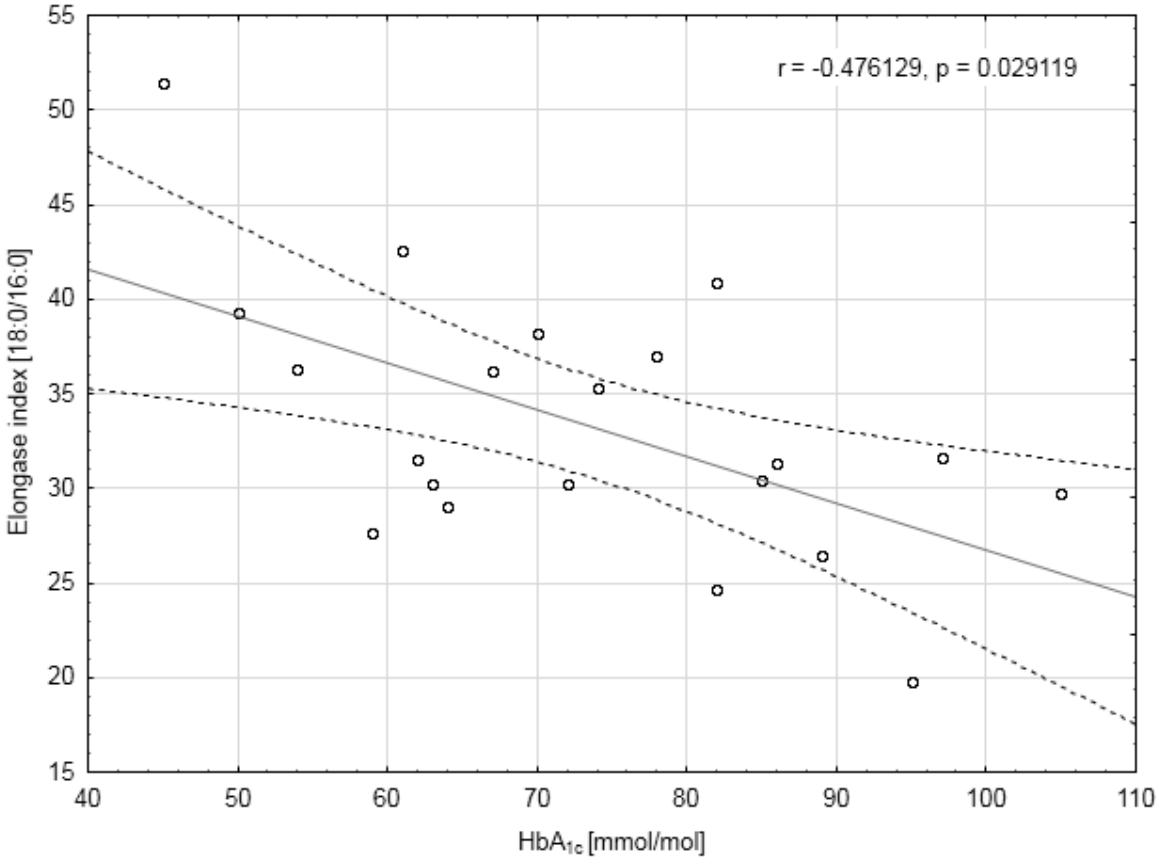

Figure 6: Cross-sectional relationship of HbA1c values with elongase index in FFA

