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Postprandial metabolic changes in healthy males and in subjects homozygous for GWAS-identified variants at lipid metabolism loci

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SUMMARY

The recent developments of metabolomics technologies enable the simultaneous measurement of various metabolites produced along a wide range of interconnected pathways of the human metabolism. Metabolism is influenced by a given genetic make-up and a number of environmental factors, including nutrition combining to shape health outcomes. This thesis addresses the human metabolism after nutritional challenges in strictly controlled human intervention studies applying time-resolved plasma metabolomics measurements. The work is divided into two major parts:

The **first part of the work** aims to further understand the postprandial plasma metabolism in healthy individuals. Six male subjects were given a high-fat, high-carbohydrate (HFHC) meal consisting of a conventional fast food meal on two independent study days (i) with their habitual diet before the HFHC challenge and (ii) with three-day food standardization before the second identical meal. In addition, at a third study day, subjects were given a healthy breakfast (HB). Plasma samples were obtained in the fasting state, and at several postprandial time-points. Samples were analyzed by targeted and non-targeted mass spectrometric methods.

In a first sub-project the need for a short-term dietary standardization at the days prior to a time-resolved nutritional challenge test was addressed. Therefore, the metabolite measurements of the HFHC meal with and without prior food standardization were compared. Significant differences in mean postprandial metabolite time-courses were identified for isobutyrylcarnitine and branched-chain amino acids. Moreover, the study revealed that the postprandial inter-individual variance in acyl-alkyl phosphatidylcholines is reduced by dietary standardization. Nonetheless, valid postprandial time-course measurements of most metabolite classes seem to be possible without standardized dietary lead-in periods in healthy subjects.

Aside, in a second sub-project differences in the postprandial metabolism of an unhealthy fast-food meal (HFHC meal) for breakfast and a healthier breakfast alternative (HB) were studied. Significant postprandial differences of both test meals were merely shown for mean metabolite time-courses of N-methyl proline, stachydrine, 3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF), as well as isoleucine and for the metabolite group of amino acids. Therefore, adverse effects of fast food consumption on plasma metabolites seem to be mainly determined by long-term exposure, whereas single fast food meals may be well compensated in healthy men due to a high metabolic flexibility.

Aside of providing insight into the postprandial metabolism, metabolic challenge tests might be able to unravel aspects of metabolic health, that would not be apparent from studying solely the fasting

metabolism. Thus, in the **second part of this work**, metabolic challenge tests were applied to further characterize the GWAS-identified gene variants rs2014355 in the acetyl-CoA-dehydrogenase short chain (*ACADS*) locus and rs174547 in the fatty acid desaturase 1 (*FADS1*) locus and targeted metabolomics were used as hypothesis free approach.

12 homozygous carriers of the minor C allele of rs2014355 and 9 homozygous carriers of the major T allele were exposed to a 24 h fasting period and an oral glucose tolerance test (OGTT). Plasma samples were obtained at baseline and at several time-points during the tests. Aside of confirming baseline differences in butyrylcarnitine (C4), the time-resolved data showed fasting-induced genotype dependent differences for acyl-alkyl phosphatidylcholine C42:0. Further challenge-genotype interactions were identified for glutamine and lyso phosphatidylcholine C20:4 during the OGTT. The results of the time-course analysis of these metabolites might indicate a less flexible metabolism in response to fasting and a glucose load in minor CC allele carriers compared to controls.

Aside, 12 homozygous carriers of the minor C allele of rs174547 and 13 homozygous carriers of the major T allele were exposed to an oral lipid tolerance test (OLTT) and an OGTT. Blood samples were taken at baseline and at several time-points after ingestion of the test meals. The results confirm previously reported genotype-dependent differences in phosphatidylcholines, lyso phosphatidylcholines and sphingomyelines, especially becoming obvious by calculation of metabolite ratios. However, further genotype-challenge interactions were not visible.

Interestingly, whereas the calculation of metabolite ratios strongly improved the genotype distinction of rs174547, it hardly improved the genotype distinction for rs2014355.

In conclusion, the results of the second part of this work show that metabolic challenge tests may contribute to a better understanding of gene function and may help to estimate the risk and progression of metabolic diseases.

ZUSAMMENFASSUNG

Technologische Fortschritte ermöglichen die zeitgleiche Messung einer Vielzahl von Metaboliten, die im menschlichen Stoffwechsel in einer Reihe verschiedener, miteinander verbundener Stoffwechselwege entstehen. Der Stoffwechsel wird sowohl durch die Gene, als auch durch eine Vielzahl an Umweltfaktoren, unter anderem der Ernährung, beeinflusst, die in ihrer Gesamtheit den menschlichen Gesundheitzustand beeinflussen und modellieren. Diese Dissertation befasst sich mit dem Stoffwechsel nach verschiedenen Nahrungsbelastungen im Rahmen von streng kontrollierten humanen Interventionsstudien mit zeitaufgelösten Metabolitenmessungen in Plasmaproben. Die Arbeit ist in zwei Hauptforschungsziele gegliedert:

Der **erste Teil der Arbeit** zielt darauf ab, den postprandialen Metabolismus bei gesunden Menschen zu untersuchen. Sechs Männer erhielten an zwei unabhängigen Studientagen jeweils eine fett- und kohlenhydratereiche (HFHC) Mahlzeit, bestehend aus einem konventionellen Fast Food Menü (i) mit der gewöhnlichen Ernährung vor der HFHC-Mahlzeit und (ii) mit einer dreitägigen Ernährungsstandardisierungsphase vor der HFHC-Mahlzeit. Zudem erhielten die Teilnehmer an einem dritten Studientag ein gesundes Frühstück (HB). Plasma-Proben wurden jeweils im Nüchternzustand, sowie zu verschiedenen Zeitpunkten nach Nahrungsaufnahme entnommen. Die Proben wurden mit verschiedenen massenspektrometrischen Methoden analysiert.

In einem ersten Teilprojekt wurde die Notwendigkeit einer Ernährungsstandardisierung vor zeitaufgelösten Nahrungsbelastungstests untersucht. Hierzu wurden die Metabolitenmessungen infolge der HFHC-Mahlzeiten mit und ohne einer vorherigen Standardisierungsphase verglichen. Signifikante Unterschiede in den mittleren Zeitverläufen nach Nahrungsbelastung zeigten sich für Isobutyrylcarnitin, sowie für die verzweigtkettigen Aminosäuren. Daneben konnte durch die Ernährungsstandardisierung die inter-individuelle Varianz in der Metabolitengruppe der acyl-alkyl Phosphatidylcholine signifikant reduziert werden. Dennoch ist eine valide zeitaufgelöste Messung nach Nahrungsaufnahme im Plasma gesunder Männer für die meisten der untersuchten Metabolitengruppen ohne eine vorherige Ernährungsstandardisierungphase möglich.

Daneben wurden in einem zweiten Teilprojekt metabolische Unterschiede nach Aufnahme eines Fast Food Menüs (HFHC-Mahlzeit) als Frühstück und einer gesunden Frühstück-Alternative (HB) untersucht. Signifikante Unterschiede in den Zeitverläufen beider Testmahlzeiten wurden lediglich für N-Methyl-Prolin, Stachydrin, 3-Carboxy-4-Methyl-5-Propyl-2-Furanpropanoat (CMPF), Isoleucin und für die Gruppe der Aminosäuren deutlich. Daher sind nachteilige Effekte von Fast Food Konsum auf den Plasma Metabolismus wahrscheinlich hauptsächlich durch wiederholten Konsum hervorgerufen. Eine einzelne

Fast Food Mahlzeit dagegen scheint beim gesunden Mann wahrscheinlich aufgrund hoher metabolischer Flexibilität gut kompensiert zu werden.

Neben der Erforschung des postprandialen Metabolismus können Nahrungsbelastungstests geeignet sein, um frühe Veränderungen im Hinblick auf metabolische Erkrankungen zu erkennen, die durch alleinige Untersuchungen im Nüchternzustand nicht deutlich werden. Daher wurden im **zweiten Teil dieser Arbeit** metabolische Belastungstests in Kombination mit Metabolomics-Messungen als hypothesen-freier Ansatz zur weitergehenden Charakterisierung der beiden GWAS-identifizierten Genvarianten rs2014355 im Kurzketten-Acyl-CoA-Dehydrogenase (*ACADS*) Locus und rs174547 im Fettsäure-Desaturase 1 (*FADS1*) Locus eingesetzt.

12 homozygote Träger des seltenen C Alleles von rs2014355 und 9 homozygote Träger des häufigen T Alleles wurden einer 24-stündigen Fastenperiode und einem oralen Glukosetoleranztest (OGTT) ausgesetzt. Während beider Tests wurde kontinuierlich Blut abgenommen. Neben der Bestätigung basaler Unterschiede in Butyrylcarnitine (C4), zeigten die zeitaufgelösten Daten Fasten-induzierte, Genotyp-abhängige Unterschiede für das acyl-alkyl Phosphatidylcholin C42:0. Weitere Unterschiede konnten für Glutamin und das lyso Phosphatidylcholine C20:4 während dem OGTT gefunden werden. Die Zeitverlaufsanalyse dieser Metabolite könnte auf eine weniger flexible metabolische Antwort der Träger des seltenen C Alleles auf den Fastenzustand und eine Glukosebelastung im Vergleich zu den Kontrollen hinweisen.

Daneben wurden 12 homozygote Träger des seltenen C Alleles von rs174547 und 13 homozygote Träger des häufigen T Alleles einem oralen Lipidtoleranztest (OLTT) und einem OGTT ausgesetzt. Plasmaproben wurden im Nüchternzustand, sowie zu mehreren Zeitpunkten nach den Testmahlzeiten entnommen. Die Ergebnisse bestätigen bereits gezeigte genotyp-spezifische Unterschiede in Phosphatidylcholinen, lyso Phosphatidylcholinen und Sphingomyelinen, die besonders durch die Analyse von Metaboliten-Verhältnissen deutlich werden.

Interessanterweise verbessert die Berechnung von Metabolitenverhältnissen deutlich die Genotyp-Zuordnung von rs174547, wohingegen sie bei der Genotyp-Zuordnung von rs2014355 kaum Vorteile zeigt.

Zusammenfassend zeigen die Ergebnisse des zweiten Teils dieser Arbeit, dass metabolische Belastungstests zu einem besseren Verständnis von Genfunktionen beitragen können, als auch helfen können Krankheitsrisiken und –Verläufe abzuschätzen.

ABBREVATIONS

ACADs	acetyl-CoA dehydrogenases
ACADS	acetyl-CoA dehydrogenase, short chain
ad	adjusted
BCAAs	branched-chain amino acids
BIA	bioelectrical impedance analysis
BMI	body mass index
C2	acetylcarnitine
C3	propionylcarnitine
C4	butyrylcarnitine
CACT	carnitine-acylcarnitine translocase
Chr	chromosome
CMPF	3-carboxy-4-methyl-5-propyl-2-furanpropanoate
CoA	coenzyme A
CPT	carnitine palmitoyltransferase
DHA	docosahexaenoic acid
EDTA	Ethylendiamintetraessigsäure
EKFZ	Else Kröner-Fresenius-Centre for Nutritional Medicine
EMA	ethylmalonic acid
EPA	eicosapentaenoic acid
FAD	flavin adenine dinucleotide
FADS	fatty acid desaturase
FAO	fatty acid oxidation
FATP	fatty acid transport protein
FDR	false discovery rate
FIA	flow injection analysis
fsd	functional standard deviation
GC	gas chromatography
GOT	glutamic oxalacetic transaminase
GPT	glutamic pyruvate transaminase
GWAS	genome-wide association study

HB	healthy breakfast
HDL	high density lipoprotein
HFHC	high-fat, high-carbohydrate
HMBD	Human Metabolome Database
HNF4	hepatocyte nuclear factor 4
HOMA	homeostasis model assessment
HPLC	high performance liquid chromatography
HWE	Hardy-Weinberg equilibrium
Ile	isoleucine
kb	kilobase
KORA	Cooperative Health Research in the Region of Augsburg
LC	liquid chromatography
LC-PUFA	long-chain polyunsatured fatty acid
LD	linkage disequilibrium
LDL	low density lipoprotein
lyso PC	lyso phosphatidylcholine
MAF	minor allele frequency
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MUFA	mono-unsatured fatty acid
NAD	nicotinamide adenine dinucleotide
NEFA	non-esterified fatty acid
NFκB	nuclear factor kappa B
NMR	nuclear magnetic resonance
OGTT	oral glucose tolerance test
OLTT	oral lipid tolerance test
PBMC	peripheral mononuclear cell
PBS	phosphate buffered saline
PC	phosphatidylcholine
PCA	principle component analysis
PC aa	diacyl phosphatidylcholine
PC ae	acyl-alkyl phosphatidylcholine
PPAR	peroxisome proliferator activated receptor

PUFA	polyunsatured fatty acid
SCAD	short chain acetyl-CoA dehydrogenase
SD	standard deviation
SFA	satured fatty acid
SM	sphingomyeline
SNP	single nucleotide polymorphism
SREBP1	sterol regulatory element binding protein 1
T2D	type 2 diabetes, diabetes mellitus
TG	triglyceride
TPDT	time-resolved paired difference test
UTR	untranslated region
VLDL	very low density lipoprotein
wc	waist circumference
WHR	waist-to-hip ratio

1 INTRODUCTION

1.1 Metabolomics

The metabolome consists of all abundant low molecular weight molecules (metabolites) in a cell, tissue or an organism in a particular physiological or developmental state (Goodacre et al. 2004; Roberts et al. 2012). It can be considered as downstream end-product of the genome, transcriptome and proteome modified by environment and, thus, represents the link between genome and the phenotype (Fiehn 2002). Metabolomics aims to study the metabolome by comprehensive profiling of a wide range of metabolites under a given set of conditions (Fiehn 2001). At present there is no complete documentation of all molecules in the human metabolome for any tissue and cell type. However, there are a few comprehensive metabolomic databases listing a huge number of metabolites in the human body. For example the comprehensive Human Metabolome Database (HMBD) comprised about 40,153 metabolites in 2013 (Wishart et al. 2013). Metabolites span a variety of chemical classes and physical properties, with significant differences in polarity and size, across a large range of concentrations. Thus, at present, no single analytical method is able to cover the chemical diversity of the entire metabolome and metabolomics is still in its shoes of infancy. However, technical advances in the last years made available various analytical methods to acquire extensive metabolomic information. Two core technologies are mainly in the focus of attention: Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectroscopy (MS).

NMR spectroscopy uses the magnetic properties of atomic nuclei for determination of abundance and structure of metabolites in biological samples. An NMR-active nucleus absorbs electromagnetic radiation at a characteristic frequency, when placed inside a strong magnetic field. The exact characteristic frequency is depending on the chemical environment of the nucleus and the coupling with adjacent nuclei and allows to identify an energetic fingerprint for that molecule (Rhee and Gerszten 2012; Griffin et al. 2011; Pohmann 2011). The most frequently used nuclei for biological metabolites are $_{1}H$, $_{13}C$, and $_{31}P$ (Griffin et al. 2011). NMR requires little sample preparation including no column chromatography and no derivatization, is non-destructive and very reproducible. Moreover, NMR enables absolute quantification without applying isotope-labeled standards. However, despite recent improvements like the cryoprobe technology, NMR is less sensitive than MS (Cox et al. 2014; Rhee and Gerszten 2012; Claus and Swann 2013).

Mass spectrometry resolves metabolites based on the mass-to charge ratio (m/z). Therefore, gas phase ions are produced i.e. by electron ionization, and placed in an electrical field (de Hoffmann and Stroobant 2007). In tandem mass spectrometry (MS/MS) three quadrupoles are arranged in series. Each quadrupole consists of 4 parallel rods creating oscillating electrical fields. The first quadrupole acts as mass filter for ions, the second quadrupole is used as collision cell to fragment ions and the third quadrupole serves as mass filter for ion fragments (de Hoffmann and Stroobant 2007; Rhee and Gerszten 2012). The greatest advantage of MS is its high sensitivity. However, disadvantages arise from destruction of samples and long time for sample preparation. MS is often combined with chromatography for analytical separation of compounds allowing a time-resolved delivery of molecules from a complex biological sample to the mass spectrometer. The two main methods for analytical separation applied for metabolite profiling are gas chromatography (GC) and liquid chromatography (LC) (Claus and Swann 2013; Lenz and Wilson 2007).
Aside of different technologies, metabolic profiling can be divided into untargeted and targeted approaches. Untargeted metabolomics is the comprehensive analysis of all measurable metabolites in a sample, including also unknown metabolites and thus, offers the opportunity for discovering novel targets (Roberts et al. 2012). Aside, targeted metabolomics quantifies predefined groups of chemically characterized and biochemically annotated metabolites. Using internal standards, analysis can take part in a quantitative or semi-quantitative way (Roberts et al. 2012).

1.2 Human metabolism

1.2.1 Influences on human metabolism

Applying metabolomics technologies enables to characterize the metabolic phenotype (metabotype) of an individual at a given time-point. The human metabotype exists in a dynamic flux shaped by a wide range of internal and external factors of influence. Such internal factors comprise the genome as well as epigenetic influences (Petersen et al. 2013). For instance, recent genome wide association studies (GWAs) have impressively shown profound impact of genetic variances on human metabolic traits (Illig et al. 2010; Gieger et al. 2008; Tanaka et al. 2009; Hicks et al. 2009; Demirkan et al. 2012; Ehrlein and Pröve 1982; Kettunen et al. 2012; Nicholson et al. 2011). Extrinsic metabotype-influencing factors combine with the genome to shape health outcomes. Such factors include physical activity (Chorell et al. 2012), gut microbiota (Wikoff et al. 2009), diurnal cycles (Slupsky et al. 2007), temperature (Westerterp-Plantenga et al. 2002), stress (Krug et al. 2012), drugs (Trupp et al. 2012), age (Yu et al. 2012) and diet (Krug et al. 2012)

(FIGURE 1). Metabolomic measurements represent the closest measurement of the phenotype compared to potential outcomes measured by transcriptomic and proteomic approaches. Hence, application of metabolomics in nutritional research is a powerful tool for exploring the influence of diet on the human metabolism and health (Claus and Swann 2013).

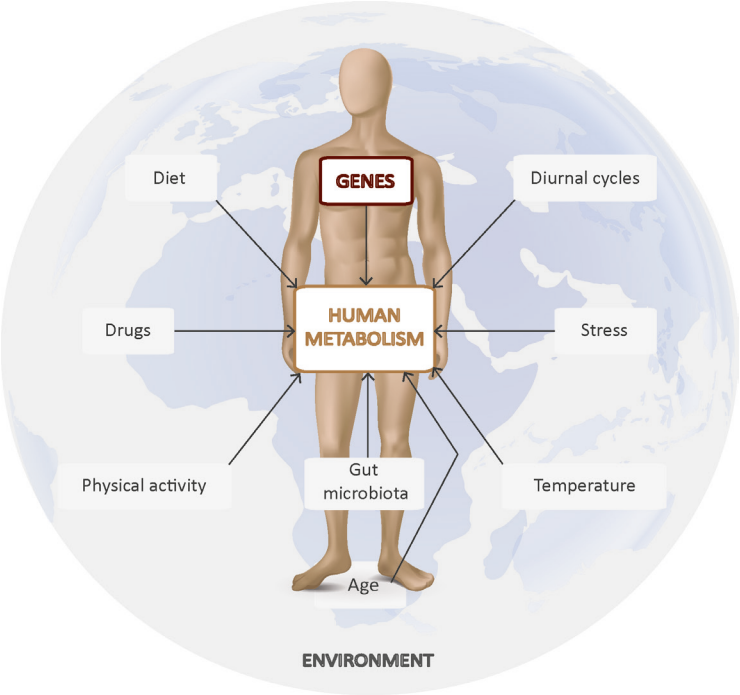

FIGURE 1: Influence factors on human metabolism

1.2.2 Dietary influences on human metabolism - the catabolic state

The catabolic state starts in the post-absorptive state, when the whole last meal is fully absorbed by the intestinal tract - in humans typically appearing after an overnight fast. Then, blood insulin concentration is low, glucagon concentrations rise and endogenous energy storages are utilized for energy requirements. During the post-absorptive period, blood glucose homeostasis is maintained by hepatic glycogenolysis and gluconeogenesis (Wahren and Ekberg 2007; Cahill 2006; Wahren et al. 1972). Glucose production covers glucose oxidation of the brain as well as the obligatory glycolytic tissues like bone marrow, red blood cells, renal medulla and peripheral nerves, whereas in other tissues like muscle and adipose tissue glucose oxidation is diminished in favor of lipid oxidation (Cahill 2006; Andres et al. 1956). Hepatic glycogen stores are depleted by the second or third day of starvation and the percentage of glucose provided by gluconeogenesis increases consecutively reaching its peak after approximately two days of fasting (Cahill 2006). Substrates for hepatic glucose production are mainly supplied by muscle proteolysis.

Additional substrates are recycled lactate and pyruvate from the cori cycle, glycerol from adipose tissue lipolysis and in small amounts from ß-hydroxybutyrate (Cahill 2006; Bao et al. 2011). After about three days of starvation, the metabolic profile is set to conserve protein and to supply greater quantities of alternate fuels. Although cells exclusively depend on glucose are still served by glucose from hepatic gluconeogenesis and by a gradually increasing component of renal gluconeogenesis, the brain metabolism switches from using glucose to ketone acids (Wahren and Ekberg 2007; Cahill 2006). Ketone bodies are produced by ketogenesis from acetyl-CoA in the liver and are a biomarker of lipolysis and fatty acid ß-oxidation in tissues. Even though the oxidation of ketone bodies preserves protein stores as well as functional proteins, plasma protein levels are not uniform during fasting. Whereas branched-chain amino acids, α-aminobutyrate and methionine show an early increase followed by a decrease in prolonged fasting periods, other amino acids like glycine, threonine and serine increase time-delayed after day 5 of fasting (Felig et al. 1969).

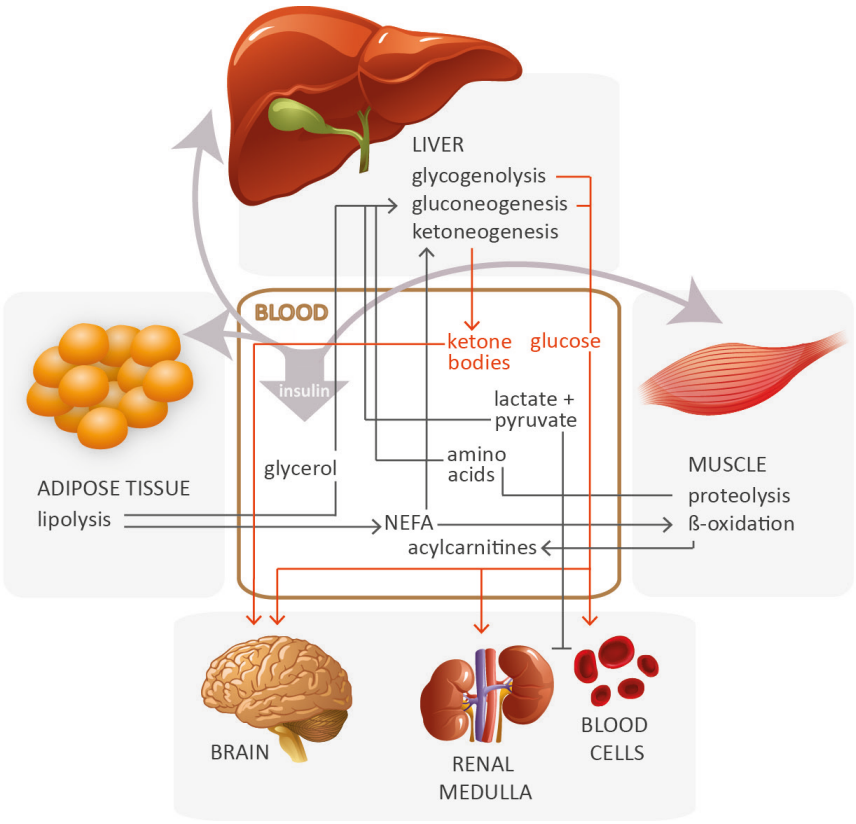

FIGURE 2: Schematic overview of key metabolic processes in the catabolic state
Red lines show metabolites released by the liver. NEFA, non-esterified fatty acids (adapted from Sailer 2013, Rubio-Aliaga et al. 2011 and Cahill 2006).

Literature shows several studies applying metabolomic technologies for detection of metabolic profiles after an overnight fast (Shaham et al. 2008; Skurk et al. 2011; Walsh et al. 2006; Wopereis et al. 2009; Lenz et al. 2003). However, studies extending the fasting period are rare (Krug et al. 2012; Rubio-Aliaga et al. 2011). Rubio-Aliaga et al. comprehensively assessed the metabolic differences of a prolonged fasting of 36 h and an overnight fast of 12 h in 10 heterogeneous subjects using a variety of state-of-the-art NMR- and MS-based methods. They identified some 100 new metabolites in blood and urine that change in the fasting state and revealed 2-hydroxybutyrate, α-aminobutyrate, methionine and the branched chain keto-acids as new fasting markers (Rubio-Aliaga et al. 2011). Aside, Krug et al. extended studies of the fasting metabolism by a time-resolved analysis including 10 sampling time-points within a 36h fasting period in 15 young healthy males (Krug et al. 2012).

FIGURE 2 gives an overview of key metabolic processes in the fasting state.

1.2.3 Dietary influences on human metabolism - the anabolic state

After food intake, glucose and amino acids are absorbed into the portal circulation and the liver takes a major role in controlling the release into the peripheral circulation. Aside, most fatty acids are absorbed into the lymphatic system as chylomicrons and are initially available for peripheral tissues. Due to the rise in blood glucose and intestinal hormones, insulin is released from the ß-cells of the pancreas, blood insulin concentrations rise, the insulin/glucagon ratio increases and the metabolism switches from the catabolic to the anabolic state. Rising glucose concentrations in the portal vein and increased insulin levels lead to an inactivation of glycogenolysis and reduction of gluconeogenesis in the liver. Glucose is metabolized to meet the demands for liver metabolism and glycogen synthesis is activated. As glycogen storages are limited compared to lipid storages, an excess of carbohydrates is used for synthesis of fatty acids that are exported in VLDL (Frayn 2010; Bender 2008). Besides glucose, the liver also extracts amino acids like alanine and glutamine arriving in the portal vein (Fouillet et al. 2002). Nevertheless, parts of glucose and amino acids, mainly branched chain amino acids, pass through the liver to reach the systemic circulation and are taken up by peripheral tissues (Bender 2008; Fouillet et al. 2002).

In adipose tissue, insulin has a direct suppressive effect on lipolysis, followed by a reduction in plasma NEFA-levels. Moreover, glucose is taken up and glycolysis is stimulated. Insulin also stimulates fatty acid uptake from chylomicrons and very low density lipoproteins. However, a study using a test meal containing [U-^{13}C]palmitate combined with intravenous infusion of [^{2}H2]palmitate to label plasma fatty acids and VLDL-triglycerides showed a greater fractional extraction of chylomicron-triglycerides compared to

VLDL-triglycerides (Bickerton et al. 2007). Once taken up, fatty acids are esterified to form new triacylglycerol for storage in adipose tissue (Frayn 2010; Bender 2008).

Further, the declining blood NEFA concentrations down-regulate the drive for the skeletal muscle to oxidize fatty acids. Instead, insulin increases glucose uptake, leading to increased glucose oxidation, glycolysis and output of lactate and pyruvate. In addition, muscle glycogen stores are replenished. Amino acids, preferentially branched chain amino acids are taken up and protein synthesis is stimulated (Frayn 2010; Bender 2008). Studies using stable isotope labeled amino acids in human leg and forearm muscle show an insulin-induced decrease in muscle protein breakdown with minor effects of insulin on protein synthesis (Fontaine-Bisson et al. 2007; Meek et al. 1998; Gelfand and Barrett 1987), which, in turn, is increased by high levels of amino acids. Additionally, an excess of amino acids that cannot be incorporated in muscle protein undergoes ureagenesis or gluconeogenesis (Rennie et al. 2002).

Increasing lactate and pyruvate levels after meal intake undergo gluconeogenesis in the liver (Rennie et al. 2002; Rathee et al. 2012; Frayn 2010; Bender 2008). Resulting glucose-6-phosphate is directed into glycogen synthesis rather than released as glucose (Frayn 2010; Bender 2008).

Taken as a whole, the postprandial metabolism of a mixed meal containing carbohydrates not only reflects a general metabolic switch to use glucose as major fuel but also to store glucose as glycogen. In addition, fatty acids are stored in adipose tissue and protein synthesis is stimulated. However, rates of the complex postprandial processes depend on both the physiological and the nutritional status of an individual as well as on the specific meal composition. Nonetheless, a schematic overview of the postprandial metabolism is illustrated in FIGURE 3.

Recently, a rising number of studies applying metabolomics technologies have been focusing on the postprandial metabolism (Bondia-Pons et al. 2014; Mathew et al. 2014; Wahl et al. 2013; Krug et al. 2012; Pellis et al. 2012; Ramos-Roman et al. 2012; Skurk et al. 2011; Zivkovic et al. 2009; Ho et al. 2013; Spégel et al. 2010; Deo et al. 2010; Shaham et al. 2008; Zhao et al. 2008). These studies extend our knowledge of the postprandial metabolism by providing understanding of the behavior of hundreds of metabolites in addition to the standard biochemistry parameters like glucose, insulin, triglycerides and NEFAs. For instance, Wahl et al. show different behaviors within the metabolite class of acylcarnitines after the intake of mixed nutrient challenges trough cluster analysis. Whereas most of the acylcarnitines decreased during the first 2h after challenge and increased thereafter, C3, C5 as well as C4, C5:1, C8:1, C10:1 and C18 clustered differentially. For example, C3 and C5 showed an opposing behavior to most of the acylcarnitines and cluster together with most amino acids, suggesting that C3 and C5 may be derived from a triggered metabolism of branched-chain amino acids after protein intake (Wahl et al. 2013; Zivkovic et al. 2009).

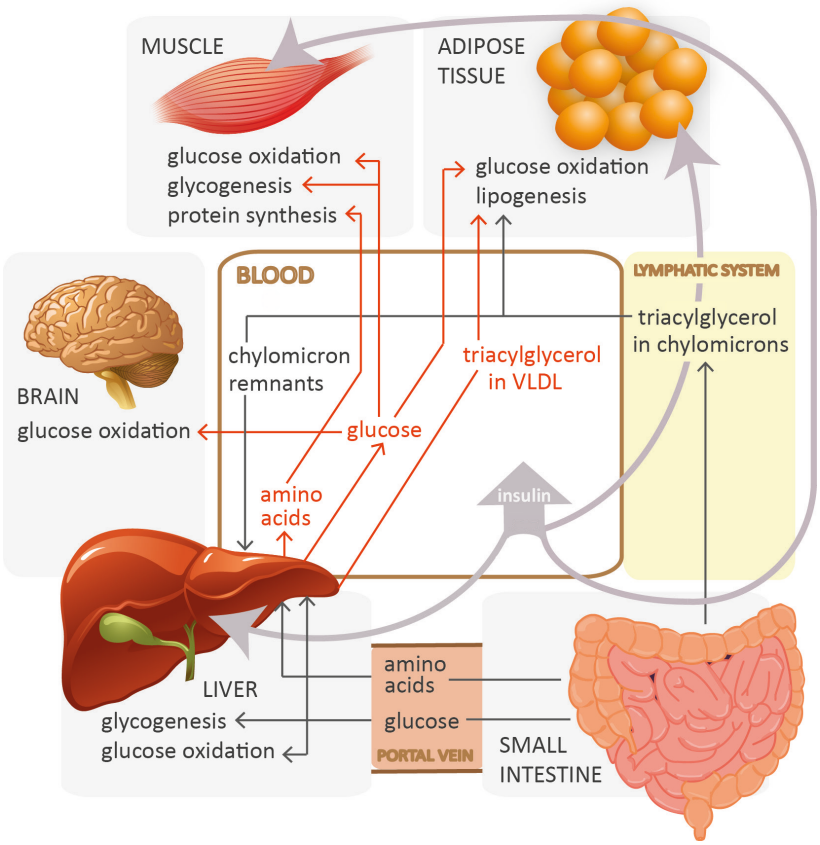

FIGURE 3: Schematic overview of key metabolic processes in the anabolic state

Red lines show metabolites released by the liver; VLDL, very low density lipoprotein.

Because of the complexity of the postprandial processes, it may be useful to examine metabolic processes primarily by pure challenges. The simplest and highest standardized method to study the condition of an anabolic state is the oral glucose tolerance test (OGTT). For decades, the OGTT has been a standard diagnostic tool in diabetology to measure the body´s ability to metabolize glucose and, thus, the test provides information on glucose tolerance (World Health Organization 2006). Recently, metabolomic studies have shown that the response to the defined amount of 75 g glucose is even more complex than considered so far (Ho et al. 2013; Skurk et al. 2011; Matysik et al. 2011; Spégel et al. 2010; Deo et al. 2010; Shaham et al. 2008; Zhao et al. 2008). Formerly unreported changes in metabolites include increases in bile acids (Shaham et al. 2008; Zhao et al. 2008; Matysik et al. 2011) and lyso phosphatidylcholines (Zhao et al. 2008), decreases of urea cycle metabolites (Ho et al. 2013), differences in the decrease of different amino acids (Deo et al. 2010; Skurk et al. 2011) as well as of fatty acids due to their degree of saturation (Zhao et al. 2008).

Although there is a rising number of studies focusing on the postprandial metabolism, studies focusing on differences between nutritional challenges that might allow a more thorough characterization of the postprandial behavior of different metabolite classes and pathways are rare (Wahl et al. 2013; Krug et al. 2012; Skurk et al. 2011).

1.2.4 Metabolic flexibility

The human metabolism needs to be well adapted to adjust fuel oxidation to fuel availability. Thus, "the capacity to switch from predominantly lipid oxidation and high rates of fatty acid uptake during fasting conditions to the suppression of lipid oxidation and increased glucose uptake, oxidation, and storage under insulin-stimulated conditions" (Kelley and Mandarino 2000) is termed as "metabolic flexibility" and characterizes the healthy state. The failure to match fuel oxidation to changes in fuel availability or metabolic inflexibility was shown to be a key dysfunction in disease states characterizing the metabolic syndrome. For instance, in diabetics and obese individuals, metabolic inflexibility becomes apparent for instance in the failure of the skeletal muscle to move between the use of lipid in the fasting state and the use of carbohydrates in the insulin-stimulated state (Storlien et al. 2004; Kelley et al. 1999; Kelley and Mandarino 2000) or impaired transition of the adipose tissue from fatty acid release in the fasting state and to fatty acid storage in the postprandial state (Storlien et al. 2004; Frayn 2002; Coppack et al. 1992). Thus, a rising number of studies in nutrition and health research focuses on the perturbation of homeostasis by metabolic challenges to study aspects of metabolic health that would not be apparent from solely studying the fasting metabolism (Shaham et al. 2008; Ramos-Roman et al. 2012; Deo et al. 2010). Aside, combining such studies with metabolomics technologies provides powerful study designs for a comprehensive characterization of the postprandial response in health and disease.

