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MASTERARBEIT

Titel der Masterarbeit

Changes in the fatty acid profile of type 2 diabetics after
an intervention with plant oil

Verfasserin

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angestrebter akademischer Grad

Master of Science (MSc)

Wien, 2012

Studienkennzahl lt.
Studienblatt:

A 066 838

Studienrichtung lt.
Studienblatt:

Masterstudium Ernährungswissenschaften

Betreuerin / Betreuer:

Univ. Prof. Dr. Karl-Heinz WAGNER

ACKNOWLEDGEMENT

I thank Prof. Karl-Heinz Wagner and Mag. Elisabeth Müllner for giving me the possibility of being part of the DIAPLANT study. Special thanks to Elisabeth for beaconing me from the first day at the laboratory until the last day of the statistical evaluations.

I thank my parents, grandparents and family for their implicit support during my whole study.

I thank my friends for supporting, motivating and believing in me and my skills in any situation.

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LIST OF ABBREVIATIONS

AA	Arachidonic acid, 20:4n6
ADA	American Diabetes Association
ALA	α -linolenic acid, 18:3n3
BHT	Butylated hydroxytoluene
BMI	Body mass index
C	Carbon
°C	Degree centigrade
DGE	Deutsche Gesellschaft für Ernährung
DHA	Docosahexaenic acid, 22:6n3
dl	Decilitre
EDTA	Ethylenediaminetetraacetate
EPA	Eicosapentaenic acid, 20:5n3
FA	Fatty acid
FAME	Fatty acid methyl esters
FFA	Free fatty acids
g	Gramme
GC	Gas chromatography
h	Hour
H	Hydrogen
HbA _{1c}	Glycated haemoglobin A _{1c}
HDL	High-density lipoprotein
Hg	Mercury
HIV	Human immunodeficiency virus
HOMA-Index	Homeostasis Model Assessment-Index
IDDM	Insulin-dependent Diabetes Mellitus
IDF	International Diabetes Federation

IV

IFG	Impaired fasting glycaemia
IGT	Impaired glucose tolerance
l	Litre
LA	Linoleic acid, 18:2n6
LDL	Low-density lipoprotein
μl	Microlitre
mg	Milligramme
min	Minute
mmol	Millimole
MUFA	Monounsaturated fatty acids
MV	Mean value
NaOH	Sodium hydroxide
NIDDM	non-Insulin-dependent Diabetes Mellitus
PUFA	Polyunsaturated fatty acids
rpm	Rotations per minute
SD	Standard deviation
SFA	Saturated fatty acids
TG	Triglycerides

1 INTRODUCTION

Changes in dietary and lifestyle patterns are the reasons for many diet-related diseases, including obesity, diabetes mellitus, cardiovascular disease, hypertension, stroke and various forms of cancer. Since the nutritional behaviour plays a pivotal factor in their development, the above mentioned diseases are partly preventable (Manco et al., 2004).

Diabetes mellitus is one of the most common chronic diseases worldwide. As recent study results show the incidence and prevalence are continuously increasing. In 2011 366 million people worldwide suffered from diabetes. The majority of those (90-95%) were affected by type 2 diabetes (IDF, 2011).

Beside antidiabetic medication a successful therapy includes the right choice of diet and physical activity (ADA, 2008).

The present master thesis was performed within the project DIAPLANT, which is a cross-border cooperation project between Austria and Slovakia, funded by EU. A human intervention study was performed at the Department of Nutritional Sciences at the University of Vienna / Emerging Field "Oxidative Stress and DNA Stability".

The aim of the thesis was to investigate the influence of an eight-week intervention with plant oil and vegetables on the fatty acid profile in the plasma of type 2 diabetics. The primary objective of this work was to verify the compliance of the subjects. Furthermore, the plasma levels of several fatty acids between type 2 diabetics, healthy subjects and those with abnormal fasting glucose concentrations were compared. The resulting changes due to the intervention and the different effects on the various states of health were analysed.

Within the DIAPLANT project the influence of the vegetable and plant oil intervention on other biochemical biomarkers (e.g. vitamins) were evaluated in other theses.

2 DIABETES

2.1 Definition of diabetes mellitus

Diabetes mellitus or simply diabetes is a group of metabolic diseases characterized by high blood glucose levels resulting from defects in the body's ability to produce and use insulin. The chronic hyperglycemia is associated with long-term damages, dysfunction and failure of several organs and blood vessels (ADA, 2012).

When glucose cannot be used for energy production, it accumulates in the blood and is excreted by the kidneys as soon as the threshold of 160 to 180 mg glucose per 100 ml blood is reached (DGE, 1998).

Since glucose is an important energy source for the cells, a lack of glucose leads to failures in energy-, fat- and protein metabolism. The reason of the abnormalities is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and / or diminished tissue responses to insulin at one or more points in the complex pathways of the hormone action (DGE, 1998).

When the glucose metabolism derails, hypo- or hyperglycemia occurs, possibly leading to secondary complications.

Symptoms of hypoglycemia include tremor, attacks of sweating, vertigo and ravenous appetite. Possible implications are disorientation and unconsciousness.

A hyperglycemia is accompanied by thirst, continual urination and tiredness. If the fettle remains untreated, complications in the blood vessels of eyes, kidneys, legs and the heart or the life-threatening diabetic coma might occur (DGE, 1998).

2.2 Epidemiology

Diabetes mellitus is one of the most common chronic diseases worldwide, with a continuously increasing number of prevalence and incidence.

According to the IDF in 2011 366 million people were suffering from type 2 diabetes, representing 8.3% of the global adult population. Forecasts assume that this number will increase to 552 million people by 2030 (figure 1). This equates to approximately three new cases every ten seconds. The IDF expects that the number of diabetics will increase to 64 million in Europe in 2030 (figure 1). In Austria about 571.000 people at the age of 20-79 years currently suffer from diabetes, which is estimated to increase to 717.000 by 2030.

Map: IDF Regions and global projections of the number of people with diabetes (20-79 years), 2011 and 2030

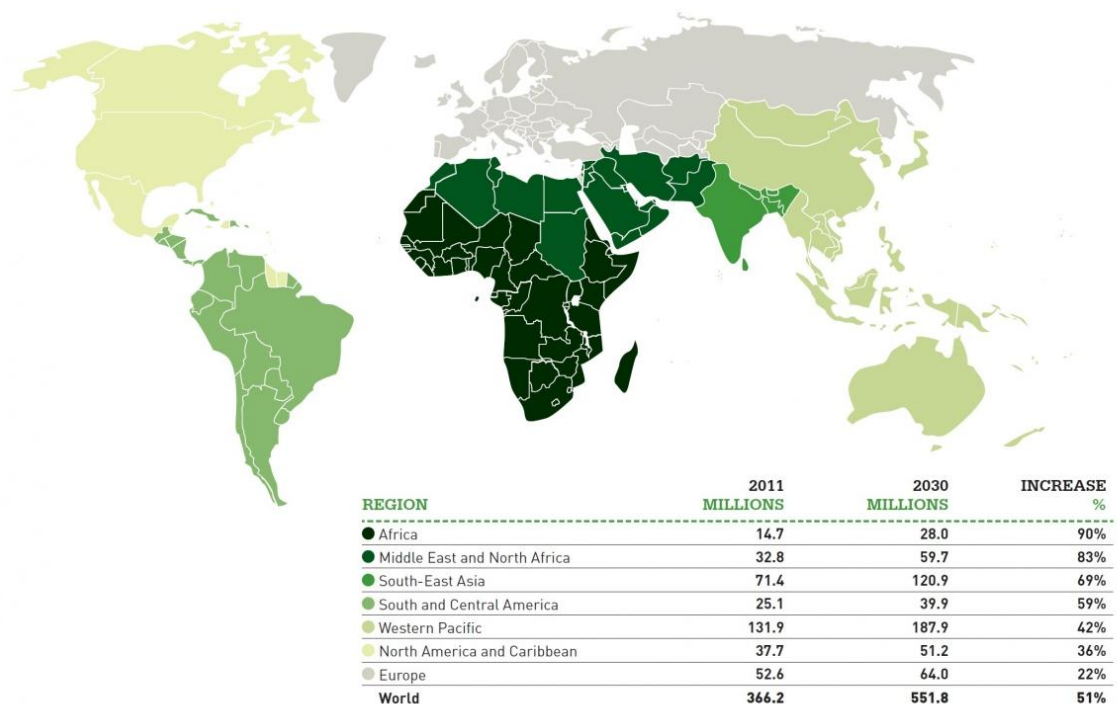


Figure 1: The number of people with diabetes by 2011 and 2030 (IDF, 2011)

More than 80% of people with diabetes live in low- and middle-income countries. In 2011 the Middle East and North Africa Region had the highest prevalence of adults with diabetes (11.0%), whereas the prevalence in Europe was the lowest (6.7%) (figure 1). In 2030 the number of African people suffering from diabetes will almost double to 28 million compared to 14,7 million in 2011. Compared to Europe the increase in Africa in 2030 will be four times as high (IDF, 2011).

2.3 Types of diabetes

2.3.1 Type 1 diabetes

Type 1 diabetes accounts for 5-10% of diabetics and is usually diagnosed in children and young adults, therefore previously known as juvenile diabetes (DGE, 1998). Type 1 diabetes results from a cellular-mediated autoimmune destruction of the β -cells of the pancreas, so that initially too little and later on no insulin at all is produced (ADA, 2012). Therefore these diabetics depend on extrinsic insulin (DGE, 1998). Autoimmune destruction of β -cells has multiple genetic predispositions and is also related to environmental factors that are still poorly understood (ADA, 2012).

2.3.2 Type 2 diabetes

Type 2 diabetes is the most common form of diabetes, accounting for 90-95% of all diabetics. It encompasses individuals who have insulin resistance and usually have relative insulin deficiency. Autoimmune destruction of β -cells does not occur (ADA, 2012).

The risk of developing this form increases with age, obesity and lack of physical activity. It is often associated with a genetic predisposition. However, the genetic background is complex and not clearly defined yet (DGE, 1998).

In type 2 diabetes either the body does not produce enough insulin or the cells are resistant to insulin. As the disease develops unapparent it is often only revealed by chance. At that point the body produces average amounts of insulin, which cannot operate properly because its emission is delayed (DGE, 1998). The higher blood glucose levels would be expected to result in even higher insulin values if their β -cell function is normal. But in these patients insulin secretion is defective and insufficient to compensate for insulin resistance (ADA, 2012).

Most often overweight and physical inactivity promote type 2 diabetes. Thus a normalization of body weight and the right choice of food help to regularise blood sugar levels. In some cases a further treatment with antidiabetic medication or insulin is necessary (DGE, 1998).

2.3.3 Gestational Diabetes Mellitus

Pregnant women with high blood glucose levels during pregnancy have established gestational diabetes. The development remains unclear, but it is hypothesized that placenta hormones which are important for the development of the baby block the function of the mother's insulin in her body. Due to this insulin resistance, the mother's body can hardly use insulin. Although insulin does not cross the placenta, glucose and other nutrients are able to. Extra blood glucose passes the placenta, resulting in high blood glucose levels in the baby.

This causes the baby's pancreas to produce extra insulin. Since the baby is getting more energy than it needs to grow and develop, the extra energy is stored as fat. This can lead to macrosomia.

Babies with macrosomia face health problems of their own, including damage to their shoulders during birth. Because of the extra insulin produced by the babies' pancreas, newborns may have very low blood glucose levels at birth and are also at higher risk for breathing problems. Babies with excessive insulin production are at higher risk for developing obesity and type 2 diabetes (ADA, 2004).

2.4 Clinical diagnostics

In order to diagnose diabetes mellitus glucose levels are measured in the blood (Leitzmann et al., 2009). It is important that higher levels are measured at least at two different days. The normal fasting blood glucose concentration in the plasma accounts for ≤ 100 mg/dl (≤ 5.6 mmol/l) (Roden, 2009).

The diagnostic criteria are (ADA, 2012):

- HbA_{1c} $\geq 6.5\%$
or
- Fasting plasma glucose ≥ 126 mg/dl (≥ 7.0 mmol/l)
or
- 2-h plasma glucose ≥ 200 mg/dl (≥ 11.1 mmol/l): The test should use a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.
or
- A random plasma glucose ≥ 200 mg/dl (≥ 11.1 mmol/l) when classic symptoms of hyperglycemia are present (ADA, 2012).

2.5 Risk factors for type 2 diabetes

As mentioned before type 2 diabetes is nowadays the most common form of diabetes mellitus. More than 90% of all cases of diabetes mellitus are assigned to this type. Type 2 diabetes is a heterogeneous disease: The focus of the pathophysiology is on the one hand a disorder of the impact of insulin on the target cells (insulin resistance) and on the other hand a failure of insulin secretion due to one or more so far not exactly known defects of the β -cells in the pancreas. At the beginning a resistance to insulin, which is caused and encouraged by many lifestyle factors, will be initially compensated by increased pancreatic insulin secretion. When the β -cells, probably due to genetic defects,

are no longer able to keep increased secretion of insulin, this results in the final manifestation of the disease.

Lifestyle factors have a major impact on the development and progression of type 2 diabetes. Important risk factors to an impaired glucose tolerance and type 2 diabetes are an age of more than 45 years, overweight and obesity (BMI > 25 kg/m²), a positive family history, physical inactivity, hypertension (140/90 mm Hg) and dyslipoproteinemia (HDL-cholesterol < 40 mg/dl, triglycerides > 250 mg/dl).