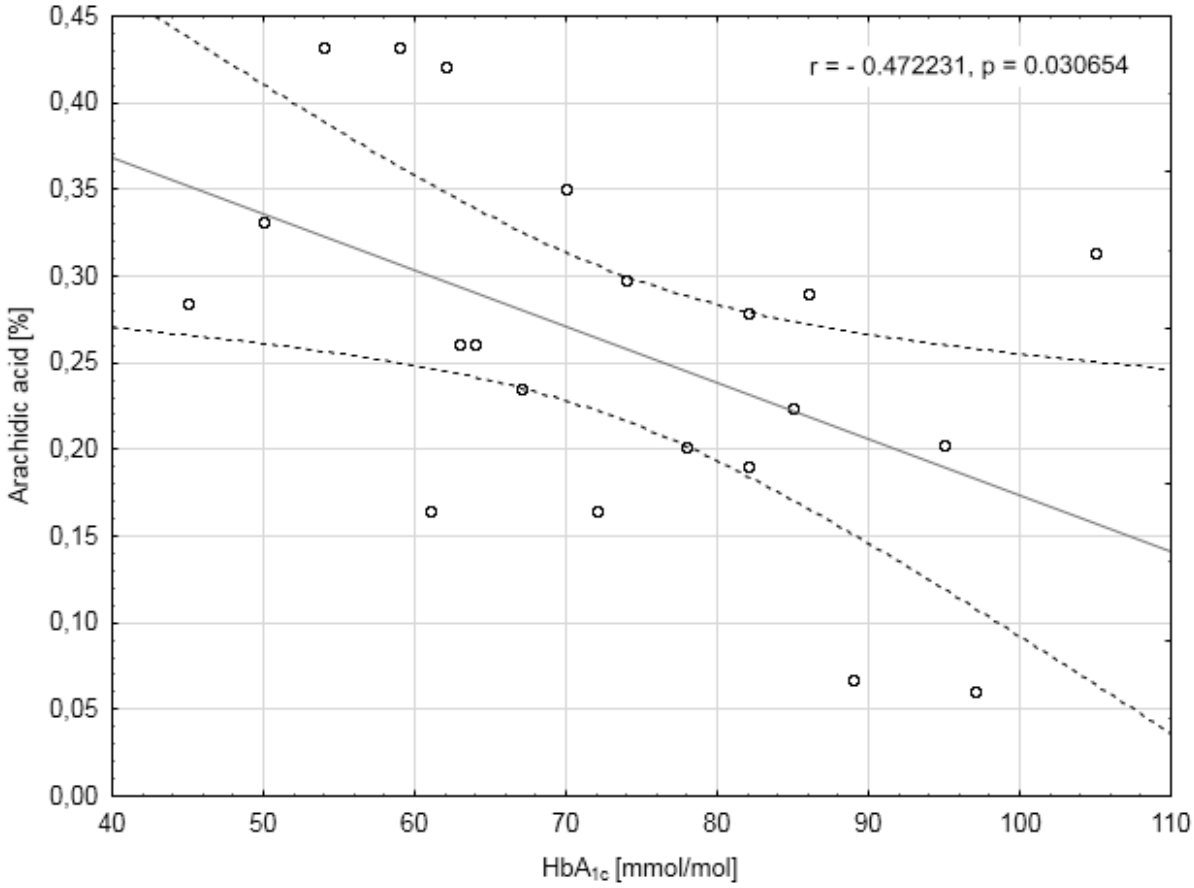

Figure 7: Cross-sectional relationship of HbA1c vlues with arachidic acid level in PL

Conclusion

Various plasmatic samples and HDL samples were analyzed in this work with a focus on discovering new findings in the field of lipid metabolism affected by diabetes of the second type. During the research, the original method was extended to determine 39 higher fatty acids, and it has been shown that even at the time of multiple mass spectrometry, simpler instrumentation in the form of a gas chromatograph with flame ionization detection can be beneficial today.

In the first group of patients, the benefits of medication and optimal dietary profile of fatty acids were shown in type 2 diabetics. In this study, we investigated the relationship between glycosidic haemoglobin (as a marker of compensation of type 2 diabetes used in real-life diabetes patients) and fatty acid composition in individual plasma fractions. Significant associations were found especially in the PL and TG fractions. As a limiting factor, age was revealed here, especially for the TG fraction. A newly discovered fact was the negative association of glycosidic haemoglobin with the 9-desaturase index.

Further we focused on the BCFA profile. These FA of bacterial origin, according to our conclusions, are not entirely suitable for diabetic patients. BCFAs are captured by the liver and further secreted only in the form of TG as part of VLDL intended to cover the energy consumption of the organism. This conclusion is supported by the fact that the increased concentration of BCFA in other liver lipid fractions (PL and CE) should lead to their recycling and accumulation in the diabetic organism. As this is not the case, BCFA must be oxidized by extrahepatic organs, mainly in muscle tissue where it is regulated by PPAR receptors less than in hepatic or adipose tissue. This knowledge has not yet been published and would have paid additional attention to the scientific groups.

With a change in lifestyle and healthier eating, more dairy products containing BCFA of bacterial origin get into the diet, resulting in the accumulation of these acids in comparison to a healthy population. Interestingly, there are no significant differences in BCFA levels between compensated and decompensated diabetics. In conclusion, it seems that the BCFA and diet issues in diabetic patients will not only be a marginal problem and should be further investigated.

In the last group, we focused on somewhat neglected HDL lipoproteins. These lipoproteins transport cholesterol away from the periphery. Because peripheral cells do not have such a sensitive enzyme equipment as the liver, we assume that lipid metabolism at this level will be impaired only in the acute phase of diabetes. The main danger for diabetics is the decline in the production of docosahexaenoic acid and arachidonic acid and thus the negatively affected fluidity of the membranes and the transport proteins embedded in them. Applying this mechanism is assumed in decompensated diabetics. A more detailed investigation would deserve the discovery of a declining trend in myristic acid.