1.3 Aim of the work

The present thesis focuses on the human metabolism during metabolic challenges in strictly controlled human intervention studies with time-resolved plasma metabolomics measurements.

The first part of the work aims to further understand the postprandial plasma metabolism in healthy individuals by using targeted and untargeted metabolomic approaches. Thereby, in a first sub-project the need for a short-term dietary standardization at the days prior to a time-resolved nutritional challenge test is addressed. In a second sub-project differences in the postprandial metabolism of an unhealthy fast-food meal for breakfast and a healthier breakfast alternative were studied aiming to get a thorough characterization of the postprandial behavior of different metabolites and metabolite classes with respect to adverse long-term effects of nutrition.

In the second part of this thesis, metabolic challenge tests were applied to further characterize the GWAS identified gene variants rs2014355 in the acetyl-CoA dehydrogenase, short chain *(ACADS)* locus and rs174547 in the fatty acid desaturase 1 *(FADS1)* locus using targeted metabolomics as hypothesis free approach. These studies aim to unravel gene-diet interactions that might give some indications about the early development of metabolic diseases.

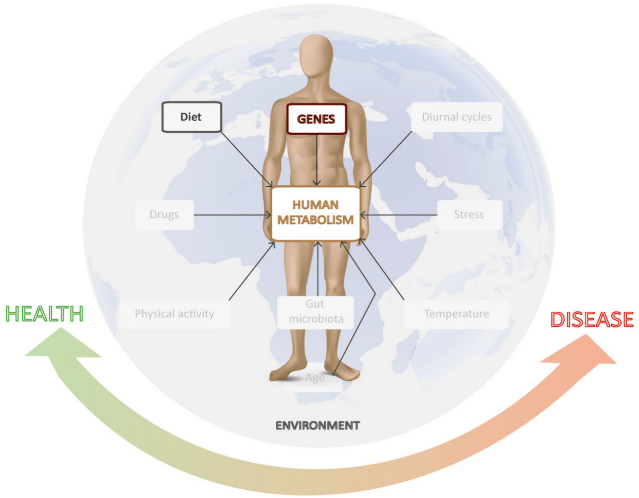

FIGURE 4: Illustration of the aim of the second part of this thesis: Unraveling gene-diet interactions at the level of the plasma metabolism that might give early indications about the development of metabolic diseases

Due to the diversity of the addressed projects and subprojects, each part of the thesis is accompanied by further background information introducing the respective topic.

2 POSTPRANDIAL METABOLISM IN HEALTHY MALES

2.1 Study design and methods

2.1.1 Study design

Six healthy, normal-weight and non-smoking males aged 40-53 years from the Munich area were recruited at the Else Kröner-Fresenius-Centre for Nutritional Medicine (EKFZ) of the Technical University of Munich. All volunteers attended a two-step screening procedure, first time by phone, second time by a screening examination which included measurement of height, weight, waist- and hip-circumference, body composition (Tanita BC-418 segmental body composition analyzer, Sindelfingen, Germany) and blood pressure using established methods. In addition, routine clinical chemistry was performed. Exclusion criteria were medication, body mass index (kg/m^2) < 20 and > 27, physical activity > 5h/week, diagnosed diabetes mellitus (T2D), immunosuppression, severe cardiovascular disease, liver disease (GOT, GPT > 3-fold of upper limits), kidney disease (creatinine > 1.2 mg/dl), psychiatric disease or unwillingness for written consent.

The study design (FIGURE 5) included three visits at the study unit separated by a “wash-out phase” of at least 2 days. The volunteers were required to abstain from taking any medication and to refrain from exertive physical activity for 24 h before each visit at the study unit. During the days before the first and the third visit, subjects were asked to maintain their normal eating and drinking habits except alcohol ingestion. Prior to the second study day, subjects were required to follow a standardized, balanced and isocaloric diet protocol according to their individual caloric requirements estimated by bioelectric impedance analysis (Tanita BC-418 segmental body composition analyzer, Sindelfingen, Germany) for three days. During this standardization phase, macronutrient intake was composed of 15% protein, 30% fat and 55% carbohydrates. On the first and the second day of standardization, subjects got an individualized diet plan elaborated according to their individual dietary habits. At the third day of standardization, diet of all subjects was completely standardized by providing subjects with identical food (breakfast: fruit muesli, milk (3.5% fat), orange juice, banana; lunch: whole grain bread, cheese, butter, tomato, gummibears; dinner: Knorr Spaghetteria Spinaci (Unilever, Hamburg, Germany)) and advising them to ingest solely provided food according to a defined time-schedule. During the three days of dietary

standardization anterior to study day 2 subjects were allowed to drink mineral water or unsweetened fruit tea ad lib.

After an overnight fast of 12 h at each study day, a venous catheter (Braun, Melsungen, Germany) was inserted into an antecubital vein and a fasting blood sample was taken. On study day 1 and 2, participants were asked to consume a high-fat, high-carbohydrate (HFHC) meal (Big Mac menu: Bic Mac, medium size French Fries, ketchup, 0.5l Orangeade, Mc Donald´s, Freising, Germany) and at study day 3 a defined healthy breakfast (HB) (100 g whole grain bread, 35 g cream cheese, 5 g margarine, 50 g boiled ham, 50 g tomatoes, 150 g yoghurt, 150 g apples, 200 g orange juice, 250 ml of herb tea). Both meals had to be eaten within 10 minutes. Details on the composition of the two test meals are given in TABLE 1. Blood samples were collected using a defined time-schedule: at 1, 2, 4, 6 and 8 h after the HFHC meal (study day 1 and 2) and at 1, 2, 4 and 6 hours after the HB (study day 3). Blood was collected into 4.9 ml EDTA K2-Gel tubes (Sarstedt, Nümbrecht, Germany), tubes were mixed thoroughly and plasma was obtained by immediate centrifugation at 3.000 x *g* for 10 min at room temperature. Plasma was aliquoted on ice, immediately frozen on dry ice and stored at -80°C until further analysis. The participants were allowed to consume mineral water, water and unsweetened fruit tea, and fluid intake was carefully recorded on all study days.

During the whole study period, dietary records were used for detailed documenting of food and fluid intake. Moreover, subjects' habitual diet was assessed by three-day dietary records.

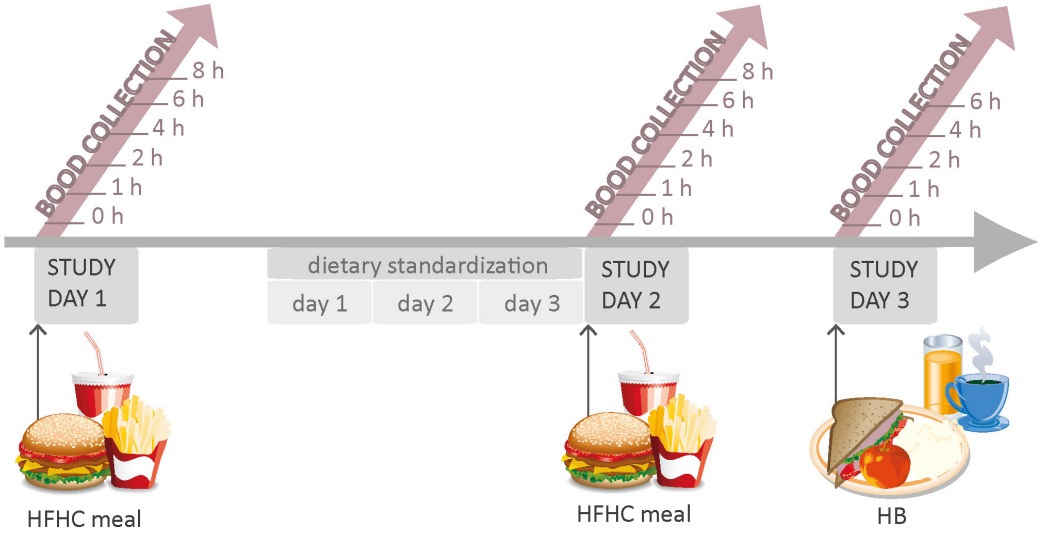

FIGURE 5: Study design of the SysMBo-Pilot study

At day 1 and 2 of the three day standardization phase percentage of macronutrient intake was standardized and at day 3 a highly defined diet was provided; HB, healthy breakfast; HFHC, high-fat, high carbohydrate.

The study was approved by the ethics committee of the Technical University München and performed in accordance with the Helsinki Declaration of 1975 as revised in 2008. Each participant had signed an informed consent. The study is registered at Deutscher Register Klinischer Studien (DRKS) as DRKS00004335.

TABLE 1: Nutrient composition of the test meals of the SysMBo pilot-study

Energy content, macro- and micronutrient intake is shown per dose; HB, healthy breakfast; HFHC, high-fat, high-carbohydrate.

Test meal	HFHC meal	HB
Composition	Big Mac menu: Big Mac, medium size French Fries, 0.5 l Orangeade, ketchup (Mc Donalds, Germany)	100 g of whole grain bread, 35 g of cream cheese, 5 g margarine, 50 g boiled ham, 50 g tomato, 150 g yoghurt, 150 g apple, 200 g orange juice, 250 ml of herb tea
	Per meal	Per meal
Energy (kcal)	1110	646
Energy density (kcal/g)	1.3	0.7
Energy density without drinking (kcal/g)	2.6	1.0
Fat (g)	49.4 (39%)	17.2 (23%)
Sattured fatty acids (g)	19.8 (16%)	8.3 (11%)
Monounsatured fatty acid (g)	11.8 (9.4%)	5.8 (7.9%)
Polyunsatured fatty acids (g)	12.5 (10%)	2.6 (3.5%)
Cholesterol (mg)	66.0	55.4
Carbohydrates (g)	132.0 (48%)	87.8 (55%)
Monosaccharides (g)	41.3	27.5
Disaccharides (g)	7.3	17.6
Oligosaccharides resororbable (g)	0.163	0.459
Oligosaccharides not resorbable (g)	0	0.002
Polysaccharides (g)	83.1	36.7
Fiber (g)	5.4	12.6
Protein (g)	31.1 (11%)	30.5 (19%)
Essential amino acids (g)	14.8	15.9
Non-essential amino acids (g)	16.3	16.1
Sodium (g)	1.6	1.1
Water (l)	0.626	0.901

2.1.2 Standard biochemistry parameters

Venous plasma glucose was determined by an enzymatic amperometric technique (Super Gl easy+, Dr. Müller Geräte Bau, Freital, Germany). Insulin was quantified by an enzyme-linked immunosorbent assay (ELISA; K6219; Dako, Glostrup, Denmark). Non-esterified fatty acids (NEFAs) and triglycerides (TG) were quantified using commercially available enzymatic methods (NEFA-HR, Wako Chemicals GmbH, Neuss, Germany and Triglycerides liquicolor mono, Human GmbH, Wiesbaden, Germany). Cholesterol, HDL-, LDL-cholesterol, triglycerides, glutamic oxalacetic transaminase (GOT), glutamic pyruvate transaminase (GPT) and creatinine were determined using established commercial tests by Synlab (Munich, Germany).

2.1.3 Analysis of dietary records

Dietary records were analyzed by a commercial nutrition software based on the official German Lebensmittelschlüssel BLS II (OptiDiet, version 5.0.0.029; GOE mbH, Linden, Germany)

2.1.4 Metabolomics analysis

Targeted and non-targeted metabolomic analyses were performed at the Genome Analysis Center of the Helmholtz Zentrum München. Liquid handling for both platforms was done on a Hamilton Microlab Star robotics system (Hamilton Bonaduz AG, Bonaduz, Switzerland).

2.1.4.1 Targeted metabolomics analysis

Targeted metabolomics measurements were carried out by using the AbsoluteIDQ^{TM} p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria) as described previously (Zukunft et al. 2013; Goek et al. 2013). 186 metabolites were analyzed by flow injection analysis and liquid chromatography tandem mass spectrometry (FIA-MS/MS and LC-MS/MS) on an API4000 mass spectrometer (AB Sciex Deutschland GmbH, Darmstadt, Germany), equipped with an 1200-Series HPLC (Agilent Technologies Deutschland GmbH, Böblingen, Germany) and a HTC PAL auto sampler (CTC Analytics, Zwingen, Switzerland). The metabolite panel includes amino acids, biogenic amines, acylcarnitines (C), sugars (H1), sphingomyelins (SM), diacylphosphatidylcholines (PC aa), acyl-alkyl phosphatidylcholines (PC ae) and lyso phosphatidylcholines (lysoPC) (SUPPLEMENTARY TABLE 1). The ratio of the median metabolite concentration in the 5 standard reference plasma samples present in every batch to the median

concentration of reference samples in all batches was used to correct inter-day variance of each metabolite. Concentrations are given in µmol/L.

2.1.4.2 Non-targeted metabolomics analysis

Non-targeted metabolomics profiles were measured using a previously described method of Metabolon Inc. (Durham, USA) (Evans et al. 2009; Boudonck et al. 2009). A brief description including some modifications is given in SUPPLEMENTARY TEXT 1. 265 metabolites as well as 136 compounds with unknown chemical structure, indicated by a X followed by a number as compound identifier were identified by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) on a LTQ mass spectrometer (Thermo Fisher Scientific GmbH, Dreieich, Germany) equipped with a Waters Acquity UPLC system (Waters GmbH, Eschborn, Germany). The metabolite panel can be divided into the following groups: amino acids, carbohydrates, cofactors and vitamins, energy, lipids, nucleotides, peptides and xenobiotics (SUPPLEMENTARY TABLE 2). Moreover, the eight groups can be subdivided into 56 biochemical pathways which are specified in SUPPLEMENTARY TABLE 2. For each identified metabolite the raw area counts were normalized to the median value of the run day to correct for inter-day variation of the measurements.

2.1.5 Statistical analysis

The statistical analysis was done by means of Ivan Kondofersky from the Institute of Computational Biology of the Helmholtz Zentrum München (German Research Center for Environmental Health (GmbH), Neuherberg, Germany) using the R statistical software (http://www.r-project.org).

2.1.5.1 Univariate t-tests

The baseline fasting levels of each metabolite of the six study participants at study day 1 and 2 as well as the energy intake and the intake of single macronutrients of the three-day dietary standardization phase and the habitual diet of the six subjects were tested for differences by using a standard univariate t-test on paired observations (Sprinthall and Fisk 1990). False discovery rate (FDR) p-value correction was used for consideration of multiple testing issues at a global significance level of 0.05 (Hochberg and Benjamini 1995).

2.1.5.2 Principal component analysis (PCA)

The baseline levels of metabolites on study day 1 and 2 were analyzed by using a PCA approach and projecting the multidimensional dataset onto two principal components. This dimension reduction was done to check whether a substantial difference was detectable between two study days under baseline conditions. The targeted metabolomic measurements were analyzed by using standard PCA since there were no missing observations. The non-targeted metabolomic measurements contained some missing data. Therefore the NIPALS algorithm (Wold and Ed. 1966) was applied.

2.1.5.3 Time-resolved paired difference test (TPDT)

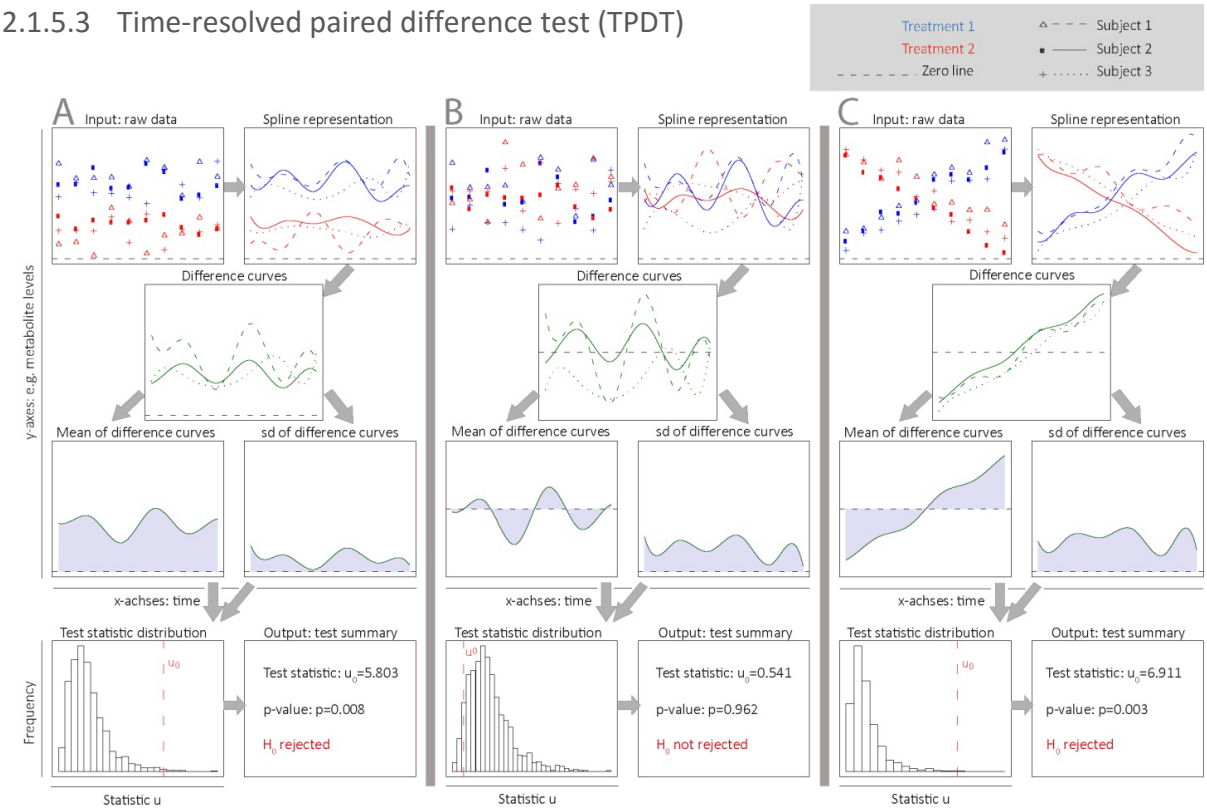

FIGURE 6: Concept figure of the time-resolved paired difference test (TPDT). The test is explained by means of three example data sets (A, B and C) including data of three subjects

For each subject (coded by different forms or lines) time-curves of two treatments (blue and red) are represented by fitted smoothing splines and difference curves of the two treatments (green curves) are calculated. The value u_0 (in each case (A, B and C) left picture of the forth row) is determined by dividing the area under the mean difference curve of all subjects (in each case (A, B and C) left picture of the third row) by the area under the standard deviation curve (in each case (A, B and C) richt picture of the third row) and correcting for the number of subjects. A large value of u_0 suggests a substantial difference between the groups (A and C), a value close to 0 suggests no difference (B). Due to unknown distribution of u_0, a resampling approach is applied to quantify the significance of the test statistic and approximate a p-value (represented by histograms). H_0, null hypothesis; sd, standard deviation.

FIGURE 6 A, B and C provide a graphical guide for the new methodology developed by Ivan Kondofersky from the Institute of Computational Biology of the Helmholtz Zentrum München (German Research Center for Environmental Health (GmbH), Neuherberg, Germany). For a brief and simplified explanation of the new methodology refer to the figure legend of FIGURE 6. The following text describes the methodology in more detail.

To test for paired time-resolved differences, it was assumed that time-resolved measurements of two variables $x_i(t_j)$ and $y_i(t_j)$ of two groups are paired over the index $i \in \{1 \dots N\}$ (top left panel of FIGURE 6). The measurements were made at discrete time-points $t_j, j \in \{0 \dots J\}$. This notation was chosen for simplicity. Since the method can deal with possibly non-synchronized and missing or repeated measurements at the same time-point, the notation would be slightly altered. It was assumed that those measurements represent local snapshots of a smooth time-course of the variables. Thus, in order to recover this time-course the discrete measurements were used and time-curves $\hat{x}_i(t)$ and $\hat{y}_i(t)$ (top right panel in FIGURE 6) identified using smoothing splines (Ramsay and Silverman 2005). This curve representation was used to calculate the difference curves $\hat{d}_i(t) = \hat{y}_i(t) - \hat{x}_i(t)$ (second row in FIGURE 6).

The TPDT was then constructed similarly to a univariate paired t-test by computing the test statistic to equal

$$u = \sqrt{N}\frac{D}{S} = \sqrt{N}\frac{\int_{t_0}^{t_n}\left|\bar{\hat{d}}(t) - \mu_0(t)\right| dt}{\int_{t_0}^{t_n}\sqrt{\frac{1}{N-1}\sum_i\left(\hat{d}_i(t) - \bar{\hat{d}}(t)\right)^2}\, dt}$$

consisting of three major parts: location measure D (third row on the left in FIGURE 6), variability measure S (third row on the right in FIGURE 6), and correction term $\sqrt{N}$. The location term was constructed by substituting a possible baseline curve $\mu_0(t)$ from a functional mean difference curve $\bar{\hat{d}}(t) = \frac{1}{N}\sum_i \hat{d}_i(t)$ with the functional difference curve $\hat{d}_i(t) = \hat{y}_i(t) - \hat{x}_i(t)$ and integrating over the considered time interval $[t_0, t_n]$. In the context of this work we used $\mu_0(t) = 0$ for all computed tests. The integral was approximated through finite differences (Ramsay and Silverman 2005). The variability measure S was computed by integrating the functional standard deviation of the difference curves $\hat{d}_i(t)$.

The test statistic u has a positive value and equals 0 only if $D = 0$, which corresponds to identical observations from both groups. A large value of u suggests a substantial difference between the two

groups. However, the distribution of u is unknown. In order to quantify the exact significance of this test statistic, a resampling approach was applied. To that end, spline curves under the assumption that there is no difference between the two groups (null hypothesis) were simulated (here we repeated for 10^6 times) and the variability in the simulated data was preserved to equal the variability of the original data. This was done by adding independent and identically distributed multivariate normal variables with 0 mean to the spline parameters with a fixed covariance matrix calculated from the already adapted spline curves on the original data. The same sample size was used for the simulated test statistics. With the resampling approach, we were able to quantify whether the observed test statistic has a significantly high value or whether this value could also be observed under the null hypothesis (considering the level of noise and the sample size of the data) by using the percentile method and counting the fraction of random test statistics which have a more extreme value than the original test statistic (bottom left in FIGURE 6). In summary, the newly developed TPDT is able to identify whether two paired groups of time-resolved measurements significantly differ in location from each other and summarizes this result in a single scalar p-value (bottom right in FIGURE 6).

In this work, the test was used on three different scenarios arising from the considered datasets. Firstly, the test was applied directly on the measurements, allowing the identification of metabolites which were significantly differing in their location. Secondly, one of the advantages of using smoothing splines was exploited, namely the easy access to the time-derivatives of the considered metabolites. Applying the test on these derivatives was straightforward, since the smoothing spline curves $x_i(t)$ and $y_i(t)$ were only replaced with $\frac{dx_i}{dt}(t)$ and $\frac{dy_i}{dt}(t)$. These time-derivatives had the same structure as the splines adapted on the discrete measurements and thus the method was directly applicable. The third scenario was the application of TPDT on groups of functional standard deviation (fsd). In this context, the question whether such fsds computed for a given group of biologically grouped metabolites (SUPPLEMENTARY TABLE 1 and SUPPLEMENTARY TABLE 2) on the first data collection day significantly differ from fsds computed for the same metabolite groups on the second data collection day was asked.

TPDT p-values were corrected for multiple testing by controlling the false discovery rate (Hochberg Y 1995) at a global significance level of 0.05 separately for each of the three test scenarios and each method of measurement (targeted and non-targeted metabolomics).

2.1.5.4 Weighted enrichment analysis

In order to assess the impact of dietary standardization or of nutritional challenges on specific biochemical groups and metabolic pathways (SUPPLEMENTARY TABLE 1 and SUPPLEMENTARY TABLE 2) we performed weighted enrichment analysis based on the statistical results from the t-tests and TPDT tests. In contrast to classical hypergeometric enrichment tests, this approach does not require a cutoff to determine which metabolites are significantly affected. The weighted enrichment analysis rather takes into account the weights (i.e. t-test or TPDT p-values) of each metabolite in its group. Specifically, it determines whether the sum of all TPDT statistics of a specific group is significantly larger than the sum of TPDT statistics based on random metabolite-group assignments. Empirical enrichment p-values were calculated by randomly shuffling metabolite-group assignments 10^6 times. A detailed description of the weighted enrichment method can be found in Krumsiek et al. (Krumsiek et al. 2012). Enrichment p-values were corrected for multiple testing by controlling the false discovery rate at a global significance level of 0.05 (Hochberg and Benjamini 1995).

2.2 Effect of dietary standardization on the plasma metabolomic response to a defined meal challenge in healthy individuals

2.2.1 Background

The metabolic phenotype of an individual provides a readout of the metabolic state at a given time point that is modified by extrinsic factors like diet (Krug et al. 2012), physical activity (Chorell et al. 2012), gut microbiota (Wikoff et al. 2009), diurnal cycles (Slupsky et al. 2007), temperature (Westerterp-Plantenga et al. 2002), stress (Krug et al. 2012), drugs (Trupp et al. 2012), age (Yu et al. 2012) and, moreover, by the endogenougs genetic (Illig et al. 2010) and epigenetic (Petersen et al. 2013) background. Therefore, metabolomic studies are influenced by distinct intra- and inter-individual variations, with the latter being considerably higher indicating a distinct metabolic phenotype of each person (Lenz et al. 2003; Walsh et al. 2006; Winnike et al. 2009; Zivkovic et al. 2009). However, this variability is a strong confounder in human studies. Therefore, the impact of diet as one modifier of the intra- and inter-individual variability in metabolic profiling was addressed in different studies. One day of dietary standardization reduced the inter-individual variation in the first void urine, but did not affect fasting plasma samples (Walsh et al. 2006). In contrast, another study reported that a normalization of the fasting serum metabolome was achieved after one day of dietary standardization, whereas the urinary metabolome was not affected (Winnike et al. 2009). Thus, the issue of dietary standardization remains a subject of controversy.

Over the last years, a rising number of metabolomics studies focused on time-resolved measurements following metabolic challenges like oral glucose tolerance testing (Ho et al. 2013; Skurk et al. 2011; Shaham et al. 2008; Wopereis et al. 2009), different other oral test meals (Krug et al. 2012; Pellis et al. 2012; Bondia-Pons et al. 2011) or physical activity tests (Krug et al. 2012). Time-resolved metabolic challenge tests can improve the identification of metabolic alterations associated with early disease states that are not detected in a homeostatic situation (Shaham et al. 2008; Ramos-Roman et al. 2012; Deo et al. 2010). However, the inter-individual variance in the fasting state was shown to be extended in the postprandial state, possibly due to the complexity of the physiological and biochemical response to a metabolic challenge (Krug et al. 2012; Zivkovic et al. 2009). This metabolic “accordion effect” (Krug et al. 2012) indicates the presence of distinct metabotypes of individuals determined by environmental factors and a given genetic and epigenetic disposition.

Aim of this project was to test the effect of a short-term dietary standardization on the postprandial time-courses of plasma metabolites after a high-fat, high-carbohydrate (HFHC) meal in healthy males. Thus, six

healthy males were provided an identical HFHC meal at two independent study days. At the days before the first study day, subjects were advised to maintain their individual eating habits and three days before the second study day, subjects had to follow a standardized, balanced and isocaloric diet protocol. On both study days, plasma samples were taken at five postprandial time-points and were analyzed by targeted and non-targeted mass spectrometric techniques. To assess both differences of the mean metabolite time-courses and the inter-individual variance of both study days, the statistical approach for the detection of paired time-resolved differences (TPDT) was applied.

2.2.2 Results

2.2.2.1 Description of the study population

The baseline characteristics including blood pressure and clinical chemical parameters of the participants demonstrate homogeneity within the study group (TABLE 2).

TABLE 2: Baseline characteristics, blood pressure and clinical chemical parameters

Data are shown as mean value and standard deviation (sd) of the six subjects and range between lowest and highest value; BIA, Bioelectrical impedance analysis; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvate transaminase; WHR, waist-to-hip ratio.

Variable	Mean ± sd	Range
Baseline characteristics		
Age (years)	44.3 ± 5.2	40 - 53
Weight (kg)	83.0 ± 10.0	72.9 - 99.9
BMI (kg/m^2)	24.8 ± 2.5	22.3 - 28.6
Waist circumference (cm)	90.7 ± 6.3	82.0 – 99.0
Lean mass BIA (kg)	69.6 ± 8.2	62.6 - 84.8
Fat mass BIA (kg)	13.4 ± 4.3	9.3 - 18.4
Body fat BIA (%)	16.1 ± 5.2	11.2 - 22.1
Blood pressure		
Systolic (mmHg)	125.5 ± 8.8	120 - 140
Diastolic (mmHg)	78.3 ± 4.1	70 - 80
Clinical chemical parameters		
Fasting blood glucose (mg/dl)	79.3 ± 6.7	70.8 - 90.9
Cholesterol (mg/dl)	193.2 ± 24.1	150 - 216
HDL (mg/dl)	60.7 ± 5.6	53 - 67
LDL (mg/dl)	122.2 ± 20.8	90 - 150
Triglycerides (mg/dl)	88.3 ± 16.9	60 - 112
GOT (U/l)	31.7 ± 9.7	26 - 49
GPT (U/l)	26.3 ± 7.4	20 - 40
Creatinine (mg/dl)	0.87 ± 0.05	0.79 - 0.94

2.2.2.2 Comparison of the diet in the standardization phase and of subjects' habitual diet

The habitual dietary intake of each subject was assessed by three-day dietary records and reflects the (unstandardized) diet before study day 1. Mean usual dietary intake of energy and macronutrients of the six subjects was compared to that in the diet ingested during the three-day dietary standardization phase before study day 2 by a paired t-test (TABLE 3). No significant differences were observable after correction for multiple testing. However, prior to the correction for multiple testing differences were observed for carbohydrate, fiber and alcohol intake. The percentage of carbohydrates was lower in the subjects' habitual diet (43.5 ± 4.0%) compared with the diet during the standardization phase (52.8 ± 3.8%; p= 0.007 prior to FDR correction). Fiber intake was also lower in the normal diet (21.6 ± 7.1 g) compared with the diet during the dietary standardization phase (35.3 ± 6.0 g; p= 0.02 prior to FDR correction). Moreover, prior to correction for multiple testing, alcohol intake was significantly higher during the normal diet compared with the three-day dietary standardization phase (17.4 ± 13.2 g vs. 0.5 ± 0.7 g/d; p= 0.02).

TABLE 3: Composition of the usual diet of subjects and the diet during the three-day dietary standardization phase assessed by dietary records

Dietary components are shown as mean value and standard deviation (sd) according to calculations with Opti Diet (Geo mbH, Linden, Germany) and a p-value of difference is calculated by a paired t-test and shown uncorrected (pval) und corrected for multiple testing by FDR correction (adj pval).

	Usual diet	Dietary standardization	p-value	
	Mean ± sd	Mean ± sd	pval	adj pval
Energy (kcal)	2366.67 ± 576.60	2641.67 ± 446.70	0.24	0.59
Protein (g)	86.03 ± 20.09	94.28 ± 16.17	0.31	0.59
Protein (%)	15.00 ± 3.35	14.67 ± 1.37	0.85	0.95
Protein/body weight (g/kg)	1.04 ± 0.21	1.14 ± 0.11	0.31	0.59
Isoleucine (g)	3.97 ± 0.92	4.27 ± 0.77	0.45	0.65
Leucine (g)	6.71 ± 1.63	7.46 ± 1.30	0.28	0.59
Valine (g)	4.71 ± 1.11	5.12 ± 0.90	0.39	0.62
Carbohydrates (g)	256.33 ± 74.42	342.67 ± 51.63	**0.03**	0.16
Carbohydrates (%)	43.50 ± 4.04	52.83 ± 3.76	**0.007**	0.14
Fat (g)	95.23 ± 29.01	95.43 ± 24.80	0.99	0.99
Fat (%)	35.33 ± 4.80	31.67 ± 4.13	0.70	0.89
SFA (g)	42.97 ± 17.68	46.12 ± 11.91	0.64	0.87
MUFA (g)	33.28 ± 7.86	29.47 ± 8.29	0.20	0.59
PUFA (g)	12.1 ± 3.25	11.80 ± 3.23	0.87	0.95
Cholesterol (g)	0.34 ± 0.14	0.47 ± 0.33	0.36	0.62
Fiber (g)	21.60 ± 7.10	35.25 ± 6.02	**0.02**	0.15
Alcohol (g)	17.39 ± 13.21	0.46 ± 0.71	**0.02**	0.15

2.2.2.3 Effect of dietary standardization on baseline plasma metabolite levels

Direct comparison of baseline plasma metabolite levels after a 12 h overnight fast with or without previous dietary standardization revealed no significant differences by a paired t-test (data not shown). Furthermore, a weighted enrichment analysis based on the results of the paired t-test was performed in order to assess whether specific metabolite groups show concerted differences after a 12 h overnight fast with or without previous dietary standardization. Thereby, metabolite groupings as predefined by the targeted and non-targeted metabolomic approaches were applied (SUPPLEMENTARY TABLE 1 and SUPPLEMENTARY TABLE 2). Results showed no significantly enriched differences (data not shown).

Next, a PCA (principle component analysis) displaying the first two principle components was performed, accounting for 44.7 % and 30.7 % of the variation of metabolite levels measured by the targeted and non-targeted metabolomics technology, respectively (FIGURE 7). PCA-plots revealed no apparent improvement of metabolite clustering with respect to the two groups, indicating that dietary standardization does not reduce the inter-individual variation of baseline plasma metabolite profiles.

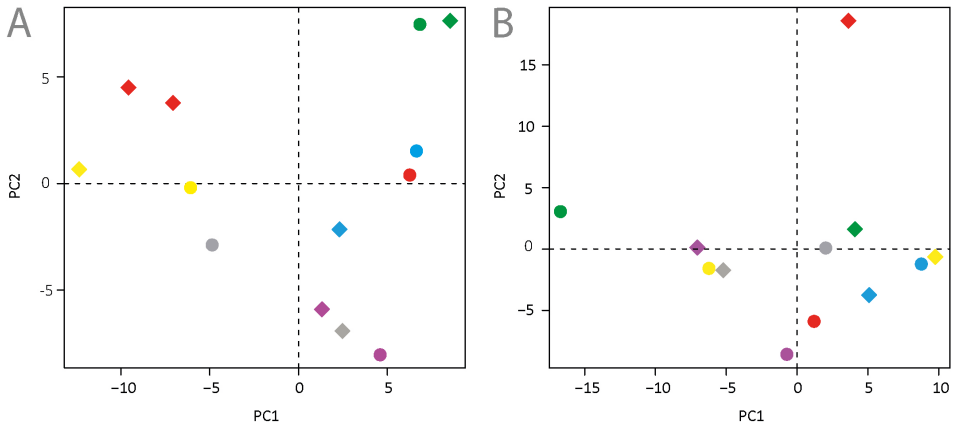

FIGURE 7: Principle component analysis of fasting samples measured with targeted (A) and the untargeted metabolomic approach (B) with and without previous dietary standardization

Rectangles show the fasting time points without dietary standardization; circles represent the fasting time points following a three day dietary standardization phase; persons are coded by color; the two red rhombi represent measurement repetitions.