However, the most significant risk factor of the manifestation of type 2 diabetes is by far obesity. Even a weight gain during adulthood increases the risk of diabetes substantially (Hauner and Schulze, 2011).

People with impaired glucose tolerance, which is defined as a fasting blood glucose level < 126 mg/dl and a 2-hour value between 140 and 199 mg/dl after a glucose load with 75 g of dextrose, have particularly high risks for diabetes (DGE, 2006).

Prevention studies have shown that intensive lifestyle modification (reducing weight, reducing total intake of fat and intake of saturated fat, increasing intake of fibre and physical activity) of these people can reduce the risk of diabetes by 58% over a period of three years. The lifestyle intervention was even significantly more effective than Metformin, which reduced the risk of diabetes by 31% (Tuomilehto et al, 2001; Knowler et al, 2002).

2.6 Nutrition therapy in type 2 diabetes

The aims of a nutrition therapy in diabetes are to achieve normal blood glucose and HbA_{1c} levels, improved blood lipid levels, normal blood pressure, normal body weight and the prevention of complications of diabetes. All these factors can be positively influenced by certain dietary measures. Diabetics are recommended to keep a diet that meets the basic outlines of the recommendations for healthy adults (ADA, 2008; DGE, 2008).

On the basis of nutrients the following measures are recommended by the DGE (2008):

- Carbohydrate intake can account for 45-60% of total energy.
- Total fat intake should not exceed 35% of the total energy; saturated and trans fatty acids together should be under 10% of total daily energy intake.
- Patients without signs of nephropathy can consume 10-20% of the total energy in form of proteins.
- Fibre intake should ideally be above 40 g/day.
- The intake of free sugars should be moderate (up to 50 g/day); free sugar intake should not exceed a total of 10% of the total energy.

The nutritional interventions at the level of food include:

- Eating enough dietary fibre and foods rich in vitamins
- Daily consumption of fruits, vegetables, salad, legumes and whole grains
- Two or three servings of fish per week, preferably fat fish
- Avoiding the consumption of fatty meats, cheeses, chocolate, cakes and pastries
- Choosing low-fat dairy products
- Using oils that are rich in monounsaturated fatty acids for cooking instead of animal fats
- Limiting the daily amount of salt
- Consuming alcoholic beverages moderately; due to the risk of hypoglycemia patients should be careful with consuming alcoholic beverages after sports and additional physical activity (DGE, 2008).

Being active is another part of living healthy and managing diabetes. Any type of physical activity can help to lower blood glucose (ADA, 2008).

2.7 Insulin resistance and sensitivity

2.7.1 Insulin resistance

Insulin resistance is the inability of a known quantity of insulin to increase glucose uptake and utilisation in an individual as much as it does in a normal population (Lebovitz, 2001).

Due to the diminished ability of cells to respond to the action of insulin in transporting glucose from the bloodstream into muscle and other tissues the person's body produces larger quantities of insulin to maintain normal levels of glucose in the blood. As long as the pancreas can produce enough insulin to overcome this resistance, blood glucose levels remain normal. Once the pancreas is no longer able to do this, blood glucose starts to rise, initially after meals, eventually even in the fasting state. This is usually the time point when type 2 diabetes is diagnosed (Lee, 2010).

There are several mechanisms suggested as possible causes for the development of insulin resistance. These include:

- ✧ genetic abnormalities of proteins of the insulin action cascade
- ✧ fetal malnutrition
- ✧ increase in visceral adiposity (Lebovitz, 2001).

Insulin resistance occurs as part of a cluster of cardiovascular-metabolic abnormalities commonly referred to as "The Metabolic Syndrome". This cluster may lead to the development of atherosclerosis, hypertension or polycystic ovarian syndrome (Lebovitz, 2001; Wilcox 2005).

Increased dietary fat intake is an important contributor to the development of insulin resistance. The underlying mechanisms could be the decreasing glucose uptake in the muscles and the reduction of the insulin-stimulated insulin receptor substrate (IRS)-1 tyrosine phosphorylation and IRS-1-associated phosphatidylinositol 3-kinase activity (Holness et al., 2003).

2.7.2 Insulin sensitivity

Insulin sensitivity is the sensitivity of the insulin receptors towards insulin. It is measured by the insulin sensitivity index and the glucose-clamp technique (Heinze et al., 2002).

The former measures the ability of endogenous insulin to reduce glucose in extracellular fluids by inhibiting glucose release from the liver and stimulating the peripheral consumption of glucose. The latter measures the effect of changes in insulin concentration on glucose clearance, which is a fictional measure for the elimination of glucose out of the blood plasma (McGraw-Hill Concise Dictionary of Modern Medicine, 2002).

2.8 Carbohydrate intake and the risk of diabetes

Carbohydrates can influence the secretion and impact of insulin in different ways, depending on their quantity and composition. A rapid increase in the blood glucose concentration due to short-chain carbohydrates causes not only a fast and strong insulin secretion but can also exert pro-oxidative and pro-inflammatory effects (Riccardi et al, 2008).

Conversely, complex carbohydrates lead to a moderate increase in blood glucose concentration, which can improve insulin resistance: Consumption of soluble dietary fibre reduces postprandial glucose responses after carbohydrate-rich meals, as well as lowering total and LDL-cholesterol levels (Jenkins et al., 2000). However, it is mainly the consumption of insoluble cereal dietary fibre and whole grains that is associated with reduced risk of type 2 diabetes (de Munter et al., 2007; Schulze et al., 2007).

An increased intake of total dietary fibre was inversely associated with markers of insulin resistance in several studies (Pi-Sunyer, 2005). Investigating different soluble and insoluble dietary fibre in randomized controlled intervention studies yielded in mixed results. Consumption of wheat bran for three months did not

change fasting glucose and glycated hemoglobin levels in diabetic subjects (Jenkins et al., 2002). Consumption of insoluble dietary fibre increased the whole body glucose disposal (Robertson et al., 2005).

Since insulin resistant subjects are more likely to eventually develop diabetes, improved insulin sensitivity could be a relevant factor contributing to reduce the risk for diabetes in subjects consuming diets high in insoluble dietary fibre (Weickert and Pfeiffer, 2008).

Furthermore a high proportion of soluble dietary fibre and a low glycemic index diet have beneficial effects on both the glucose and lipid metabolism. The underlying mechanism is probably a delay of the intestinal glucose absorption. Thus the type and amount of carbohydrates and dietary fibre as part of a mixed meal have considerable influence on the postprandial glucose and insulin responses (Riccardi et al, 2008).

The postprandial rise of the blood glucose concentration seems to control not only the insulin secretion significantly, but also influences many steps in the utilization of glucose, which is under the control of insulin and other hormones. However, what remains unclear is the role of the absolute amount of carbohydrate intake in connection with the risk for type 2 diabetes (Hauner and Schulze, 2011).

2.9 Fat consumption and the risk of type 2 diabetes

The primary objective of a lifestyle and nutrition intervention is the removal of hyperglycemia as a risk factor for micro- and macroantipathic complications. Therefore relations between fat consumption and insulin resistance are to the fore of the assessment of evidence (Hauner and Schulze, 2006).

There are further biological mechanisms that could produce an effect of fatty acids on the risk of diabetes. Accumulation of triglycerides in the muscle tissue reduces the insulin-stimulated glucose absorption and glucose oxidation,

whereas the accumulation of triglycerides in β -cells of the pancreas leads to a reduction of insulin secretion (Manco et al., 2004). Furthermore chronic exposure to high levels of lipids initially boosts the insulin response in hyperinsulinemic conditions as a result of a glucose load (Warnotte et al., 1994). Later, it selectively desensitises the β -cell to glucose and causes β -lipotoxicity (Capito et al., 1992).

In order to normalise the blood or tissue lipid profile a β -cell compensation for insulin resistance may be adapted (Marchesini et al., 2001). Though, β -cell compensation may be inadequate during the development and progression of type 2 diabetes, since it is insufficient to maintain blood glucose and blood and tissue lipid levels within the normal physiological range (Kubota et al., 2000). The β -cell response depends on the chain length of fatty acids and on their degree of saturation (Stein et al., 1997). Thus, for any given level of glucose, insulin secretion will be greatly influenced by free fatty acid (FFA) circulating levels and also by the percentage of unsaturation of the fatty acids. The mechanisms leading to a decrease in insulin secretion are still unclear. The decrease is likely to occur via other mechanisms such as induction of glucokinase and insulin gene expression (Gremlich et al., 1997).

On the contrary, polyunsaturated fatty acids (PUFA), in particular ω -3 fatty acids, could improve the insulin sensitivity (Manco et al., 2004). Eicosanoids, which are metabolic products of PUFA, could influence the activity of insulin in muscle cells (Pegorier et al. 2004). Thus PUFA could immediately contribute to the regulation of enzymes of lipolysis and lipogenesis in muscle cells (Manco et al., 2004).

Although data of animal experiments mostly confirm these assumptions (Hu et al. 2001; Rivellese and Lilli, 2003), several investigations in human subjects (Vessby et al. 2001, Salméron et al. 2001, Van Dam et al. 2002) failed to yield conclusive results regarding the effects of dietary saturated fatty acids (SFA) and PUFA on insulin sensitivity and secretion, because many confounding dietary and non-dietary factors directly affect insulin sensitivity, such as physical activity, smoking habits or amount of fibres in the diet. (Manco et al., 2004).

2.9.1 Impacts of total fat

An increasing body of evidence suggests that lipid overflow causes excessive storage of triglycerides (TG) in non-adipocyte cells, leading to impairment of cellular metabolism through the phenomenon of lipotoxicity (Manco et al., 2004).

Muscle and hepatic insulin resistance are two major defects of non-insulin-dependent diabetes mellitus. Since dietary factors may be important in the etiology of insulin resistance, Kraegen et al. (1991) studied progressive changes in the development of high-fat-diet-induced insulin resistance in tissues of adult male Wistar rats. Muscle triglyceride accumulation due to fat feeding was not significantly different after three days, but had doubled after three weeks in red muscle cells compared to starch-fed controls. Fat feeding significantly reduced suppressibility of hepatic glucose output by insulin. After three weeks high-fat-fed animals had developed significant glucose intolerance. The authors concluded that fat feeding induces insulin resistance in liver and adipose tissue before skeletal muscle with early metabolic changes favoring an oversupply of energy substrate to skeletal muscle relative to metabolic needs. This may generate later muscle insulin resistance (Kraegen et al., 1991).

Conclusion:

It lacks studies investigating the effect of the total fat intake on insulin sensitivity and glucose metabolism in patients with type 2 diabetes mellitus. It is important that a reduction in fat intake is not accompanied by additional dietary modifications. Therefore the relative content of the individual fatty acid fractions should remain unchanged (Hauner and Schulze, 2006). The Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition (2008) defines the relationship between the total fat intake and the risk for diabetes as insufficient.

2.9.2 Impacts of ω -3 fatty acids

Controlled intervention studies in subjects with type 2 diabetes could not find an improvement in insulin sensitivity after supplementation with ω -3 fatty acids (Rivellese and Lilli, 2003; Storlien et al., 1996). Therefore Mostad et al. (2006) investigated short-term (one week) and longer-term (nine weeks) effects of ω -3 fatty acids in subjects with type 2 diabetes which had no hypertriglyceridemia by supplementing fish oil, while participants of the control group got the same amount of corn oil (20 ml/day for nine weeks, given as liquid). Concentrations of both eicosapentaenic acid (EPA) and docosahexaenic acid (DHA) increased in adipose tissue and plasma phospholipids in the fish oil group. Opposite to this the ratio of ω -6 to ω -3 fatty acids decreased during the intervention. The increased intake of linoleic acid (LA) in the corn oil group had no effect on the fatty acid profile in plasma phospholipids and adipose tissue. However, the LA decreased in the phospholipids in the fish oil group.

The average daytime concentrations as well as the fasting concentrations of blood glucose were higher in the fish oil group after nine weeks of intervention. Glycated hemoglobin was not affected by the intervention with ω -3 fatty acids. Nevertheless, glucose utilisation, a measure of insulin sensitivity, was lowered in the fish oil group. On the other hand the insulin secretion did not significantly differ but tended to be higher in the fish oil group. Furthermore, ω -3 fatty acids first (after one week) decreased and later on increased the fatty acid utilisation. The authors conclude that a high intake of fish oil (20 ml/day) has moderate but negative effects on glycemia like increased blood glucose concentrations and decreased insulin sensitivity (Mostad et al., 2006).

The beneficial effects of ω -3 fatty acids on insulin action are well documented in animals but not in humans (Hu et al. 2001; Rivellese and Lilli, 2003). Giacco et al. (2007) evaluated whether a supplementation with ω -3 fatty acids is able to modulate insulin sensitivity and secretion in healthy individuals. Subjects were randomised into groups consuming a diet rich in either saturated or monounsaturated fatty acids. Within these two groups there was a second

randomisation to fish oil (3.6 g ω -3 fatty acids/day) or placebo. EPA and DHA concentrations increased in the subjects supplemented with ω -3 fatty acids. In the placebo groups a decrease in these long-chain PUFA could be observed. Insulin sensitivity did not change in any of the four groups as well as insulin secretion was not affected by supplementation with ω -3 fatty acids in both diets. Consequently, the disposition index, which is an integrated parameter of glucose tolerance accounting for insulin sensitivity and secretion, was not affected by ω -3 fatty acid supplementation in either diet.