List of abbreviations

5D	5 desaturase
6D	6 desaturase
9D	9 desaturase
BCFA	branched chain fatty acids
CE	cholesterol esters
CETP	cholesterol ester transfer protein
DG	diglycerides
FA	fatty acid
FATP	fatty acid translocase
FFA	free fatty acid
HbA1c	glycosidic haemoglobin
HDL	high-density lipoproteins
LDL	low-density lipoproteins
MUFA	monounsaturated fatty acids
NIDDM	non-insulin dependent diabetes mellitus
PL	phospholipids
PPAR	peroxisome proliferator-activated receptor
PUFA	polyunsaturated fatty acids
R_F	retardation factor
SFA	saturated fatty acids
T2D	type 2 diabetics
TG	triglycerides
VLDL	very low-density lipoproteins

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List of Students' Published Works

The results of this thesis are contained in 2 publications:

- ČERMÁK, Tomáš, LAŠTOVIČKA, Petr, MUŽÁKOVÁ, Vladimíra, LÍBALOVÁ, Martina, KOUKALOVÁ, Lucie, KANĎÁR, Roman, ČEGAN, Alexander. Association of fatty acid profile in plasma lipid fractions with HbA1c in type 2 diabetic patients. *International Journal of Diabetes in Developing Countries*, 2016, vol. 36, no. 1, p. 23-33.

- LAŠTOVIČKA, Petr, ČERMÁK, Tomáš, HÁJEK, Filip, LÍBALOVÁ, Martina, MUŽÁKOVÁ, Vladimíra, SKALICKÝ, Jiří, ČEGAN, Alexander. Determination of branched chain fatty acids in plasma of type 2 diabetics. *Scientific Papers of the University of Pardubice Series A* 22(2016).

I also cooperated on other projects the results of that are included in 2 publications:

- SARTORIUS, Tina, DRESCHER, Andrea, PANSE, Madhura, LAŠTOVIČKA, Petr, PETER, Andreas, WEIGERT, Cora,KOSTENIS, Evi, ULLRICH, Susanne, HÄRING, Hans-Ulrich. Mice Lacking Free Fatty Acid Receptor 1 (GPR40/FFAR1) are Protected Against Conjugated Linoleic Acid-Induced Fatty Liver but Develop Inflammation and Insulin Resistance in the Brain. *Cellular Physiology and Biochemistry*, 2015, vol. 35, no. 6, p. 2272-2284.

- ČERMÁK, Tomáš, MUŽÁKOVÁ, Vladimíra, MATĚJKA, Jan, SKALICKÝ, Jiří, LAŠTOVIČKA, Petr, LÍBALOVÁ, Martina, KANĎÁR, Roman, NOVOTNÝ, Vojtěch, ČEGAN, Alexander. Fatty Acid Profile in Erythrocyte Membranes and Plasma Phospholipids Affects Significantly the Extent of Inflammatory Response to Coronary Stent Implantation. *Physiological Research*, 2015, vol. 65, no. 6, p. 941-951.

Some of the results were reported at international conferences:

- LAŠTOVIČKA, Petr, KOUKALOVÁ, Lucie, ČERMÁK, Tomáš, MUŽÁKOVÁ, Vladimíra, ŠTRAMOVÁ, Xenie, ČEGAN, Alexander. Determination of activities of desaturases and elongases in type 2 diabetics, *12th International Nutrition and Dignostics Conference*, 27. – 30.8.2012, Praha, Collection p. 96, ISBN 978-80-7395-456-7.

- LAŠTOVIČKA, Petr, KOUKALOVÁ, Lucie, ČERMÁK, Tomáš, ŠTRAMOVÁ, Xenie, MUŽÁKOVÁ, Vladimíra, ČEGAN, Alexander. Plasma fatty acids and lipidomic enzymes levels in diabetic patients, *3rd European*

Lipidomic Meeting, 2. – 4. 7. 2013, Pardubice, p. P-28, ISBN 978-80-7395-648-6.

- LAŠTOVIČKA, Petr, LÍBALOVÁ, Martina, ČERMÁK, Tomáš, MUŽÁKOVÁ, Vladimíra, ČEGAN, Alexander. Analysis of fatty acids composition in HDL lipoproteins of type 2 diabetic patients, *14th International Nutrition and Diagnostics Conference*, Praha, 2. – 5. 9. 2014, Praha, p. 147, ISBN 978-80-7395-776-6.