2.2.2.4 Effect of dietary standardization on postprandial plasma metabolite levels

To study the influence of dietary standardization on postprandial metabolite responses, time-course experiments were performed by measuring postprandial plasma metabolite levels 1, 2, 4, 6 and 8 h after a HFHC meal for each individual on two independent study days with or without prior dietary standardization. The baseline and postprandial plasma levels were plotted over time for all metabolites using smoothing splines.

To quantify potential differences in mean postprandial time-curves of metabolites after a defined HFHC meal with and without prior dietary standardization, a new statistical method (chapter 2.1.5.3) to test for differences in paired time-resolved observations by taking the whole time-scale of postprandial metabolic changes into account was used. The time-resolved paired difference test (TPDT) was applied to the metabolite measurements of the HFHC meal with or without prior three-day dietary standardization and results for both metabolites measured with the targeted and non-targeted metabolomics approach are shown in the upper left part of TABLE 4.

A significant difference was identified for isobutyrylcarnitine (non-targeted metabolomics). Metabolite time-courses of the six subjects (FIGURE 8A) indicated lower baseline and postprandial isobutyrylcarnitine levels after the HFHC meal with previous dietary standardization as compared to the identical challenge test without previous standardization. Of note, with the targeted metabolomics approach we measured acylcarnitines with a chain length of 4 carbons (C4), potentially also including isobutyrylcarnitine. However, C4 did not reach significance after FDR correction for multiple testing ($p= 0.55$), although differences were indicated prior to the correction for multiple testing ($p= 0.01$).

Next, the TPDT was applied to compare the first time-derivative of postprandial time-courses for each metabolite. Significant inequalities on this derivative level would reveal differences in the rate of change of the considered metabolite time-course. In contrast to the application of TPDT on the original time-courses, the focus was hereby put on the curve gradients and not on the location of the two groups. The upper right part of TABLE 4 indicates a negligible effect of dietary standardization influences on the gradient of time-courses, but rather on their location as shown for lower postprandial isobutyrylcarnitine levels (FIGURE 8A) after the HFHC meal with previous dietary standardization compared to the HFHC meal without standardization.

TABLE 4: Paired time-resolved differences of the HFHC meal with previous three-day dietary standardization and without standardization

Results for the TPDT and weighted enrichment analysis are shown for the targeted and non-targeted metabolomic measurements based on the zeroth derivative (f(x)) and the first derivative (f´(x)). Weighted enrichment analysis was performed based on the results of the TPDT using metabolite groupings and sub-groupings as predefined by the targeted and non-targeted metabolomic approaches (SUPPLEMENTARY TABLE 1 and SUPPLEMENTARY TABLE 2). HFHC, high-fat, high-carbohydrate; TPDT, time-resolved paired difference test.

		F (x)			F´(x)		
TPDT		Metabolite	u_0	p-value	Metabolite	u_0	p-value
	Targeted	No differences			No differences		
	Non-targeted	Isobutyrylcarnitine	5.4994	**< 0.0305**	No differences		

		Metabolite group	p-value	Metabolite group	p-value
Weighted enrichment analysis	Targeted	Acylcarnitines	0.9824	Acylcarnitines	0.9764
		Amino acids	0.5043	Amino acids	0.3699
		Biogenic amines	0.9881	Biogenic amines	0.9764
		Phosphatidylcholines acyl-alkyl	0.1767	Phosphatidylcholines acyl-alkyl	0.3699
		Phosphatidylcholines diacyl	0.9881	Phosphatidylcholines diacyl	0.9451
		Sphingolipids	0.9678	Sphingolipids	0.7193
		Lyso-phosphatidylcholines	0.5314	Lyso-phosphatidylcholines	0.3699
	Non-targeted	Amino acids	**0.0011**	Amino acids	0.7124
		Sub-group: branched chain amino acid metabolism	**0.0105**		
		Carbohydrates	0.6691	Carbohydrates	0.1386
		Cofactors and vitamins	0.6691	Cofactors and vitamins	0.8119
		Energy	0.9993	Energy	0.9776
		Lipids	0.9993	Lipids	0.9776
		Nucleotides	0.6691	Nucleotides	0.7124
		Peptides	0.6691	Peptides	0.7124
		Xenobiotics	0.6040	Xenobiotics	0.1386

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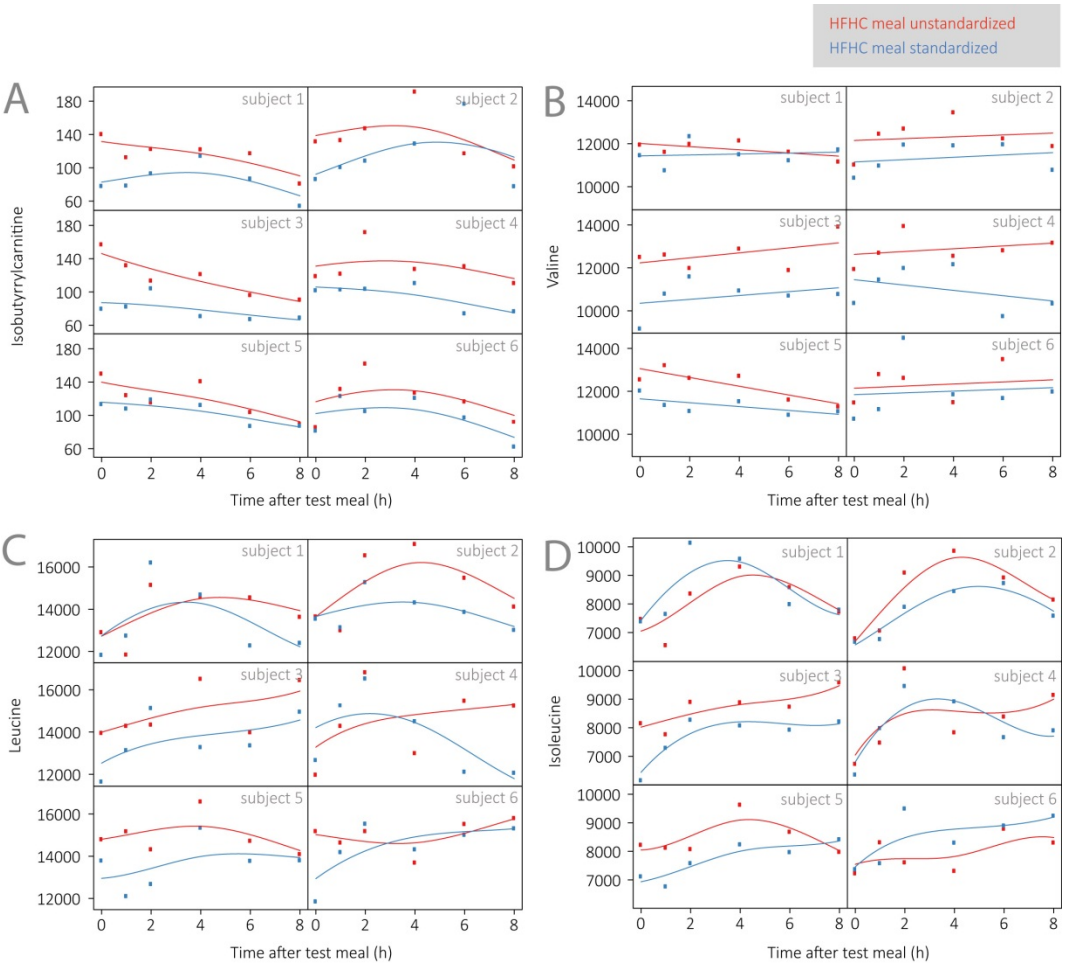


FIGURE 8: Postprandial time-courses of isobutyrylcarnitine (A) and the branched-chain amino acids valine (B), leucine (C) and isoleucine (D) (non-targeted metabolomics measurements) of the six subjects after ingestion of the HFHC meals

Red lines show the postprandial time-courses of the HFHC meal without previous dietary standardization (unstandardized), blue lines after three-day dietary standardization (standardized). Dots represent single measurements, lines show fitted smoothing splines with the degree of smoothness chosen with leave-one-out cross validation. HFHC, high-fat, high-carbohydrate.

Furthermore, a weighted enrichment analysis based on the results of the TPDT was performed in order to assess whether specific metabolite groups show concerted differences. Therefore, metabolite groupings as defined by the targeted and non-targeted metabolomic approaches (SUPPLEMENTARY TABLE 1 and SUPPLEMENTARY TABLE 2) were applied. Significantly enriched differences for the amino acid group (non-targeted metabolomics) that could be further specified as a specific difference in the branched-chain amino acid metabolism (BCAA) sub-group (lower part of TABLE 4) were observed. The BCAA metabolism sub-group includes the three BCAA valine, leucine and isoleucine and degradation products arising during BCAA metabolism including also isobutyrylcarnitine. Metabolite time-courses indicate lower basal levels

for valine after dietary standardization (FIGURE 8B) maintained over postprandial time-courses. Similar trends were observed in three subjects for leucine (FIGURE 8C) and isoleucine (FIGURE 8D). Noteably, amino acids measured with the targeted metabolomics approach did not reach statistical significance (p= 0.5043). This difference in the weighted enrichment analysis between targeted and untargeted metabolomics methods might be explained by the different composition of the metabolite group of amino acids defined by the targeted and non-targeted metabolomics approaches. Including a total of 68 metabolites, the amino acid group defined by the non-targeted metabolomics approach comprises amino acids as well as metabolites involved in amino acid metabolism, whereas the amino acid group defined by the targeted metabolomics approach includes merely 20 amino acids. Therefore, amino acid groups of both methods are hardly comparable, despite an overlap of 15 amino acids.

Overall, differences in mean time-courses of the HFHC meal with previous three-day dietary standardization and without previous standardization are shown for isobutyrylcarnitine as well as in the metabolite groups amino acids and BCAA metabolism. Differences in time-courses are mainly determined by lower metabolite levels with prior three-day dietary standardization compared to time-courses without previous standardization. Noteably, the comparison of fasting metabolite levels of isobutyrylcarnitine (p= 0.65) and of metabolite groups of amino acids (p= 0.27) as well as BCAAs metabolism (p= 0.43) did not reach significance after correction for multiple testing. However, a difference was indicated prior to the correction for multiple testing (p= 0.02, p= 0.03 and p= 0.03, respectively).

To quantify differences in postprandial inter-individual variations comparing HFHC meals with and without previous dietary standardization, we applied TPDT (chapter 2.1.5.3) based on the comparison of the standard deviations over all subjects over time (functional standard deviation (fsd)) of both test meals. Analysis of differences in functional standard deviations was performed separately for both metabolites measured by the targeted and non-targeted metabolomics technology and, additionally, for specific metabolite groups (SUPPLEMENTARY TABLE 1 and SUPPLEMENTARY TABLE 2). No significant differences were found for metabolites measured with the targeted and non-targeted metabolomic approaches (TABLE 5), indicating negligible effects of dietary standardization on the plasma metabolome. However, assessing specific metabolite groups elucidated significant different functional standard deviations for acyl-alkyl phosphatidylcholines (PC ae) measured with the targeted metabolomics approach (TABLE 5). Specifically, the mean functional standard deviations of the PC ae group showed lower postprandial levels for the HFHC meal after dietary standardization as compared to the HFHC meal without dietary standardization (FIGURE 9), indicating an effect of dietary standardization on inter-individual variation

solely for metabolite levels of PC ae (single plots of the 37 PC ae are shown in SUPPLEMENTARY FIGURE 1).

TABLE 5: Paired time-resolved differences in standard deviations after the HFHC meal with previous three-day dietary standardization and without standardization

The TPDT based on functional standard deviations (fsd) is shown for all metabolites measured by the targeted and the non-targeted metabolomic approach, respectively and for specific metabolite groups. HFHC, high-fat, high-carbohydrate.

	Metabolite group	p-value
Targeted	**All groups (all metabolites)**	0.30
	Acetylcarnitines	0.5702
	Amino acids	0.1552
	Biogenic amines	0.7778
	Lyso Phosphatidylcholine	0.1552
	Phosphatidylcholine acyl-alkyl	**0.0217**
	Phosphatidylcholine diacyl	0.2066
	Sphingolipids	0.1552
Non-targeted	**All groups (all metabolites)**	0.07
	Carbohydrate	0.5153
	Lipid	0.2840
	Amino acid	0.6636
	Xenobiotics	0.5153
	Cofactors and vitamins	0.5153
	Peptide	0.5153
	Energy	0.5178
	Nucleotide	0.5153

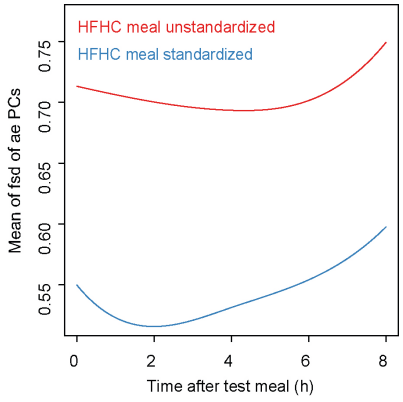


FIGURE 9: Mean of the functional standard deviations (fsd) of the acyl –alkyl phosphatidylcholines (PC ae) measured with the targeted metabolomic approach after the two HFHC meals

The red line shows the mean of the fsd after the HFHC meal without dietary standardization (unstandardized), the blue line shows the mean of the fsd after the HFHC with previous three-day dietary standardization (standardized); functional standard deviations of the single PC ae are shown in SUPPLEMENTARY FIGURE 1; HFHC, high-fat, high-carbohydrate.

2.2.3 Discussion

The aim of this study was to assess if a dietary standardization prior to a defined test meal is necessary to reduce variations of postprandial plasma metabolite time-courses.

As a main result, differences in postprandial time-courses of isobutyrylcarnitine as well as of the metabolite groups of amino acids and branched chain amino acids (BCAAs) were found. Time-courses show lower postprandial plasma-isobutyrylcarnitine and valine levels after dietary standardization and similar trends in some subjects for leucine and isoleucine. An explanation may be the trend for higher carbohydrate and fiber intake in the standardization phase compared with the habitual diet of the study participants. Dietary fiber is discussed to delay nutrient absorption and, therefore, might lead to a higher local protein synthesis and oxidation in the small intestine (Pirman et al. 2007; ten Have et al. 2007). Consequently, amino acid levels in the portal vein and plasma might be reduced (ten Have et al. 2007). BCAAs are not degraded in the liver and therefore may directly influence plasma concentrations, as shown under a prolonged protein-enriched diet (Jakobsen et al. 2011). Isobutyryl-CoA is known to be an intermediate of valine metabolism (Luís et al. 2011), therefore, valine levels might be associated with isobutyryl-CoA levels measured as isobutyrylcarnitine levels in plasma. Although current literature shows a reduction of BCAA levels in the postprandial state (Tovar et al. 1996) and after long-term intervention of a fiber enriched diet (Moazzami et al. 2012), a three-day fiber enriched diet shows a trend, but no significant reduction of fasting BCAA levels (Tovar et al. 1996). Thus, due to the current literature and the fact that fiber intake was not significantly different after correction for multiple testing, it is not clear whether the difference in BCAA metabolism can be fully explained by fiber intake. It is noteworthy that the difference observed in time-courses for isobutyrylcarnitine and in the group of amino acids, specifically BCAA, was not statistically significant after correction for multiple testing by merely comparing baseline measurements, showing the potential benefit of postprandial time-course experiments compared to a single fasting metabolite profile.

In addition to the specific effects of the three-day dietary standardization on mean time-courses of single metabolites, the effects on the inter-individual variation after a defined HFHC meal were assessed. Studying inter-individual variance in time-courses for all metabolites measured either by the targeted or untargeted metabolomic approach showed no effect of dietary standardization on the plasma metabolome. However, assessing single metabolite categories revealed that dietary standardization reduced variance in PC ae. Consistent with our findings, a recent study also applying targeted metabolomics in fasted serum samples showed that the proportion of explained variation by habitual diet

was highest for PC ae (5.7%) (Floegel et al. 2013a). Studying fasting plasma samples, we observed no apparent effect of standardization on inter-individual variance, indicating that time-resolved tests are able to overcome this limitation. At present, clinical trials investigating the effect of dietary standardization on variation in fasting plasma or serum metabolite profiles have been rare and controversial (Walsh et al. 2006; Winnike et al. 2009), and the effects on postprandial time-resolved metabolite profiles have not been studied so far. Altmaier et al. showed that dietary fiber intake is associated with a shift towards more saturation and smaller chain length of the fatty acid residues of phosphatidylcholines (Altmaier et al. 2013). Therefore, an increased fiber intake during dietary standardization might be associated with the reduced inter-individual variance in PC ae levels, however, the underlying mechanisms remain to be elucidated.

The effect of dietary standardization on postprandial plasma metabolomics profiles was comprehensively investigated by two commercial platforms using sensitive mass spectrometry. However, the 45 overlapping metabolites of both methods did not reach the same significance levels, although high correlations were found for most metabolites (data not shown). Thus, differences across technological platforms have to be considered in future studies. Another shortcoming of our study was the limited number of subjects analyzed. Although the smooth curves were fitted using cross validation techniques, the considered time-series consisted of six subsequent measurements, which is possibly at the lower limit for a spline representation of a time-depending variable and, thus, outliers may represent a serious obstacle and may lead to result modification.

In conclusion, the current study shows that dietary standardization prior to a defined high-fat, high-carbohydrate test meal results in significant differences in mean postprandial time-courses for isobutyrylcarnitine and BCAAs compared to an identical meal without dietary standardization. Moreover, our study revealed that the postprandial inter-individual variance in PC ae is reduced by dietary standardization. Nonetheless, valid postprandial time-course measurements of most metabolite classes seem to be possible without standardized dietary lead-in periods in healthy subjects.

2.3 Comparative analysis of postprandial plasma metabolic changes to a fast-food meal and a healthy breakfast

2.3.1 Background

The modern lifestyle is characterized by time scarcity for food preparation and a growing trend towards consumption of fast and convenience foods away from home (Jabs and Devine 2006; Guthrie et al. 2002). Frequent takeaway and fast food consumption was shown to be associated with higher intake of energy, fat, saturated fatty acids, trans fatty acids, added sugar and sodium and lower intake of fiber, vitamins and micronutrients compared with the consumption of self-prepared food eaten at home (Jaworowska et al. 2013; Orfanos et al. 2007; Bowman and Vinyard 2004; Paeratakul et al. 2003; French et al. 2001). This changing dietary pattern was shown to result in elevated plasma triglycerides, total cholesterol and low-density lipoprotein cholesterol, as well as decreased high-density lipoprotein cholesterol concentrations and, moreover, was associated with overweight as well as increased risk of insulin resistance and T2D (Jaworowska et al. 2013; Pereira et al. 2005; Duffey et al. 2009).

Recent studies suggested acute impairments of metabolic risk factors after intake of a single meal. For instance, fat load was shown to induce postprandial inflammatory responses that might be associated with endothelial dysfunction and atherosclerosis (Alipour et al. 2008; van Oostrom et al. 2004; van Oostrom et al. 2003a; van Oostrom et al. 2003b). Moreover, high glycemic index food is well known to induce postprandial hyperglycemia (Brynes et al. 2003; Liu et al. 2012) discussed to contribute to the etiology of obesity, cardiovascular disease and T2D (Blaak et al. 2012). There are only few studies directly focusing on the postprandial plasma metabolism after a single conventional fast food meal (Bray et al. 2007; Ramel et al. 2012; Rudolph et al. 2007). Comparing a fast food meal to unconventional fast food alternatives or a healthier meal, such studies have shown higher postprandial glucose as well as insulin concentrations (Ramel et al. 2012) and higher LDL area under the curve after the fast food meal (Bray et al. 2007).

To our knowledge, studies analyzing postprandial plasma metabolism of a fast food meal compared to a healthy alternative are limited on studying classic clinical chemical parameters. However, addressing this topic by a comprehensive metabolomics-based approach might enable to unravel further acute impairments in plasma metabolism. Thus, the aim of this sub-project was to compare postprandial metabolic changes upon a defined fast food meal for breakfast and a healthier breakfast alternative in healthy males applying a comprehensive metabolomics approach. Due to the valid metabolomics measurements without lead-in periods of dietary standardization shown in paragraph 2.2, previously

unstandardized postprandial metabolite measurements of the HFHC meal (fast food) on study day 1 and of the HB (healthy breakfast) at study day 3 were choosen for comparison. Thereby, plasma samples taken at baseline and at four postprandial time-points up to 6 h after the challenges, analyzed by targeted and non-targeted mass spectrometric techniques, were analyzed regarding differences in mean metabolite time-courses by the time-resolved paired difference test (TPDT).

2.3.2 Results

2.3.2.1 Description of the study population

The baseline characteristics including blood pressure and clinical chemical parameters of the six participants are shown in TABLE 2 in paragraph 2.2.2.1.

2.3.2.2 Composition of test meals

For the HFHC meal and the HB normal meal size of an adult person was chosen to study "real-life conditions". The composition of the test meals as well as their energy, micro- and macronutrient content is shown in TABLE 1 in paragraph 2.1.1. Energy intake and energy density was higher in the HFHC meal (1110 kcal, 1.3 kcal/g (without drinking: 915 kcal, 2.6 kcal/g)) than in the HB (646 kcal, 0.7 kcal/g (without drinking: 560 kcal, 1.0 kcal/g)). The difference in energy density was determined by higher content of fat in the HFHC meal compared to the HB (49.4 g (39%) and 17.2 g (23%), respectively). Due to the high total fat content, the HFHC meal had more saturated fatty acids (SFA), monounsatured fatty acids (MUFA) but also polyunsatured fatty acids (PUFA) than HB (TABLE 1). Moreover, cholesterol intake was higher in the HFHC meal compared to the HB (66 mg and 55.4 mg, respectively). Total carbohydrate intake was 1.5 times higher in the HFHC meal compared to the HB (132 g and 87.8 g, respectively), due to a higher content of mono- and polysaccharides, though fiber intake was 2.3 times higher in the HB (HFHC meal 5.43 g and HB 12.6 g). Total protein content as well as the composition of amino acids was similar in both test meals, however percentage of protein intake was higher in the HB compared to HFHC meal (19% and 11%, respectively). Sodium intake was higher in the HFHC meal than in the HB (1.64 g and 1.11 g, respectively), whereas intake of most vitamins was lower in the HFHC meal compared to the HB.

2.3.2.3 Time-resolved differences of test meals

To compare the postprandial metabolite responses of the HFHC meal and the HB, time-course experiments were performed by measuring metabolite levels in the fasting state and 1, 2, 4 and 6 h after both test meals. The baseline and postprandial plasma levels were plotted over time for each metabolite using smoothing splines. The quantification of differences in postprandial time-courses of each metabolite after the defined HFHC meal and HB was done by the time-resolved paired differences test (TPDT). TPDT was applied to the metabolite measurements of both test meals and results are shown in the upper left part of TABLE 6. The test revealed significant functional differences between the HFHC meal and the HB in

N-methyl proline, stachydrine, 3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF), isoleucine and three unknown metabolites measured by the untargeted metabolomics approach.

TABLE 6: Time-resolved paired differences of the HFHC meal and the HB

Results for the TPDT and weighted enrichment analysis are shown for the targeted and non-targeted metabolomic measurements based on the zeroth derivative (f(x)) and the first derivative (f´(x)). Weighted enrichment analysis was performed based on the results of the TPDT using metabolite groupings and subgroupings as predefined by the targeted and non-targeted metabolomic approaches (SUPPLEMENTARY TABLE 1 and SUPPLEMENTARY TABLE 2). HB, healthy breakfast; HFHC, high-fat, high carbohydrate; TPDT, time-resolved paired difference test.

		F (x)			F´(x)		
TPDT		Metabolite	u_0	p-value	Metabolite	u_0	p-value
	Targeted	No differences			No differences		
	Non-targeted	N-methyl proline	7.2591	**< 0.0061**	stachydrine	6.0002058	**0.0306**
		stachydrine	8.3542	**< 0.0061**	N-methyl proline	3.46807	**0.0459**
		3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	6.1331	**0.0061**			
		isoleucine (Ile)	4.1428	**0.0061**			
		X_09789	6.3784	**0.0061**			
		X_11360	4.5137	**0.0102**			
		X_18913	4.4468	**0.0481**			

		Metabolite group	p-value	Metabolite group	p-value
Weighted Enrichment Analysis	Targeted	Acylcarnitines	0.7009	Acylcarnitines	0.9448
		Amino acids	0.1149	Amino acids	**0.0054**
		Biogenic amines	0.9999	Biogenic amines	0.7319
		Phosphatidylcholines acyl-alkyl	0.9999	Phosphatidylcholines acyl-alkyl	0.9448
		Phosphatidylcholines diacyl	0.7009	Phosphatidylcholines diacyl	0.7319
		Sphingolipids	0.1672	Sphingolipids	0.9448
		Lyso-phosphatidylcholine	0.1672	Lyso-phosphatidylcholine	0.7319
	Non-targeted	Amino acids	0.3041	Amino acids	0.8124
		Carbohydrates	0.9827	Carbohydrates	0.9659
		Cofactors and vitamins	0.9411	Cofactors and vitamins	0.6855
		Energy	0.9827	Energy	0.9659
		Lipids	0.9827	Lipids	0.9659
		Nucleotides	0.9827	Nucleotides	0.9659
		Peptides	0.9827	Peptides	0.8282
		Xenobiotics	0.3041	Xenobiotics	0.6855

In addition, TPDT was applied to compare the first time-derivative of postprandial time-courses of both test meals for each metabolite. Significant differences in the time-derivatives would unveil inequalities in the rate of change of the postprandial metabolite responses of a considered metabolite. In contrast to the test application on original time-courses, TPDT on the first time-derivative enables to focus on the curve gradients and not on the location of postprandial time-courses of the two test meals. Significant differences in the first time-derivative were revealed for stachydrine and N-methylproline (upper right part of TABLE 6).

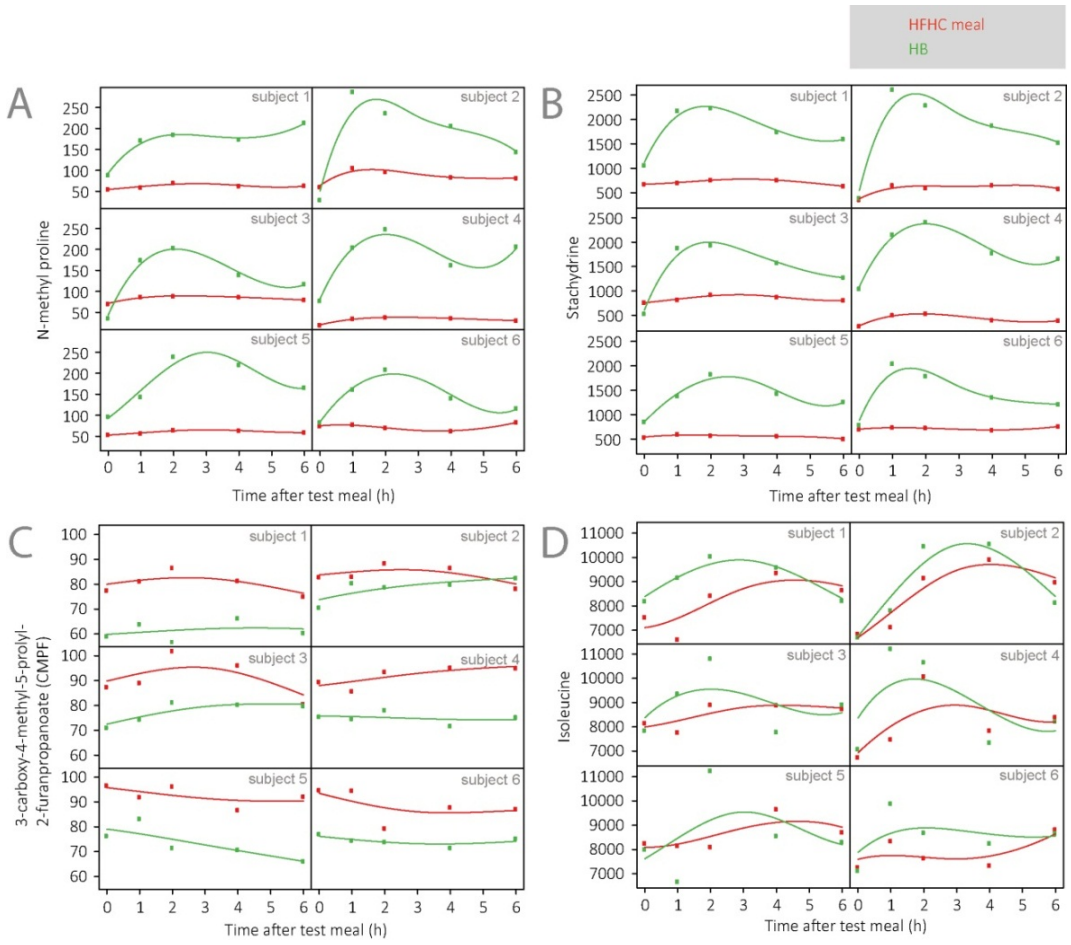


FIGURE 10: Postprandial time-courses of significant different metabolites by comparison of the HFHC meal and the HB

(A) N-methylproline; (B) stachydrine; (C) 3-carboxy-4-methyl-5-prolyl-2-furanpropanoate (CMPF); (D) isoleucine (non-targeted metabolomics measurements); the red lines show the time-courses after the HFHC meal, the green lines after the HB. Dots represent single measurements, lines show fitted smoothing splines with the degree of smoothness chosen with leave-one-out cross validation; HB, healthy breakfast; HFHC, high-fat, high-carbohydrate.

Time-courses of the HFHC meal and the HB are shown for significant different metabolites, separately for the six subjects in FIGURE 10. Statistically significant differences in original time-courses as well as in the first time-derivative of the time-courses indicate the most distinct differences between the test meals in N-methylproline and stachydrine. Time-courses of both metabolites (FIGURE 10A and B) showed a precise postprandial increase in metabolite levels in the first 2 h due to the HB and stable levels following the HFHC meal. 3-carboxy-4-methyl-5-prolyl-2-furanpropanoate (CMPF) (FIGURE 10C) showed lower baseline and postprandial levels for the HB than for the HFHC meal. Isoleucine rose in response to both challenges, showing an earlier and steeper increase induced by the HB (FIGURE 10D).

Moreover, we performed a weighted enrichment analysis based on the results of TPDT to assess if specific metabolite groups show concerted differences. We used metabolite groupings defined by the targeted and non-targeted metabolomic approaches (SUPPLEMENTARY TABLE 1 and SUPPLEMENTARY TABLE 2). Significantly enriched differences of the HFHC meal and the HB in the first deviation were identified for amino acids measured with the targeted metabolomics approach (lower part of TABLE 6). Time-curves of single amino acids (e.g. isoleucine, threonine, asparagine, proline) implied postprandial increases after both test meals, tending to be more distinct for the HB and more delayed for the HFHC meal as shown for isoleucine measured with non-targeted metabolomics approach.

Although most amino acids measured with targeted and the non-targeted metabolomics approaches showed close correlations (e.g. isoleucine: r= 0.74), the targeted measurement of isoleucine did not reach significance by TPDT after correction for multiple testing (p-value= 0.7), although differences were indicated prior to the correction for multiple testing (p-value= 0.02). Moreover, weighted enrichment analysis revealed significant differences of both test meals in the metabolite group of amino acids measured with the targeted approach, but did not reach significance for the non-targeted measurements (TABLE 6). These differences might partially be explained by different composition of the metabolite group of amino acids defined by the targeted and non-targeted metabolomics technologies. The amino acids group of the non-targeted metabolomics approach includes a total of 68 metabolites, implicating amino acids as well as metabolites involved in amino acid metabolism, whereas the amino acid group defined by the non-targeted metabolomics approach includes merely 21 amino acids and no metabolites of the amino acid metabolism. Thus, the amino acid groups of both methods are hardly comparable, despite an overlap of 15 metabolites.

Overall, distinct differences of the HFHC meal and the HB were identified in postprandial time-courses of N-methyl proline, stachydrine, 3-carboxy-4-methyl-5-propyl-2-furanpropanoate, isoleucine and of three

unknown metabolites measured with the non-targeted metabolomics approach. Moreover, concerted differences were shown in the metabolite group of amino acids measured with the targeted metabolomics approach.

2.3.3 Discussion

Aim of this study was to compare the dynamic postprandial metabolite responses of two defined meal challenges, the first one representing a western fast food meal and the second one a healthy alternative. For both meals a “normal” meal size of an adult person was chosen instead of an isocaloric state to be close to “real life conditions”. The comprehensive metabolomic analysis applying targeted and untargeted metabolomics methods in the six healthy males showed rather modest postprandial differences between the fast food meal and the healthy breakfast. Statistically significant differences were identified in time-courses of N-methyl-proline, stachydrine, 3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF), isoleucine, three unknown metabolites and in the group of amino acids.

N-methyl-proline and stachydrine are betaines found in many citrus foods and juices (Servillo et al. 2011). The postprandial increases of both metabolites after the HB were induced by the 200 ml of orange juice included. In contrast, plasma levels of both metabolites following the HFHC meal remained almost stable, despite containing 500 ml Fanta including orange juice and orange extract in addition to water, sugar and other compounds (McDonalds Germany 2013). The current knowledge on the physiological role of stachydrine is limited. In human studies, stachydrine was shown to increase urinary loss of glycine-betaine and is, therefore, discussed to be associated with an increased risk of cardiovascular disease (Lever et al. 2007; Lever et al. 2005). In contrast, in vitro studies demonstrated beneficial functions of stachydrine for endothelial cell injury (Yin et al. 2010).

3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF) plasma levels were shown to be associated with the intake of fish and greens (Hanhineva et al. 2015; Guertin et al. 2014). Thus, higher baseline plasma levels of CMPF during the HFHC meal compared to the HB, might imply an increased intake of fish or greens in the days prior to the HFHC meal. Aside, Prentice et al. showed elevated plasma CMPF levels in individuals with gestational diabetes, T2D and prediabetes (Prentice et al. 2014). Moreover, CMPF was shown to be one of the major uremic toxins (Miyamoto et al. 2012). However, as we recruited healthy persons with normal renal function and glucose tolerance, we did not expect an elevation or accumulation of CMPF due to such reasons.