The study showed that a supplementation with ω -3 fatty acids was not able to modulate insulin action, insulin secretion disposition index or glucose tolerance, regardless of the fatty acid composition of the background diet. However, the authors assume that the inverse relation between ω -3 fatty acids and the incidence of type 2 diabetes has to be explained by other effects of ω -3 fatty acids (Giacco et al., 2007).

In the KANWU study Vessby et al. (2001) examined whether a change in the dietary fat quality influences insulin sensitivity and if so, whether these supposed effects were affected by addition of ω -3 fatty acids. Experimental studies have already indicated that fat quality could affect insulin sensitivity (Storlien et al., 1996). However, this could not be shown in human intervention studies.

The healthy study population was divided into two groups: One group received a diet containing a high proportion of SFA (SFA diet), the other one received a diet containing a high proportion of monounsaturated fatty acids (MUFA diet). Within these two groups there was another splitting into those who obtained capsules containing fish oil (3.6 g ω -3 fatty acids/day for 90 days) and those who received placebo capsules containing the same amount of olive oil.

The study could show that a shift from SFA to MUFA improves insulin sensitivity, which was 12.5% lower on the SFA diet and 8.8% higher on the MUFA diet. Nevertheless the beneficial effects of the MUFA diet on insulin sensitivity could not be seen when the fat intake was high (> 37% of total energy intake).

Improvements in insulin sensitivity were only observed in subjects with a lower fat intake. Hence not only the quality but also the proportion of total fat in a diet is relevant. The addition of ω -3 fatty acids did not affect the insulin sensitivity in both diets. The authors suggest that it will take years to see a change in insulin actions as effects of ω -3 fatty acids. Furthermore the ratio of ω -6: ω -3 of skeletal muscle cell membranes might be important to determine the insulin sensitivity. Moreover the addition of ω -3 fatty acids may affect the insulin sensitivity only in people with very low initial concentrations of ω -3 fatty acids. The insulin secretion was not affected by a change of dietary fat quality (Vessby B. et al, 2001).

Beside other foods nuts are rich in unsaturated fatty acids, vitamin E and fibre. What is unique about walnuts is their high content in both ω -6 and ω -3 fatty acids. Since unsaturated fatty acids and fibre may improve plasma lipids, Almario et al. (2001) investigated the effects of walnut consumption in subjects with high plasma total cholesterol (> 5.17 mmol/l) and TG (> 2.26 mmol/l) concentrations. On the basis of two different background diets (habitual diet and low-fat diet) they wanted to determine whether supplementation of 48 g walnuts (3.3 g ω -3 fatty acids) or restriction with other dietary fats was responsible for the improvements in plasma lipids.

The addition of walnuts to both diets increased LA and α -linolenic acid (ALA) and decreased arachidonic acid (AA) concentrations in the plasma, whereas concentrations of EPA and DHA did not change. The authors suggest these findings as a result of the competition between LA and ALA for the δ -6-desaturase.

In summary this study shows that walnuts, which are a rich source of both ω -3 and ω -6 fatty acids, have different metabolic effects on weight, plasma fatty acid composition, plasma lipids and apoproteins than the individual effects of either ω -3 or ω -6 fatty acids (Almario et al., 2001).

Conclusion:

The pictured intervention studies provide convincing evidence that long-chain ω -3 fatty acids have no effect on the long-term blood glucose control and the insulin sensitivity in diabetic patients. Thus the available data appear to be insufficient for recommendations concerning the supplementation with ω -3 fatty acids in subjects with type 2 diabetes (Hauner and Schulze, 2006).

2.9.3 Impacts of Saturated Fatty Acids

Several studies have been conducted to demonstrate that a high fat intake, particularly if rich in SFA, is associated with impaired insulin sensitivity and secretion and the development of type 2 diabetes (Lovejoy, 1999; Marshall et al., 1997). However, no large modification of the type of fatty acids in the diet (saturated vs. unsaturated) is needed in order to prevent the adverse effect of dietary saturated fat on insulin action (Kraeger et al., 1991; Jucker et al., 1999).

Reducing the intake of SFA on a 10% maximum of total energy intake was part of the intervention in the Finnish Diabetes Prevention Study and the American Diabetes Prevention Program (Tuomilehto et al., 2001; Diabetes Prevention Program Research Group, 2002). In both studies the risk of developing diabetes was reduced by 58%. Due to the multifactorial nature of the studies (weight-commissioning, physical activity, changes in other nutritional parameters, especially the total fat intake) an allocation of the observed effect to the changes in SFA intake is not possible (Hauner and Schulze, 2006).

In the San Luis Valley diabetes study subjects with incident diabetes mellitus did not have a significantly higher intake of SFA than adults with normal glucose tolerance (Marshall et al., 1994). Even in the Iowa Women's Study no relationship between SFA intake and diabetes risk was observed. However, the replacement of SFA with PUFA was associated with a significantly lower risk of developing diabetes (Meyer et al., 2001). In the Health Professionals Follow-up Study no relationship between SFA and the risk for diabetes could be observed (Van Dam et al., 2002). In the Kuopio Ischaemic Heart Disease Risk Factor

Study the intake of SFA in individuals who developed diabetes mellitus was not significantly different from individuals staying healthy (Laaksonen et al., 2002).

In Wistar-rats fed with high saturated fat peripheral insulin resistance was accompanied by increased insulin secretion such that glucose tolerance was maintained (Chalkley et al., 2002). This finding confirms the concept that insulin resistance generates hyperinsulinemia (Holness et al., 2003).

Holness et al. (2003) substituted a small amount of dietary lipid with long-chain ω -3 fatty acids during four weeks of high-saturated fat feeding. They investigated the relationship between amelioration of insulin resistance and glucose-stimulated insulin secretion. They demonstrate that, despite dietary delivery of saturated fat, this manipulation prevents insulin resistance and reverses insulin hypersecretion in response to glucose in vivo.

Effects of long-chain ω -3 fatty acid supplementation to lower the insulin secretion were also observed in perfused islets suggesting a direct effect on islet function. However, this supplementation led to hepatic insulin resistance. The data demonstrate that the insulin response to glucose is suppressed to a greater extent than insulin sensitivity is enhanced by intake of a high SFA diet with long-chain ω -3 fatty acids. Furthermore, reduced insulin secretion indicates that long-chain ω -3 fatty acids directly impair the β -cell response to SFA. Therefore insulin secretion cannot be augmented to normalise glucose tolerance. Moreover, β -cell compensatory hypersecretion represents a response to insulin resistance.

It seems reasonable that peripheral insulin resistance and increased insulin secretion during high SFA feeding arise simultaneously. The reason could be a common action on skeletal muscle and the pancreatic β -cell that is counteracted by small quantities of dietary long-chain ω -3 fatty acids (Holness et al., 2003).

Conclusion:

Cohort studies on the question of whether there is an association between SFA intake and the risk for type 2 diabetes mellitus could not confirm this in the majority (Meyer et al., 2001; Van Dam et al., 2002). The Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition (2008) rates the

relationship between SFA and the risk for diabetes as possible. However, cohort studies allow us to conclude that the exchange of SFA by PUFA lowers the risk for type 2 diabetes (Hauner and Schulze, 2006).

2.9.4 Impacts of Monounsaturated Fatty Acids

Results of the Finnish and Dutch cohorts of the Seven Countries Study showed that men with newly diagnosed diabetes mellitus had a higher MUFA intake at baseline than men with normal glucose tolerance (Feskens et al., 1995).

In the San Luis Valley Diabetes Study people with glucose intolerance and developing diabetes mellitus had even consumed a larger amount of MUFA than those who returned to a normal glucose tolerance after the observation period (Marshall et al., 1994). In the more recent cohort studies [Iowa Women's Study (Meyer et al., 2001), Nurses' Health Study (Salmeron et al., 2001), Health Professionals Follow-up Study (Van Dam et al., 2002), Kuopio Ischaemic Heart Disease Risk Factor Study (Laaksonen et al., 2002) and the Women's Health Study (Song et al., 2004)] no association between the intake of MUFA and the risk for diabetes could be observed.

Conclusion:

In cohort studies (Meyer et al., 2001; Salmeron et al., 2001; Van Dam et al., 2002; Laaksonen et al., 2002; Song et al., 2004) no association was observed between MUFA intake and risk for diabetes. The Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition (2008) rates the connection between MUFA and the risk for diabetes as insufficient.

2.9.5 Impacts of Polyunsaturated Fatty Acids

In order to examine the effects of safflower oil versus fish oil feeding on intramuscular glucose metabolism Jucker et al. (1999) pair-fed rats on diets consisting of 59% safflower oil, 59% menhaden fish oil or 59% carbohydrate (control) in calories.

The insulin resistance in skeletal muscle associated with chronic safflower oil feeding could primarily be attributed to a reduction in glycolysis. By contrast, fish oil feeding had no effect on any of the major intramuscular pathways of glucose metabolism. Additionally, FFA and ketone oxidation were greater in the safflower oil group than in the control or fish oil group.

These data suggest that the elevated intramuscular TG observed in the rats fed safflower oil correlates with increased fat oxidation. Moreover the protective effect of fish oil feeding may occur through its ability to limit TG deposition in skeletal muscle. However, a relative increase in fat oxidation in the fish oil group versus controls during hyperinsulinemic conditions could not be observed. Thus, the protective effect of fish oil on insulin-stimulated muscle glucose disposal seems to occur via a relative reduction of FFA oxidation compared to the rats fed safflower oil. Possibly this is a result of lowered intramuscular TG and FFA availability.

The results demonstrate that the decreased release of glucose in muscle cells in rats fed with safflower oil can be primarily ascribed to a reduction in glycolysis, which is associated with decreased intramuscular pyruvate oxidation and increased fat oxidation. Furthermore chronic fish oil feeding saves the muscle from becoming insulin resistant by lowering intramuscular TG availability for oxidation relative to safflower oil feeding (Jucker et al., 1999).

In the San Luis Valley Diabetes Study no relationship was found between the PUFA intake and the development of diabetes mellitus. Subjects who developed diabetes mellitus did not have a different intake of PUFA compared to those who kept their impaired glucose tolerance (IGT) or those who returned to a normal glucose tolerance (Marshall et al., 1995).

After an observation period of 11 years in the Iowa Women's Health Study a negative relationship between the PUFA intake and the incidence of diabetes was found, but it did not reach statistical significance. However, the replacement of SFA with PUFA was associated with a significantly lower risk (Meyer et al., 2001).

In the Nurses' Health Study an increased proportion of PUFA in the diet correlated negatively with the risk for diabetes (Salmeron et al., 2001). In the Kuopio Ischaemic Heart Disease Risk Factor Study the PUFA intake in subjects who developed diabetes mellitus was not significantly different to healthy staying individuals. Moreover the PUFA content in the serum was no significant predictor for the development of a reduced glucose tolerance or diabetes mellitus (Laaksonen et al., 2002).

Conclusion:

Altogether studies on the role of PUFA in the prevention of type 2 diabetes are inconsistent, but assume that a higher PUFA intake, especially as replacement for SFA, is associated with a reduced risk for type 2 diabetes (Hauner and Schulze, 2006). Additionally, according to the Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition (2008), there is convincing evidence that PUFA, when replacing SFA, reduce the risk for coronary heart diseases. Furthermore the association between PUFA and a reduced risk for diabetes is seen as possible (Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition, 2008).

3 FATTY ACIDS

3.1 General Facts

Fatty acids are saturated or unsaturated aliphatic monocarbon acids (Vostrowsky, 2005). The most common naturally occurring forms are unbranched with an even number of C atoms, since they are synthesised by C_2 -units (Miebach, 2008).

SFA are aliphatic monocarbon acids with the general chemical formula $C_nH_{2n+1}-COOH$ (n representing the amount of carbon atoms without the C of the carboxyl group).

Unsaturated fatty acids are fatty acids with the general chemical formula $C_nH_{2n-1,3,5}-COOH$ and having at least one double bond in their chain. They are known as mono and poly unsaturated fatty acids.

Unsaturated fatty acids are named after position and number of double bonds. Their specific name arises from the position of the carbon atom of the methyl group, which is opposite to the carboxyl group at the distal end of the chain. This carbon atom is also referred to as the omega carbon atom (abbreviated with ω or n). When indicating the position of the double bond the omega carbon atom is the starting point for counting and is therefore provided with number 1 (Doenecke et al., 2005).

The common SFA in the diet are palmitic and stearic acids - which are present in lard, mutton and beef - and lauric and myristic acids, which are present in coconut and palm oils. The major sources of MUFA are olive and rapeseed oil, which contain oleic acid (Manco et al., 2004).

3.2 Polyunsaturated fatty acids (PUFA)

3.2.1 Biosynthesis of PUFA

PUFA are of major importance for development and maintenance of health of humans and animals. The double bonds are hereby in the cis-configuration and mostly isolated, which means that they are separated by a CH₂-group (Doenecke et al., 2005)

Since double bonds at the ω -3 and ω -6 position can only be inserted by plant enzymes, fatty acids with double bonds at these positions belong to the essential fatty acids (LA and ALA). The requirements of PUFA like LA (starting compound of the ω -6 fatty acid family) or ALA (starting compound of the ω -3 fatty acid family) therefore have to be covered from animal and vegetable sources (Bitsch and Bitsch, 2010; Löffler, 2007).

Due to structural differences there is no possibility to convert ω -3 into ω -6 fatty acids and vice versa. Nevertheless, conversions within the ω -3 and ω -6 fatty acid family can be followed by elongation of the chain and the implementation of further double bonds, called desaturation (Fiebig, 2010).