In addition, significantly different postprandial metabolite levels after both test meals were found for isoleucine and in the metabolite group of amino acids. Most amino acids increased after both challenges due to the substantial protein load, but there was a lower and delayed increase after the HFHC meal compared to the HB. Plasma levels of amino acids were mainly determined by the amount of

protein/amino acids ingested by a diet/meal, but may also depend on factors like gastric emptying, utilization by gut epithelial cells, liver and peripheral tissues like muscle. In view of the rather similar protein content of the HFHC meal (31.1 g) and the HB (30.5 g) (TABLE 1) and of lacking postprandial differences in plasma insulin levels, this effect may be explained by differences in gastric emptying or intestinal absorption. A high energy density of food was shown to be associated with a lower rate of gastric emptying (Calbet and MacLean 1997; Hunt and Stubbs 1975). Thus, there might be a slower gastric emptying of the HFHC meal (1.3 kcal/g, without drinking 2.6 kcal/g) compared to the HB (0.7 kcal/g, without drinking 1.0 kcal/g). It is interesting to note that only negligible differences in plasma free fatty acids, triglycerides as well as glucose and insulin were observed between both test meals despite of substantial differences in macronutrient and energy intake (HFHC meal contained an almost 3-times higher amount of fat and an 1.5 times higher amount of carbohydrates than HB) (TABLE 1). This surprising finding may also be explained by potentially slower gastric emptying due to the high energy density of the HFHC meal (Calbet and MacLean 1997; Hunt and Stubbs 1975). However, one has to keep in mind the low time-resolution of glucose and insulin measurements after the two meals. It is known that, depending on the composition of meals, postprandial increases in blood glucose and insulin concentrations may be detectable within about 15 minutes and peak at around 45 to 90 minutes after a meal (Krug et al. 2012; Wahl et al. 2013; Ramel et al. 2012).

There are only a few studies in the literature which investigated differences in postprandial metabolism after a fast food compared to a balanced healthy meal or fast food alternatives (Ramel et al. 2012; Bray et al. 2007; Rudolph et al. 2007) and, to our knowledge, there are no studies comparing postprandial metabolic changes by a comprehensive large-scale metabolomics approach. Ramel et al. (Ramel et al. 2012) reported higher postprandial glucose and insulin levels after a conventional hamburger meal compared with an isocaloric salmonburger meal. Several explanations were discussed by the authors, mainly differences in the composition of both meals such as fiber content. In the study of Rudolf et al. (Rudolph et al. 2007), the acute effects of a conventional burger meal was compared to two vegetarian alternatives. No differences in plasma glucose and insulin concentrations and in flow-mediated endothelium-dependent dilatation were found at baseline 2 and 4 h after the meals. Using closer intervals for blood collection, Bray et al. were also unable to detect significant differences in postprandial glucose and insulin levels in overweight subjects after consumption of a fast food meal, an organic beef meal and a turkey meal (Bray et al. 2007).

Based on a comprehensive metabolomics approach using two commercial technologies, rather modest differences in the plasma metabolomic response to a fast food meal compared to a healthy breakfast could

be shown, despite of an 1.7 times higher amount of calories and considerably different compositions of macro- and micronutrients in the HFHC meal. This surprising result may be attributable to the high metabolic flexibility of the human volunteers, although they were at an age in which metabolic diseases are common. However, they were healthy according to usual criteria and may have retained the capacity to perfectly adapt to substantial differences in meal composition. Thus, short-term exposure of healthy individuals to varying meals might be fully balanced by this metabolic flexibility. It is likely that adverse health effects of fast food consumption including changes in plasma metabolites (Jaworowska et al. 2013; Pereira et al. 2005; Duffey et al. 2009) are predominantly determined by long-term and repeated exposure to fast food or may be more rapidly visible in individuals who are particularly susceptible to metabolic disturbances due to genetic or environmental factors. As such individuals were excluded from this study, it remains to be elucidated as to whether this comparison would result in similar or other findings in subjects at risk of metabolic diseases.

In conclusion, comparing a conventional fast food meal and a healthy breakfast, only modest differences in the postprandial plasma metabolite profiles measured by a comprehensive metabolomics approach were found. Differences were merely shown for N-methyl-proline, stachydrine, CMPF, as well as for amino acids. Therefore, adverse effects of fast food consumption on plasma metabolites seem to be mainly determined by long-term exposure, whereas single fast food meals may be well compensated by healthy men due to a high metabolic flexibility. Additional studies are needed to better define the conditions under which the analysis of the postprandial metabolomic profile may provide an improvement of the early detection of individuals at risk of diet-related metabolic diseases.

3 POSTPRANDIAL METABOLISM IN SUBJECTS HOMOZYGOUS FOR GWAS-IDENTIFIED VARIANTS AT LIPID METABOLISM LOCI

3.1 Background

Recent genome-wide association studies (GWASs) identified thousands of single nucleotide polymorphisms (SNPs) associated with an increased risk of common diseases (Zeggini et al. 2008; Hindorff et al. 2009; Samani et al. 2007). However, this approach does not allow insight into the biological processes underlying these associations. Metabolomics has become a powerful tool to define specific metabotypes potentially linking gene variants and their potential contribution to disease-causing processes. The linkage of GWASs with metabotypes identified genetic variants in genes encoding transporter proteins and enzymes with profound impact on human metabolic traits (Illig et al. 2010; Gieger et al. 2008; Tanaka et al. 2009; Hicks et al. 2009; Demirkan et al. 2012; Kettunen et al. 2012; Nicholson et al. 2011). Moreover, few GWASs using metabolite concentration ratios as proxies for enzymatic reaction rates identified several genetic loci highly associated with metabolite pairs (Gieger et al. 2008; Illig et al. 2010; Nicholson et al. 2011). These studies found strongest associations for the SNP rs2014355 in the acetyl-CoA dehydrogenase short chain *(ACADS)* gene locus (OMIM: 606885), with the C3/C4-acylcarnitine ratio and of the SNP rs174547 in the fatty acid desaturase 1 *(FADS1)* gene locus (OMIM: 606148) with the diacyl phosphatidylcholine (PC aa) ratio C36:3/C36:4 (Illig et al. 2010; Nicholson et al. 2011) in the fasting state. The variants rs2014355 in the ACADS-locus and rs174547 in the FADS1 locus explained 21.5 - 29% of the inter-individual variance of the metabolite ratio of C3/C4 and 28.62 - 36.5% of the inter-individual variance of the metabolite ratio of PC aa C36:3/C36:4, respectively (Gieger et al. 2008; Illig et al. 2010; Nicholson et al. 2011). In addition, applying a longitudinal twin design Nicholson et al. reported a familial component of variation of 51% and 12% in metabolite levels of C3/C4 and PC aa C36:3/C36:4, respectively. This familial component of variation is determined i.e. by common environmental and heritable factors (Nicholson et al. 2011). Nutrition seems to be the most important environmental factor in this context as the composition of food may strongly modulate circulating lipid parameters in humans and may also play a major role in the development of frequent chronic metabolic diseases. Defined nutritional challenge tests were reported to uncover early metabolic changes in carriers of genotypes associated with a higher risk

for metabolic diseases (Franks et al. 2007; Tan et al. 2006; Weickert et al. 2007). Thus, genotype effects of rs2014355 and rs174547 may be more easily unmasked by using challenge tests compared to analyzing merely fasting state conditions.

Aim of this second part of the work was to characterize the functional role of the gene variants rs2014355 in the *ACADS* gene locus and rs174547 in the *FADS1* locus in strictly controlled human studies using targeted metabolomics as hypothesis-free approach. Homozygous carriers of the minor C and major T allele of the gene variant rs2014355 were exposed to a 24 h fasting period and a standardized oral glucose tolerance test (OGTT), homozygous carriers of the minor C and major T alleles of the variant rs174547 were exposed to an oral lipid tolerance test (OLTT) and a standardized OGTT. Potentially novel genotype-dependent differences were studied by a logistic regression model using bootstrap randomized performance. Details about the studied variants and the associated genes are given prior to the respective results.

3.2 Study design and methods

3.2.1 Study design

Subjects were recruited from the population-based KORA (Cooperative Health Research in the Region of Augsburg) S1-S3 and follow-up F3 and F4 cohort in the region of Augsburg, southern Germany (Rathmann et al. 2009) based on existing imputed genome-wide association data using Affymetrix 6.0 chip (Kolz et al. 2009), Affymetrix 500K chip (Voight et al. 2010) and Illumina Cardio-Metabochip (Morris et al. 2012). From all male participants of Caucasian origin aged 18 to 69 years with BMI < 35 kg/m^2 and available genotype data, 13 subjects carrying the CC genotype of the variant rs2014355 in the ACADS locus and 13 subjects carrying the TT genotype (controls) were recruited. In addition, 13 subjects carrying the CC genotype of the variant rs174547 in the FADS1 locus and 13 subjects carrying the TT genotype (controls) were recruited. Seven of the recruited subjects carried the TT genotype of rs2014355 and rs174547, and thus, overlap as control for both genotypes. Subjects with known diabetes mellitus, immune-suppressive therapy, cardiovascular disease, liver disease (GOT (glutamic oxalacetic transaminase), GPT (glutamic pyruvate transaminase) >3-fold above normal range), kidney disease (creatinine >1.2 mg/dl) and psychiatric disease were excluded from the study. All participants gave written informed consent. The study is registered as DRKS00006202 at the Deutsches Register Klinischer Studien, was approved by the ethics committee of the Bavarian Medical Association (Bayerische Landesärztekammer) and performed in accordance with the Helsinki Declaration of 1975 as revised in 2008.

The study design (FIGURE 11) included two days with two overnight stays at the Else Kröner-Fresenius-Centre for Nutritional Medicine (EKFZ) of the Technische Universität München. Volunteers were carefully advised to refrain from exertive physical activity and alcohol for 24 h before the first study day. After arrival at the study center in the late afternoon, subjects were given a standardized supper (REWE Bio Schlemmer Spätzle, (REWE, Köln, Germany), 0.1 l orange juice (461 kcal, 23.9 g protein, 64.4 g carbohydrates, 11.8 g fat)) at 8 pm. Following an overnight fast of 12 h, a venous catheter (Braun, Melsungen, Germany) was inserted into an antecubital vein and a first fasting blood sample (baseline) was taken. In addition, an anthropometric examination was performed including measurement of height, weight, waist circumference and blood pressure using established methods. For minor CC allele carriers of rs2014355 in the ACADS locus and controls further blood samples were taken after 14, 16, 18, 20, 22 and 24 h of fasting. The fasting period was terminated by another standardized supper (2 pretzels, 16.7 g butter, 25 g liver sausage, 16.7 g cream cheese, 1 canned peach, 1 Kinder Riegel (Ferrero, Frankfurt am Main, Germany),

0.1 l orange juice (869 kcal, 20.5 g protein, 121.6 g carbohydrates, 32.2 g fat). In parallel, the minor CC allele carriers of rs174547 and controls were asked to consume an oral lipid tolerance test (OLTT) consisting of three parts Fresubin Energy Drink (Fresenius Kabi, Bad Homburg, Germany) and one part Calogen (Nutricia, Zoetemeer, The Netherlands) (TABLE 7). The volume of the liquid meal was calculated for each volunteer to provide 35 g fat/m^2 body surface area. Additional blood samples were taken at 1, 2, 3, 4, 6 and 8 h after the OLTT. At 8 pm, subjects were given a standardized supper identical to that provided to subjects of the ACADS-study at study day 1. At 8 am of the second study day, all subjects underwent a standardized oral glucose tolerance test (OGTT, 75 g glucose, Dextro O. G. T., Roche Diagnostics, Mannheim, Germany). During OGTT, venous blood samples were taken at baseline (after 12 h overnight fast) and 15, 30, 60, 120 and 240 min after the glucose load.

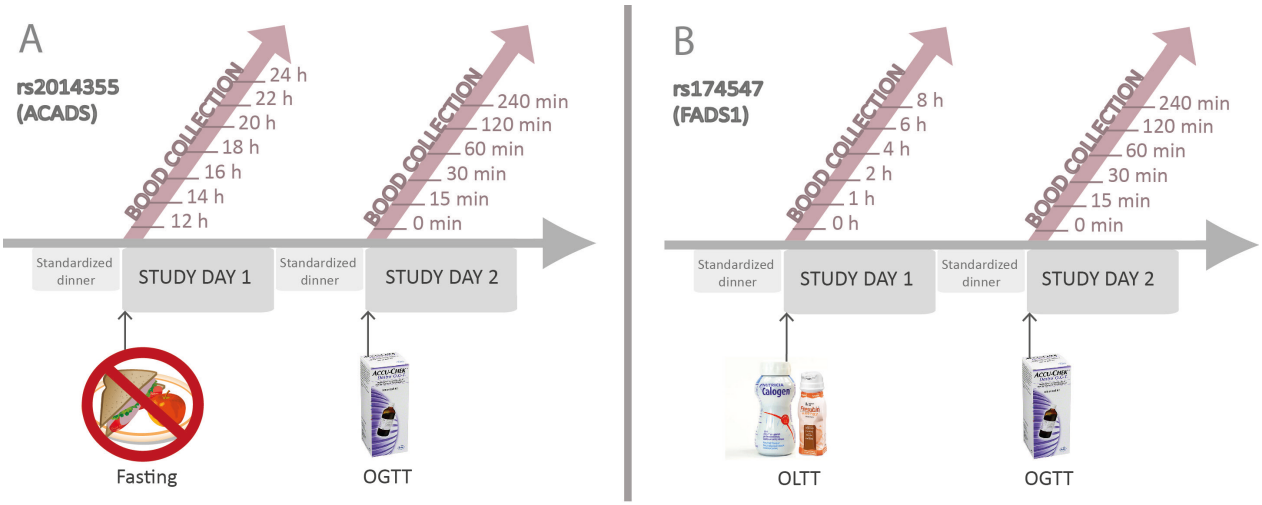


FIGURE 11: Study design of the SysMBo-study

A: study design for carriers of the minor CC allele of rs2014355 in the acetyl-CoA dehydrogenase, short chain (*ACADS*) locus and carriers of the major TT alleles for controls; B: study design for minor CC allele carriers of rs174547 in the fatty acid desaturase 1 (*FADS1*) locus and carriers of the major TT alleles for controls; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test.

Blood was collected into 4.9 ml EDTA K2-Gel tubes (Sarstedt, Nümbrecht, Germany). Tubes were mixed thoroughly and plasma was obtained by immediate centrifugation (10 min at 3.000 x *g*, 20°C). Plasma was aliquoted on ice, immediately frozen on dry ice, stored at -80°C and defrosted only once before metabolite measurement. In addition, at 8 am of the first study day, a further blood sample was collected into a 9 ml

heparin-monovette (Sarstedt, Nümbrecht, Germany) for isolating genomic DNA from peripheral mononuclear cells (PBMC). Blood was diluted 1:2 with phosphate buffered saline (PBS). 16 ml Ficoll (Biocoll #L 6115, Biochrome, Berlin, Germany) were overlaid with the diluted blood sample. Tubes were subsequently centrifuged for 25 min at 400 x *g* and 20°C. PBMC were harvested from the interface above the Ficoll solution and were washed twice with PBS. Supernatants were discarded and cell pellets were immediately (shock) frozen in liquid nitrogen. Cell pellets were stored at -80°C until isolation of genomic DNA with the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

The participants were allowed to consume tap water, mineral water, and unsweetened fruit tea ad lib, and fluid intake was carefully recorded over the study period. To control nutrient intake and activity, participants stayed at the study center at both study days. Study personnel and volunteers were blinded for the genotype during the study.

TABLE 7: Nutrient composition of the test meals of the SysMBo study

Energy content, macro- and micronutrient intake is shown per meal; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test.

Test meal	OLTT	OGTT
Composition	Three parts Fresubin Energy Drink (Fresenius Kabi, Bad Homburg, Germany) + one part Calogen (Nutricia, Zoetemeer, The Netherlands)	75 g glucose, Dextro O. G. T., (Roche Diagnostics, Mannheim, Germany)
	Per meal (mean ± SD)	Per meal
Amount (ml)	425.9 ± 31.0	300
Energy (kcal)	958.2 ± 69.8	300
Fat (g)	71.8 ± 5.2 (67.5%)	0
Sattured fatty acids (g)	7.2 ± 0.5	0
Monounsatured fatty acid (g)	44.2 ± 3.2	0
Polyunsatured fatty acids (g)	20.3 ± 1.5	0
Linoleic acid (C18:2 n6) (g)	15.5 ± 1.1	0
α-linoleic acid (C18:3 n3) (g)	3.7 ± 0.3	0
Cholesterol (mg)	<0.01	0
Carbohydrates (g)	60.0 ± 4.4 (25%)	75
Fiber (g)	1.6 ± 0.1	0
Protein (g)	17.9 ± 1.3 (7.5%)	0
Sodium (g)	0.3 ± 0.02	0

3.2.2 Standard biochemistry parameters

Venous plasma glucose concentrations were determined by an enzymatic amperometric method (Super Gl easy+, Dr. Müller Geräte Bau, Freital, Germany). Insulin was quantified by an enzyme-linked immunosorbent assay (ELISA; K6219; Dako, Glostrup, Denmark). Non-esterified fatty acids (NEFAs) and triglycerides (TG) were quantified using commercially available enzymatic methods (NEFA-HR, Wako Chemicals GmbH, Neuss, Germany and Triglycerides liquicolor mono, Human GmbH, Wiesbaden, Germany). Cholesterol, HDL-, LDL-cholesterol, triglycerides, glutamic oxalacetic transaminase (GOT), glutamic pyruvate transaminase (GPT), creatinine, hemoglobin, thrombocytes and leucocytes were determined using established commercial tests by Synlab (Munich, Germany). HOMA-B and HOMA-IR were calculated using the following formulas: HOMA-B (%) = 20 x fasting insulin (mU/l) / (fasting glucose (mmol/l) – 3.5) and HOMA-IR = fasting insulin (mU/l) x fasting glucose (mmol/l) / 22.5.

3.2.3 Linkage disequilibrium-block analysis

Linkage disequilibrium block analysis (LD, r^2= 1.0) of the lead SNPs rs2014355 and rs174547 was done using public data bases: HapMap release 22 and 1,000 Genome Pilot I: CEU (Utah residents with ancestry from northern and western Europe) population with SNAP (Broad institute) (Johnson et al. 2008) and 1,000 Genome Phase 1: European population with HaploReg (Broad institute) (Ward and Kellis 2012).

3.2.4 Genotype analysis

The genotypes of rs2014355 were verified by allelic discrimination (TaqMan® SNP Genotyping Assay # C_8713836_20, Life Technologies) using standard protocols on an ABI 7900 HT instrument (Applied Biosystems) with automated calling by the SDS 2.3 software.

Genotypes of the missence variant rs1799958 in perfect linkage disequilibrium block with rs2014355 were sequenced by standard Sanger sequencing on an ABI3730 instrument (Applied Biosystems) using standard protocols after PCR amplification of the SNP containing DNA fragment with genomic DNA as template and with the following primers: for-5'-tgggctgctgtcatttct, rev-5'-agtcctcaaagatgaggtt.

Genotypes of the variant rs174547 were verified by genotyping with the MassARRAY system using the iPLEX Gold Chemistry (Sequenom, San Diego, CA, USA). The samples were analyzed in a matrix-assisted laser desorption ionization time of flight mass spectrometer (MALDI TOF MS, Bruker Daltonik, Leipzig,

Germany). χ2 test was used to test for deviation from the Hardy–Weinberg equilibrium (HWE). The single nucleotide polymorphism (SNP) rs174547 fulfilled HWE (P>0.05), and the genotyping success rate was 99%.

3.2.5 Metabolomics analysis

Targeted metabolomic measurements were performed at the Genome Analysis Center of the Helmholtz Zentrum München using the AbsoluteIDQ™ p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria) as described previously (Zukunft et al. 2013; Goek et al. 2013). Liquid handling was done on a Hamilton Microlab Star robotics system (Hamilton Bonaduz AG, Bonaduz, Switzerland). 186 metabolites were analyzed by flow injection analysis and liquid chromatography tandem mass spectrometry (FIA-MS/MS and LC-MS/MS). The metabolite panel includes amino acids, biogenic amines, acylcarnitines (C), sugars (H1), sphingomyelins (SM), diacyl phosphatidylcholines (PC aa), acyl-alkyl phosphatidylcholines (PC ae) and lyso phosphatidylcholines (lysoPC) (SUPPLEMENTARY TABLE 1). The ratio of the median metabolite concentration in the 5 standard reference plasma samples present in every batch to the median concentration of reference samples in all batches was used to correct for inter-day variance of each metabolite. Concentrations are given in µmol/L.

3.2.6 Statistical analysis

The statistical analysis was done by means of Ivan Kondofersky from the Institute of Computational Biology of the Helmholtz Zentrum München (German Research Center for Environmental Health (GmbH), Neuherberg, Germany) using the R statistical software (http://www.r-project.org).

3.2.6.1 Univariate t-tests

The baseline characteristics of carriers and non-carriers of both genotypes were tested for differences by using a standard univariate t-test on unpaired observations.

3.2.6.2 Logistic regression with bootstrap randomized performance

In this project a potential connection between a binary outcome (homozygous carriers of the minor C allele of rs2014355 (carriers) and homozygous carriers of the major T allele (non-carriers)) and metabolic variables was investigated. We selected logistic regression to quantify this connection. Thereby, a series

of logistic regression models was performed instead of one overall model. The corresponding equations can be summarized as:

$$P(y|x_i) = \frac{1}{1 + \exp(-(\alpha_i + x_i * \beta_i))}$$

y describes the binary outcome (1 denoting carriers and 0 non-carriers), x_i describes the i-th metabolic measurement, α_i and β_i are regression coefficients that are estimated in the model using the data y and x_i and $P(y|x_i)$ describes the probability of the binary outcome to have the value of 1 given the covariate x_i.

Two different logistic regression models were applied for analyzing the data depending on the data structure. The simple case of analyzing data recorded at a single time point (after 12 hours of fasting) was tackled by a standard logistic regression model (Lenz and Wilson 2007). Analyzing the connection between a binary outcome and a whole time-series is more challenging and was tackled by functional data analysis (Ramsay and Silverman 2005). To that end, an altered version of the standard logistic regression model was used:

$$P(y|x_i(t)) = \frac{1}{1 + \exp(-(\alpha_i + x_i(t) * \beta_i(t)))}$$

The biggest change was introduced by considering the time variable t and thus also extending the covariates $x_i(t)$ and the coefficients $\beta_i(t)$ to be both time-resolved. The theory and software for fitting logistic regression models with time-resolved covariates has recently been developed (de Hoffmann and Stroobant 2007).

The estimated $\widehat{P(y|x_i)}$ could then be compared to the true outcome and different diagnostic goodness-of-fit measures could be used to establish the quality of the i-th model. Especially for low sample sizes, these diagnostic measures contain a high amount of optimism. This can be explained by the fact that diagnostics are computed by evaluating the data a second time after having used it for the model calibration and thus the predictive quality of the model is biased. To correct for this optimism, Steyerberg et al. (Steyerberg et al. 2001) proposed and evaluated several diagnostic measures and concluded that using bootstrap techniques to estimate the optimism leads to a more realistic and stable assessment of the performance of logistic regression models. Following this finding, a bootstrap approach for estimating an optimism-corrected concordance statistic c_i was applied for each of the computed regression models.

For binary outcomes c_i is identical to the area under the receiver operating characteristic (ROC) curve. The optimism-corrected concordance index was computed as

$$c_i = c_i^o - \frac{1}{B}\sum_{b=1}^{B}(c_i^b - c_i^{ob})$$

In this notation, c_i^o is the concordance index computed on the original data that is too optimistic. c_i^{ob} is the concordance index computed on the original data by using a model that was calibrated on the b-th bootstrap sample. Finally, c_i^b is the concordance index computed on the b-th bootstrap sample by using a model that was calibrated on this b-th bootstrap sample. In this part of the work, a total of $B = 200$ bootstrap samples were used.

The described method was applied by using the metabolite measurements as covariates. Furthermore, we also analyzed enzymatic reactions, which are appropriately described by computing metabolite ratios of various fatty acid concentrations (Illig et al. 2010).

3.3 The impact of rs2014355 in the *ACADS* locus on the plasma metabolism in the anabolic and catabolic state

3.3.1 The *ACADS* (Acetyl-dehydrogenase, short chain) – gene

The *ACADS* gene spans approximately 13 kb on the terminal region of the long arm of chromosome 12 and consists of 10 exons (Corydon et al. 1997). Its transcription product is the enzyme acetyl-CoA dehydrogenase short chain (ACADS) that belongs to a large family of acyl-CoA dehydrogenases (ACADs), flavoproteins that are involved in the mitochondrial fatty acid oxidation (FAO, ß-oxidation).

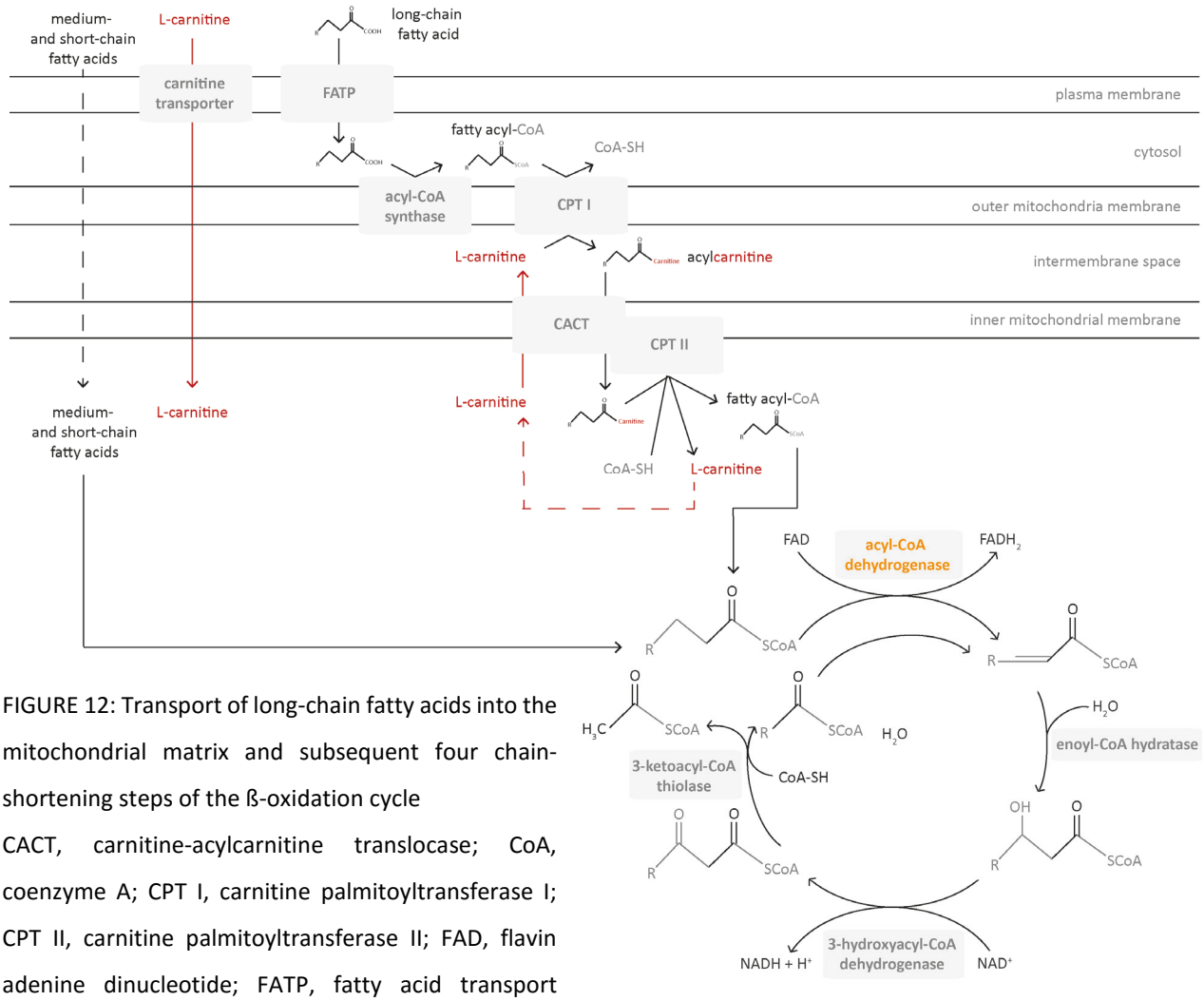


FIGURE 12: Transport of long-chain fatty acids into the mitochondrial matrix and subsequent four chain-shortening steps of the ß-oxidation cycle

CACT, carnitine-acylcarnitine translocase; CoA, coenzyme A; CPT I, carnitine palmitoyltransferase I; CPT II, carnitine palmitoyltransferase II; FAD, flavin adenine dinucleotide; FATP, fatty acid transport protein; NAD, nicotinamide adenine dinucleotide; (adapted from Ehlers 2014 and Dokoupil and Ensenauer 2008).

Substrates of the FAO are mainly fatty acids released during adipose tissue lipolysis during exercise or fasting. Released fatty acids are imported into various tissues. Thereby, long-chain fatty acids are dependent on fatty acid transporters. Inside the cytosol, long-chain fatty acids are rapidly esterified to fatty acyl-CoAs. Whereas short- and medium-chain fatty acids can enter the mitochondria passively, the transport of long-chain fatty acids depends on the carnitine shuttle consisting of three interdependent enzymatic reactions (FIGURE 12). Inside the mitochondrion, acyl-CoAs are released and can be degraded by the mitochondrial FAO. Thereby, fatty acid chains are shortened by repetitive ß-oxidation cycles including four enzymatic reactions and resulting in the release of an acetyl-CoA (FIGURE 12). ACADs are the first enzymes in this four-step ß-oxidation cycle, catalyzing the dehydrogenation of CoA-conjugated fatty acids. ACADS is specific for fatty acids with a chain length of 4 to 6 carbons, with C4-CoA being the favorite substrate (Ghisla and Thorpe 2004). Thus, ACADS catalyses the initial step of the final ß-oxidation cycle, resulting in the production of two acetyl-CoA molecules. The acetyl-CoA molecules can enter the tricarboxylic acid cycle and the respiratory chain for adenosine triphosphate production (Houten and Wanders 2010; Eaton et al. 1996).

In the literature, a number of cases of SCAD (short chain acetyl-CoA dehydrogenase) deficiency (OMIM: 201470) are described with variable genetic, biochemical and clinical characteristics (Pedersen et al. 2008a; Gallant et al. 2012; Tein et al. 2008). About 70 rare mutations (van Maldegem et al. 2010) and two common missense variants c.625G>A (rs1799958; G185S) and c.511 C>T (rs1800556; R147W) (Gregersen et al. 1998; Kristensen et al. 1994) were reported to be associated with SCAD deficiency (van Maldegem et al. 2006). The two missense variants have been found with a prevalence of homozygosity and heterozygosity of approximately 0.3% and 5.6% for the c.511C>T and 5.5% and 31.3% for the c.625G>A variant, respectively (van Maldegem et al. 2006). The common variants as well as most of the reported deleterious mutations cause single amino acid substitutions leading to protein miss-folding and aggregation (Pedersen et al. 2008b; Pedersen et al. 2003). Clinical symptoms reported in patients with SCAD deficiency are variable, ranging from hypotension, mental retardation, behavioral disorders and epilepsy to ketotic hypoglycaemia. However, in some cases, symptoms ameliorate or disappear and many individuals diagnosed with SCAD deficiency remain fully asymptomatic. Therefore, there is discussion about co-incidence of symptoms or other factors like genetic or environmental, that may be needed to develop from susceptibility to clinical disease (van Maldegem et al. 2010). Moreover, the high frequency of the common gene variants might indicate a potential involvement of SCAD deficiency in the pathogenesis of relatively common disorders. Biochemical symptoms of SCAD deficiency are determined by an accumulation of the substrate of SCAD. Accumulating C4-CoA can be converted into different

metabolites including the corresponding carnitineester butyrylcarnitine (C4), the corresponding glycine ester butyrylglycine, butyrate and ethylmalonic acid (EMA) (van Maldegem et al. 2006). Thus, the accumulation of potentially toxic metabolites like EMA was supposed to be the most likely mechanism involved in the pathophysiology of SCAD deficiency (van Maldegem et al. 2006). However, highest levels of C4 and EMA were not shown to be stronger related to clinical symptoms than moderately elevated levels (van Maldegem et al. 2006; van Maldegem et al. 2010).

The variant rs2014355 (minor allele frequency: 27.7%) has been shown to cause a mild biochemical phenotype (Gieger et al. 2008; Illig et al. 2010; Nicholson et al. 2011) mainly characterized by increased C4 levels (Illig et al. 2010) in minor C allele carriers compared to the average population. Though this phenotype does not lead to clinical symptoms, it might be that carriers of the minor allele are more sensitive to metabolic stress like fasting and thus potentially are more susceptible to the development of metabolic diseases during lifetime (Illig et al. 2010). In this context, there is a first study showing an association of rs2014355 with reduced measures of glucose-stimulated insulin release during an oral glucose tolerance test (OGTT) (Hornbak et al. 2011).

3.3.2 Results

3.3.2.1 Genotype verification and baseline characteristics of the study population

Based on the imputed genome-wide association data of the KORA cohort, 13 homozygous carriers of the minor C allele of rs2014355 (carriers) and 13 homozygous carriers of the major T allele (non-carriers, controls) were recruited. Genotype verification revealed four of the recruited "non-carriers" as heterozygous carriers of the minor C allele of rs2014355. Thus, these four subjects were excluded from further analysis. LD-block analysis based on 1,000 Genome Pilot I data (SUPPLEMENTARY TABLE 3) revealed the missense variant rs1799958 to be in perfect LD with rs2014355. Sequencing of rs1799958 showed 100% compliance with rs2014355. During OGTT, one of the carriers showed a 2 h-glucose concentration higher than 200 mg/dl as a prove of overt diabetes and was excluded from further analysis.

Baseline characteristics and clinical chemical parameters of the remaining 12 carriers and 9 non-carriers included in the statistical analysis are shown in TABLE 8. Carriers and non-carriers did not differ significantly in age and BMI and in most of the clinical chemical parameters. Of note, participants carrying the CC genotype showed a significantly higher waist circumference compared with the major TT genotype ($p= 0.004$). In addition, homozygous minor allele carriers had significantly higher NEFAs ($p= 0.027$), hemoglobin ($p= 0.008$) as well as thrombocytes ($p= 0.002$) and significantly lower GOT ($p= 0.032$) and creatinine ($p= 0.009$).

TABLE 8: Baseline characteristics and clinical chemical parameters of the study population per genotype

Data are given as mean value and standard deviation (sd) and range between lowest and highest values. p-values were derived from an unpaired t-test; OGTT, oral glucose tolerance test; NEFA, non-esterified fatty acids; GOT, glutamic oxalacetic transaminase; GPT, glutamic pyruvate transaminase.

	Carriers (n=12)			Non-Carriers (n=9)			
Variable	Mean	sd	Range	Mean	sd	Range	p-value
Baseline characteristics							
Age (years)	60.8	6.9	46.0 - 68.0	62.1	3.0	57.0 - 64.0	0.586
BMI (kg/m²)	27.4	1.9	24.1 - 30.5	25.8	1.7	22.5 - 27.9	0.053
Waist circumference (cm)	98.6	4.9	92.0 - 107.0	91.1	5.4	80.0 - 100.1	**0.004**
Blood pressure (mmHg)							
Systolic	125.8	15.1	100.0 - 145.0	121.1	14.3	100 - 140.0	0.477
Diastolic	76.3	8.3	60.0 - 85.0	71.7	15.0	50.0 - 90.0	0.381
Clinical chemical parameters							
Glucose (mg/dl)	97.8	11.8	78.1 - 116.6	95.3	8.7	84.3 - 108.4	0.603
Glucose 2h after OGTT (mg/dl)	140.1	40.7	92.3 - 198.5	112.3	27.2	78.4 - 156.5	0.093
Insulin (µIU/ml)	4.0	1.7	2.1 - 7.9	3.3	1.0	1.5 - 5.0	0.319
Cholesterol (mg/dl)	228.3	24.0	194.0 - 276.0	218.2	24.9	193.0 - 275.0	0.359
HDL (mg/dl)	60.4	13.5	38.0 - 84.0	61.2	9.6	47.0 - 79.0	0.881
LDL (mg/dl)	145.0	29.0	95.0 - 197.0	139.3	22.4	111.0 - 180.0	0.632
LDL/HDL	2.5	0.7	1.1 - 3.6	2.3	0.6	1.5 - 3.7	0.549
Triglycerides (mg/dl)	116.7	51.0	57.0 - 240.0	113.7	35.8	79.0 - 196.0	0.882
NEFA (mmol/l)	0.6	0.2	0.4 - 1.0	0.4	0.2	0.3 - 0.8	**0.027**
GOT (U/l)	23.6	5.8	16.0 - 32.0	29.0	4.5	24.0 - 38.0	**0.032**
GPT (U/l)	24.8	8.6	12.0 - 42.0	29.3	22.4	12.0 - 86.0	0.531
Creatinine (mg/dl)	1.0	0.1	0.8 -1.2	1.1	0.1	1.0 - 1.2	**0.009**
Hemoglobin (g/dl)	15.4	0.6	14.7 - 16.4	14.3	1.1	13.3 - 16	**0.008**
Thrombocytes (thou/µl)	244.8	35.6	188 - 312	196.8	22.8	171 - 246	**0.002**
Leucocytes (nl)	6.1	1.2	4.4 - 7.4	5.7	0.5	5.2 - 6.7	0.423

3.3.2.2 Baseline and time-resolved measurements during metabolic challenges

To study genotype effects on metabolite responses after the catabolic and anabolic challenge, time-course experiments were performed by measuring time-resolved metabolite levels during fasting with the first measurement after a 12h overnight fast (baseline) and further measurements every 2h up to 24h fasting on study day 1, as well as after a 12h overnight fast (baseline) and at 5 postprandial time-points after an OGTT on study day 2 (FIGURE 11).

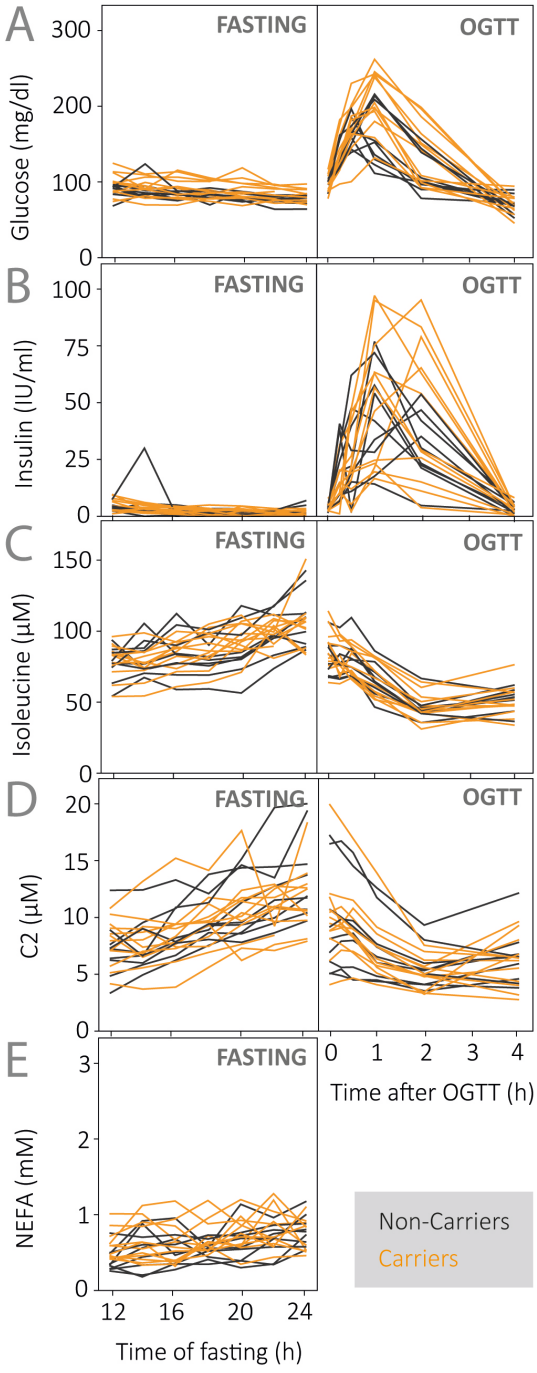


FIGURE 13 shows time-courses of glucose (A), insulin (B), isoleucine (C), acetylcarnitine (C2) (D) and NEFA (E) during the fasting period (left part of FIGURE 13) and during the OGTT (right part of FIGURE 13). Plasma concentrations of glucose and insulin were low during fasting and increased after the glucose load. However, some of the subjects showed more delayed and higher peaks during the OGTT than others, indicating impaired glucose tolerance of some of the subjects. Time-courses of other selected metabolites also showed dynamic changes characteristic of an anabolic and catabolic state (Krug et al. 2012). Fasting for 24 h increased branched chain amino acids (e.g. shown for isoleucine (FIGURE 13C)), C2 as well as NEFAs. In contrast, up to 2 h after the OGTT, levels of isoleucine and C2 decreased and showed a mirror-like behavior in the anabolic as compared to the catabolic state.

FIGURE 13: Time-courses of selected plasma metabolites during the fasting period and the OGTT

Glucose (A), insulin (B), isoleucine (C), C2 (D) are shown during the fasting period and the OGTT and NEFAs (E) during the OGTT. Single black lines show homozygous carriers of the major T allele of rs2014355 (non-carriers), single orange lines show homozygous carriers of the minor C allele of rs2014355 (carriers). C2, acetylcarnitine; NEFA, non-esterified fatty acids; OGTT, oral glucose tolerance test.

3.3.2.3 Effect of rs2014355 on baseline and time-resolved metabolite-levels

To study genotype effects on fasting metabolite levels and on time-resolved metabolite responses during the catabolic and anabolic challenge, logistic regression with bootstrap randomized performance was applied. Using this model, we studied the accuracy by which baseline metabolite levels or the metabolite responses to both challenges predict the respective alleles of rs2014355. A C-index for estimation of prediction was calculated for each metabolite and a cutoff of 0.9 was defined for determination of highly predictive metabolites (Vanagas 2004). A C-index of 0.5 indicates random group assignment, whereas an index of 1.0 shows 100% group assignment.

3.3.2.3.1 Baseline differences after a 12h overnight fast

Applying logistic regression on baseline metabolite levels after an overnight fast of 12h, unraveled solely C4 (butyrylcarnitine) as highly predictive for the genotype at both study days (left part of TABLE 9).

TABLE 9: Results of the logistic regression model

Results are shown for baseline (after 12h overnight fasting) and time-resolved metabolite levels after OGTT and during fasting; top-ten results are given in SUPPLEMENTARY TABLE 4; adj. wc., analysis adjusted for waist circumference; C4, butyrylcarnitine; lysoPC a C20:4, lyso phosphatidylcholine C20:4; OGTT, oral glucose tolerance test; PC ae C42:0, acyl-alkyl phosphatidylcholine C42:0.

	Metabolite	C-index (adj. wc.)	Metabolite	C-index (adj. wc.)
study day 1	12 h fasting		Time-resolved analysis (fasting)	
	C4	0.950 (0.969)	C4	0.999 (0.978)
			PC ae C42:0	0.906 (0.914)
study day 2	12 h fasting		Time-resolved analysis (OGTT)	
	C4	0.969 (0.970)	C4	0.964 (0.976)
			lysoPC a C20:4	0.917 (0.926)
			Glutamine	0.909 (0.912)

3.3.2.3.2 Time-resolved analysis during fasting

Next, logistic regression was applied to the time-resolved metabolite measurements during the fasting period. Thereby, a good group assignment was also shown for C4 (upper right part of TABLE 9). FIGURE 14A illustrates smoothed time-courses of single subjects as well as the mean curve and standard deviation over time for carriers and non-carriers, respectively. It clearly shows higher baseline and time-resolved concentrations of C4 in carriers of the minor CC allele. Aside, the time-resolved analysis of metabolite levels during fasting shows high genotype prediction for PC ae C42:0 (acyl-alkyl phosphatidylcholine C42:0; upper right part of TABLE 9). FIGURE 14B indicates a trend for an increase of plasma levels of PC ae C42:0 with prolonged fasting for non-carriers, whereas plasma levels of carriers remained rather stable over time.

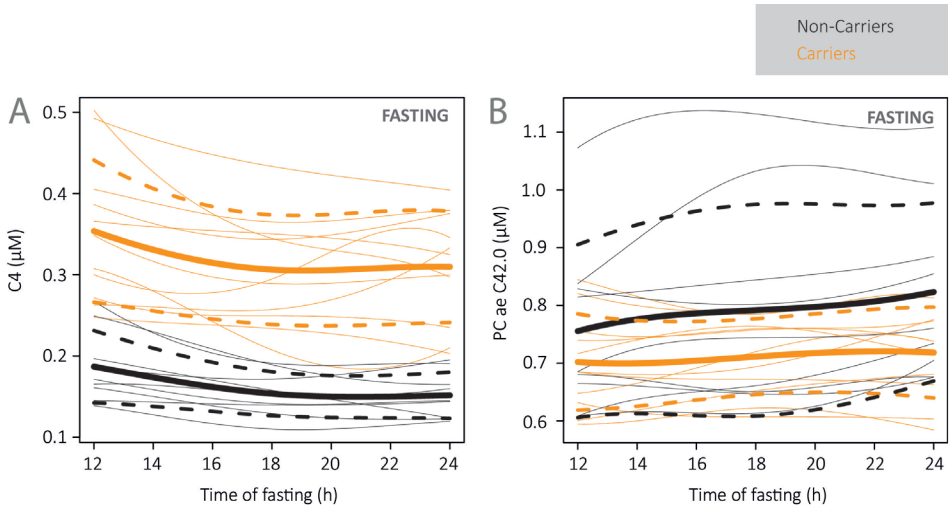


FIGURE 14: Time-courses of C4 (A) and PC ae C42:0 (B) during the 24h fasting period

Black lines show homozygous carriers of the major T allele of rs2014355 (non-carriers), orange lines show homozygous carriers of the minor C allele of rs2014355 (carriers); light lines show time-courses of single subjects fitted by smoothing splines with the degree of smoothness chosen with leave-one-out cross validation; the bold line shows mean values of time-courses of all subjects per genotype; the bold and dashed line shows the standard deviation of time-courses of all subjects per genotype; C4, butyrylcarnitine; PC ae C42:0, acyl-alkyl phosphatidylcholine C42:0.

In addition, we calculated selected metabolite ratios of time-resolved metabolites during the fasting challenge as proxies for enzymatic activities of ß-oxidation enzymes and also applied logistic regression with bootstrap randomized performance (SUPPLEMENTARY TABLE 5). The metabolite ratio of acetylcarnitine and butyrylcarnitine (C2/C4) (left part of FIGURE 15) resembling most likely the enzyme activity of ACADS was highly predictive for the respective allele of rs2014355 (C-index= 0.96), however, less predictive than analyses including solely time-courses of C4 (C-index= 0.999). Nevertheless, the ratio of propionylcarnitine to butyrylcarnitine (C3/C4) (right part of FIGURE 15), that showed highest associations with rs2014355 in GWAS (Illig et al. 2010), showed a C-index of 0.999 for the time-resolved analysis during fasting and, thus, showed equal prediction of the respective allele of rs2014355 like C4. The metabolite ratio of butyrylcarnitine and hexanoylcarnitine/fumarylcarnitine (C4/C6 (C4.1 DC) resulted in a C-index of 1.0 and, thus, a slightly better group assignment than C4 and C3/C4. Calculations of the same metabolite ratios after an overnight fast of 12 h on study day 1 (left part of SUPPLEMENTARY TABLE 5) indicated that the same ratios were highly predictive for the respective allele of rs2014355 like the time-resolved measurements. However, the time-resolved measurements showed a slightly better group assignment for the respective ratios. In conclusion, there was no remarkably better group assignment by calculating metabolite ratios at baseline and during the fasting period than by analyzing exclusively the levels of C4.

As expected, the metabolite ratio of C2/C4 that might approximate the enzyme activity of ACADS clearly showed increasing levels during fasting for carriers and non-carriers that were less pronounced in carriers (FIGURE 15). In line with the literature, C2 and most higher-chained acylcarnitines showed an increase of levels during fasting. Nevertheless, C4 levels of carriers and non-carriers rather tended to decrease over the fasting period (Krug et al. 2012). Remarkably, in spite of the genotype difference in C4 levels that might approximate C4-CoA levels (substrate of ACADS), time-courses showed no genotype-difference in C2 approximating the ACADS product acetyl-CoA (FIGURE 15). Moreover, there were also no genotype-dependent differences in higher-chained acylcarnitines.

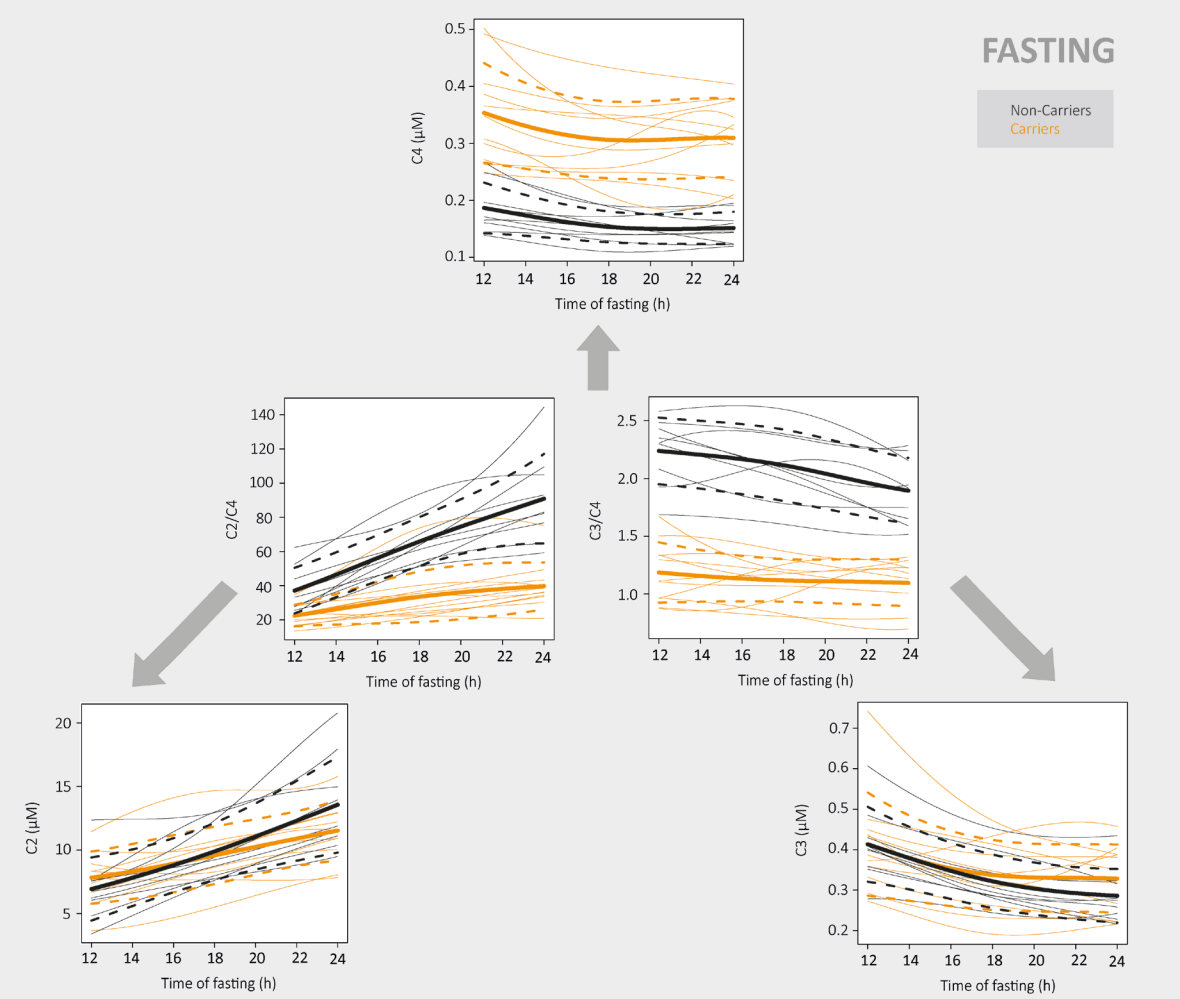


FIGURE 15: Timelines of the metabolite ratios C2/C4 and C3/C4 during fasting and corresponding metabolites over time during the fasting period

Black lines show homozygous carriers of the major T allele of rs2014355 (non carriers), orange lines show homozygous carriers of the minor C allele of rs2014355 (carriers); light lines show time-courses of single subjects fitted by smoothing splines with the degree of smoothness chosen with leave-one-out cross validation; the bold line shows mean values of time-courses of all subjects per genotype; the bold and dashed line shows the standard deviation of time-courses of all subjects per genotype; C2, acetylcarnitine; C3, propionylcarnitine; C4, butyrylcarnitine.

3.3.2.3.3 Time-resolved analysis after the OGTT

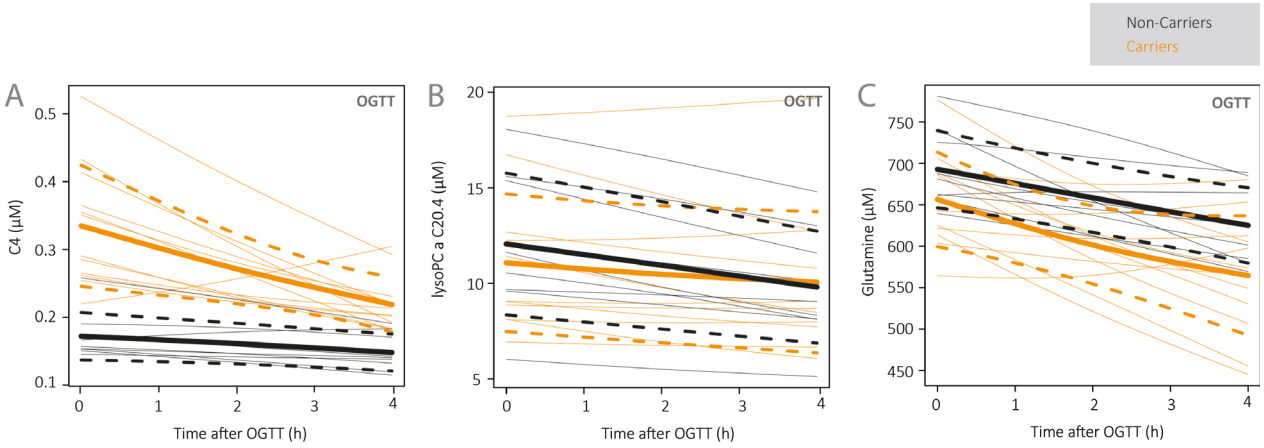


FIGURE 16: Postprandial time-courses of C4 (A), PC a C20:4 (B) and glutamine (C) after OGTT

Black lines show homozygous carriers of the major T allele of rs2014355 (non carriers), orange lines show homozygous carriers of the minor C allele of rs2014355 (carriers); Light lines show time-courses of single subjects fitted by smoothing splines with the degree of smoothness chosen with leave-one-out cross validation; the bold line shows mean values of time-courses of all subjects per genotype; the bold and dashed line shows the standard deviation of time-courses of all subjects per genotype; C4, butyrylcarnitine; lyso PC a C20:4, lyso phosphatidylcholine C20:4; OGTT, oral glucose tolerance test.

The logistic regression model with bootstrap randomized performance was also applied for the time-resolved metabolite levels during OGTT. Again, the analysis revealed C4 as highly predictive for the respective allele of rs2014355 (lower right part of TABLE 9). Time-courses of C4 showed higher baseline concentrations (after a 12h overnight fast) in carriers of the minor CC allele (FIGURE 16A) which approached the levels of the non-carriers over time. The time-resolved analysis further unraveled lyso PC a C20:4 (lyso phosphatidylcholine C20:4) and glutamine as highly distinctive metabolites for the respective alleles of rs2014355 (lower right part of TABLE 9). Time-courses of lyso PC a C20:4 (FIGURE 16B) indicated a decrease of metabolite level in non-carriers, whereas metabolite levels of carriers were not uniform. Time-courses of glutamine (FIGURE 16C) generally showed lower levels for carriers than for non-carriers during OGTT with a trend for a stronger decrease over time in carriers.

3.3.2.3.4 Adjusted analysis for waist circumference

Due to the baseline difference between minor CC allele compared to major TT allele carriers in waist circumference, we additionally applied logistic regression with bootstrap randomized performance adjusted for waist circumference and, again, set the cutoff for highly predictive metabolites and metabolite ratios to 0.9. Including waist circumferences into the analysis and comparing the adjusted C-indices to the unadjusted C-indices allowed us to assess the strength of genotype-prediction that can be explained by waist circumference. TABLE 9 compares results of the logistic regression analysis adjusted for waist circumference (C-index in brackets) with C-indices of highly predictive unadjusted metabolites and clearly shows comparable genotype prediction. Thus, waist-circumference seems to have no relevant influence on genotype prediction by C4, PC ae C42:0, lyso PC a C20:4 and glutamine in the baseline state as well as during the respective metabolic challenge tests. Moreover, results of the metabolite ratios at baseline and during the fasting period also showed equal genotype-prediction by adjusting waist circumference (SUPPLEMENTARY TABLE 5).

3.3.3 Discussion

Using targeted metabolomics as a hypothesis-free approach, we investigated whether a 24h fasting period and an OGTT might unravel novel metabolic effects for the GWAS-identified variant rs2014355 in the ACADS locus. The idea was that defined challenges may unmask early metabolomic changes which are not detectable in the fasting state.

Logistic regression identified butyrylcarnitine as highly genotype-distinctive metabolite after an overnight fast of 12h (baseline) and during a prolonged fasting period of 24h as well as during 4h following an OGTT. Further genotype-fasting interaction was shown for PC ae C42:0 and a genotype-OGTT interaction for lysoPC a C20:4 as well as for glutamine.

Acylcarnitines measured in plasma or serum approximate intra-mitochondrially accumulating acyl-CoAs which are exported out of the mitochondria as their corresponding carnitine esters (Noland et al. 2009; Ventura et al. 1999; ter Veld et al. 2009). However, the exact mechanism of the transport of acylcarnitines across the mitochondrial and plasmalemmal membranes into the extracellular space is still elusive (Violante et al. 2013). Butyryl-CoA is the major substrate of ACADS (Ghisla and Thorpe 2004), and thus, changes in plasma levels of butyrylcarnitine (C4) probably indicate an altered enzyme activity of ACADS. Time-courses of C4 during the fasting challenge as well as after the OGTT showed higher baseline concentrations for homozygous carriers of the minor C allele of rs2014355 (carriers) compared to the homozygous carriers of the major T allele (non-carriers). Higher C4 concentrations of carriers remained stable during the fasting challenge and approached levels of non-carriers during the OGTT. Our baseline data are in line with the GWAS identified association of rs2014355 with decreased C3/C4 acetylcarnitine ratios (Gieger et al. 2008; Illig et al. 2010; Nicholson et al. 2011), mainly explained by increased C4 levels in carriers (Shin et al. 2014; Illig et al. 2010). Thus, there might be an accumulation of the major substrate of ACADS (Ghisla and Thorpe 2004) reflecting a reduced ACADS activity in minor C allele carriers of rs2014355. Our analysis showed rs2014355 to be in perfect LD with rs1799958, one of the two common variants described in association with SCAD deficiency (Gregersen et al. 1998). Biochemical symptoms of SCAD deficiency are determined by an accumulation of the substrate of SCAD. Accumulating C4-CoA can be converted into different metabolites including the corresponding carnitineester C4, the corresponding glycine ester butyrylglycine, butyrate and ethylmalonic acid (EMA) that is excreted via urine (van Maldegem et al. 2006). Including homozygous carriers of rs1799958 in perfect LD with rs2014355, Maldegem et al. conducted fasting tests up to 46h and showed that EMA levels in urine increased during fasting, whereas consistent with our findings, plasma C4 levels remained stable over time (van Maldegem

et al. 2010). Thus, potentially increasing butyryl-CoA levels during fasting might be converted into EMA and excreted via urine and thus are not becoming apparent as increased C4 in our plasma samples. Aside, the decrease in plasma C4 levels observed during OGTT in our study might probably be based on insulin-induced inhibition of ß-oxidation in the catabolic state.

Hornbaek et al. hypothesized that elevated circulating C4 levels in subjects homozygous for the minor allele of rs2014355 increase basal insulin secretion thereby causing hyerinsulinemia that might exhaust ß-cells and desensitize insulin receptors in the long run (Hornbak et al. 2011). In glucose-tolerant individuals carrying the minor C allele of rs2014355, authors showed a reduced glucose-stimulated insulin release during an OGTT prior to the correction for multiple testing. However, the minor allele was not directly associated with T2D (Hornbak et al. 2011). This finding is in line with previously published meta-analyses (Zeggini et al. 2008; Morris et al. 2012) showing that the ACADS variant rs2014355CC does not associate with an increased risk for development of T2D. Moreover, GWAS showed no significant association between fasting glucose (Dupuis et al. 2010; Manning et al. 2012), fasting insulin (Dupuis et al. 2010; Manning et al. 2012) and 2 h glucose after OGTT (Saxena et al. 2010) for rs2014355 as well as for SNPs in high linkage disequilibrium (LD) with rs2014355. Though there was a trend for an association (ß= 0.234, $p = 3.70 \times 10^{-3}$) of rs2066938 (LD: r2= 0.91 with rs2014355, European population, 1,000 Genome Phase 1) and homeostatic model assessment-B (HOMA-B) (Dupuis et al. 2010) (Data on glycaemic traits have been contributed by MAGIC investigators and have been downloaded from www.magicinvestigators.org). Aside, the data of our study did not indicate differences of carriers of rs2014355 and controls in basal glucose, 2 h glucose after OGTT and basal insulin levels (TABLE 8) as well as no genotype destinction for basal and time-resolved metabolite levels of insulin and glucose (TABLE 9). Therefore, the association of rs2014355 with T2D is still controversial and remains subject for further investigation.

In addition to increased C4 levels, we found a moderate genotype-fasting and genotype-OGTT interaction in phosphatidylcholines that was not identified in the analysis of baseline samples in our study as well as in GWAS (Shin et al. 2014; Gieger et al. 2008; Illig et al. 2010). During fasting, plasma levels of PC ae C42:0 showed an increase in non-carriers, whereas concentrations of carriers remained stable over time. A study with healthy young males also showed a slight increase in PC ae C42:0 levels in the catabolic state (Krug et al. 2012). Thereupon, PC ae C42.0 seems to be mobilized during fasting in healthy males, whereas there might be less mobilization in homozygote carriers of the minor C allele of rs2014355. Moreover, during the OGTT, lyso PC a C20:4 levels remained rather stable or were not uniform over time in carriers, whereas levels were reduced in plasma of non-carriers. Consequently, the stronger reduction of lyso PC a C20:4 in

the anabolic state as well as the higher increase in PC ae C40:2 in the catabolic state in control subjects may indicate a less flexible reaction of lipid metabolism in carriers of the minor C allele in response to these conditions. Altered PC/lyso PC metabolism was shown to be associated with atherosclerosis (Lusis 2000), obesity (Pietilainen et al. 2007), impaired glucose tolerance and T2D (Zhu et al. 2011; Floegel et al. 2013b; Wang-Sattler et al. 2012; Ha et al. 2012). However, to our knowledge, PC ae C42.0 has not been reported to be associated with diabetes and lyso PC C20.4 levels were shown to be increased in T2D compared to controls (Zhu et al. 2011).

Moreover, we observed a genotype-OGTT interaction in glutamine levels, that was also not identified in our analysis of baseline data as well as in published GWAS (Gieger et al. 2008; Illig et al. 2010). We found a decrease in glutamine concentrations over 4h after OGTT with a stronger manifestation in homozygous carriers of the minor C allele. A study measuring metabolites during 180 minutes after OGTT in healthy subjects showed a minor increase of glutamine 30 min after OGTT and levels returned to baseline after 180 minutes (Skurk et al. 2011). Aside, another study measuring metabolites at baseline and 240 min after OGTT also showed a decrease of glutamine after 240 min compared to baseline (Ho et al. 2013). Alterations in the glutamine-glutamate cycle have also been shown in epilepsy (Petroff et al. 2002; Pan et al. 2008) and, hence, there might be a potential link between the altered postprandial glutamine metabolism in minor C allele carriers of rs2014355 and epilepsy as one of the clinical symptoms reported in patients with SCAD deficiency (van Maldegem et al. 2010). In addition, an inverse association of glutamine levels with multiple metabolic risk factors like insulin, triglycerides and lower HDL was reported (Cheng et al. 2012; Menge et al. 2010). Decreased plasma glutamine levels were also shown in early T2D (Menge et al. 2010). However, Xu et al. described a significant increase in glutamine levels in subjects with impaired fasting glucose, whilst no significant changes could be shown in subjects with T2D (Xu et al. 2013).

Our study revealed significantly higher mean waist circumferences in homozygous minor C allele carriers of rs2014355 compared to homozygous major T allele carriers. Waist circumference was found to be inversely associated with lyso PCs as well as PCs in overweight males (Szymanska et al. 2012) and visceral adiposity was inversely associated with ae PCs in obese females (Martin et al. 2013). In contrast, glutamine levels were shown to be rather increased with increasing visceral adiposity (Martin et al. 2013). Due to the potential influence of waist-circumference on concentrations of selected metabolites, we additionally analyzed data adjusted for waist circumference. As genotype prediction of C4, PC ae C42:0, lyso PC a C20:4 as well as glutamine adjusted for waist circumference was similar to the unadjusted prediction, we assume no marked influence of waist circumference on these metabolites in our study.

A limitation of the study is its small sample size, thus results should be considered as preliminary findings that need verification by other studies. Moreover, impaired glucose tolerance after OGTT, might potentially be due to the advanced age of participants (61.3 ± 5.5 years) and, hence, verification in a younger study cohort might be useful. Furthermore, our analysis only includes male subjects to increase the homogeneity within the study population. However, men and women differ in their postprandial response (Ho et al. 2013), thus, generalization of the finding to both sexes should be avoided. We used targeted metabolomics mainly focusing on lipids. Hence, associations of rs2014355 with other metabolites cannot be excluded. In addition, the exact biochemical mechanisms leading to the observed changes of metabolites remain unknown and further investigations are needed to clarify the biological significance of the findings.

To our knowledge, this is the first study clearly underlining the link between the GWAS tag SNP rs2014355 and the variant rs1799958 (625G>A) frequently reported in association with SCAD deficiency. Due to using a comprehensive metabolomics approach to analyze gene-environment interactions, our data clearly show that studies of rs2014355 phenotypes in the basal state after a 12h overnight fast deliver incomplete information and metabolic challenge tests are essential for detecting moderate differences in plasma metabolism. In addition to genotype-dependent baseline differences in C4 levels, we found moderate genotype-fasting interactions in PC ae C42:0 and genotype-OGTT interactions in lyso PC a C20:4 as well as glutamine. Further studies are needed to unravel how gene variants in the ACADS locus affect metabolomics responses to anabolic and catabolic challenges and to find out if there is susceptibility for the development of a multifactorial disease like T2D.

3.4 The impact of rs174547 in the *FADS1* locus on the plasma metabolism in the anabolic state during nutritional challenges

3.4.1 The *FADS* (Fatty acid desaturase) – genes

The *FADS1* gene spans 17.2 kb on chromosome 11 and is located in a gene cluster including also *FADS2* (OMIM 606149, 39.1 kb) and *FADS3* (OMIM: 606150, 18.0 kb). *FADS1* and *FADS2* are oriented head-to-head, with the exons 1 of both genes being separated by an 11.4 kb region. FADS3 is located in the 6 kb telomeric side from FADS2 in tail-to-tail orientation. *FADS1*, *FADS2* and *FADS3* show the same exon/intron organization (12 exons and 11 introns) suggesting that they have arisen evolutionary from gene duplication (Nakamura and Nara 2004; Lattka et al. 2010). Whereas the function of the transcripts of the *FADS3* gene is still an open question (Lee and Park 2014), *FADS1* and *FADS2* encode for fatty acids desaturases (delta-5 desaturase and delta-6 desaturase, respectively), nonheme iron-containing enzymes that introduce a double bound between carbons of fatty acid acyl chains during the biosynthesis long-chain fatty acids (Nakamura and Nara 2004).

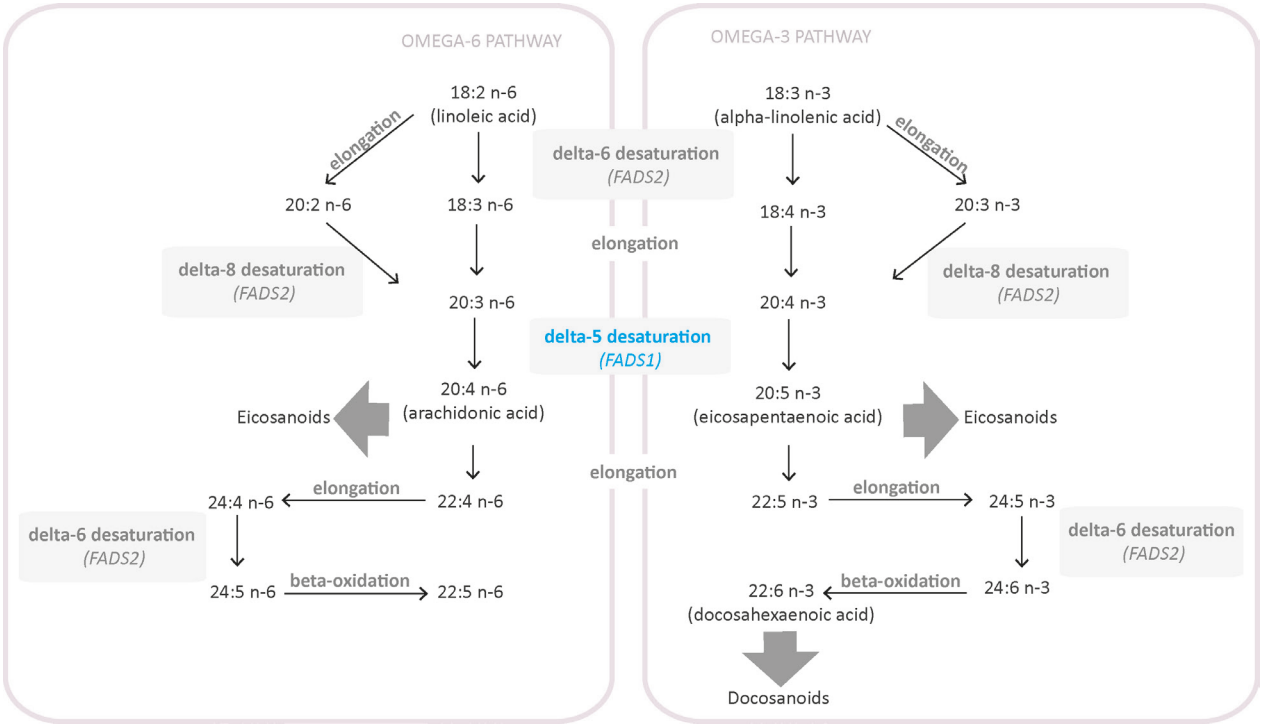


FIGURE 17: Mammalian pathway for omega-6 and omega-3 long-chain polyunsatured fatty acid synthesis from essential fatty acids by enzymatic desaturation and chain elongation

Modified from Sprecher 1981.