3.2.2 Elongation of the chain

The elongation of LA to ALA and further happens by a number of different desaturases (δ -6-, δ -5- and δ -4-desaturase). These enzymes implement a new double bond between the carboxyl terminus and the already existing double bond (Rehner and Daniel, 2010). In the course of this process the position of the double bonds before the ω -9 C-atom remains unchanged. This is the reason why the ω -notation is suitable to track the paths of polyunsaturated fatty acids in the metabolism (Doenecke et al., 2005). Onwards there are further steps of elongation, whereas two carbon atoms are added each time. Thereby it becomes important that both LA and ALA compete for the same enzymes, namely the δ -4- and δ -6-desaturase (Figure 3). However, both enzymes prefer

ω -3 fatty acids to ω -6 fatty acids (Rehner and Daniel, 2010). The conversion from ALA to EPA is estimated to be 10 to 15 %, to DHA at 4% (Trautwein, 1999).

3.2.3 Eicosanoids

Eicosanoids are a group of autocrine and paracrine acting signalling substances that arise from C₂₀-fatty acids and emerge in most animal tissues.

As mediators they affect many physiological processes and modulate hormonal and other signals. Additionally they are essentially involved in the development of inflammation. Thus the metabolism of eicosanoids is an important working point of medication (Doenecke et al., 2005).

Eicosanoids are produced from EPA or AA. EPA and DHA are formed by ALA, whereas the ω -6 fatty acids dihomo- γ -linolenic acid and AA are synthesised from LA (Figure 2) (Biesalski and Grimm, 2011).

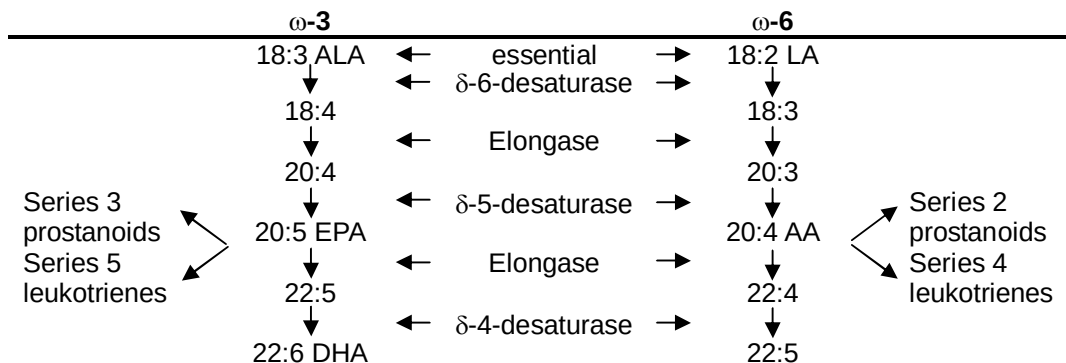


Figure 2: Synthesis of eicosanoids (modified after De Caterina et al., 2007)

The eicosanoids derived from AA, including series 2 prostanoids and series 4 leukotrienes, are more inflammatory than those produced from EPA, which are series 3 prostanoids and series 5 leukotrienes (Figure 3) (Calder, 1998). Thus a shift to a diet rich in ω -3 fatty acids can reduce inflammatory processes (De Caterina et al., 2007).

3.2.4 Sources of PUFA

PUFA are fundamental components of phospholipids in cell membranes and the source for the synthesis of eicosanoids (De Caterina et al., 2007).

The prevalent source of ω -3 fatty acids in the diet are plant oils and fish. Some seeds, for example those of cereals, cotton, safflower and soy beans are rich in ALA. Linseed oil and soya oil are particularly rich in ALA, whereas safflower oil has the highest content of LA of all plant oils with 75-80%, followed by sunflower oil (up to 63%) and hemp oil with 50-70%.

Fatty fishes like salmon, mackerel and herring are the best sources of EPA and DHA. Other minor sources are nuts, seeds, egg yolk, some fruits, vegetables and poultry meat (Heckel, 2009). The fatty acid pattern of some vegetable oils is given in table 1.

*Table 1: Fatty acid profile of different plant oils (modified after Fiebig HJ, 2011)
percent by weight, based on total fatty acids, u = unverifiable defined as $\leq 0.05\%$*

Fatty acid	Cottonseed oil	Linseed oil	Safflower oil	Sunflower oil	Walnut oil
8:0	u	u	u	u	u
10:0	u	u	u	u	u
12:0	u-0.2	u	u	u	u
14:0	0.6-0.1	u	u-0.2	u-0.1	u-0.1
16:0	21.4-26.4	4.0-6.0	5.3-8.0	2.6-5.0	6.0-8.0
16:1	u-1.2	u-0.5	u-0.2	u-0.1	u-0.2
17:0	u-0.1	U	u-0.1	u-0.1	u-0.1
17:1	u-0.1	U	u-0.1	u-0.1	u
18:0	2.1-3.3	2.0-3.0	1.9-2.9	2.9-6.2	1.0-3.0
18:1	14.7-21.7	10.0-22.0	8.4-21.3	75.0-90.7	14.0-21.0
18:2	46.7-58.2	12.0-18.0	67.8-83.2	2.1-17.0	54.0-65.0
18:3	u-0.4	56.0-71.0	u-0.1	u-0.3	9.0-15.0
20:0	0.2-0.5	u-0.5	0.2-0.4	0.2-0.5	u-0.3
20:1	u-0.1	u-0.6	0.1-0.3	0.1-0.5	u-0.3
20:2	u-0.1	u	u	u	u
22:0	u-0.6	u	u-1.0	0.5-1.6	u-0.2
22:1	u-0.3	u	u-1.8	u-0.3	u
22:2	u-0.1	u	u	u	u
24:0	u-0.1	u	u-0.2	u-0.5	u
24:1	u	u	u-0.2	u	u

3.2.5 Recommendations

Fats are important energy storages for human and animal organisms, they should account for 25-30% of the daily energy intake.

The amount of SFA should not exceed 10% of the total energy, MUFA should account for 10-15% of the total energy and PUFA should be around 7% of the total daily energy intake. Important is the ratio of ω -6 to ω -3 fatty acids, which shall amount to 5:1, but is in reality around 8-10:1 (ÖGE, 2011).

Nevertheless, the maximal intake of ω -3 fatty acids shall not exceed 2.5-3% of the total energy intake since they are able to result in bleedings or increased lipid oxidation in a very high dose. Furthermore they can unfavourably affect the function of leucocytes and the immune system (DGE, 2004). Increased requirements exist during pregnancy, lactation and growth period, after stages of stress and in old age.

4 MATERIALS AND METHODS

4.1 Study design and population

The EU project DIAPLANT is a program for a cross-border cooperation between Austria and Slovakia ("creating the future") with a view to investigate the influence of a plant oil and plant foods on biomarkers of secondary diseases of type 2 diabetics. The DIAPLANT-study is an epidemiological, prospective, randomized, controlled intervention study, which was approved by the Ethics Committee of the City of Vienna.

The recruitment was done by three physicians at the health center Wien South of the Viennese regional medical insurance and a supplement in the journal "Diabetes- Info" of the Austrian Diabetic Association taking account of the inclusion and exclusion criteria.

The following criteria had to be fulfilled:

- Men and women with and without type 2 diabetes between 40 and 85 years
- glycated hemoglobin $\leq 9.5\%$
- total cholesterol < 300 mg/dl
- triglycerides in the serum < 500 mg/dl
- creatinine < 2.5 mg/dl
- steady medication concerning glucose, fat and uric acid metabolism
- steady eating habits and physical activity
- steady body weight

Exclusion criteria were:

- type 1 diabetes
- people younger than 40 and older than 85 years
- smoker

- pregnant and breastfeeding women
- participation in other clinical studies
- recently changed medication concerning glucose, fat and uric acid metabolism
- intake of fish oil capsules or other fatty acids
- cardiovascular diseases
- hepatic diseases
- chronic renal insufficiency
- gastrointestinal malabsorption
- organ transplantation
- systemic steroids
- cancer
- HIV
- intake of glitazone

Interested people were invited to information sessions at the health center Wien South and informed about the health benefits of a diet rich in vegetables and the advantages of plant oils over animal fat. Afterwards the DIAPLANT study was presented and the study process was explained. Once the subjects had signed the statement of agreement, the randomization of the study participants in the intervention (Iv) and the information group (Inf) followed. Both the intervention and the information group included the four health states of the participants, namely insulin-dependent diabetes mellitus (IDDM), non-insulin-dependent diabetes mellitus (NIDDM), impaired fasting glucose (IFG) and healthy (figure 3). Since some of the participants first allocated to the healthy group turned out to have impaired fasting blood glucose concentrations during the examinations an extra group (IFG) was formed. Different than originally planned this resulted in four groups with various health status.

All subjects (Iv and Inf group) were informed about the advantages of a healthy diet. Additionally, the subjects of the intervention group received 300 g of vegetables and 25 ml of plant oil every day of the intervention phase, which lasted eight weeks.

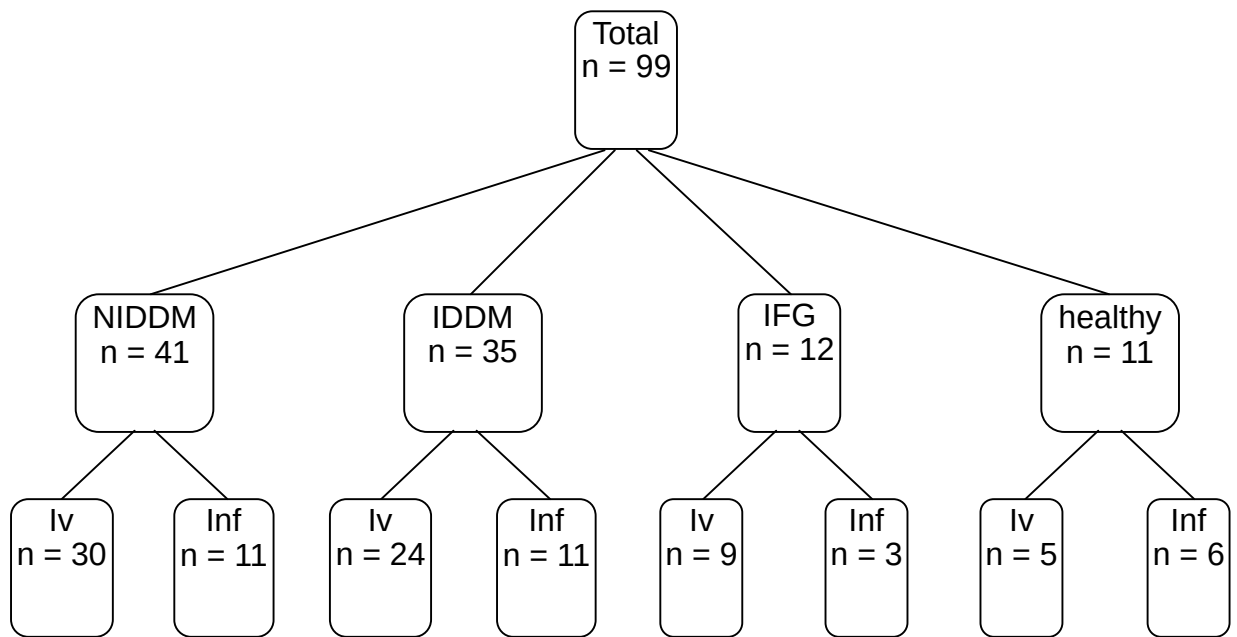


Figure 3: Study design and classification of the participants

4.2 Study process over time

During the study period (16 weeks) there were four blood collection dates. Blood was collected from fasting subjects, blood pressure was measured, urine was collected, a swab of buccal mucosa was done and anthropometric measurements were conducted at each examination for the entire study population. The last examination took place eight weeks after the end of the intervention. At T0 additionally the height was measured in order to determine the BMI (body mass index) of all the participants.

Furthermore the subjects had to fill several questionnaires: a 24-hour protocol one day before each examination day, a quality of life questionnaire (QLQ) and a food frequency questionnaire (FFQ). Type 2 diabetics (IDDM and NIDDM) delivered their blood sugar profile. Study participants of the IDDM group also kept an insulin protocol.

The intervention group was characterized by an eight-week lasting intervention phase (active phase), which was defined by two blood samplings, one after four weeks of intervention (T1) and one after eight weeks of intervention (T2). After

the active phase a further eight-week lasting period followed (T3), in which subjects did not get any oil and vegetables and returned to their original diet (figure 4).

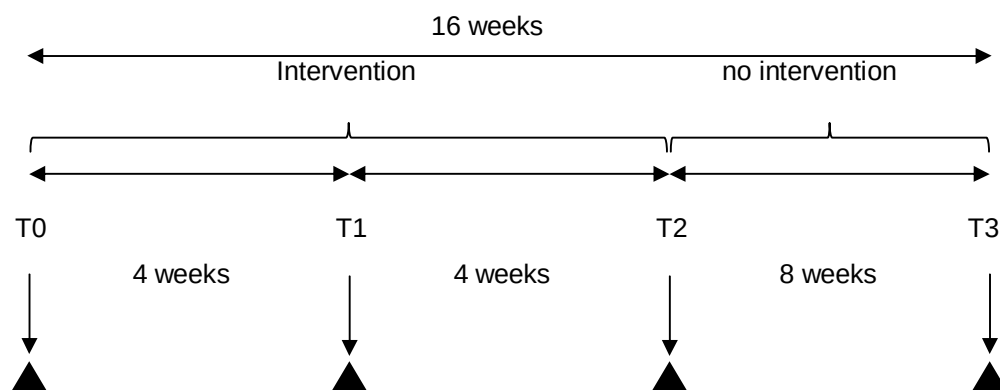


Figure 4: Intervention phase and timing of the examinations

▲ Blood sampling, blood pressure, urine collection, swab of buccal mucosa, anthropometric measurements, QLQ, FFQ, 24-hour protocol, blood sugar profile, insulin protocol

T0	Start of the study
T1	after four weeks of intervention
T2	after eight weeks of intervention
T3	eight weeks after end of intervention

4.3 Characteristics of the intervention phase

During the eight-week lasting intervention phase (active phase) participants consumed 300 g frozen vegetables of *figlo* and 25 ml plant oil daily. Subjects were given a receipt book to demonstrate the right intake of the intervention oil and to facilitate the preparation of food. The recipes should demonstrate the

possibilities of the wide range of dishes based on vegetables. The required 300 g of frozen vegetables per day could be consumed at once or spread throughout the day. Beyond that participants of the intervention group were asked to keep a food diary during the duration of the intervention.