The delta-6 destaurase converts the essential fatty acid 18:2 n-6 (linoleic acid) to 18:3 n-6 in the omega-6 pathway and the essential fatty acid 18:3 n-3 (α-linoleic acid) to 18:4 n-3 in the omega-3 pathway (FIGURE 17). After an elongation step, the delta-5 desaturase desaturates 20:3 n-6 to 20:4 n-6 (arachidonic acid) in the omega-6 pathway and 20:4 n-3 to 20:5 n-3 (eicosapentaenoic acid (EPA)) in the omega-3 pathway (FIGURE 17). These molecules are either converted into eicosanoids or further elongated and desaturated (again with a delta-6 desaturase (Nakamura and Nara 2004)), resulting in several long chain polyunsaturated fatty acids (LC-PUFAs), e.g. 22:6 n-3 (docosahexaenoic acid (DHA)). Thus, the levels of LC-PUFAs in the human body are highly dependent on their intake of precursor fatty acids that are endogenously elongated and desaturated to physiologically active LC-PUFAS or the intake of LC-PUFAS by diet (Lattka et al. 2009b). Thereby, the ratio of n-6 to n-3 fatty acid intake is of special importance as a high ratio of omega-6 to omega-3 fatty acids is considered as a major contributor to the pathogenesis of many diseases, such as diabetes, cardiovascular disease and cancers (Lee and Park 2014).

LC-PUFAS perform a variety of physiological functions in the human metabolism. For example, the fluidity and integrity of cell membranes is influenced by the LC-PUFA composition in phospholipids. Further, LC-PUFAS have several other central functions on the molecular level, e.g. by acting as second messengers in intracellular signaling pathways or regulating transcription. Aside, they are precursors of eicosanoids and play an important role in inflammatory processes (Nakamura and Nara 2004; Lattka et al. 2009a). Moreover, LC-PUFAS, especially DHA, are very important for early stage brain development (Martinez 1992).

Emerging research has demonstrated that genetic variation in *FADS1* and *FADS2* are associated with alterations in fatty acid composition of different biological sources that may subsequently modify an individual's propensity for disease. Common genetic variants of *FADS1* and *FADS2* were shown to be in high LD, with one LD block (including rs174547) spanning over FADS1, the intergenic region and the promotor region of FADS2 (Zietemann et al. 2010). Association studies on FADS polymorphisms clearly showed significant associations with an accumulation of desaturase substrates and a decline of desaturase products due to the minor alleles of the associated SNPs in serum (Schaeffer et al. 2006; Malerba et al. 2008; Gieger et al. 2008), plasma (Lemaitre et al. 2011; Martinelli et al. 2008; Baylin et al. 2007; Rzehak et al. 2009; Xie and Innis 2008; Tanaka et al. 2009), erythrocyte membranes (Zietemann et al. 2010; Martinelli et al. 2008; Malerba et al. 2008; Rzehak et al. 2009; Xie and Innis 2008), adipose tissue (Baylin et al. 2007) and breast milk phospholipids (Xie and Innis 2008). Most significant associations were observed for the majority of SNPs in FADS1 and FADS2 genes and arachidonic acid (Schaeffer et al. 2006; Malerba et al. 2008; Xie and Innis 2008; Tanaka et al. 2009). Aside, GWAs on complex lipid traits reported decreased total

cholesterol (Aulchenko et al. 2009; Tanaka et al. 2009), LDL (Aulchenko et al. 2009; Sabatti et al. 2009; Tanaka et al. 2009) and HDL levels (Kathiresan et al. 2009) and increased triglyceride levels (Kathiresan et al. 2009) in carriers of the minor alleles of SNPs in the *FADS*-locus (Sabatti et al. 2009). Moreover, *FADS* gene cluster polymorphisms are disscused to be associated with several diseases. It is discussed that *FADS* genotypes have a modulating effect on fatty acid related phenotypes such as mental ability (Caspi et al. 2007; Brookes et al. 2006), atopic disease (Lattka et al. 2009b), coronary artery disease (Liu et al. 2015; Martinelli et al. 2008), the metabolic syndrome (Truong et al. 2009) and T2D (Kroger and Schulze 2012; Dupuis et al. 2010).

3.4.2 Results

3.4.2.1 Genotype verification and baseline characteristics of the study population

Based on the imputed genome-wide association data of the KORA cohort 13 homozygous carriers of the minor C allele of rs174547 (carriers) and 13 homozygous carriers of the major T allele (non-carriers, controls) were recruited. Genotype verification showed 100% compliance with assumed genotypes of rs174547. The results of the LD-block analysis of rs174547 are shown in SUPPLEMENTARY TABLE 6. During the OGTT, one of the carriers showed a 2 h-glucose concentration higher than 200 mg/dl as a sign of overt diabetes and, thus, was excluded from further analysis.

Baseline characteristics and clinical chemical parameters of the 12 carriers and 13 non-carriers included in the statistical analysis are shown in TABLE 10. Carriers and non-carriers did not differ significantly in age, BMI, waist circumference, blood pressure and in most of the clinical chemical parameters. Of note, participants carrying the CC genotype showed significantly higher fasting insulin concentrations, HOMA-B and HOMA-IR compared to the major TT genotype (p= 0.017, 0.020 and 0.033, respectively). Moreover, homozygous minor allele carriers were significantly higher in triglycerides (p= 0.034).

TABLE 10: Baseline characteristics and clinical chemical parameters of the study population per genotype
Data are given as mean value and standard deviation (sd) and range between lowest and highest values. p-values were derived from unpaired t-tests; GOT, glutamic oxalacetic transaminase; GPT, glutamic pyruvate transaminase; HOMA, homeostatic model assessment; OGTT, oral glucose tolerance test.

	Carriers (n= 12)			Non-Carriers (n= 13)			
Variable	Mean	sd	Range	Mean	sd	Range	p-value
Baseline characteristics							
Age (years)	57.7	6.5	43.0 - 65.0	61.8	3.5	53.0 - 65.0	0.056
BMI (kg/m²)	26.6	3.6	19.1 - 30.2	27.0	3.2	22.6 - 33.6	0.745
Waist circumference (cm)	94.6	10.2	76.0 - 109.0	93.8	8.1	82.0 - 110.0	0.828
Blood pressure (mmHg)							
Systolic	123.8	17.1	100.0 -150.0	122.4	14.7	100.0 -140.0	0.832
Diastolic	70.0	7.7	60.0 - 80.0	71.5	13.0	50.0 - 90.0	0.725
Clinical chemical parameters							
Glucose (mg/dl)	94.5	10.2	80.0 - 113.6	94.9	10.2	74.3 - 110.5	0.923
Glucose 2h after OGTT (mg/dl)	104.0	18.7	66.3 - 132.0	105.3	20.5	78.4 - 156.5	0.867
Insulin (µIU/ml)	4.8	2.3	1.2 - 9.7	3.0	1.1	1.5 - 5.0	**0.017**
HOMA-B	56.1	23.1	24.4 - 104.6	36.7	15.6	14.4 - 60.4	**0.020**
HOMA-IR	1.2	0.6	0.2 - 2.7	0.7	0.3	0.3 - 1.2	**0.033**
Cholesterol (mg/dl)	234.3	35.9	197.0 - 320.0	216.4	30.7	177.0 - 275.0	0.193
HDL (mg/dl)	53.9	14.5	37.0 - 79.0	59.4	13.1	38.0 - 85.0	0.331
LDL (mg/dl)	152.5	24.6	120.0 - 204.0	138.3	30.2	103.0 - 188.0	0.213
LDL/HDL	3.1	1.1	1.7 - 5.5	2.5	0.8	1.3 - 3.7	0.134
Triglycerides (mg/dl)	177.8	98.9	93.0 - 453.0	110.7	40.9	45.0 - 196.0	**0.034**
GOT (U/l)	24.8	4.2	20.0 - 33.0	25.2	5.4	16.0 - 38.0	0.840
GPT (U/l)	29.3	10.2	10.0 - 43.0	31.1	20.3	12.0 - 86.0	0.782
Creatinine (mg/dl)	1.0	0.1	0.8 - 1.2	1.0	0.1	0.8 - 1.3	0.097
Hemoglobin (g/dl)	15.7	1.2	13.8 - 17.5	14.8	1.0	13.3 - 16.1	0.069
Thrombocytes (thou/µl)	230.4	48.6	174.0 - 350.0	200.8	53.3	148.0 - 317.0	0.162
Leucocytes (nl)	6.3	1.6	3.6 - 8.9	5.9	1.0	4.6 - 8.5	0.413

3.4.2.2 Baseline and time-resolved measurements during metabolic challenges

To study genotype effects on metabolite responses after two anabolic metabolic challenges, time-course experiments were performed by measuring time-resolved metabolite levels during the OLTT and the OGTT with the first measurement after a 12h overnight fast (0h, baseline) and further measurements at 5 postprandial time-points after the OLTT and the OGTT, respectively.

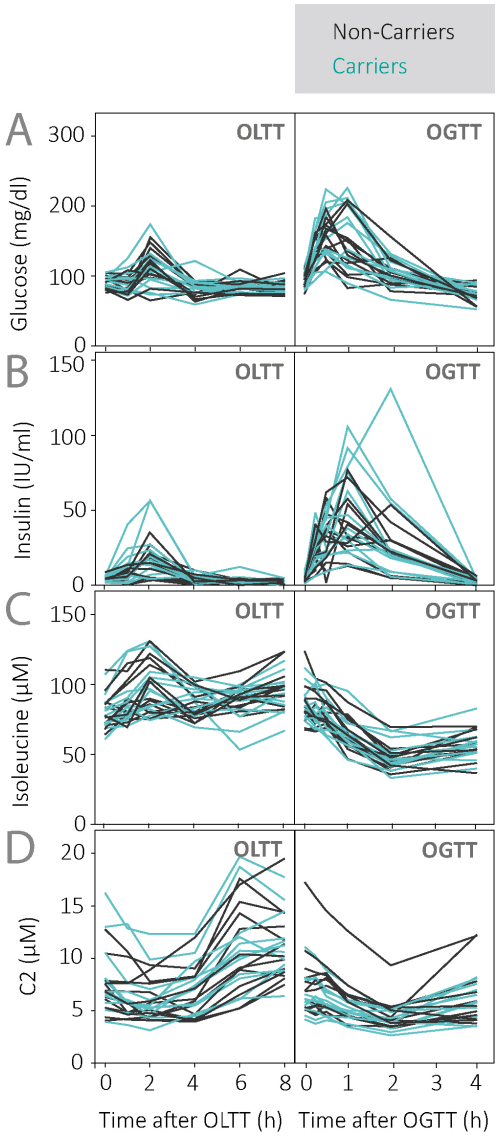


FIGURE 18 shows time-courses of glucose (A), insulin (B), isoleucine (C) and acetylcarnitine (C2) (D) during the OLTT and the OGTT. Plasma concentrations of glucose and insulin increased after the OGTT (FIGURE 18A, B, right part). However, some of the subjects showed more delayed and higher peaks than others, indicating impaired glucose tolerance of some of the subjects. Aside, glucose and insulin levels also increased after the OLTT with a peak at about 2 h (FIGURE 18A, B, left part). This increase was expected, because the OLTT includes a substantial amount of carbohydrates (60.0 ± 4.4 g) aside from the high amount of fatty acids (71.8 ± 5.2 g). Time-courses of other selected metabolites (FIGURE 18C, D) showed dynamic changes characteristic for an OLTT and an OGTT (Krug et al. 2012). Due to the OLTT, isoleucine (FIGURE 18C, left part) levels increased within 2 h, decreased thereafter within about 4 h and approximated baseline levels after 8 h. In contrast, concentrations of C2 (FIGURE 18D, left part) initially decreased within 2 to 4 h, thereafter increased with a peak at 6 h and in most cases approximated baseline levels 8 h after challenge. Aside, during the OGTT, plasma concentrations of

FIGURE 18: Time-courses of selected plasma metabolites during the OLTT and the OGTT

Glucose (A), insulin (B), isoleucine (C) and C2 (D) concentrations are shown as time-resolved measurements during the OLTT (left part) and the OGTT (right part). Single black lines show homozygous carriers of the major T allele of rs174547 (non-carriers), single blue lines show homozygous carriers of the minor C allele of rs174547 (carriers). C2, acetylcarnitine; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test.

both isoleucine (FIGURE 18C, right part) and C2 (FIGURE 18D, right part) decreased within 2 h and approximated baseline thereafter.

3.4.2.3 Effect of rs174547 on baseline and time-resolved metabolite-levels

To study genotype effects on fasting metabolite levels and on time-resolved metabolite responses during the OLTT and the OGTT, logistic regression with bootstrap randomized performance was applied. Using this model, the accuracy by which the baseline metabolite levels or the challenge responses of metabolites predict the respective alleles of rs174547 was studied. A C-index for estimation of prediction was calculated for each metabolite and a cutoff of 0.9 was defined for determination of highly predictive metabolites (Vanagas 2004). A C-index of 0.5 indicates random group assignment, an index of 1.0 shows 100% group assignment.

3.4.2.3.1 Baseline differences after a 12h overnight fast

Applying logistic regression on baseline metabolite levels after an overnight fast of 12h did not unravel highly predictive metabolites for the respective genotype at both study days (left part of TABLE 11).

TABLE 11: Highly distinctive metabolites revealed by logistic regression

Results are shown for baseline (after 12h overnight fast) and time-resolved metabolite levels after the OLTT and the OGTT; top-ten results are given in SUPPLEMENTARY TABLE 7; lyso PC a C 20.4, lyso phosphatidylcholine C20:4; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test; SM C20:2, shingomyeline C20:2.

	Metabolite	C-index	Metabolite	C-index
study day 1	12 h fasting		Time-resolved analysis (OLTT)	
			SM C20:2	**0.903**
study day 2	12 h fasting		Time-resolved analysis (OGTT)	
			lysoPC a C20:4	**0.900**

3.4.2.3.2 Time-resolved analysis during the OLTT

Next, logistic regression was applied to the time-resolved metabolite measurements during the OLTT. Thereby, shingomyeline C20:2 (SM C20:2) was identified as the only highly predictive metabolite for the genotype of rs174547 (upper right part of TABLE 11). FIGURE 19 illustrates the smoothed time-couses of single subjects as well as the mean curve and standard deviation over time for carriers and non-carriers, respectively. Although a difference between carriers and non-carriers is hardly visible, carriers show a trend for a slight decrease over time, while non-carriers rather show a slight increase prior to approaching baseline levels after 8 h.

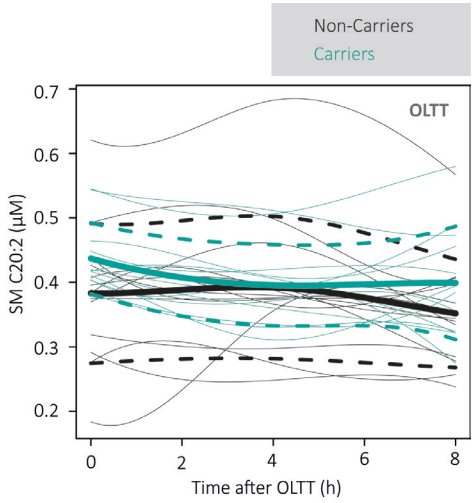


FIGURE 19: Time-courses of SM C20:2 during the OLTT
Black lines show homozygous carriers of the major T allele of rs174547 (non-carriers), blue lines show homozygous carriers of the minor C allele of rs174547 (carriers); light lines show time-courses of single subjects fitted by smoothing splines with the degree of smoothness chosen with leave-one-out cross validation; the bold line shows mean values of time-courses of all subjects per genotype; the bold and dashed line shows the standard deviation of time-courses of all subjects per genotype; SM C20:2, sphingomyeline C20:2; OLTT, oral lipid tolerance test.

3.4.2.3.3 Time-resolved analysis after the OGTT

The logistic regression model with bootstrap randomized performance was also applied for the time-resolved metabolite levels during the OGTT. The analysis revealed solely lyso phosphatidylcholine C20:4 (lyso PC a C20:4) as highly predictive for the respective allele of rs174547 (lower right part of TABLE 11). Time-courses of lyso PC a C20:4 (FIGURE 20B) showed lower concentrations for carriers than for non-carriers at baseline as well as during 4 h after the OGTT. Moreover, there was a general trend towards a decrease in lyso PC a C20:4 levels over time being slightly more pronounced for non-carriers than for carriers. Although lyso PC a C20:4 was not identified as highly genotype-distinctive during the OLTT, it showed a good C-index of 0.883. Time-courses during the OLTT (FIGURE 20A) also showed lower concentrations for carriers than for non-carriers. However, there were higher inter-subject variations at baseline in non-carriers at the day of the OLTT compared to the day of the OGTT.

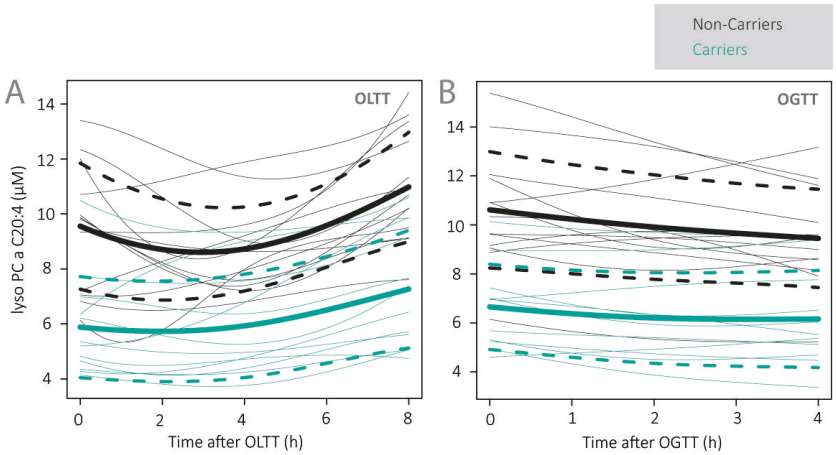


FIGURE 20: Postprandial time-courses of lyso PC a C20:4 after the OLTT (A) and OGTT (B)

Black lines show homozygous carriers of the major T allele of rs2014355 (non-carriers), blue lines show homozygous carriers of the minor C allele of rs2014355 (carriers); light lines show time-courses of single subjects fitted by smoothing splines with the degree of smoothness chosen with leave-one-out cross validation; the bold line shows mean values of time-courses of all subjects per genotype; the bold and dashed line shows the standard deviation of time-courses of all subjects per genotype; lyso PC a C20:4; lyso phosphatidylcholine C20:4; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test.

3.4.2.3.4 Analysis of selected metabolite ratios

In addition to the analysis of single metabolites, metabolite ratios of metabolites that were previously associated with SNPs in high LD with rs174547 (Illig et al. 2010; Hicks et al. 2009; Gieger et al. 2008) as well as metabolite ratios approximating enzyme activity of the delta-5 and delta-6 desaturase were calculated (SUPPLEMENTARY TABLE 8). Logistic regression with bootstrap randomized performance was applied on these selected metabolite ratios at baseline and on time-resolved metabolite ratios during the OGTT and OLTT (SUPPLEMENTARY TABLE 9). Results showed a number of ratios previously reported to be associated with rs174547 or SNPs in high LD with rs174547 in fasting blood samples (Illig et al. 2010; Gieger et al. 2008) to be highly distinctive for the respective allele of rs174547 at baseline as well as during the OLTT and OGTT. Most of the distinctive metabolite ratios contain an acyl-alkyl, diacyl or lyso phoshatidylcholine with four double bonds as numerator or denumerator and an acyl-alkyl, diacyl or lyso phoshatidylcholine with less than four double bonds on the repective other side of the fraction line. FIGURE 21 illustrates the metabolite ratio of lyso PC a C20:4 / lyso PC a C20:3 during the OLTT (left part) and the OGTT (right part). The ratio shows a clear distinction between carriers of the minor CC allele (carriers) and of the major TT allele (non-carriers) at baseline (0 h) of both study days as well as during the OLTT and the OGTT. The

C-indices (SUPPLEMENTARY TABLE 9) confirmed the high genotype distinction of the ratio (baseline OLTT: c= 0.947; OLTT: c= 0.904; baseline OGTT: c= 0.928; OGTT: c= 0.886). Aside, the upper and lower part of FIGURE 21 shows the time-courses of the corresponding metabolites lyso PC a C20.3 (upper part) and lyso PC a C20:4 (lower part) during the OLTT (left part) and the OGTT (right part). The time-courses elucidated that lower levels of lyso PC a C20:4 in carriers at baseline as well as over time during the OLTT and OGTT were mainly responsible for genotype-distinctive characteristics of the ratio of lyso PC a C 20:4 / lyso PC a C20:3. However, there were also slightly higher lyso PC a C20:3 levels in carriers that might contribute to the good genotype distinction of the ratio of both metabolites.

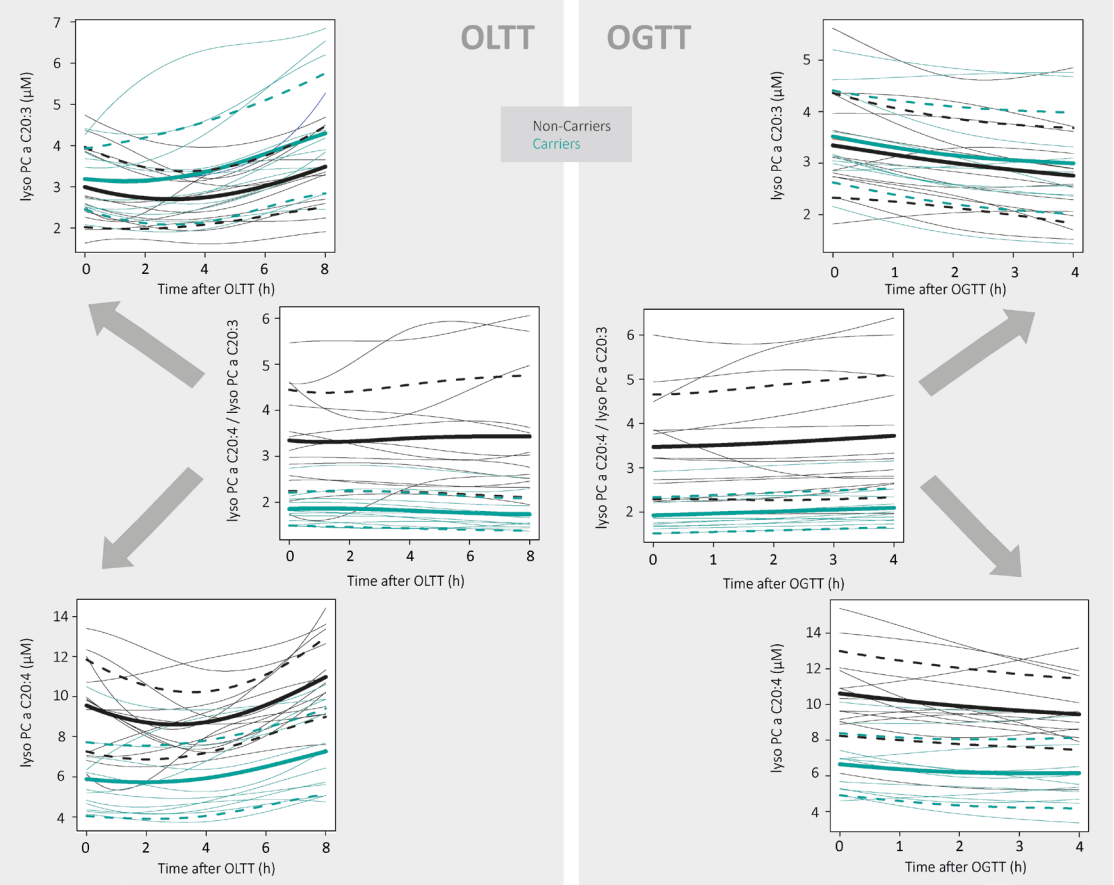


FIGURE 21: Time-courses of the metabolite ratio of lyso PC a C20:4 / lyso PC a C20:3 as well as the time-courses of lyso PC a C20:3 (upper part) and lyso PC a C20:4 (lower part) shown during the OGTT (left part) and the OGTT (right part) Black lines show homozygous carriers of the major T allele of rs174547 (non carriers), blue lines show homozygous carriers of the minor C allele of rs174547 (carriers); light lines show time-courses of single subjects fitted by smoothing splines with the degree of smoothness chosen with leave-one-out cross validation; the bold line shows mean values of time-courses of all subjects per genotype; the bold and dashed line shows the standard deviation of time-courses of all subjects per genotype; lyso PC a, lyso phosphatidylcholine; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test.

FIGURE 22 illustrates time-courses of the metabolite ratio of PC aa C36:4 / PC aa C36:3 that was reported to have highest association with rs174547 in fasting serum samples by Illig et al. (Illig et al. 2010). In line with the findings of Illig et al, the ratio showed a very good distinction between the respective genotype (baseline OLTT: c= 0.966; OLTT: c= 0.949; baseline OGTT: c= 0.974; OGTT: c= 0.97). The upper and lower part of FIGURE 22 show the time-courses of the corresponding metabolites PC aa C36:3 (upper part) and PC aa C36:4 (lower part) during the OLTT (left part) and the OGTT (right part). Both metabolites were not highly genotype distinctive by their own. However, time-courses of both metabolites indicated differences

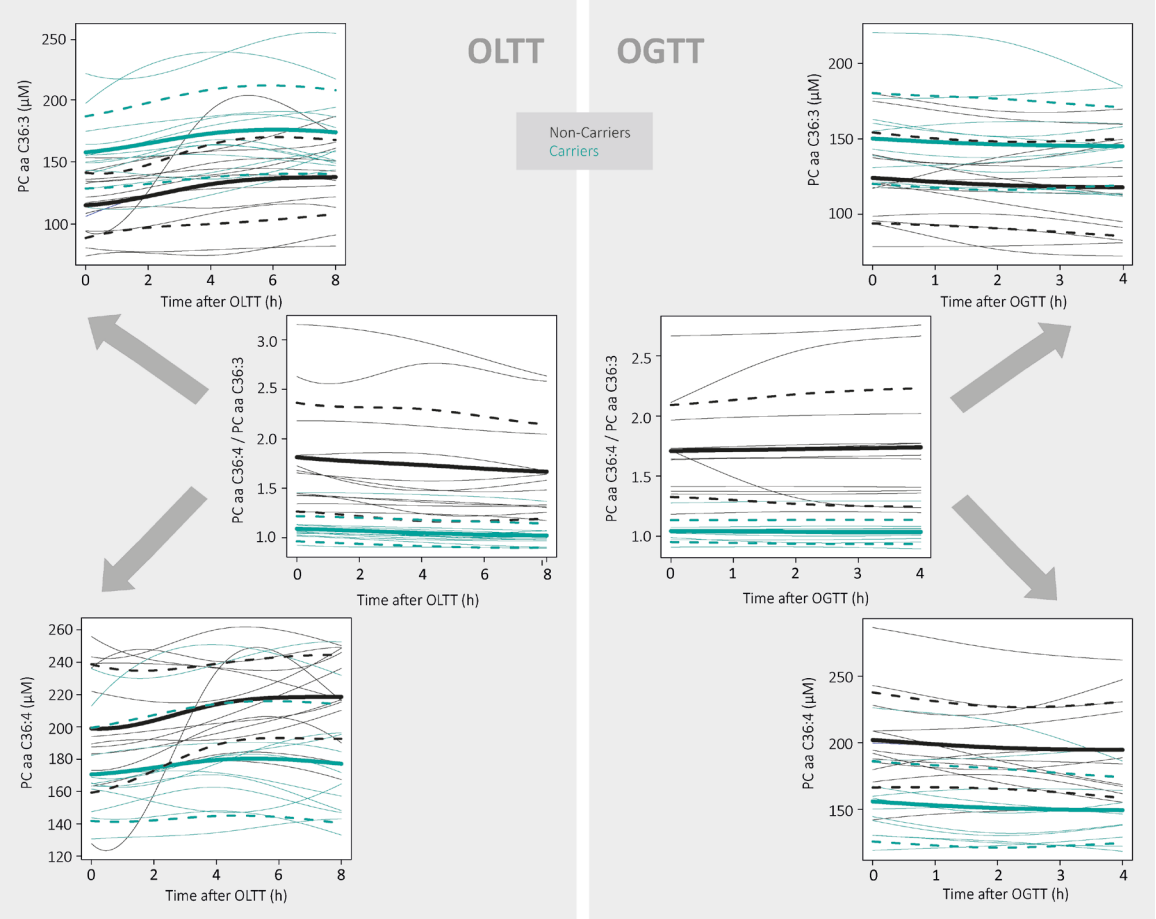


FIGURE 22: Time-courses of the metabolite ratio of PC aa C36:4 / PC aa C36:3 as well as the time-courses of PC aa C36:3 (upper part) and PC aa C36:4 (lower part) shown during the OGTT (left part) and the OGTT (right part)
Black lines show homozygous carriers of the major T allele of rs174547 (non carriers), blue lines show homozygous carriers of the minor C allele of rs174547 (carriers); light lines show time-courses of single subjects fitted by smoothing splines with the degree of smoothness chosen with leave-one-out cross validation; the bold line shows mean values of time-courses of all subjects per genotype; the bold and dashed line shows the standard deviation of time-courses of all subjects per genotype; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test; PC aa, diacyl phosphatidylcholine.

between carriers and non-carriers at baseline of both study days as well as during the OLTT and the OGTT that might combine and become highly genotype-distinctive by calculating the ratio of both metabolites.

Aside of a number of further metabolite ratios (SUPPLEMENTARY TABLE 9), the ratio of SM C18:0 to SM C 18:1 (FIGURE 23) was highly distinctive for the respective genotype of rs174547 (baseline OLTT: c= 0.901; OLTT: c= 0.862; baseline OGTT: c= 0.950; OGTT: c= 0.916). Interestingly, the ratio was highly distinctive although no genotype effect was visible in the included metabolites (upper and lower part of FIGURE 23).

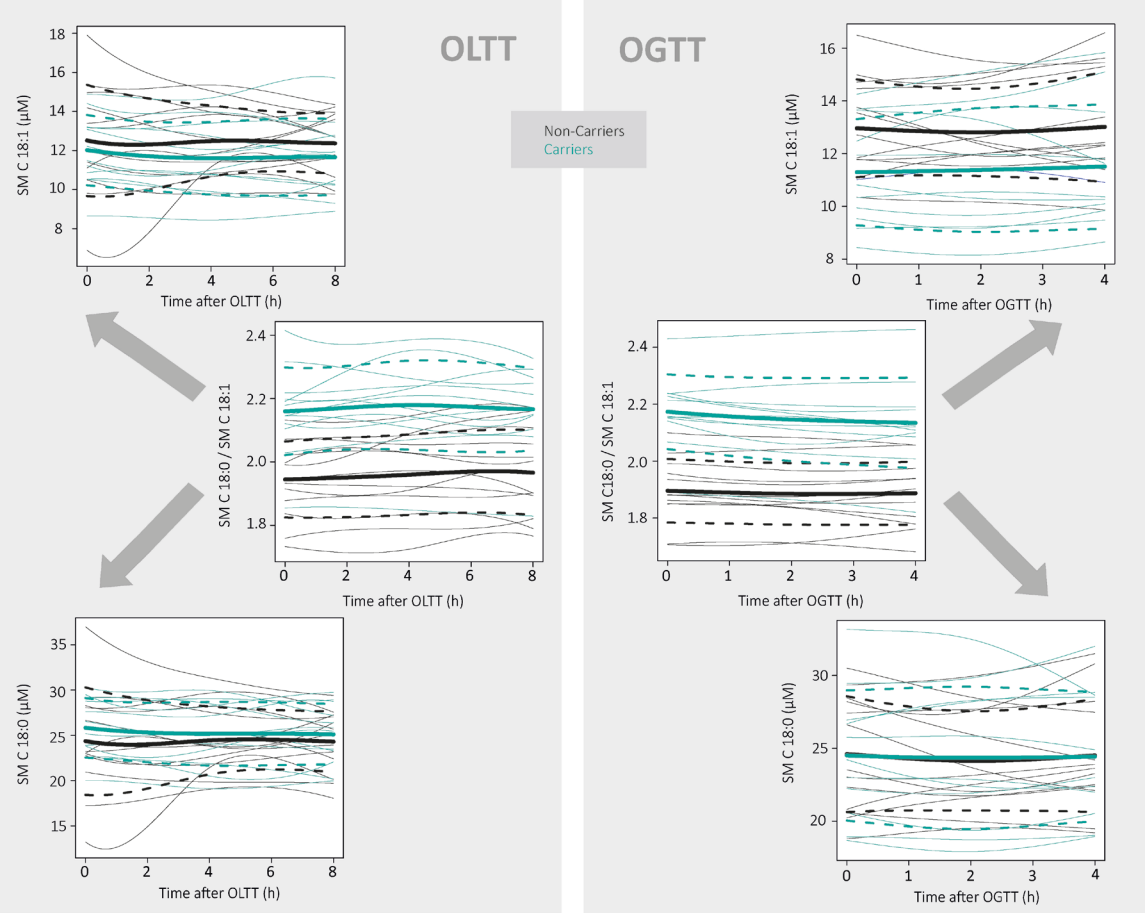


FIGURE 23: Time-courses of the metabolite ratio of SM C18:0 / SM C18:1 as well as the time-courses of SM C18:1 (upper part) and SM C18:0 (lower part) shown during the OGTT (left part) and the OGTT (right part)
Black lines show homozygous carriers of the major T allele of rs174547 (non carriers), blue lines show homozygous carriers of the minor C allele of rs174547 (carriers); light lines show time-courses of single subjects fitted by smoothing splines with the degree of smoothness chosen with leave-one-out cross validation; the bold line shows mean values of time-courses of all subjects per genotype; the bold and dashed line shows the standard deviation of time-courses of all subjects per genotype; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test; PC aa, diacyl phosphatidylcholine.