The following frozen products were provided by *flglo*

- *Spinat passiert*
- *Blattspinat Zwutschgerl*
- *Cremespinat mit Mini-Rösti*
- *Junge Fisolen*
- *Kochsalat mit Erbsen*
- *Broccoli*
- *Kohlsprossen*
- *Zarte Sojabohnen*
- *Junge Erbsen*
- *Karfiol Broccoli DUO*
- *Buntes Gemüse Trio*
- *Junges Sommergemüse* *Zarte Mischung*
- *Suppengemüse*
- *Röstgemüse Korsika*
- *Röstgemüse Gärtnerin*

The intervention oil was a nutritionally high-quality plant oil, which was rich in PUFA and contained a high amount of α -tocopherol. Due to the fatty acid profile the oil should not be heated but stirred in ready-to-eat, cooked (vegetable soup, mashed potatoes, spinach) and cold foods (herb spread). When using the oil for making salads, it was important not to leave it in the marinade behind.

The composition of the intervention oil, which was rich in ω -3 and ω -6 fatty acids and γ -tocopherol, is shown in the tables 2 and 3:

Table 2: Fatty acid profile of the intervention oil

% of total fatty acids	C16:0	C18:0	C18:1n9c	C18:1n7c	C18:2n6c	C18:3n3
Mean value	7.28	2.06	16.4	0.95	61.76	11.49
Standard deviation	0.09	0.07	0.99	0.03	1.00	0.43

Table 3: Tocopherol content of the intervention oil

mg/100 g oil	γ -tocopherol	α -tocopherol
Mean value	32.98	2.67
Standard deviation	1.82	0.14

4.4 Study subjects

The statement of agreement was signed by 120 people. At baseline the DIAPLANT collective consisted of 111 participants, 99 of them also completed the study. Lack of compliance, intolerance of intervention food and oil as well as hospitalizations were responsible for the loss of 10.8%. In table 4 general base data of the study population (n = 99) are shown.

Table 4: Base data of the study population

	Intervention group MW $\pm$ SD	Information group MW $\pm$ SD
Total population	68	31
IDDM	25	11
NIDDM	29	11
IFG	9	3
Healthy	5	6
Sex (male/female)	29/39	12/19
Age (years)	65.76 $\pm$ 7.29	61.65 $\pm$ 6.66
Body weight (kg)	89.38 $\pm$ 16.41	90.02 $\pm$ 18.98
BMI (kg/m ²)	32.50 $\pm$ 5.71	32.21 $\pm$ 7.03
Duration of diabetes (years)	12.01 $\pm$ 10.81	8.94 $\pm$ 9.65
HbA _{1c} (%)	7.23 $\pm$ 1.08	7.01 $\pm$ 1.05

4.5 Blood preparation

Blood samples taken from fasting patients were put into centrifuge tubes which were coated with lithium-heparin or EDTA. After ten min. centrifugation at 3000 rpm the plasma was aliquoted into Eppendorf tubes, purged with nitrogen and stored at -80°C until further analyses. Analyses of fatty acids were performed in heparin plasma.

4.6 Determination of the fatty acid profile

4.6.1 Method

The plasma fatty acid profile in the plasma was determined by gas chromatography after previous hydrolysis under alkaline conditions with methanolic sodium hydroxide and esterification with boron trifluoride-methanol to fatty acid methyl esters (FAME).

4.6.2 Background

Gas chromatography denotes a chromatographic technique for the segregation of mixtures of substances which can be vaporised without composition. The separation line is a column, where the stationary phase is adhered to the wall of the capillary and the mobile gas phase.

Two substances can be separated when they show different retention times at the stationary phase. The separated substances are then detected by the detector and identified based on their individual retention time (Brinkert, 2005).

4.6.3 Reagents

- Methanol p.a (Merck)
- Sodium hydroxide (NaOH) (Riedel-de-Haën)

- Butylated hydroxytoluene (BHT) (Sigma)
- Methanolic sodium hydroxide solution (1 g NaOH and 3 mg BHT in a 50 ml round-bottom flask, padded with methanol and dissolved in ultrasonic bath)
- Boron trifluoride-methanol, 14% (Sigma)
- n-Hexan p.a (Merck)
- 37-component standard SUPELCO 18919 AMP, F.A.M.E. Mix, C4-C24 (Sigma)
- PUFA No.2 SUPELCO 47015-u, Animal Source

4.6.4 Appliances

- Pyrex test tubes with screw-cap and teflon-coated
- glass test tubes
- Shaker (IKA Labortechnik, KS 125 basic)
- Water bath (40°C and 100°C)
- Autosystem Gas chromatograph and gas chromatography vials

4.6.5 Implementation

35 µl of heparinised plasma was transferred into Pyrex test tubes and laced with newly produced methanolic sodium hydroxide solution. Samples were saponified during five minutes boiling at 100°C in a water bath. After cooling to room temperature the released fatty acids were esterified by addition of 1 ml boron trifluoride-methanol and five minutes boiling at 100°C.

After addition of 1 ml hexan it was shaken for five minutes. In order to gain all fatty acid methyl esters (FAME) this step was carried out four times. The hexan phase was transferred into glass test tubes and evaporated under nitrogen at 40°C. 150 µl hexan were added to the dry FAME, transferred into gas chromatography vials and closed densely.

FAME were separated by gas chromatography and detected by a flame ionization detector.

4.6.6 Instrument adjustments

- GC: Perkin Elmer Autosystem Gas Chromatograph
- Detector: Flame ionization detector
- Pillar: Restek 30 m, 0.25 mm ID, 0.2 μm df, 90% Biscyanopropyl, 10% Phenylcyanopropylpolysiloxane
- Temperature of injector: 250°C
- Temperature of detector: 275°C
- Carrier gas: Helium
- Gas of detector: Hydrogen and air
- Injection volume: 0.5 μl
- Split: 1:50
- Software: TotalChrom Workstation

4.6.7 Temperature program and duration

Start temperature was 90°C and increased by 13°C/min until 155°C/min and further increased by 5°C/min up to 175°C. This temperature was held for 12 minutes. Then the temperature increased by 2.9°C/min to 225°C.

4.6.8 Evaluation

The qualitative evaluation was performed through the retention times of a 37-component standard (figure).

4.6.9 Quality management

In order to check the precision of the analyses a control plasma sample which was taken before the actual analyses, was analysed every workday. Every eighth sample was a control sample.

4.7 Statistical evaluation

Statistical evaluation was carried out with SPSS for Windows, Version 17.0. After exclusion of outliers, the data were checked for normal distribution with the Kolmogorov-Smirnov-Test. Data, which were normally distributed, were evaluated by the unpaired t-tests and one-way analysis of variance. Not normally distributed data were tested with the non-parametric Mann-Whitney-Test or Kruskal-Wallis-Test. At T0 measures of correlations were realised with the Pearson's correlation. Changes during the intervention were evaluated by repeated-measures analysis of variance (ANOVA). Time was used as repeating factor, treatment (information or intervention) and state of health (IDDM, NIDDM, healthy, IFG) as between subject factors.

Statistical differences were considered to be significant at a value of $p \leq 0.05$.

5 RESULTS AND DISCUSSION

To make a statement about the subjects' compliance the fatty acid profile in plasma was determined. The major fatty acids in the intervention oil were LA and ALA. Furthermore, the oil was rich in γ -tocopherol (tables 2 and 3).

5.1 Important key findings at baseline

5.1.1 Gender-related differences of fatty acids

At baseline (T0) there were no differences between men and women in those fatty acids shown on the left side of table 5. The right part of the table shows the fatty acids which were significantly different between men and women.

Table 5: Gender-related differences of fatty acids (% of total fatty acids) at baseline

Fatty acid	Mean value	p	Fatty acid	Mean value	p
16:0 male female	21.04±1.32 21.27±1.80	0.503	16:1n7 male female	1.66±0.42 2.01±0.63	0.010*
18:1n9 male female	21.43±2.64 21.27±2.93	0.781	18:0 male female	7.11±0.65 7.44±0.68	0.019*
18:2n6 male female	24.84±3.46 24.99±4.31	0.860	18:1n7 male female	2.01±0.26 1.85±0.28	0.004*
18:3n3 male female	0.55±0.29 0.48±0.15	0.207	18:3n6 male female	0.38±0.13 0.46±0.16	0.013*
20:5n3 male female	0.70±0.28 0.70±0.31	0.968	20:4n6 male female	7.58±1.56 6.89±1.51	0.033*
22:5n3 male female	0.54±0.10 0.51±0.11	0.213			
22:6n3 male female	1.68±0.45 1.61±0.47	0.426			
SFA male female	30.01±1.51 30.59±2.03	0.132			
MUFA male female	25.77±3.03 25.74±3.46	0.965			
PUFA male female	38.08±3.76 37.62±4.77	0.620			

* p -value ≤ 0.05

Differences in AA probably occurred due to men's higher consumption of meat and meat products. According to Prättälä et al. (2007) men's diet contains more often meat while women prefer fruits and vegetables. The data of the present study support this finding. The evaluation of food frequency questionnaires, completed within this study, also confirms that men consume more pork (44% 2-3 times a week and 24% rarely) than women (31% 2-3 times a week and 36% rarely). γ -linolenic acid (18:3n6), which is generated from LA by the δ -6-desaturase, is higher in women at baseline, indicating a higher enzyme activity in females (Watkins and German, 2008).

There is no specific explanation for the gender-related differences of palmitoleic acid (16:1n7), stearic acid (18:0) and vaccenic acid (18:1n7) since these fatty acids occur in almost every fat and oil (European Food Information Council, 2006).

5.1.2 Differences of fatty acids between the intervention and the information group

There were no significant differences between the intervention and the information group at baseline in any of the fatty acids presented in table 6.

Table 6: Differences of fatty acids (% of total fatty acids) between the information and the intervention group at baseline

Fatty acid		Mean value	p	Fatty acid		Mean value	p
16:0	Information Intervention	20.83±1.46 21.34±1.68	0.145	18:3n6	Information Intervention	0.45±0.15 0.42±0.15	0.277
16:1n7	Information Intervention	1.88±0.53 1.86±0.61	0.869	18:3n3	Information Intervention	0.51±0.19 0.51±0.23	0.979
18:0	Information Intervention	7.35±0.69 7.29±0.68	0.675	20:4n6	Information Intervention	7.29±1.68 7.11±1.51	0.592
18:1n9	Information Intervention	21.26±2.69 21.37±2.87	0.856	20:5n3	Information Intervention	0.68±0.24 0.70±0.32	0.748
18:1n7	Information Intervention	1.91±0.28 1.91±0.29	0.988	22:5n3	Information Intervention	0.53±0.10 0.51±0.11	0.330
18:2n6	Information Intervention	24.86±3.77 24.97±4.09	0.901	22:6n3	Information Intervention	1.62±0.39 1.65±0.49	0.754
SFA	Information Intervention	30.06±1.66 30.50±1.93	0.279	MUFA	Information Intervention	25.71±3.21 25.77±3.34	0.940
PUFA	Information Intervention	37.95±3.75 37.74±4.66	0.829				

5.1.3 Differences of fatty acids between the various groups with different health status

Table 7 summarises all the fatty acids that were significantly differed at baseline between IDDM, NIDDM, IFG and healthy subjects.

Table 7: Significant differences of fatty acids (% of total fatty acids) in the various groups with different health status at baseline

Fatty acid		Mean value	p-value
16:0	IDDM	20.91±1.44 ^a	0.001*
	NIDDM	21.86±1.45	
	healthy	19.98±1.97 ^a	
	IFG	20.71±1.45	
18:1n9	IDDM	21.73±1.98	0.010*
	NIDDM	21.92±2.75	
	healthy	19.96±3.08	
	IFG	19.28±3.78 ^a	
18:2n6	IDDM	24.08±3.50	0.001*
	NIDDM	23.99±3.19	
	healthy	27.62±4.17 ^{ab}	
	IFG	28.23±5.08 ^{ab}	
SFA	IDDM	30.07±1.63 ^a	0.007*
	NIDDM	31.08±1.86	
	healthy	29.39±1.76 ^a	
	IFG	29.62±1.81	
MUFA	IDDM	26.31±2.32	0.011*
	NIDDM	26.33±3.27	
	healthy	24.43±3.82	
	IFG	23.19±4.13 ^{ab}	
PUFA	IDDM	37.32±3.41	0.001*
	NIDDM	36.57±4.10	
	healthy	40.05±4.35	
	IFG	41.57±5.64 ^{ab}	

* p-value ≤ 0.05

^a significantly different from NIDDM

^b significantly different from IDDM

The higher plasma levels of palmitic acid (16:0) and SFA in NIDDM compared to healthy subjects indicate a diet based on higher saturated fat intake. Especially diabetic subjects should reduce their intake of saturated fat due to the increased risk for cardiovascular diseases and stroke (Toeller, 2000). These findings go well together with the higher levels of LA and PUFA in the plasma of healthy and IFG subjects. These results indicate that the nutritional behaviour of healthy subjects is better than that of diabetics.

δ9-desaturase activity is known to be high in conditions like diabetes, atherosclerosis and obesity (Warnesjö et al., 2006). This explains the higher levels of oleic acid (18:1n9) and MUFA in subjects with IDDM and NIDDM.

5.1.4 Correlations between PUFA and plasma lipids

At baseline there was a positive correlation between the plasma PUFA content of all participants and HDL ($r=0.524$, $p=0.000$) and a negative correlation between PUFA and the ratio of total to HDL-cholesterol ($r=-0.271$, $p=0.007$). The Strong Heart Study has found the ratio of total to HDL-cholesterol to be a good predictor of cardiovascular risk in both men and women with type 2 diabetes (Lu et al., 2003). The study of Tapsell et al. (2004) demonstrated a significant decrease in the ratio of total to HDL-cholesterol in patients with diabetes by including 30g walnuts per day to the habitual diet, indicating the antiatherogenic potential of PUFA.

Furthermore the correlation between PUFA and the HOMA Index for insulin resistance tended to be negative ($r=-0.216$, $p=0.071$) in diabetic subjects at baseline. These interesting findings point to the fact that PUFA might be supportive to decrease the blood glucose levels. On the other hand Mostad et al. (2006) found negative effects of a high fish oil intake (20 ml/day for nine weeks) on glucose metabolism.

In the present study TG were negatively correlated with PUFA ($r=-0.694$, $p=0.000$) at baseline. Loschiavo et al. (1990) reported similar results in subjects consuming a diet with an elevated PUFA/SFA ratio. Conversely, in the present study the correlation between TG and SFA was positive, ($r=0.546$, $p=0.000$), which was also found by Cao et al. (2009).

5.2 Change over time in the intervention group

5.2.1 Total population

In the total intervention group the following fatty acids changed significantly over time (table 8):

Table 8: Changes of fatty acids over time in the intervention group. * p value ≤ 0.05

Fatty acid	Change	p-value
16:0	↓	0.000*
16:1n7	↓	0.000*
18:1n9	↓	0.000*
18:1n7	↓	0.004*
18:2n6	↑	0.000*
18:3n3	↑	0.000*
22:6n3	↓	0.018*
SFA	↓	0.000*
MUFA	↓	0.000*
PUFA	↑	0.000*

The consumption of 25 ml PUFA rich plant oil, providing approximately 3 g ALA and 15 g LA, led to significant changes in SFA, MUFA and PUFA levels as well as in those fatty acids predominant in the plant oil, indicating a good dietary compliance.

ALA significantly increased in the intervention group by 58.8% at T1 ($0.81 \pm 0.32\%$) and 62.8% at T2 ($0.83 \pm 0.36\%$) compared with baseline levels ($0.51 \pm 0.24\%$). Also LA significantly increased by 15.1% at T1 ($28.62 \pm 4.53\%$) and 14.5% at T2 ($28.47 \pm 4.28\%$) in comparison to baseline ($24.87 \pm 4.11\%$). Consequently, PUFA also increased significantly by 8.8% at T1 ($40.96 \pm 4.70\%$) and by 8.4% T2 ($40.84 \pm 4.47\%$) compared to baseline ($37.66 \pm 4.72\%$) (figure 5).

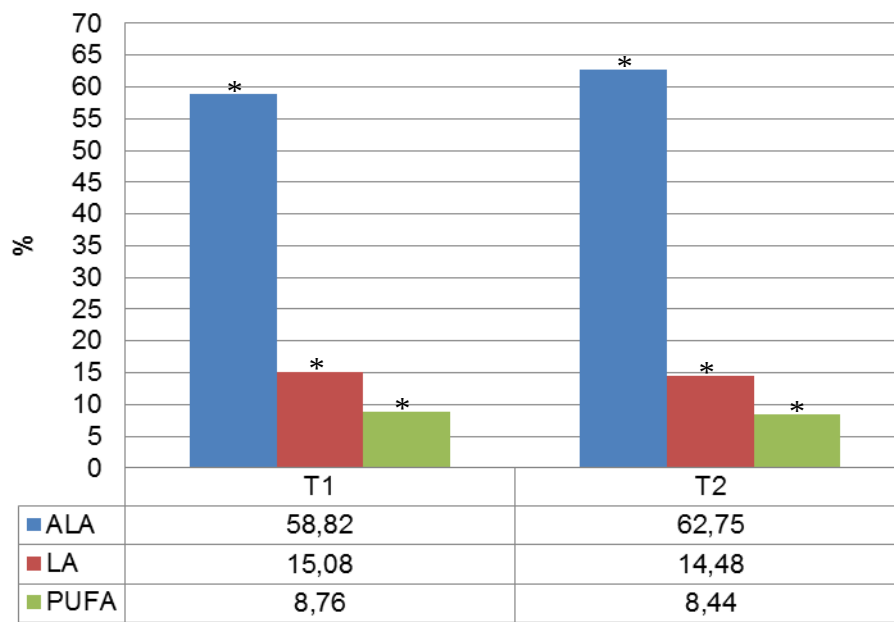


Figure 5: Changes (%) of ALA and LA compared to T0 during the intervention (T1 and T2).
 * Significant changes compared to T0. T0=0%

The decreases of LA ($25.26 \pm 4.05\%$) and ALA ($0.57 \pm 0.25\%$) at T3 in comparison to baseline indicate that their changes were due to the intake of the intervention oil. It should be mentioned that the participants had to return the oil bottles at T2, so the intervention oil was not available any longer.

Although LA and ALA are precursors of EPA and DHA, EPA remained constant and DHA decreased slightly ($p=0.012$) over time ($T0=1.66 \pm 0.50$, $T1=1.53 \pm 0.42$, $T2=1.53 \pm 0.42$). These findings are similar to the results of Almario et al. (2001). They found increased LA and ALA plasma concentrations after the addition of 48 g walnuts to the habitual diet, whereas concentrations of EPA and DHA did not change. The authors suggest these findings as a result of the competition between LA and ALA for the δ -6-desaturase.

The plasma levels of AA did not show significant changes during the intervention. Other intervention studies demonstrated an increase in LA and a decrease in AA after interventions with a plant oil rich in PUFA (Karvonen et al., 2002; Moloney et al., 2004). These findings are inconsistent with the results of Hodson et al. (2001), who showed an increase in both LA and AA after a shift from a diet rich in SFA to a diet rich in PUFA. During the SFA diet subjects used

butter for baking and cooking, consumed high-fat dairy foods and avoided the intake of vegetable oils. Within the ω -6 PUFA diet participants used low-fat dairy foods, minimised the intake of foods high in SFA and avoided the consumption of butter. Furthermore they were provided with safflower oil (Hodson et al., 2001).

SFA significantly decreased over time in the intervention group ($T_0=30.53\pm1.95\%$, $T_1=29.56\pm2.00\%$, $T_2=29.68\pm1.78\%$) due to a significant reduction of palmitic acid ($T_0=21.40\pm1.69\%$, $T_1=20.52\pm1.89\%$, $T_2=20.61\pm1.59\%$). Also MUFA showed a significant decrease over time ($T_0=25.78\pm3.39\%$, $T_1=23.71\pm3.44\%$, $T_2=23.56\pm3.38\%$). In this respect the significant decreases of palmitoleic acid ($T_0=1.86\pm0.60\%$, $T_1=1.62\pm0.51\%$, $T_2=1.60\pm0.50\%$), vaccenic acid ($T_0=1.90\pm0.29\%$, $T_1=1.81\pm0.27\%$, $T_2=1.82\pm0.32\%$) and oleic acid ($T_0=21.18\pm2.75\%$, $T_1=19.44\pm2.71\%$, $T_2=19.42\pm2.79\%$) should be mentioned. The decrease in SFA and MUFA could result from the replacement of butter, margarine or oils rich in MUFA by the intervention oil, which contains high contents of PUFA.

Results of Hodson et al. (2001) showed a significant decrease in palmitic acid after a replacement of SFA by a plant oil rich in PUFA. Changes in SFA were also found after supplementation with long-chain ω -3 fatty acids in NIDDM subjects (Haban et al., 2000). Karvonen et al. (2002) chose camelina oil for the intervention and showed a significant decrease in palmitic acid and oleic acid. A compensatory decrease in palmitic acid and oleic acid to a higher PUFA intake and a significant reduction in SFA could also be shown after an addition of 48g walnuts to the habitual diet (Almario et al., 2001).

Peck et al. (1996) observed a significant reduction of oleic acid in the plasma after an intervention with safflower oil.

At T_3 none of the investigated fatty acids showed any significance compared to baseline, indicating that the subjects of the intervention group returned to their usual diet.

As shown in the present study moderate changes in the diet by addition of 25 ml PUFA rich plant oil lead to an elevation of PUFA and a reduction of SFA and MUFA.

Several studies could demonstrate that a high fat intake, particularly if rich in SFA, is associated with impaired insulin sensitivity and secretion and the development of type 2 diabetes (Lovejoy, 1999; Marshall et al., 1997). Although studies on the role of PUFA in the prevention of type 2 diabetes are inconsistent (Jucker et al., 1999; Marshall et al., 1995; Meyer et al., 2001; Salméron et al., 2001; Laaksonen et al., 2002), it is assumed that a higher PUFA intake, especially as replacement for SFA, is associated with a reduced risk for type 2 diabetes (Hauner and Schulze, 2006).

5.2.2 IDDM and NIDDM

The two predominant PUFA of the intervention oil, LA and ALA, significantly increased in the IDDM and NIDDM groups. Since both fatty acids were characteristic for the intervention oil, the increase confirms a good dietary compliance. In the IDDM group the LA significantly differed after four weeks of intervention. At T2 the levels were slightly lower than at T1 and therefore not significantly different from T0. Furthermore ALA significantly rose at T1 and T2 (table 9). In patients with NIDDM, both LA and ALA were significantly different at T1 and T2 in comparison to T0. Furthermore 16:0 significantly decreased at T2 (table 10).

Table 9: Fatty acid profile (% of total fatty acids) in the plasma of patients with **IDDM** in the intervention group. * Significant changes compared to T0. ^x Tendency of changes compared to T0 ($p=0.05-0.1$)

Fatty acid	T0	T1	T2	T3
16:0	21.22±1.55	20.79±1.77	20.90±1.51	21.96±2.08
16:1n7	1.95±0.52	1.75±0.51	1.65±0.43	1.99±0.52
18:0	7.29±0.58	7.1±0.44	7.19±0.71	7.16±0.60
18:1n9	21.67±2.03	20.17±2.60	19.94±2.59	21.78±2.04
18:1n7	1.94±0.32	1.86±0.29	1.84±0.32	1.89±0.31
18:2n6	24.59±3.67	28.31±4.08*	28.07±3.59 ^x	24.21±3.10
18:3n6	0.41±0.17	0.44±0.15	0.44±0.19	0.47±0.18
18:3n3	0.43±0.19	0.74±0.22*	0.74±0.21*	0.50±0.12
20:4n6	7.17±1.49	6.72±1.21	6.79±1.26	6.94±1.44
20:5n3	0.65±0.25	0.66±0.25	0.77±0.44	0.78±0.34
22:5n3	0.53±0.12	0.52±0.11	0.51±0.10	0.51±0.10
22:6n3	1.42±0.23	1.37±0.28	1.43±0.39	1.47±0.40
SFA	30.46±1.75	29.74±2.00	30.04±1.61	31.07±2.67
MUFA	26.22±2.31	24.40±3.06	24.06±3.03	26.37±2.30
PUFA	37.14±3.82	40.18±4.39	40.05±3.97	36.48±3.31

Table 10: Fatty acid profile (% of total fatty acids) in the plasma of patients with **NIDDM** in the intervention group. * Significant changes compared to T0

Fatty acid	T0	T1	T2	T3
16:0	21.97±1.56	20.93±1.75	20.77±1.42*	21.62±1.62
16:1n7	1.84±0.62	1.61±0.52	1.62±0.55	1.82±0.49
18:0	7.08±0.58	7.24±0.68	7.20±0.51	7.04±0.54
18:1n9	21.74±2.76	19.84±2.60	19.82±2.82	21.09±2.99
18:1n7	1.92±0.27	1.82±0.26	1.87±0.34	2.00±0.30
18:2n6	23.75±3.28	27.49±4.43*	27.43±4.28*	24.16±3.91
18:3n6	0.39±0.14	0.40±0.13	0.41±0.15	0.38±0.13
18:3n3	0.51±0.16	0.86±0.27*	0.82±0.31*	0.60±0.26
20:4n6	6.84±1.52	6.64±1.47	6.78±1.65	6.81±1.68
20:5n3	0.68±0.30	0.71±0.27	0.73±0.28	0.70±0.26
22:5n3	0.49±0.11	0.50±0.13	0.54±0.08	0.49±0.09
22:6n3	1.75±0.51	1.61±0.44	1.55±0.33	1.61±0.39
SFA	31.05±1.98	30.06±1.79	29.80±1.55	30.54±1.78
MUFA	26.54±3.55	24.32±3.58	24.10±3.55*	26.98±4.23
PUFA	36.23±4.32	39.85±4.66*	39.97±4.55*	36.82±4.96

In the **IDDM** group the relative increase of LA was 15.1% at T1 and 14.1% at T2 (figure 6).