Overall, genotype-distinction of metabolite ratios potentially approximating enzyme activity of the delta -5 and delta-6 desaturases at baseline as well as during the anabolic challenges showed much better prediction of the respective allele of rs174547 than the baseline metabolite levels and postprandial time-courses of single metabolites.

3.4.3 Discussion

Using targeted metabolomics as a hypothesis free approach, it was investigated whether an OLTT and an OGTT might unravel novel metabolic effects for the GWAS-identified variant rs174547 in the FADS1 locus. The idea was that defined challenges may unmask early metabolic changes which are not detectable in the fasting state.

Logistic regression identified shingomyeline C20:2 (SM C20:2) as a highly genotype-distinctive metabolite following an OLTT as well as lyso phosphatidylcholine C20:4 (lyso PC a C20:4) as highly genotype-distinctive following an OGTT. Moreover, a number of selected metabolite ratios were highly distinctive at baseline as well as during the OLTT and OGTT.

Phosphatidylcholines (PCs) contain two fatty acid side chains esterified to a glycerol backbone and a phosphodiester linkage connecting the third hydroxylgroup to choline. PCs contain a range of fatty acids varying in position of double bonds and length (Cole et al. 2012). PCs are physiologically important as main component of eukaryotic cell membranes, as precursor of signaling molecules (van Meer et al. 2008; Robinson et al. 1989) and as key element of bile (Alvaro et al. 1986), lung surfactant (Perez-Gil 2008) and lipoproteins (Skipski et al. 1967). Lysophosphatidylcholines (lyso PCs) are the primary product of phosphatidylcholine hydrolysis catalyzed by phospholipase A2 (Nishizuka 1992). Lyso PC C20:4 includes only one fatty acid with a chain length of twenty carbons and four double bonds that might be C20:4 n-6 (arachidonic acid) or C20:4 n-3. Time-courses of lyso PC C20:4 generally showed lower concentrations for carriers of the minor CC alleles of rs174547 than for non-carriers. Although the c-index was solely highly distinctive for the respective allele of rs174547 during the OGTT (FIGURE 20B), the same trend was indicated in time-courses during the OLTT (FIGURE 20A) as well as at baseline of both study days. Our observations are in line with the findings of the GWA by Illig et al. (Illig et al. 2010). The authors showed the highest association of rs174547 with single metabolites (not ratios) for lyso PC 20:4 in baseline serum samples ($p= 2.6 \times e^{-51}$). 20:4 n-6 is the product of the delta-5 desaturase in the omega-6 pathway (FIGURE 17). Thus, a lower enzyme activity of the delta-5 desaturase would result in lower levels of 20:4 n-6. Aside, 20:4 n-3 is the substrate of the delta-5 desaturase in the omega-3 pathway as well as the elongated product of the first delta-6 desaturation (FIGURE 17). Hence, decreased delta-6 desaturase activity might also be responsible for the decreased levels of lyso PC C20:4. Given that the intake of omega-6 fatty acids is generally higher than that of omega-3 fatty acids, with a ratio of 15/1– 16.7/1 in typical Western diets (Simopoulos 2003), omega-6 fatty acids are likely to be the predominant fatty acids. Therefore, lower levels of lyso PC a C20:4 are probably mainly determined by lower levels of arachidonic

acid. This assumption correlates with previous studies that directly measured n-3 and n-6 polyunsatured fatty acids and showed the highest association of SNPs in high LD with rs174547 with arachidonic acid in baseline blood samples (Schaeffer et al. 2006; Malerba et al. 2008; Tanaka et al. 2009).

The findings could be an indication for a decline in the conversion rates of desaturases or of changes occurring at the transcriptional level. If functional polymorphisms exist in the FADS gene cluster, it can affect the expression of the delta-6 as well as the delta-5 desaturase. In a genome-wide association study of global gene expression, rs174546 in LD 1.0 with rs174547 was associated with FADS1 expression but not with FADS2 expression in lymphoblastoid cells (Dixon et al. 2007). This would explain highest impact on arachidonic acid as the product of the delta-5 desturase encoded by FADS1. Moreover, a further expression study in human liver tissue samples showed correlation of rs174547 with expression of both FADS1 and FADS3 genes (Kathiresan et al. 2009).

Aside, our findings show a number of metabolite ratios that were highly distinctive at baseline as well as during the OLTT and OGTT. Metabolite concentration ratios were shown to reduce the variance and noise in the dataset and yield robust statistical associations (Illig et al. 2010; Gieger et al. 2008; Altmaier et al. 2008). Thereby, they were shown to increase the power of GWA studies by reducing the p-values of association by several orders of magnitude (Gieger et al. 2008). Moreover, if a pair of metabolites is related to the direct substrates and products of an enzymatic conversion, the ratio between their concentrations can be applied as an approximation of the enzymatic activity (Gieger et al. 2008). Most of the distinctive metabolite ratios in our study contained an acyl-alkyl, diacyl or lyso phoshatidylcholine with four double bonds as numerator or denumerator and an acyl-alkyl, diacyl or lyso phoshatidylcholine with less than four double bonds on the respective other side of the fraction line. Therefore, they might approximate enzyme activity of the delta-5 desaturase. Exemplarily, we show the metabolite ratio of lyso PC a C20:4 and lyso PC a C20:3 (FIGURE 21) and of PC aa C36:4 and PC aa C36:3 (FIGURE 22), showing highest association with rs174547 in baseline blood samples in GWAS (Illig et al. 2010; Nicholson et al. 2011) and also being highly distinctive at baseline as well as during the challenges in our study. C20:4n-6 and C16:0 represent major fatty acids in membranes and thus are the most common fatty acid side chains in PCs. Thus, PC aa C36:4 most likely comprises the product of the delta-5 desaturase C20:4 n-6 and C16:0 as second incorporated fatty acid, while PC aa C36:3 on the other hand might contain the substrate of the delta-5 desaturase C20:3 n-6 and C16:0 (Lattka et al. 2010). Thus, both ratios can be considered as modified substrate and product of the delta-5 desaturase reaction. Time-courses of the single metabolites included in both ratios indicated higher levels of substrates (upper part of FIGURE 21 and FIGURE 22) and lower levels of products of the delta-5 desaturase (lower part of FIGURE 21 and FIGURE 22). However, compared

to these time-courses of single metabolites, ratios clearly showed a more pronounced distinction of carriers and non-carriers over time.

Time-courses of lyso PC a C20:4, lyso PC a C20:3 as well as PC aa C36:3 and PC aa C36:4 slightly increased during the OLTT. Although phosphatidylcholines showed a high inter-individual variability, the finding most widely correlates with a previous study applying identical test meals in healthy, young males (Krug et al. 2012). The OLTT contains 20.3 g ± 1.5 g of polyunsatured fatty acids consisting of 15.5 g ± 1.1 g of C18:2 n-6 (Linoleic acid) and 3.7 g ± 0.3 g of C18:3 n-3 (alpha-linoleic acid). Hence, almost no higher unsatured fatty acids are included. Fatty acids in the OLTT were desaturated and elongated and might be incorporated into phosphatidylcholines and as a result explain increasing levels of lyso PC a C20:4, lyso PC a C20:3, PC aa C36:3 and PC aa C36:4 during the OLTT. In contrast, during the OGTT, PCs rather remained stable or decreased slightly over time. This finding is generally in line with previous findings in healthy young males (Krug et al. 2012).

Phosphatidylcholine and the shingolipide ceramide are converted to diacylglycerol and sphingomyeline (SM) by the sphingomyelin synthase. Aside, SM may be generated from lysosphingomyelin (lyso SM) by fatty acid acylation or by direct transfer of phosphocholine to ceramide without PC (Taniguchi and Okazaki 2014; Bielawski et al. 2010). SMs are sphingophospholipids consisting of a sphingosine bound to a phosphorylcholine and are amide-linked with an acyl-chain differing widely in length (from 16 to 24 carbons). Amongst others, SMs function as a structural component in biological membranes. Recently, novel functions of SM have been revealed, such as a regulating microdomain structure, attenuating of in–out/out–in signal through SM-containing microdomains, exocytosis and endocytosis, intracellular vesicular trafficking and nuclear function (Taniguchi and Okazaki 2014). Aside, products of the SM metabolism like sphingosine, sphingosine-1 phosphate, ceramide and diacylglycerol are important cellular effectors functioning, for instance in apoptosis, ageing and development (Ramstedt and Slotte 2002). SM C20:2 is a shingomyeline with an acyl-chain of twenty carbons and two double-bondings. Levels of SM C20:2 were highly distinctive during the OLTT with carriers showing a trend for a slight decrease over time. Whereas non-carriers rather showed a slight increase prior to approaching baseline levels after 8 h. However, the difference was very slight and might also be random. Applying an identical OLTT, a study in young, healthy males showed rather stable metabolite levels of SM C20:2 over time confirming our assumption of a random effect (Krug et al. 2012). However, the GWA of Illig et al. showed a significant association of rs174547 with SM C20:2 in baseline serum samples ($p= 2.5 \times 10^{-10}$) determined by lower metabolite levels in carriers of the minor C allele. Aside, associations with further sphingomyelines were shown (Illig et al. 2010). Moreover, other GWAS in baseline blood samples also showed associations of FADS gene variants with sphingomyeline levels (Hong et al. 2013; Gieger et al. 2008; Hicks et al. 2009). Further, we calculated

the metabolite ratio SM C16:0/ SM C16.1 that was previously shown to be associated with SNPs in perfect LD with rs174547 as well as SM C18:0/ SM C18:1 that was previously shown to be associated with a SNP in LD 0.8 with rs174547 (Hicks et al. 2009). Our results also showed SM C18:0 to SM C18:1 to be highly distinctive for the respective allele of rs174547 at baseline of both study days as well as during the OGTT. Thus, results of our study as well as the literature shows an association of rs174547 with altered sphingomyeline levels.

Alterations in sphingomyelines and PC/lyso PCs are discussed to be associated with metabolic diseases including atherosclerosis (Fan et al. 2010; Dong et al. 2006; Lusis 2000), coronary artery disease and T2D (Yano et al. 2011; Taniguchi and Okazaki 2014; Zhu et al. 2011; Floegel et al. 2013; Wang-Sattler et al. 2012; Ha et al. 2012). Thus, there might be a potential link of the altered sphingomyeline as well as phosphatidylcholine metabolism in carriers of the minor allele of rs174547 and common complex disease processes.

In addition to the genotype-dependent differences in the targeted metabolomics measurements, our study showed genotype-depedent differences in the baseline characteristics in triglycerides, insulin, HOMA-B and HOMA-IR.

Higher triglyceride levels of carriers compared to non-carriers are in line with a meta-analysis of seven GWAS of blood lipoprotein and lipid phenotypes by Kathiresan et al. (association of rs174547 with triglycerides: $p= 2.0 \times 10^{-14}$) (Kathiresan et al. 2009). However, the authors also showed lower HDL levels in minor allele carriers of rs174547. Moreover, further GWAs showed associations of SNPs in high LD with rs174547 with LDL and total cholesterol levels with minor allele carriers showing lower concentrations of both lipid traits (Tanaka et al. 2009; Sabatti et al. 2009). Thereby, recent work observed that FADS variants potentially interact with dietary omega-3 and omega-6 PUFA intake to affect cholesterol levels (Hellstrand et al. 2012; Lu et al. 2010). However, we did not assess the usual dietary PUFA intake of our subjects which might be a reason for no significance in baseline cholesterol levels in our study aside of the low number of participants. The underlying biological mechanisms between FADS gene variants and blood lipoprotein and lipid phenotypes are not entirely clear (Standl et al. 2012). It is likely that the variations in LC-PUFAS are the direct link between the observed associations. Tanaka et al. (Tanaka et al. 2009) supposed that higher concentrations of the precursor fatty acids shown in minor allele carriers might result in increased membrane fluidity and, thus, in lower LDL. In addition, decreases in LC-PUFAs in minor allele carriers might lead to reduction in PPARα (peroxisome proliferator activating receptor alpha) activation as endogenous LC-PUFAS are natural ligands of PPARα (Fruchart et al. 1999). PPARα activation has been shown to lower triglyceride levels and elevate HDL levels by inducing the expression of ApoAI, Apo-AII, lipoprotein lipase

and by suppressing ApoCIII (Hertz et al. 1995; Schoonjans et al. 1996; Vu-Dac et al. 1994; Vu-Dac et al. 1995). Therefore, lower levels of LC-PUFAs might result in lower PPARα activation and increased TG as well as decreased HDL levels. However, PPARα is also known to increase LDL-C clearance (Guerin et al. 1996). Thus, one would also expect higher LDL levels in carriers of the minor C allele. Therefore, other regulatory mechanisms are likely (Tanaka et al. 2009). Overall, blood lipid concentrations are well known to be associated with cardiovascular disease (Kannel et al. 1961; Gordon et al. 1977; Kareinen et al. 2001). Thus, altered lipoproteins are very likely to be one link to metabolic diseases associated with FADS gene variants. Aside, our findings showed significantly higher baseline insulin concentrations as well as higher HOMA-B and HOMA-IR in carriers of the minor C allele of rs174547 by a paired t-test compared with controls. Interestingly, results of the logistic regression model did not classify insulin levels as highly distinctive for the respective allele of rs174547 (baseline OLTT: c= 0.499; OLTT: c= 0.703; baseline OGTT: c= 0.247; OGTT: c= 0.624) indicating the severity of the selected statistical test. GWAS showed a significant association between fasting glucose and rs174547 ($p= 1.72 \times 10^{-8}$ (Dupuis et al. 2010) and $p= 1.33 \times 10^{-17}$ (Scott et al. 2012)) as well as for SNPs in high linkage disequilibrium (LD) and a trend for an association with fasting insulin ($p= 1.8 \times 10^{-2}$ (Scott et al. 2012)) and HOMA-B ($p= 4.31 \times 10^{-5}$ (Dupuis et al. 2010)). However, there was no association for HOMA-IR and for 2 h glucose after OGTT (Dupuis et al. 2010). Although the genome-wide significance level was not reached, a meta-analysis by Morris et al. (Morris et al. 2012) showed a trend for an association of rs174547 with an increased risk for the development of T2D ($p= 3.30 \times 10^{-3}$). (Data on glycaemic traits have been contributed by MAGIC investigators and have been downloaded from www.magicinvestigators.org). A number of studies with comprehensive confounder adjustment showed, that the estimated delta-5-desaturase and delta-6-desaturase activity by metabolite ratios is associated with T2D risk. Thereby, most of these studies observed a strong inverse relation of the estimated delta-5 desaturase activity and a strong direct relation of the estimated delta-6 desaturease activity to T2D risk (Hodge et al. 2007; Krachler et al. 2008; Kroger et al. 2011; Patel et al. 2010). However, as SNPs in the FADS1 and FADS2 genes are in high LD, opposing effects might counterbalance each other resulting in a weak overall association between the variance and T2D risk. The biological mechanisms underlying the relation of fatty acid desaturases activity are not well understood yet. However, effects are likely to be mediated by changes in fatty acids. The fatty acid composition of cell membranes might affect cellular functions, insulin receptor affinity and binding, translocation of glucose transporters as well as intracellular signaling (Storlien et al. 1996; Ginsberg et al. 1981). Moreover, LC-PUFAs might act as ligands for transcription factors like SREBP1 (sterol regulatory element binding protein 1), HNF4 (hepatocyte nuclear factor 4), NFκB (nuclear factor κB), and PPARs (peroxisome proliferators activating receptors), which are

participating in lipogenesis and fatty acid oxidation (Jump and Clarke 1999; Kroger and Schulze 2012). Thus, more research is needed to understand the biological mechanisms underlying the observed associations.

A limitation of the study is the small sample size of 12 carriers and 13 non-carriers. Aside, impaired glucose tolerance after OGTT, might be based on generally increased age of participants (MV= 59.8 ± 5.5). Therefore, further studies should be based on a younger study cohort. We used targeted metabolomics mainly focusing on amino acids, biogenic amines, acylcarnitines, sphingomyelins, diacyl phosphatidylcholines, acyl-alkyl phosphatidylcholines and lyso phosphatidylcholines. However, omega-3 and omega-6 fatty acids were not directly measured, which would be very useful in further studies. Moreover, the measurement of eicosanoids as arachidonic acid derived metabolites might shed new light on the impact of genetics on inflammatory processes in the context of nutritional challenges. Of note, we only calculated metabolite ratios of metabolites that were previously associated with SNPs in high LD with rs174547 (Illig et al. 2010; Hicks et al. 2009; Gieger et al. 2008) as well as metabolite ratios approximating enzyme activity of the delta-5 and delta-6 desaturase for proof of concept. Thus, results for metabolite ratios should be considered as preliminary findings. For a detailed discussion of metabolite ratios, ratios of all measured metabolites have to be calculated which was not possible in the time-frame of this thesis.

In conclusion, the study confirms previously reported genotype-specific effects of the variant rs174547 in the FADS1 locus on triglycerides, insulin and HOMA-B at baseline. Aside, minor allele carriers of rs174547 were significantly higher in HOMA-IR than carriers of the major allele. Our time-resolved analysis identified genotype-OLTT interactions in SM C 20:2. Nevertheless, time-courses were not able to show a clear distinction by the respective genotype of rs174547. Moreover, lyso PC a C20:4 showed a high genotype-distinction during the OGTT. Time-courses confirmed previously reported lower metabolite levels of carriers of the minor alleles at baseline and showed that this difference is maintained during the time-course. However, the number of subjects seems to be too low to confirm reported baseline differences in our baseline samples. No further genotype-challenge interactions could be identified for the measured metabolites. However, calculation of selected metabolite ratios showed a number of metabolite ratios to be highly distinctive for the respective genotype at baseline as well as during the OLTT and OGTT. Thus, further studies should include calculations of metabolite ratios aside a higher sample size and direct measurements of omega-3 and 6 fatty acids and eicosanoids.

4 GENERAL DISCUSSION

The present thesis focuses on the postprandial plasma metabolism by time-resolved studies in healthy subjects and in subjects homozygous for the GWAS-identified variants rs2014355 and rs174547 in the acetyl-CoA dehydrogenase, short chain (*ACADS*) and the fatty acid desaturase 1 (*FADS1*) locus.

The statistical analysis of all sub-projects mainly focused on time-resolved analyses of the plasma metabolism during metabolic challenges. The time-dependence was taken into account by using smooth functions as a representation of the time-series data. After the smooth functions were fixed, parameters describing the functions were used for the comparison of two groups. This kind of analysis taking the whole time-interval during a metabolic challenge into account is advantageous compared to usually applied analyses based on single snapshots because it is able to deal with missing or repeated values and non-synchronized measurements. Thus, such kind of analyses, for example allow to include precise time-points of blood taking and thus are able to overcome limitations of human studies like delayed blood sampling, for instance due to occluded vein catheters. For the study in healthy males, we compared postprandial time-courses of identical subjects, thus a statistical test for paired time-resolved differences was needed. The case of paired time-resolved measurements was considered in a recent publication (Crainiceanu et al. 2012). The authors propose the usage of bootstrap methods for the detection of certain intervals of the considered time-scale with a high contrast between the two considered groups. Although this approach is suitable for examination of a low number of variables in a very detailed manner, its application on a large number of variables as in this thesis (approx. 600 variables) is not feasible. Therefore, in the context of the data generated by the study in healthy males, the time-resolved paired difference test (TPDT) that is applicable on paired time-resolved data including a large number of variables was developed by Ivan Kondofersky at the Institute of Computational Biology at the Helmhotz Zentrum München. Aside, the second part of this thesis aimed to study a potential connection between carriers of the minor CC allele and the major TT alleles of rs2014355 and rs174547, respectively, and time-resolved metabolic variables. Hence, a statistical analysis that is able to deal with time-courses of two groups including different subjects was needed. We decided for logistic regression with bootstrap randomized performance that is also based on smoothing functions and, thus, also utilizes the advantages of a time-resolved analysis based on smoothed functions named to be able to deal with missing and repeated values, non-synchronized measurements or multiple time-series per group.

Combined with recent advances in metabolomics technologies, time-resolved metabolic challenge tests provide further understanding of the human metabolism (Ho et al. 2013; Skurk et al. 2011; Shaham et al. 2008; Wopereis et al. 2009; Krug et al. 2012; Pellis et al. 2012; Bondia-Pons et al. 2011) and, moreover, were shown to improve the identification of metabolic alterations associated with early disease states that were not detected in a homeostatic situation (Shaham et al. 2008; Ramos-Roman et al. 2012; Deo et al. 2010). However, the variance between healthy individuals in the fasting state (Lenz et al. 2003; Walsh et al. 2006; Winnike et al. 2009) appreared to be extended in the postprandial state (Krug et al. 2012; Zivkovic et al. 2009) indicating the presence of distinct metabotypes of individuals determined by environmental factors and a given genetic and epigenetic disposition.

Thus, one aim of the study in healthy males was to investigate the effect of a three-day dietary standardization on the postprandial time-courses of plasma metabolites after a high-fat, high-carbohydrate (HFHC) meal. Thereby, lower mean postprandial metabolite levels were unveiled for branched chain amino acids and isobutyrylcarnitine after dietary standardization. Aside, standardization-induced reduction in the inter-individual variation was achieved for the metabolite group of acyl-alkyl phosphatidylcholines. However, postprandial time-course measurement of most metabolite classes was shown to be feasible without lead-in periods of dietary control in healthy males. Therefore, depending on the study design and the research question, it should be considered, whether a dietary standardization previous to nutritional challenge tests could be advantageous or not. Nevertheless, diet at the days prior to postprandial time-course measurements does not seem to have a major impact on the determinded metabolites.

The second part of the study in healthy males compared the postprandial metabolism of a HFHC meal consisting of a conventional fast food meal for breakfast and a healthier breakfast alternative to assess potential differences in the metabolic profile and to identify potential specific markers that might be associated with fast food consumption. Despite considerably different compositions of macro- and micronutrients of both test meals, few differences (in N-methyl proline, stachydrine, CMPF, isoleucine and in generally in the group of amino acids) were apparent in the measured metabolites in plasma samples within 6 h after test meals. Therefore, short-term exposure of healthy individuals to varying meals mostly seems to be balanced by metabolic flexibility.

To conclude, such studies in healthy individuals are of great importance for further understanding the nutritional impact on the human metabolism. Aside, distinct metabotypes after an overnight fast (Lenz et al. 2003; Walsh et al. 2006; Winnike et al. 2009) as well as in the postprandial state (Krug et al. 2012; Zivkovic et al. 2009) are determined by the individual genetic make-up. GWAS using basal metabolite concentrations have shown a number of genetic variants in genes encoding transporter proteins and

enzymes with profound impact on human metabolic traits (Shin et al. 2014; Illig et al. 2010; Gieger et al. 2008; Tanaka et al. 2009; Hicks et al. 2009; Demirkan et al. 2012; Kettunen et al. 2012; Nicholson et al. 2011). The observed metabotypes might therefore be partially caused by gene variants and by complex interaction of gene variants with environmental factors including nutrition. In this context, defined nutritional challenge tests were reported to uncover early metabolic changes in carriers of genotypes associated with a higher risk for metabolic diseases (Franks et al. 2007; Tan et al. 2006; Weickert et al. 2007).

Thus, in the second part of the work, the functional role of the gene variants rs2014355 in the *ACADS* gene locus and rs174547 in the *FADS1* locus should be characterized in strictly controlled human intervention studies using targeted metabolomics as hypothesis free approach.

The study of rs2014355 in the *ACADS* locus showed previously unreported fasting-induced genotype dependent differences for acyl-alkyl phosphatidylcholine C42:0 during a prolonged fasting of 24 h and further challenge-genotype interactions for glutamine and lyso phosphatidylcholine C20:4 during an OGTT that might indicate a less flexible metabolism in response to fasting and to a glucose load in minor CC allele carriers compared to controls.

Besides, the study of rs174547 in the *FADS1* locus confirmed previously reported genotype-dependent differences in phosphatidylcholines, lyso phosphatidylcholines and sphingomyelines, especially becoming obvious through calculation of metabolite ratios. However, further genotype-challenge interactions were not visible.

Interestingly, whereas calculations of metabolite ratios strongly improved the genotype distinction of rs174547, it did hardly improve the genotype distinction for rs2014355. A reason might be the coding SNP rs1799958 in perfect LD with rs2014355 that might induce stronger variations in the plasma metabolism of minor CC allele carriers than the non-coding variant rs174547 and various non-coding SNPs in LD with rs174547. This assumption might be confirmed by the GWA of Illig et al. (Illig et al. 2010) that showed a lower p-value of association for rs2014355 with the metabolite C4 of 2.5×10^{-78} than for rs174547 with the metabolite PC aa C36:4 of 2.3×10^{-43} in the KORA cohort. We calculated c-indices that show group assignment of metabolites or metabolite time-courses to the respective genotypes, no p-values. Thus, the group assignment of solely C4 seems to be good enough for distinction of the respective alleles of rs2014355. Moreover, a closer investigation of the time-courses of C3 showed no obvious genotype distinction for rs2014355, while time-courses of PC aa C36:3 indicated a trend for lower levels in carriers of the minor CC alleles of rs174547. Thus, there seems to be an accumulation of substrates of the enzyme acetyl-CoA dehydrogenase short chain and fatty acid desaturase 1 in carriers of the minor alleles of rs2014355 and rs174547, respectively, whereas metabolite levels of enzyme products seem to be

decreased solely for the fatty acid desaturase 1. This might be the reason for the notably added value of the calculation of metabolite ratios for rs174547 compared to rs2014355 in our study, which was also confirmed by the GWA of Illig et al. showing a p-value of association for rs2014355 and C3/C4 of 5.1×10^{-96} compared to a lower p-value of association of rs174547 and PC aa C36:3/PC aa C36:4 of 6.5×10^{-179}. However, it seems to be advantageous to include calculations of metabolite ratios in all kinds of metabolomics studies.

In summary, the studies in the second part of this thesis indicate that metabolic challenge tests may contribute to a better understanding of gene function and may help to estimate the risk and progression of metabolic diseases. However, such studies are rather proof-of-concept for recruitment by genotype and further studies are needed to unravel how gene variants affect metabolic responses to metabolic challenges and whether there is susceptibility to the development of common diseases. Such nutrigenetic studies as well as nutrigenomic studies are extremely important and valuable to detect early disturbances in chronic disease processes which may be accessible to individualized preventive interventions. Although there are some examples of personalized nutrition in monogenetic disorders, like lactose intolerance (Lomer et al. 2008; Swallow 2003) and phenylketonuria (Blau et al. 2010), the application of personalized nutrition in polygenetic disorders like hypertention or T2D is much more challenging and far from practice as the interaction of the genetic make-up, diet and health is far more complex and subtle than originally assumed (Minihane 2013). For instance, a number of loci, identified primarily through large-scale GWAS, have been found to be associated with susceptibillity to T2D (Mahajan et al. 2014; Morris et al. 2012). At the same time, development of T2D is known to be modified by exogenous factors including diet (Schulze and Hu 2005) and the extent to which exogenous factors affect disease outcome in individuals can be influenced by individual genotypes. Thus, we have learned that the prediction of a single genotype is small compared to that of a family history of an individual, risk scores and other risk factors in complex diseases (de Roos 2013). To clear up this complexity, e.g., a more detailed understanding of the penetrance of genotypes in population subgroups, the identity of biological mechanisms behind variants and interactions of multiple variants with environmental factors is needed (Rimbach and Minihane 2009). Thereby, metabolomics along with genomics, epigenomics, transcriptomics, and proteomics combined with health phenotyping are very powerful by development of integrative system biology approaches with robust computational and statistical approaches. Such kind of analysis enables the development of integrative models required for personalized diet in complex diseases. Thus, not only from the bioinformatic point of view, personalized nutrition is one of the major challenges in nutrition research of the twenty-first century.

REFERENCES

Alipour A., van Oostrom A. J. H. H. M., Izraeljan A., Verseyden C., Collins J. M., Frayn K. N., et al. (2008). Leukocyte activation by triglyceride-rich lipoproteins. Arterioscler. Thromb Vasc Biol. doi:10.1161/ATVBAHA.107.159749

Altmaier E., Emeny R. T., Krumsiek J., Lacruz M. E., Lukaschek K., Häfner S., et al. (2013). Metabolomic profiles in individuals with negative affectivity and social inhibition: a population-based study of Type D personality. Psychoneuroendocrinology. doi:10.1016/j.psyneuen.2012.11.014

Altmaier E., Ramsay S. L., Graber A., Mewes H.-W., Weinberger K. M., Suhre K. (2008). Bioinformatics analysis of targeted metabolomics--uncovering old and new tales of diabetic mice under medication. Endocrinology. doi:10.1210/en.2007-1747

Alvaro D., Cantafora A., Attili A. F., Ginanni Corradini S., Luca C. de, Minervini G., et al. (1986). Relationships between bile salts hydrophilicity and phospholipid composition in bile of various animal species. Comp Biochem Physiol B, 83, 551–554.

Andres R., Cader G., Zierler K. L. (1956). The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state; measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. J Clin Invest. doi:10.1172/JCI103324

Aulchenko Y. S., Ripatti S., Lindqvist I., Boomsma D., Heid I. M., Pramstaller P. P., et al. (2009). Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. Nat Genet. doi:10.1038/ng.269

Bao J., Atkinson F., Petocz P., Willett W. C., Brand-Miller J. C. (2011). Prediction of postprandial glycemia and insulinemia in lean, young, healthy adults: glycemic load compared with carbohydrate content alone. Am J Clin Nutr. doi:10.3945/ajcn.110.005033

Baylin A., Ruiz-Narvaez E., Kraft P., Campos H. (2007). alpha-Linolenic acid, Delta6-desaturase gene polymorphism, and the risk of nonfatal myocardial infarction. Am J Clin Nutr, 85, 554–560.

Bender D. A. (2008). Introduction to nutrition and metabolism, 4th ed. CRC Press, Broca Raton, FL

Bickerton A. S. T., Roberts R., Fielding B. A., Hodson L., Blaak E. E., Wagenmakers A. J. M., et al. (2007). Preferential uptake of dietary Fatty acids in adipose tissue and muscle in the postprandial period. Diabetes. doi:10.2337/db06-0822

Bielawski J., Pierce J. S., Snider J., Rembiesa B., Szulc Z. M., Bielawska A. (2010). Sphingolipid analysis by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Adv Exp Med Biol, 688, 46–59.

Blaak E. E., Antoine J.-M., Benton D., Björck I., Bozzetto L., Brouns F., et al. (2012). Impact of postprandial glycaemia on health and prevention of disease. Obes Rev. doi:10.1111/j.1467-789X.2012.01011.x

Blau N., van Spronsen F. J., Levy H. L. (2010). Phenylketonuria. Lancet. doi:10.1016/S0140-6736(10)60961-0

Bondia-Pons I., Maukonen J., Mattila I., Rissanen A., Saarela M., Kaprio J., et al. (2014). Metabolome and fecal microbiota in monozygotic twin pairs discordant for weight: a Big Mac challenge. FASEB J. doi:10.1096/fj.14-250167

Bondia-Pons I., Nordlund E., Mattila I., Katina K., Aura A.-M., Kolehmainen M., et al. (2011). Postprandial differences in the plasma metabolome of healthy Finnish subjects after intake of a sourdough fermented endosperm rye bread versus white wheat bread. Nutr J. doi:10.1186/1475-2891-10-116

Boudonck K. J., Mitchell M. W., Wulff J., Ryals J. A. (2009). Characterization of the biochemical variability of bovine milk using metabolomics. Metabolomics. doi:10.1007/s11306-009-0160-8

Bowman S. A. & Vinyard B. T. (2004). Fast food consumption of U.S. adults: impact on energy and nutrient intakes and overweight status. J Am Coll Nutr, 23, 163–168.

Bray G. A., Most M., Rood J., Redmann S., Smith S. R. (2007). Hormonal responses to a fast-food meal compared with nutritionally comparable meals of different composition. Ann Nutr Metab. doi:10.1159/000103277

Brookes K. J., Chen W., Xu X., Taylor E., Asherson P. (2006). Association of fatty acid desaturase genes with attention-deficit/hyperactivity disorder. Biol Psychiatry. doi:10.1016/j.biopsych.2006.04.025

Brynes A. E., Mark Edwards C., Ghatei M. A., Dornhorst A., Morgan L. M., Bloom S. R., et al. (2003). A randomised four-intervention crossover study investigating the effect of carbohydrates on daytime

profiles of insulin, glucose, non-esterified fatty acids and triacylglycerols in middle-aged men. Br J Nutr. doi:10.1079/BJN2002769

Cahill G. F. (2006). Fuel Metabolism in Starvation. Annu Rev Nutr. doi:10.1146/annurev.nutr.26.061505.111258

Calbet J. A. & MacLean D. A. (1997). Role of caloric content on gastric emptying in humans. J Physiol, 498 (Pt 2), 553–559.

Caspi A., Williams B., Kim-Cohen J., Craig I. W., Milne B. J., Poulton R., et al. (2007). Moderation of breastfeeding effects on the IQ by genetic variation in fatty acid metabolism. Proc Natl Acad Sci U S A. doi:10.1073/pnas.0704292104

Cheng S., Rhee E. P., Larson M. G., Lewis G. D., McCabe E. L., Shen D., et al. (2012). Metabolite profiling identifies pathways associated with metabolic risk in humans. Circulation. doi:10.1161/CIRCULATIONAHA.111.067827

Chorell E., Svensson M. B., Moritz T., Antti H. (2012). Physical fitness level is reflected by alterations in the human plasma metabolome. Mol Biosyst. doi:10.1039/c2mb05428k

Claus S. P. & Swann J. R. (2013). Nutrimetabonomics:applications for nutritional sciences, with specific reference to gut microbial interactions. Annu Rev Food Sci Technol. doi:10.1146/annurev-food-030212-182612

Cole L. K., Vance J. E., Vance D. E. (2012). Phosphatidylcholine biosynthesis and lipoprotein metabolism. Biochim Biophys Acta. doi:10.1016/j.bbalip.2011.09.009

Coppack S. W., Evans R. D., Fisher R. M., Frayn K. N., Gibbons G. F., Humphreys S. M., et al. (1992). Adipose tissue metabolism in obesity: lipase action in vivo before and after a mixed meal. Metabolism, 41, 264–272.

Corydon M. J., Andresen B. S., Bross P., Kjeldsen M., Andreasen P. H., Eiberg H., et al. (1997). Structural organization of the human short-chain acyl-CoA dehydrogenase gene. Mamm Genome, 8, 922–926.