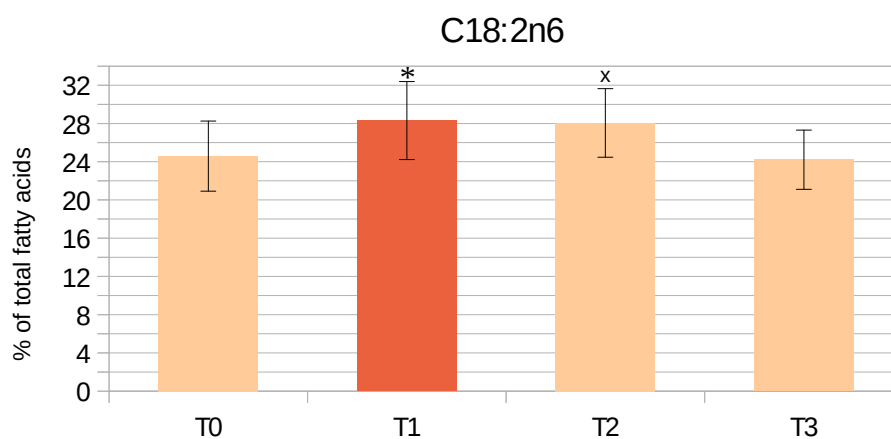


Figure 6: Changes of LA in the plasma of patients with **IDDM** in the intervention group.

* Significant changes compared to T0. x Tendency of changes compared to T0 ($p=0.05-0.1$)

ALA relatively increased by 72.1% at T1 and T2 compared with T0 (figure 7).

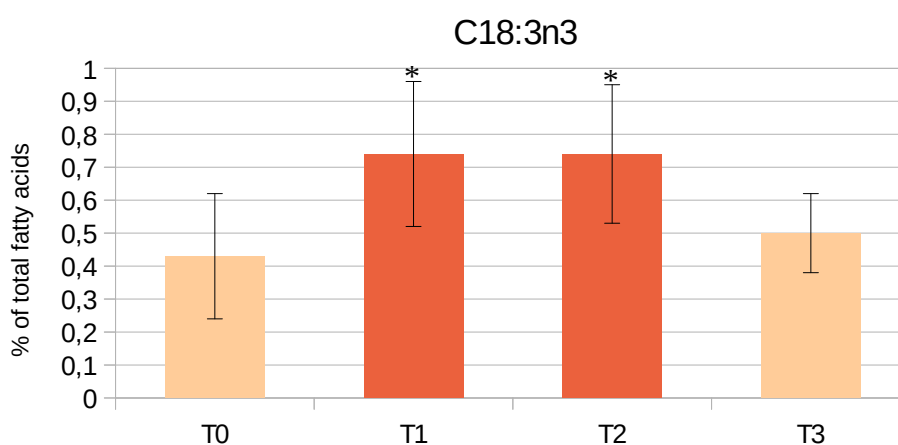


Figure 7: Changes of ALA in the plasma of patients with **IDDM** in the intervention group.

* Significant changes compared to T0

In the **NIDDM** group LA increased by 13.6% at T1 and 13.4% at T2 (figure 8).

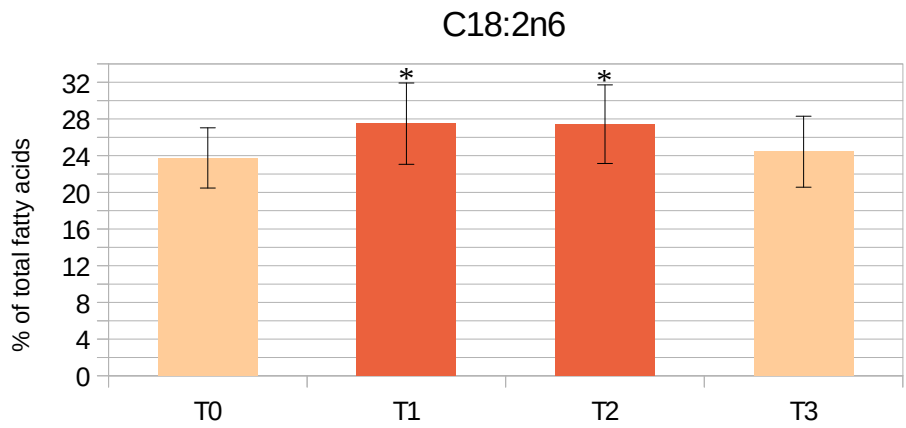


Figure 8: Changes of LA in the plasma of patients with **NIDDM** in the intervention group.
* Significant changes compared to T0

The rise of ALA accounted for 40.7% at T1 and 37.8% at T2 (figure 9).

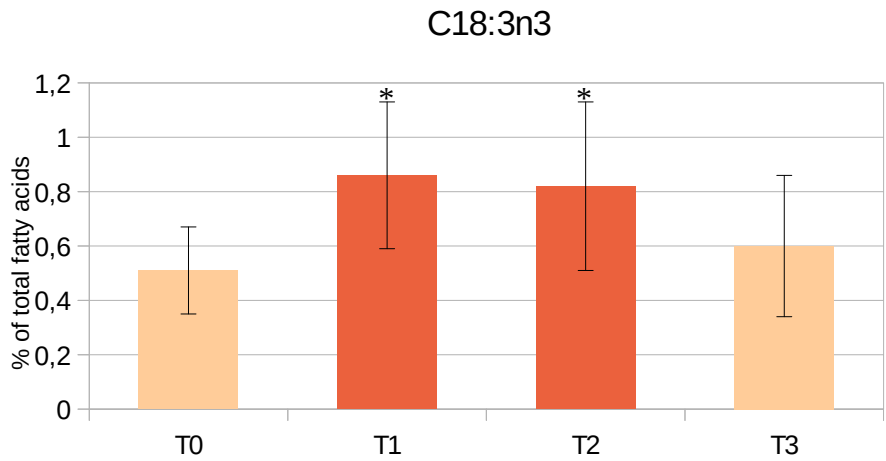


Figure 9: Changes of ALA in the plasma of patients with **NIDDM** in the intervention group.
* Significant changes compared to T0

5.2.3 Healthy and IFG subjects

Although the mean values of the characteristic fatty acids LA and ALA were higher at T1 and T2, no significant changes were observed in the healthy and the IFG-subjects (table 11 and 12). The reason could be the small sample number of only five healthy and nine IFG subjects and the large standard deviations.

Table 11: Fatty acid profile (% of total fatty acids) in the plasma of **healthy participants** in the intervention group

Fatty acid	T0	T1	T2	T3
16:0	20.39±2.51	18.51±1.88	18.84±1.62	19.27±1.05
16:1n7	2.10±0.79	1.52±0.50	1.49±0.62	1.64±0.43
18:0	7.56±1.14	7.48±1.08	7.32±0.92	7.36±0.87
18:1n9	21.26±2.11	18.95±1.23	19.23±2.82	20.95±2.72
18:1n7	1.81±0.29	1.81±0.29	1.85±0.22	1.87±0.20
18:2n6	26.12±4.61	30.77±4.43	31.49±5.08	29.07±2.34
18:3n6	0.55±0.20	0.44±0.20	0.44±0.28	0.51±0.26
18:3n3	0.62±0.29	0.90±0.74	1.05±0.88	0.56±0.18
20:4n6	6.41±1.28	7.09±2.04	6.31±1.32	6.66±1.61
20:5n3	0.86±0.38	0.71±0.25	0.63±0.27	0.92±0.58
22:5n3	0.57±0.10	0.54±0.08	0.49±0.06	0.54±0.12
22:6n3	1.64±0.70	1.58±0.66	1.43±0.50	1.67±0.68
SFA	29.90±1.99	27.58±1.79	27.74±1.96	28.13±1.04
MUFA	25.93±2.74	22.89±1.28	23.21±3.09	25.08±2.55
PUFA	38.79±3.97	43.73±2.21	43.54±3.55	41.82±1.59

Table 12: Fatty acid profile (% of total fatty acids) in the plasma of participants with **IFG** in the intervention group

Fatty acid	T0	T1	T2	T3
16:0	20.32±1.23	19.38±1.86	20.36±1.99	20.62±2.26
16:1n7	1.58±0.56	1.38±0.46	1.46±0.48	1.50±0.46
18:0	7.91±0.71	7.74±0.57	7.65±0.48	7.61±0.70
18:1n9	17.96±2.92	16.49±2.30	16.82±2.07	17.94±2.26
18:1n7	1.81±0.26	1.67±0.21	1.57±0.14	1.67±0.18
18:2n6	29.46±5.40	32.66±4.31	31.77±3.64	28.96±5.04
18:3n6	0.42±0.14	0.40±0.17	0.45±0.20	0.39±0.17
18:3n3	0.65±0.44	0.81±0.32	0.93±0.31	0.67±0.43
20:4n6	7.85±1.47	7.20±1.16	7.13±1.82	7.58±1.74
20:5n3	0.88±0.49	0.70±0.21	1.04±0.51	0.71±0.13
22:5n3	0.53±0.07	0.52±0.10	0.57±0.05	0.53±0.07
22:6n3	1.93±0.68	1.56±0.45	1.73±0.66	1.71±0.50
SFA	29.26±1.89	28.52±2.05	29.53±2.38	29.99±3.12
MUFA	21.81±3.32	20.16±2.87	20.50±2.44	21.75±2.53
PUFA	43.53±4.76	45.30±4.18	44.35±4.25	42.82±5.43

Pooling healthy and IFG subjects shows a significant increase of ALA at T2 compared to T0. Increases of LA (T1 and T2) and ALA (T1) are indeed recognisable, but not significantly different from T0 (table 13).

Table 13: Fatty acid profile (% of total fatty acids) in the plasma of **healthy and IFG** participants in the intervention group. * Significant changes compared to T0

Fatty acid	T0	T1	T2	T3
16:0	20.35±1.77	19.02±1.84	19.73±1.93	20.06±1.92
16:1n7	1.78±0.68	1.43±0.46	1.47±0.52	1.55±0.43
18:0	7.76±0.88	7.63±0.79	7.51±0.68	7.50±0.75
18:1n9	19.23±3.04	17.44±2.27	17.75±2.58	19.10±2.79
18:1n7	1.81±0.26	1.73±0.25	1.69±0.22	1.75±0.21
18:2n6	28.07±5.16	31.87±4.27	31.66±4.08	29.01±3.98
18:3n6	0.46±0.17	0.41±0.17	0.45±0.22	0.43±0.20
18:3n3	0.64±0.37	0.84±0.49	0.97±0.57*	0.63±0.35
20:4n6	7.29±1.53	7.16±1.47	6.81±1.64	7.23±1.68
20:5n3	0.87±0.44	0.70±0.21	0.88±0.46	0.79±0.37
22:5n3	0.55±0.08	0.53±0.09	0.54±0.06	0.53±0.09
22:6n3	1.82±0.67	1.57±0.51	1.61±0.60	1.70±0.55
SFA	29.51±1.87	28.16±1.94	28.84±2.33	29.27±2.63
MUFA	23.39±3.65	21.21±2.69	21.54±2.92	23.03±2.96
PUFA	41.71±4.92	44.70±3.52	44.04±3.86	42.44±4.28

5.3 Change over time in the information group

5.3.1 Total population

In the fatty acid profiles of subjects of the information group no significant changes occurred.

5.3.2 IDDM and NIDDM

The fatty acid profile of subjects with IDDM did not significantly change over time (table 14) as well as that of subjects with NIDDM (table 15).

Table 14: Fatty acid profile (% of total fatty acids) in the plasma of patients with **IDDM** in the information group

Fatty acid	T0	T1	T2	T3
16:0	20.55±1.25	21.25±1.90	21.45±1.73	21.21±1.73
16:1n7	2.03±0.39	2.08±0.35	2.19±0.52	2.23±0.53
18:0	7.02±0.45	7.20±0.40	7.17±0.45	7.16±0.60
18:1n9	22.15±2.14	22.14±2.22	22.27±1.91	23.37±2.01
18:1n7	2.07±0.28	2.01±0.28	1.93±0.22	2.09±0.28
18:2n6	22.36±2.52	22.38±2.86	22.86±3.31	22.29±3.10
18:3n6	0.48±0.15	0.56±0.17	0.55±0.17	0.51±0.14
18:3n3	0.46±0.16	0.47±0.18	0.47±0.14	0.45±0.20
20:4n6	8.16±1.40	8.05±1.45	7.69±1.08	7.72±1.05
20:5n3	0.72±0.13	0.79±0.20	0.82±0.25	0.69±0.22
22:5n3	0.57±0.10	0.56±0.05	0.58±0.08	0.59±0.10
22:6n3	1.75±0.25	1.76±0.32	1.68±0.40	1.60±0.29
SFA	29.31±1.22	30.45±2.06	30.53±1.66	30.16±1.95
MUFA	26.90±2.57	26.89±2.63	27.08±2.35	28.35±2.39
PUFA	36.93±2.64	36.26±2.55	36.42±2.87	25.63±2.98

Table 15: Fatty acid profile (% of total fatty acids) in the plasma of patients with **NIDDM** in the information group

Fatty acid	T0	T1	T2	T3
16:0	21.42±1.08	21.96±1.66	21.60±1.25	21.95±1.39
16:1n7	1.74±0.63	1.97±0.83	1.80±0.76	2.00±0.84
18:0	7.65±0.71	7.33±0.33	7.37±0.75	7.27±0.58
18:1n9	21.06±1.56	22.56±1.74	22.23±2.34	21.81±1.59
18:1n7	1.90±0.20	1.87±0.23	2.02±0.27	1.97±0.20
18:2n6	25.10±2.66	24.20±2.29	24.78±3.23	24.37±3.80
18:3n6	0.46±0.16	0.48±0.22	0.40±0.15	0.46±0.16
18:3n3	0.48±0.11	0.54±0.17	0.43±0.12	0.49±0.14
20:4n6	7.35±1.97	6.75±1.81	7.32±1.84	6.90±1.65
20:5n3	0.69±0.34	0.56±0.22	0.54±0.14	0.63±0.21
22:5n3	0.51±0.11	0.44±0.11	0.47±0.08	0.51±0.11
22:6n3	1.58±0.53	1.46±0.55	1.48±0.39	1.47±0.62
SFA	30.95±1.49	31.36±2.04	30.84±0.87	31.24±1.92
MUFA	25.34±2.12	27.01±2.16	26.64±2.81	26.41±2.10
PUFA	38.11±2.93	36.18±3.01	37.21±3.14	36.85±3.34

5.3.3 Healthy and IFG subjects

The fatty acid profile of healthy subjects did not significantly differ between T0 and any time point during (T1) or at the end the intervention (T2) (table 16). In the IFG group the ALA significantly decreased at T1 (table 17), maybe due to the high baseline level.

Table 16: Fatty acid profile (% of total fatty acids) in the plasma of **healthy participants** in the information group

Fatty acid	T0	T1	T2	T3
16:0	19.63±1.55	19.58±1.49	19.82±1.56	20.14±1.59
16:1n7	1.95±0.70	2.02±0.76	2.20±1.11	2.28±1.13
18:0	7.62±0.70	7.44±0.75	7.17±0.96	7.32±0.87
18:1n9	18.88±3.52	20.16±3.75	19.97±3.43	20.21±2.81
18:1n7	1.72±0.34	1.80±0.31	1.89±0.31	1.86±0.32
18:2n6	28.88±3.69	28.28±3.41	27.55±3.56	26.56±3.23
18:3n6	0.44±0.23	0.46±0.16	0.43±0.12	0.46±0.17
18:3n3	0.46±0.15	0.51±0.19	0.52±0.10	0.59±0.15
20:4n6	6.48±1.36	6.53±1.37	6.47±1.63	6.55±1.33
20:5n3	0.57±0.17	0.73±0.29	0.70±0.22	0.76±0.23
22:5n3	0.50±0.05	0.53±0.08	0.54±0.07	0.55±0.10
22:6n3	1.73±0.29	1.65±0.35	1.54±0.44	1.59±0.30
SFA	28.96±1.59	28.76±1.04	28.87±1.02	29.39±1.03
MUFA	23.17±4.35	24.63±4.70	24.75±4.92	25.05±4.16
PUFA	41.09±4.73	40.83±4.91	39.83±5.20	39.14±4.34

Table 17: Fatty acid profile (% of total fatty acids) in the plasma of **participants with IFG** in the information group. * Significant changes compared to T0

Fatty acid	T0	T1	T2	T3
16:0	21.62±1.80	21.85±0.95	21.99±1.26	21.65±0.99
16:1n7	1.61±0.28	1.78±1.88	1.76±0.40	1.73±0.08
18:0	6.62±0.78	6.94±0.47	6.59±0.76	6.91±0.86
18:1n9	22.79±4.01	22.14±2.17	20.01±1.85	20.25±0.85
18:1n7	1.77±0.11	1.64±0.11	1.67±0.11	1.63±0.24
18:2n6	25.06±3.77	24.57±3.26	26.40±3.48	25.24±3.43
18:3n6	0.38±0.04	0.47±0.11	0.44±0.15	0.47±0.22
18:3n3	0.91±0.28	0.51±0.15*	0.68±0.20	0.60±0.26
20:4n6	5.59±0.40	6.30±1.06	6.52±1.42	6.38±0.43
20:5n3	0.59±0.17	0.71±0.32	0.61±0.19	0.59±0.07
22:5n3	0.56±0.13	0.64±0.14	0.57±0.11	0.59±0.10
22:6n3	1.18±0.12	1.40±0.33	1.36±0.29	1.26±0.14
SFA	30.57±1.41	31.10±0.90	31.02±1.10	31.04±1.32
MUFA	26.89±4.31	26.25±2.21	24.09±2.27	24.29±0.86
PUFA	36.36±4.86	36.65±1.42	38.46±2.55	37.00±2.92

6 CONCLUSION

Diabetes mellitus is one of the most common chronic diseases worldwide, with a continuously increasing number in prevalence and incidence. In 2011 366 million people were affected by type 2 diabetes. Estimations assume that this number will increase to 552 million people by 2030. In Austria about 571.000 of the adult population currently suffer from diabetes (IDF, 2011).

Due to the great number of patients and the high risk for coronary heart diseases and other long-term complications nutrition programs are of great importance. Therefore a human intervention study entitled "DIAPLANT" was performed at the Department of Nutritional Sciences at the University of Vienna within the Emerging Field "Oxidative Stress and DNA Stability". The study aimed to investigate the influence of a plant oil rich in PUFA and vegetables on biomarkers of secondary diseases in type 2 diabetics. The total study population consisted of 99 healthy and diabetic participants.

The aim of the present master thesis was to investigate the influence of an eight-week intervention with plant oil and vegetables on the fatty acid profile in the plasma of type 2 diabetics. The plasma fatty acid profile was determined by gas chromatography. The primary objective of this work was to verify the compliance. Furthermore, the plasma levels of several fatty acids between type 2 diabetics, healthy subjects and those with abnormal fasting glucose concentrations were compared. Moreover, changes due to the intervention and the different effects on the various health states were investigated.

At baseline

At baseline, there were no significant differences between the intervention and the information group in any of the investigated fatty acids. Most fatty acids did not show any gender-related differences. Those differing can be explained by a higher meat consumption of men (AA) and a higher enzyme activity in females (γ -linolenic acid) (Prättälä et al. 2007; Watkins and German, 2008).

Comparisons between the various groups with different health status (IDDM, NIDDM, healthy and IFG subjects) showed significant differences in several

fatty acids. Higher plasma levels of palmitic acid and SFA in diabetics indicate a usual diet higher in saturated fat, which is accompanied with the lower levels of LA and PUFA. These results assume a better nutritional behaviour of healthy subjects. Higher levels of oleic acid and MUFA in diabetic subjects can be explained by a higher activity of the δ -9-desaturase. The activity of this enzyme is known to be high in conditions like diabetes, atherosclerosis and obesity (Warnesjö et al., 2006).

Significant correlations for all subjects were found between PUFA and plasma lipids. There was a positive correlation between PUFA and HDL ($r=0,524$, $p=0.000$). The correlation between PUFA and the ratio of total to HDL-cholesterol was negative ($r=-0,271$, $p=0.007$). This association is important as the ratio of total to HDL-cholesterol is a good predictor of cardiovascular risk in subjects with type 2 diabetes (Lu et al., 2003). TG were negatively correlated with PUFA ($r=-0.694$, $p=0.000$). Opposite to this the correlation between TG and SFA was positive ($r=0.546$, $p=0.000$). A trend towards a negative correlation ($r=-0.216$, $p=0.071$) was observed between plasma PUFA and the HOMA Index for insulin resistance in diabetic subjects.

Similar correlations as found in the present intervention study were reported in several other studies (Tapsell et al., 2004; Loschiavo et al., 1990; Cao et al., 2009). Due to these findings one can assume that a diet containing high-quality fat has positive impacts on glucose and fat metabolism.

Changes over time

The consumption of 25 ml PUFA rich plant oil led to significant changes in SFA, MUFA and PUFA levels as well as their predominant forms (palmitic acid, palmitoleic acid, vaccenic acid, oleic acid, LA, ALA) in the intervention group, indicating a good dietary compliance. These significant changes result from the replacement of butter, margarine or oils rich in MUFA by the PUFA-rich intervention oil.

ALA significantly increased in the intervention group by 58.8% at T1 and 62.8% at T2 compared with baseline levels. LA significantly increased by 15.1% at T1

and 14.5% at T2 in comparison to baseline. Consequently, PUFA increased significantly by 8.8% at T1 and by 8.4% T2 compared with baseline.

Results of other studies showed similar changes of the fatty acid profiles after an intervention with an oil or food rich in PUFA (Hodson et al., 2001; Haban et al., 2000; Karvonen et al., 2002; Peck et al., Almario et al., 1996).

In the **IDDM** group the relative increase of LA was 15.13% at T1 and 14.11% at T2. ALA relatively increased by 72.09% at T1 and T2 compared with T0.

In the **NIDDM** group LA increased by 13.61% at T1 and 13.42% at T2. The rise of ALA accounted for 40.70% at T1 and 37.81% at T2.

In the **healthy** and **IFG**-subjects no significant changes could be observed due to the small sample number and the large standard deviations.

The fatty acid profiles of the information group did not change significantly.

As shown in the present study moderate changes in the diet by addition of 25 ml PUFA rich plant oil lead to an elevation of PUFA and a reduction of SFA and MUFA in the plasma of healthy and diabetic subjects.

Although studies on the role of PUFA in the prevention of type 2 diabetes are inconsistent (Jucker et al., 1999; Marshall et al., 1995; Meyer et al., 2001; Salméron et al, 2001; Laaksonen et al., 2002), it is assumed that a higher PUFA intake, especially as replacement for SFA, is associated with a reduced risk for type 2 diabetes (Hauner and Schulze, 2006).

7 SUMMARY

Background: Diabetes mellitus is one of the most common chronic diseases worldwide, with a continuously increasing number of prevalence and incidence. In 2011 the estimated number of adults living with diabetes has reached 366 million, representing 8.3% of the global adult population.

A higher PUFA intake, especially as replacement for SFA, is associated with a reduced risk for type 2 diabetes.

Methods: The study investigated the effect of an intervention with a plant oil rich in ω -3 and ω -6 fatty acids and vegetables on biomarkers of secondary diseases of diabetic patients. Therefore 99 diabetic and non-diabetic persons were randomly assigned to either the information or the intervention group. Participants of the intervention group received 25 ml of plant oil and 300 g vegetables each day for eight weeks. After blood sampling and blood preparation the plasma fatty acid profile was determined by gas chromatography. The aim of this master thesis was to investigate the influence of the eight-week intervention with a plant oil and vegetables on the fatty acid profile in the plasma of type 2 diabetics in order to prove their study compliance.

Results: The intervention with 25 ml plant oil rich in ω -3 and ω -6 fatty acids and 300 g vegetables per day showed significant increases in LA, ALA and PUFA in the plasma of the participants, confirming a good dietary compliance. Simultaneously, SFA and MUFA significantly decreased.

Conclusions:

Intake of PUFA-rich plant oil significantly increased PUFA in the plasma of diabetic subjects and led at the same time to significant reductions of SFA and MUFA.

8 ZUSAMMENFASSUNG

Hintergrund: Diabetes mellitus ist weltweit eine der am häufigsten auftretenden chronischen Erkrankungen, wobei Prävalenz und Inzidenz stetig ansteigen. 2011 betrug die geschätzte Anzahl an erwachsenen Diabetikern 366 Millionen, entsprechend 8,3% der erwachsenen Weltbevölkerung.

Eine erhöhte Aufnahme von mehrfach ungesättigten Fettsäuren, besonders als Ersatz für gesättigte Fettsäuren, wird mit einem verringerten Risiko für Typ 2 Diabetes assoziiert.

Methoden: Die Studie untersuchte den Effekt einer Intervention mit einem ω -3 und ω -6 fettsäurereichen Pflanzenöl und Gemüse auf die Sekundärerkrankungen von Diabetikern. Dazu erfolgte eine Randomisierung von 99 diabetischen und nicht-diabetischen Studienteilnehmern in eine Informations- und eine Interventionsgruppe. Die Teilnehmer der Interventionsgruppe erhielten 25 ml Pflanzenöl und 300 g Gemüse pro Tag. Nach Blutabnahmen und in situ aufbereitung wurde das Fettsäuremuster im Plasma mittels Gaschromatographie bestimmt.

Das Ziel dieser Arbeit war es, den Einfluss einer achtwöchigen Intervention mit dem Pflanzenöl und Gemüse auf das Fettsäuremuster im Plasma von Typ-2 Diabetikern zu untersuchen und deren Studiencompliance zu bestätigen.

Ergebnisse: Die Intervention mit 25 ml eines Pflanzenöls, das reich an ω -3 und ω -6 Fettsäuren war, sowie 300 g Gemüse pro Tag zeigte signifikante Steigerungen von Linolsäure, α -Linolensäure und der Summe an mehrfach ungesättigten Fettsäuren im Plasma der Studienteilnehmer. Dies bestätigt eine gute Ernährungscompliance. Gleichzeitig wurde eine signifikante Reduktion der gesättigten und einfach ungesättigten Fettsäuren beobachtet.

Conclusio:

Die Einnahme eines Pflanzenöls, das reich an ungesättigten Fettsäuren ist, erhöht mehrfach ungesättigte Fettsäuren im Plasma von Diabetikern signifikant und führt auch zu signifikanten Reduktionen von gesättigten und einfach ungesättigten Fettsäuren.

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