Cox D. G., Oh J., Keasling A., Colson K. L., Hamann M. T. (2014). The utility of metabolomics in natural product and biomarker characterization. Biochim Biophys Acta. doi:10.1016/j.bbagen.2014.08.007

Crainiceanu C. M., Staicu A.-M., Ray S., Punjabi N. (2012). Bootstrap-based inference on the difference in the means of two correlated functional processes. Stat Med. doi:10.1002/sim.5439

Demirkan A., van Duijn C. M., Ugocsai P., Isaacs A., Pramstaller P. P., Liebisch G., et al. (2012). Genome-Wide Association Study Identifies Novel Loci Associated with Circulating Phospho- and Sphingolipid Concentrations. PLoS Genet. doi:10.1371/journal.pgen.1002490

Deo R. C., Hunter L., Lewis G. D., Pare G., Vasan R. S., Chasman D., et al. (2010). Interpreting Metabolomic Profiles using Unbiased Pathway Models. PLoS Comput Biol. doi:10.1371/journal.pcbi.1000692

Dixon A. L., Liang L., Moffatt M. F., Chen W., Heath S., Wong K. C. C., et al. (2007). A genome-wide association study of global gene expression. Nat Genet. doi:10.1038/ng2109

Dokoupil K. & Ensenauer R. (2008). Disorders of Mitochondrial Beta-Oxidation of Fatty Acids: A Growing Challenge For Pediatric Dietetics. Aktuelle Ernährungsmedizin, 195–200.

Dong J., Liu J., Lou B., Li Z., Ye X., Wu M., et al. (2006). Adenovirus-mediated overexpression of sphingomyelin synthases 1 and 2 increases the atherogenic potential in mice. J Lipid Res. doi:10.1194/jlr.M600040-JLR200

Duffey K. J., Gordon-Larsen P., Steffen L. M., Jacobs D. R., Popkin B. M. (2009). Regular consumption from fast food establishments relative to other restaurants is differentially associated with metabolic outcomes in young adults. J. Nutr. doi:10.3945/jn.109.109520

Dupuis J., Langenberg C., Prokopenko I., Saxena R., Soranzo N., Jackson A. U., et al. (2010). New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet. doi:10.1038/ng.520

Eaton S., Bartlett K., Pourfarzam M. (1996). Mammalian mitochondrial beta-oxidation. Biochem J, 320 (Pt 2), 345–357.

Ehlers K. (2014). Dissertation: From GWAS to functionality: association of rs2014355 in the ACADS gene locus with acylcarnitine ratio and postprandial metabolic and inflammatory activation of human PBMC. Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt

Ehrlein H. J. & Pröve J. (1982). Effect of viscosity of test meals on gastric emptying in dogs. Q J Exp Physiol, 67, 419–425.

Evans A. M., DeHaven C. D., Barrett T., Mitchell M., Milgram E. (2009). Integrated, Nontargeted Ultrahigh Performance Liquid Chromatography/Electrospray Ionization Tandem Mass Spectrometry Platform for the Identification and Relative Quantification of the Small-Molecule Complement of Biological Systems. Anal Chem. doi:10.1021/ac901536h

Fan Y., Shi F., Liu J., Dong J., Bui H. H., Peake D. A., et al. (2010). Selective reduction in the sphingomyelin content of atherogenic lipoproteins inhibits their retention in murine aortas and the subsequent development of atherosclerosis. Arterioscler Thromb Vasc Biol. doi:10.1161/ATVBAHA.110.213363

Felig P., Owen O. E., Wahren J., Cahill G. F. (1969). Amino acid metabolism during prolonged starvation. J. Clin Invest. doi:10.1172/JCI106017

Fiehn O. (2001). Combining Genomics, Metabolome Analysis, and Biochemical Modelling to Understand Metabolic Networks. Comp Funct Genomics. doi:10.1002/cfg.82

Fiehn O. (2002). Metabolomics--the link between genotypes and phenotypes. Plant Mol Biol, 48, 155–171.

Floegel A., Ruesten A. von, Drogan D., Schulze M. B., Prehn C., Adamski J., et al. (2013a). Variation of serum metabolites related to habitual diet: a targeted metabolomic approach in EPIC-Potsdam. Eur J Clin Nutr. doi:10.1038/ejcn.2013.147

Floegel A., Stefan N., Yu Z., Muhlenbruch K., Drogan D., Joost H.-G., et al. (2013b). Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. Diabetes. doi:10.2337/db12-0495

Fontaine-Bisson B., Wolever T. M. S., Chiasson J.-L., Rabasa-Lhoret R., Maheux P., Josse R. G., et al. (2007). Tumor necrosis factor alpha -238GA genotype alters postprandial plasma levels of free fatty acids in obese individuals with type 2 diabetes mellitus. Metab Cli. Exp. doi:10.1016/j.metabol.2006.12.013

Fouillet H., Bos C., Gaudichon C., Tome D. (2002). Approaches to quantifying protein metabolism in response to nutrient ingestion. J Nutr, 132, 3208S-18S.

Franks P. W., Ekelund U., Brage S., Luan J., Schafer A. J., O'Rahilly S., et al. (2007). PPARGC1A coding variation may initiate impaired NEFA clearance during glucose challenge. Diabetologia. doi:10.1007/s00125-006-0580-1

Frayn K. N. (2002). Adipose tissue as a buffer for daily lipid flux. Diabetologia. doi:10.1007/s00125-002-0873-y

Frayn N. F. (2010). Metabolic regulation: A human perpective. A human perpective, 3rd ed. Wiley-Blackwell, Chichester, West Sussex, UK

French S. A., Story M., Neumark-Sztainer D., Fulkerson J. A., Hannan P. (2001). Fast food restaurant use among adolescents: associations with nutrient intake, food choices and behavioral and psychosocial variables. Int J Obes Relat Metab Disord. doi:10.1038/sj.ijo.0801820

Fruchart J. C., Duriez P., Staels B. (1999). Peroxisome proliferator-activated receptor-alpha activators regulate genes governing lipoprotein metabolism, vascular inflammation and atherosclerosis. Curr Opin Lipidol, 10, 245–257.

Gallant N. M., Leydiker K., Tang H., Feuchtbaum L., Lorey F., Puckett R., et al. (2012). Biochemical, molecular, and clinical characteristics of children with short chain acyl-CoA dehydrogenase deficiency detected by newborn screening in California. Mol Genet Metab. doi:10.1016/j.ymgme.2012.02.007

Gelfand R. A. & Barrett E. J. (1987). Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. J Clin Invest. doi:10.1172/JCI113033

Ghisla S. & Thorpe C. (2004). Acyl-CoA dehydrogenases. A mechanistic overview. Eur J Biochem, 271, 494–508.

Gieger C., Geistlinger L., Altmaier E., Hrabé Angelis M. de, Kronenberg F., Meitinger T., et al. (2008). Genetics Meets Metabolomics: A Genome-Wide Association Study of Metabolite Profiles in Human Serum. PLoS Genet. doi:10.1371/journal.pgen.1000282

Ginsberg B. H., Brown T. J., Simon I., Spector A. A. (1981). Effect of the membrane lipid environment on the properties of insulin receptors. Diabetes, 30, 773–780.

Goek O.-N., Prehn C., Sekula P., Römisch-Margl W., Döring A., Gieger C., et al. (2013). Metabolites associate with kidney function decline and incident chronic kidney disease in the general population. Nephrol Dial Transplant. doi:10.1093/ndt/gft217

Gomez-Casati D. F., Zanor M. I., Busi M. V. (2013). Metabolomics in plants and humans: applications in the prevention and diagnosis of diseases. Biomed Res Int. doi:10.1155/2013/792527

Goodacre R., Vaidyanathan S., Dunn W. B., Harrigan G. G., Kell D. B. (2004). Metabolomics by numbers: acquiring and understanding global metabolite data. Trends Biotechnol. doi:10.1016/j.tibtech.2004.03.007

Gordon T., Castelli W. P., Hjortland M. C., Kannel W. B., Dawbert T. R. (1977). High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med, 62, 707–714.

Gregersen N., Winter V. S., Corydon M. J., Corydon T. J., Rinaldo P., Ribes A., et al. (1998). Identification of four new mutations in the short-chain acyl-CoA dehydrogenase (SCAD) gene in two patients: one of the variant alleles, 511C--T, is present at an unexpectedly high frequency in the general population, as was the case for 625G--A, together conferring susceptibility to ethylmalonic aciduria. Hum Mol Genet, 7, 619–627.

Griffin J. L., Atherton H., Shockcor J., Atzori L. (2011). Metabolomics as a tool for cardiac research. Nat Rev Cardiol. doi:10.1038/nrcardio.2011.138

Guerin M., Bruckert E., Dolphin P. J., Turpin G., Chapman M. J. (1996). Fenofibrate reduces plasma cholesteryl ester transfer from HDL to VLDL and normalizes the atherogenic, dense LDL profile in combined hyperlipidemia. Arterioscler Thromb Vasc Biol, 16, 763–772.

Guertin K. A., Moore S. C., Sampson J. N., Huang W.-Y., Xiao Q., Stolzenberg-Solomon R. Z., et al. (2014). Metabolomics in nutritional epidemiology: identifying metabolites associated with diet and quantifying their potential to uncover diet-disease relations in populations. Am J Clin Nutr. doi:10.3945/ajcn.113.078758

Guthrie J. F., Lin B.-H., Frazao E. (2002). Role of food prepared away from home in the American diet, 1977-78 versus 1994-96: changes and consequences. J Nutr Educ Behav, 34, 140–150.

Ha C. Y., Kim J. Y., Paik J. K., Kim O. Y., Paik Y.-H., Lee E. J., et al. (2012). The association of specific metabolites of lipid metabolism with markers of oxidative stress, inflammation and arterial stiffness in men with newly diagnosed type 2 diabetes. Clin Endocrinol (Oxf). doi:10.1111/j.1365-2265.2011.04244.x

Hanhineva K., Lankinen M. A., Pedret A., Schwab U., Kolehmainen M., Paananen J., et al. (2015). Nontargeted metabolite profiling discriminates diet-specific biomarkers for consumption of whole grains, fatty fish, and bilberries in a randomized controlled trial. J Nutr. doi:10.3945/jn.114.196840

Have G. A. M. ten, Engelen M. P. K. J., Luiking Y. C., Deutz N. E. P. (2007). Absorption kinetics of amino acids, peptides, and intact proteins. Int J Sport Nutr Exerc Metab, 17, S23-36.

Hellstrand S., Sonestedt E., Ericson U., Gullberg B., Wirfalt E., Hedblad B., et al. (2012). Intake levels of dietary long-chain PUFAs modify the association between genetic variation in FADS and LDL-C. J Lipid Res. doi:10.1194/jlr.P023721

Hertz R., Bishara-Shieban J., Bar-Tana J. (1995). Mode of action of peroxisome proliferators as hypolipidemic drugs. Suppression of apolipoprotein C-III. J Biol Chem, 270, 13470–13475.

Hicks A. A., Pramstaller P. P., Johansson A., Vitart V., Rudan I., Ugocsai P., et al. (2009). Genetic determinants of circulating sphingolipid concentrations in European populations. PLoS Genet. doi:10.1371/journal.pgen.1000672

Hindorff L. A., Sethupathy P., Junkins H. A., Ramos E. M., Mehta J. P., Collins F. S., et al. (2009). Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci U S A. doi:10.1073/pnas.0903103106

Ho J. E., Larson M. G., Vasan R. S., Ghorbani A., Cheng S., Rhee E. P., et al. (2013). Metabolite Profiles During Oral Glucose Challenge. Diabetes. doi:10.2337/db12-0754

Hochberg Y B. Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc Series B, 289–300.

Hochberg Y. & Benjamini Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Series B (Methodological). doi:10.2307/2346101

Hodge A. M., English D. R., O'Dea K., Sinclair A. J., Makrides M., Gibson R. A., et al. (2007). Plasma phospholipid and dietary fatty acids as predictors of type 2 diabetes: interpreting the role of linoleic acid. Am J Clin Nutr, 86, 189–197.

Hoffmann E de & Stroobant V (eds). (2007). Mass spectrometry. Principles and applications, third edition. Willey, Chichester, West Sussex, UK

Hong S. H., Kwak J. H., Paik J. K., Chae J. S., Lee J. H. (2013). Association of polymorphisms in FADS gene with age-related changes in serum phospholipid polyunsaturated fatty acids and oxidative stress markers in middle-aged nonobese men. Clin Interv Aging. doi:10.2147/CIA.S42096

Hornbak M., Banasik K., Justesen J. M., Krarup N. T., Sandholt C. H., Andersson Å., et al. (2011). The minor C-allele of rs2014355 in ACADS is associated with reduced insulin release following an oral glucose load. BMC Med Genet. doi:10.1186/1471-2350-12-4

Houten S. M. & Wanders R. J. A. (2010). A general introduction to the biochemistry of mitochondrial fatty acid beta-oxidation. J Inherit Metab Dis. doi:10.1007/s10545-010-9061-2

Hunt J. N. & Stubbs D. F. (1975). The volume and energy content of meals as determinants of gastric emptying. J Physiol, 245, 209–225.

Illig T., Gieger C., Zhai G., Römisch-Margl W., Wang-Sattler R., Prehn C., et al. (2010). A genome-wide perspective of genetic variation in human metabolism. Nat Genet. doi:10.1038/ng.507

Jabs J. & Devine C. M. (2006). Time scarcity and food choices: an overview. Appetite. doi:10.1016/j.appet.2006.02.014

Jakobsen L. H., Kondrup J., Zellner M., Tetens I., Roth E. (2011). Effect of a high protein meat diet on muscle and cognitive functions: A randomised controlled dietary intervention trial in healthy men. Clinical Nutrition. doi:10.1016/j.clnu.2010.12.010

Jaworowska A., Blackham T., Davies I. G., Stevenson L. (2013). Nutritional challenges and health implications of takeaway and fast food. Nutr Rev. doi:10.1111/nure.12031

Johnson A. D., Handsaker R. E., Pulit S. L., Nizzari M. M., O'Donnell C. J., Bakker P. I. W. de. (2008). SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics. doi:10.1093/bioinformatics/btn564

Jump D. B. & Clarke S. D. (1999). Regulation of gene expression by dietary fat. Annu Rev Nutr. doi:10.1146/annurev.nutr.19.1.63

KANNEL W. B., DAWBER T. R., KAGAN A., REVOTSKIE N., STOKES J. 3. (1961). Factors of risk in the development of coronary heart disease--six year follow-up experience. The Framingham Study. Ann Intern Med, 55, 33–50.

Kareinen A., Viitanen L., Halonen P., Lehto S., Laakso M. (2001). Cardiovascular risk factors associated with insulin resistance cluster in families with early-onset coronary heart disease. Arterioscler Thromb Vasc Biol, 21, 1346–1352.

Kathiresan S., Willer C. J., Peloso G. M., Demissie S., Musunuru K., Schadt E. E., et al. (2009). Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet. doi:10.1038/ng.291

Kelley D. E. & Mandarino L. J. (2000). Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes, 49, 677–683.

Kelley D. E., Goodpaster B., Wing R. R., Simoneau J. A. (1999). Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol., 277, E1130-41.

Kettunen J., Tukiainen T., Sarin A.-P., Ortega-Alonso A., Tikkanen E., Lyytikäinen L.-P., et al. (2012). Genome-wide association study identifies multiple loci influencing human serum metabolite levels. Nat Genet. doi:10.1038/ng.1073

Kolz M., Johnson T., Sanna S., Teumer A., Vitart V., Perola M., et al. (2009). Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. PLoS Genet. doi:10.1371/journal.pgen.1000504

Krachler B., Norberg M., Eriksson J. W., Hallmans G., Johansson I., Vessby B., et al. (2008). Fatty acid profile of the erythrocyte membrane preceding development of Type 2 diabetes mellitus. Nutr Metab Cardiovasc Dis. doi:10.1016/j.numecd.2007.04.005

Kristensen M. J., Kmoch S., Bross P., Andresen B. S., Gregersen N. (1994). Amino acid polymorphism (Gly209Ser) in the ACADS gene. Hum Mol Genet, 3, 1711.

Kroger J. & Schulze M. B. (2012). Recent insights into the relation of Delta5 desaturase and Delta6 desaturase activity to the development of type 2 diabetes. Curr Opin Lipidol. doi:10.1097/MOL.0b013e32834d2dc5

Kroger J., Zietemann V., Enzenbach C., Weikert C., Jansen E. H., Doring F., et al. (2011). Erythrocyte membrane phospholipid fatty acids, desaturase activity, and dietary fatty acids in relation to risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Am J Clin Nutr. doi:10.3945/ajcn.110.005447

Krug S., Kastenmuller G., Stuckler F., Rist M. J., Skurk T., Sailer M., et al. (2012). The dynamic range of the human metabolome revealed by challenges. FASEB J, 26, 2607–2619.

Krumsiek J., Suhre K., Illig T., Adamski J., Theis F. J. (2012). Bayesian independent component analysis recovers pathway signatures from blood metabolomics data. J. Proteome Res. doi:10.1021/pr300231n

Lattka E., Illig T., Heinrich J., Koletzko B. (2009a). FADS gene cluster polymorphisms: important modulators of fatty acid levels and their impact on atopic diseases. J Nutrigenet Nutrigenomics. doi:10.1159/000235559

Lattka E., Illig T., Heinrich J., Koletzko B. (2009b). FADS gene cluster polymorphisms: important modulators of fatty acid levels and their impact on atopic diseases. J Nutrigenet Nutrigenomics. doi:10.1159/000235559

Lattka E., Illig T., Heinrich J., Koletzko B. (2010). Do FADS genotypes enhance our knowledge about fatty acid related phenotypes? Clin Nutr. doi:10.1016/j.clnu.2009.11.005

Lee H. & Park W. J. (2014). Unsaturated fatty acids, desaturases, and human health. J Med Food. doi:10.1089/jmf.2013.2917

Lemaitre R. N., Tanaka T., Tang W., Manichaikul A., Foy M., Kabagambe E. K., et al. (2011). Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. PLoS Genet. doi:10.1371/journal.pgen.1002193

Lenz E. M. & Wilson I. D. (2007). Analytical strategies in metabonomics. J Proteome Res. doi:10.1021/pr0605217

Lenz E. M., Bright J., Wilson I. D., Morgan S. R., Nash A. F. P. (2003). A 1H NMR-based metabonomic study of urine and plasma samples obtained from healthy human subjects. J Pharm Biomed Anal, 33, 1103–1115.

Lever M., Atkinson W., George P. M., Chambers S. T. (2007). Sex differences in the control of plasma concentrations and urinary excretion of glycine betaine in patients attending a lipid disorders clinic. Clin Biochem. doi:10.1016/j.clinbiochem.2007.05.021

Lever M., George P. M., Dellow W. J., Scott R. S., Chambers S. T. (2005). Homocysteine, glycine betaine, and N,N-dimethylglycine in patients attending a lipid clinic. Metabolism, 54, 1–14.

Liu A. G., Most M. M., Brashear M. M., Johnson W. D., Cefalu W. T., Greenway F. L. (2012). Reducing the glycemic index or carbohydrate content of mixed meals reduces postprandial glycemia and insulinemia over the entire day but does not affect satiety. Diabetes Care. doi:10.2337/dc12-0329

Liu F., Li Z., Lv X., Ma J. (2015). Dietary n-3 polyunsaturated fatty acid intakes modify the effect of genetic variation in fatty acid desaturase 1 on coronary artery disease. PLoS ONE. doi:10.1371/journal.pone.0121255

Llorach R., Garcia-Aloy M., Tulipani S., Vazquez-Fresno R., Andres-Lacueva C. (2012). Nutrimetabolomic strategies to develop new biomarkers of intake and health effects. J Agric Food Chem. doi:10.1021/jf301142b

Lomer M. C. E., Parkes G. C., Sanderson J. D. (2008). Review article: lactose intolerance in clinical practice--myths and realities. Aliment Pharmacol Ther. doi:10.1111/j.1365-2036.2007.03557.x

Lu Y., Feskens E. J., Dolle M. E., Imholz S., Verschuren W. M., Muller M., et al. (2010). Dietary n-3 and n-6 polyunsaturated fatty acid intake interacts with FADS1 genetic variation to affect total and HDL-cholesterol concentrations in the Doetinchem Cohort Study. Am J Clin Nutr. doi:10.3945/ajcn.2009.29130

Luís P. B. M., Ruiter J. P. N., Ijlst L., Tavares Almeida I. de, Duran M., Mohsen A.-W., et al. (2011). Role of isovaleryl-CoA dehydrogenase and short branched-chain acyl-CoA dehydrogenase in the metabolism of valproic acid: implications for the branched-chain amino acid oxidation pathway. Drug Metab. Dispos. doi:10.1124/dmd.110.037606

Lusis A. J. (2000). Atherosclerosis. Nature. doi:10.1038/35025203

Mahajan A., Go M. J., Zhang W., Below J. E., Gaulton K. J., Ferreira T., et al. (2014). Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nat Genet. doi:10.1038/ng.2897

Malerba G., Schaeffer L., Xumerle L., Klopp N., Trabetti E., Biscuola M., et al. (2008). SNPs of the FADS gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease. Lipids. doi:10.1007/s11745-008-3158-5

Manning A. K., Hivert M.-F., Scott R. A., Grimsby J. L., Bouatia-Naji N., Chen H., et al. (2012). A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. Nat Genet. doi:10.1038/ng.2274

Martin F.-P. J., Montoliu I., Collino S., Scherer M., Guy P., Tavazzi I., et al. (2013). Topographical body fat distribution links to amino acid and lipid metabolism in healthy non-obese women. PLoS ONE. doi:10.1371/journal.pone.0073445

Martinelli N., Girelli D., Malerba G., Guarini P., Illig T., Trabetti E., et al. (2008). FADS genotypes and desaturase activity estimated by the ratio of arachidonic acid to linoleic acid are associated with inflammation and coronary artery disease. Am J Clin Nutr, 88, 941–949.

Martinez M. (1992). Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr, 120, S129-38.

Mathew S., Krug S., Skurk T., Halama A., Stank A., Artati A., et al. (2014). Metabolomics of Ramadan fasting: an opportunity for the controlled study of physiological responses to food intake. J Transl Med. doi:10.1186/1479-5876-12-161

Matysik S., Martin J., Bala M., Scherer M., Schaffler A., Schmitz G. (2011). Bile acid signaling after an oral glucose tolerance test. Chem Phys Lipids. doi:10.1016/j.chemphyslip.2011.05.003

McDonalds Germany. (2013). McDonald's Standardprodukte: Zutaten und Inhaltsstoffe. http://www.mcdonalds.de/produkte/unsere-zutaten

Meek S. E., Persson M., Ford G. C., Nair K. S. (1998). Differential regulation of amino acid exchange and protein dynamics across splanchnic and skeletal muscle beds by insulin in healthy human subjects. Diabetes, 47, 1824–1835.

Menge B. A., Schrader H., Ritter P. R., Ellrichmann M., Uhl W., Schmidt W. E., et al. (2010). Selective amino acid deficiency in patients with impaired glucose tolerance and type 2 diabetes. Regulatory Peptides. doi:10.1016/j.regpep.2009.08.001

Minihane A. M. (2013). The genetic contribution to disease risk and variability in response to diet: where is the hidden heritability? Proc Nutr Soc. doi:10.1017/S0029665112002856

Miyamoto Y., Iwao Y., Mera K., Watanabe H., Kadowaki D., Ishima Y., et al. (2012). A uremic toxin, 3-carboxy-4-methyl-5-propyl-2-furanpropionate induces cell damage to proximal tubular cells via the generation of a radical intermediate. Biochemical Pharmacology. doi:10.1016/j.bcp.2012.07.033

Moazzami A. A., Bondia-Pons I., Hanhineva K., Juntunen K., Antl N., Poutanen K., et al. (2012). Metabolomics reveals the metabolic shifts following an intervention with rye bread in postmenopausal women--a randomized control trial. Nutr J. doi:10.1186/1475-2891-11-88

Morris A. P., Voight B. F., Teslovich T. M., Ferreira T., Segre A. V., Steinthorsdottir V., et al. (2012). Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet. doi:10.1038/ng.2383

Nakamura M. T. & Nara T. Y. (2004). Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. Annu Rev Nutr. doi:10.1146/annurev.nutr.24.121803.063211

Nicholson G., Rantalainen M., Li J. V., Maher A. D., Malmodin D., Ahmadi K. R., et al. (2011). A Genome-Wide Metabolic QTL Analysis in Europeans Implicates Two Loci Shaped by Recent Positive Selection. PLoS Genet. doi:10.1371/journal.pgen.1002270

Nicholson J. K., Lindon J. C., Holmes E. (1999). 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica. doi:10.1080/004982599238047

Nishizuka Y. (1992). Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science, 258, 607–614.

Noland R. C., Koves T. R., Seiler S. E., Lum H., Lust R. M., Ilkayeva O., et al. (2009). Carnitine insufficiency caused by aging and overnutrition compromises mitochondrial performance and metabolic control. J Biol Chem. doi:10.1074/jbc.M109.032888

Orfanos P., Naska A., Trichopoulos D., Slimani N., Ferrari P., van Bakel M., et al. (2007). Eating out of home and its correlates in 10 European countries. The European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr. doi:10.1017/S1368980007000171

Paeratakul S., Ferdinand D. P., Champagne C. M., Ryan D. H., Bray G. A. (2003). Fast-food consumption among US adults and children: dietary and nutrient intake profile. J Am Diet Assoc, 103, 1332–1338.

Pan J. W., Williamson A., Cavus I., Hetherington H. P., Zaveri H., Petroff O. A. C., et al. (2008). Neurometabolism in human epilepsy. Epilepsia. doi:10.1111/j.1528-1167.2008.01508.x

Patel P. S., Sharp S. J., Jansen E., Luben R. N., Khaw K.-T., Wareham N. J., et al. (2010). Fatty acids measured in plasma and erythrocyte-membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes: a pilot study in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort. Am J Clin Nutr. doi:10.3945/ajcn.2010.29182

Pedersen C. B., Bross P., Winter V. S., Corydon T. J., Bolund L., Bartlett K., et al. (2003). Misfolding, degradation, and aggregation of variant proteins. The molecular pathogenesis of short chain acyl-CoA dehydrogenase (SCAD) deficiency. J Biol Chem. doi:10.1074/jbc.M309514200

Pedersen C. B., Kolvraa S., Kolvraa A., Stenbroen V., Kjeldsen M., Ensenauer R., et al. (2008a). The ACADS gene variation spectrum in 114 patients with short-chain acyl-CoA dehydrogenase (SCAD) deficiency is dominated by missense variations leading to protein misfolding at the cellular level. Hum Genet. doi:10.1007/s00439-008-0521-9

Pedersen C. B., Kolvraa S., Kolvraa A., Stenbroen V., Kjeldsen M., Ensenauer R., et al. (2008b). The ACADS gene variation spectrum in 114 patients with short-chain acyl-CoA dehydrogenase (SCAD) deficiency is dominated by missense variations leading to protein misfolding at the cellular level. Hum Genet. doi:10.1007/s00439-008-0521-9

Pellis L., van Erk M. J., van Ommen B., Bakker G. C. M., Hendriks H. F. J., Cnubben N. H. P., et al. (2012). Plasma metabolomics and proteomics profiling after a postprandial challenge reveal subtle diet effects on human metabolic status. Metabolomics. doi:10.1007/s11306-011-0320-5

Pereira M. A., Kartashov A. I., Ebbeling C. B., van Horn L., Slattery M. L., Jacobs D. R., et al. (2005). Fast-food habits, weight gain, and insulin resistance (the CARDIA study): 15-year prospective analysis. Lancet. doi:10.1016/S0140-6736(04)17663-0

Perez-Gil J. (2008). Structure of pulmonary surfactant membranes and films: the role of proteins and lipid-protein interactions. Biochim Biophys Acta. doi:10.1016/j.bbamem.2008.05.003

Petersen A.-K., Zeilinger S., Kastenmuller G., Romisch-Margl W., Brugger M., Peters A., et al. (2013). Epigenetics meets metabolomics: an epigenome-wide association study with blood serum metabolic traits. Human Molecular Genetics. doi:10.1093/hmg/ddt430

Petroff O. A. C., Errante L. D., Rothman D. L., Kim J. H., Spencer D. D. (2002). Glutamate-glutamine cycling in the epileptic human hippocampus. Epilepsia, 43, 703–710.

Pietilainen K. H., Sysi-Aho M., Rissanen A., Seppanen-Laakso T., Yki-Jarvinen H., Kaprio J., et al. (2007). Acquired obesity is associated with changes in the serum lipidomic profile independent of genetic effects--a monozygotic twin study. PLoS ONE. doi:10.1371/journal.pone.0000218

Pirman T., Ribeyre M. C., Mosoni L., Remond D., Vrecl M., Salobir J., et al. (2007). Dietary pectin stimulates protein metabolism in the digestive tract. Nutrition. doi:10.1016/j.nut.2006.09.001

Pohmann R. (2011). Physical basics of NMR. Methods Mol Biol. doi:10.1007/978-1-61779-219-9_1

Prentice K. J., Luu L., Allister E. M., Liu Y., Jun L. S., Sloop K. W., et al. (2014). The furan fatty acid metabolite CMPF is elevated in diabetes and induces beta cell dysfunction. Cell Metab. doi:10.1016/j.cmet.2014.03.008

Ramel A., Gudmundsdottir F. D., Thorsdottir I. (2012). Effects of two different types of fast food on postprandial metabolism in normal and overweight subjects. Eur J Clin Nutr. doi:10.1038/ejcn.2012.125

Ramos-Roman M. A., Sweetman L., Valdez M. J., Parks E. J. (2012). Postprandial changes in plasma acylcarnitine concentrations as markers of fatty acid flux in overweight and obesity. Metabolism. doi:10.1016/j.metabol.2011.06.008

Ramsay JO & Silverman B (eds). (2005). Functional data analysis. Springer, New York

Ramstedt B. & Slotte J. P. (2002). Membrane properties of sphingomyelins. FEBS Lett, 531, 33–37.

Rathee P., Rathee D., Rathee D., Rathee S. (2012). In vitro anticancer activity of stachydrine isolated from Capparis decidua on prostate cancer cell lines. Nat Prod Res. doi:10.1080/14786419.2011.608673

Rathmann W., Strassburger K., Heier M., Holle R., Thorand B., Giani G., et al. (2009). Incidence of Type 2 diabetes in the elderly German population and the effect of clinical and lifestyle risk factors: KORA S4/F4 cohort study. Diabet Med. doi:10.1111/j.1464-5491.2009.02863.x

Rennie M. J., Bohé J., Wolfe R. R. (2002). Latency, duration and dose response relationships of amino acid effects on human muscle protein synthesis. J Nutr., 132, 3225S-7S.

Rhee E. P. & Gerszten R. E. (2012). Metabolomics and cardiovascular biomarker discovery. Clin Chem. doi:10.1373/clinchem.2011.169573

Rimbach G. & Minihane A. M. (2009). Nutrigenetics and personalised nutrition: how far have we progressed and are we likely to get there? Proc Nutr Soc. doi:10.1017/S0029665109001116

Roberts L. D., Souza A. L., Gerszten R. E., Clish C. B. (2012). Targeted metabolomics. Curr Protoc Mol Biol. doi:10.1002/0471142727.mb3002s98

Robinson B. S., Yao Z. M., Baisted D. J., Vance D. E. (1989). Lysophosphatidylcholine metabolism and lipoprotein secretion by cultured rat hepatocytes deficient in choline. Biochem J, 260, 207–214.

Roos B. de. (2013). Personalised nutrition: ready for practice? Proc Nutr Soc. doi:10.1017/S0029665112002844

Rubio-Aliaga I., Roos B., Duthie S. J., Crosley L. K., Mayer C., Horgan G., et al. (2011). Metabolomics of prolonged fasting in humans reveals new catabolic markers. Metabolomics. doi:10.1007/s11306-010-0255-2

Rudolph T. K., Ruempler K., Schwedhelm E., Tan-Andresen J., Riederer U., Böger R. H., et al. (2007). Acute effects of various fast-food meals on vascular function and cardiovascular disease risk markers: the Hamburg Burger Trial. Am J Clin Nutr., 86, 334–340.

Rzehak P., Heinrich J., Klopp N., Schaeffer L., Hoff S., Wolfram G., et al. (2009). Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster and the fatty acid composition of erythrocyte membranes. Br J Nutr. doi:10.1017/S0007114508992564

Sabatti C., Service S. K., Hartikainen A.-L., Pouta A., Ripatti S., Brodsky J., et al. (2009). Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. Nat Genet. doi:10.1038/ng.271

Sailer M. (2013). Dissertation: Metabolic interrelationships of amino acid metabolism in health and disease. Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt

Samani N. J., Erdmann J., Hall A. S., Hengstenberg C., Mangino M., Mayer B., et al. (2007). Genomewide association analysis of coronary artery disease. N. Engl. J. Med. doi:10.1056/NEJMoa072366

Saxena R., Hivert M.-F., Langenberg C., Tanaka T., Pankow J. S., Vollenweider P., et al. (2010). Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. Nat Genet. doi:10.1038/ng.521

Schaeffer L., Gohlke H., Muller M., Heid I. M., Palmer L. J., Kompauer I., et al. (2006). Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. Hum Mol Genet. doi:10.1093/hmg/ddl117

Schoonjans K., Peinado-Onsurbe J., Lefebvre A. M., Heyman R. A., Briggs M., Deeb S., et al. (1996). PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. EMBO J, 15, 5336–5348.

Schulze M. B. & Hu F. B. (2005). Primary prevention of diabetes: what can be done and how much can be prevented? Annu Rev Public Health. doi:10.1146/annurev.publhealth.26.021304.144532

Scott R. A., Lagou V., Welch R. P., Wheeler E., Montasser M. E., Luan J., et al. (2012). Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. Nat Genet. doi:10.1038/ng.2385

Servillo L., Giovane A., Balestrieri M. L., Bata-Csere A., Cautela D., Castaldo D. (2011). Betaines in fruits of Citrus genus plants. J Agric Food Chem. doi:10.1021/jf2014815

Shaham O., Wei R., Wang T. J., Ricciardi C., Lewis G. D., Vasan R. S., et al. (2008). Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity. Mol Syst Biol. doi:10.1038/msb.2008.50

Shin S.-Y., Fauman E. B., Petersen A.-K., Krumsiek J., Santos R., Huang J., et al. (2014). An atlas of genetic influences on human blood metabolites. Nat Genet. doi:10.1038/ng.2982

Simopoulos A. P. (2003). Importance of the ratio of omega-6/omega-3 essential fatty acids: evolutionary aspects. World Rev Nutr Diet, 92, 1–22.

Skipski V. P., Barclay M., Barclay R. K., Fetzer V. A., Good J. J., Archibald F. M. (1967). Lipid composition of human serum lipoproteins. Biochem J, 104, 340–352.

Skurk T., Rubio-Aliaga I., Stamfort A., Hauner H., Daniel H. (2011). New metabolic interdependencies revealed by plasma metabolite profiling after two dietary challenges. Metabolomics. doi:10.1007/s11306-010-0258-z

Slupsky C. M., Rankin K. N., Wagner J., Fu H., Chang D., Weljie A. M., et al. (2007). Investigations of the effects of gender, diurnal variation, and age in human urinary metabolomic profiles. Anal Chem. doi:10.1021/ac0708588

Spégel P., Danielsson A. P. H., Bacos K., Nagorny C. L. F., Moritz T., Mulder H., et al. (2010). Metabolomic analysis of a human oral glucose tolerance test reveals fatty acids as reliable indicators of regulated metabolism. Metabolomics. doi:10.1007/s11306-009-0177-z

Sprecher H. (1981). Biochemistry of essential fatty acids. Prog Lipid Res, 20, 13–22.

Sprinthall RC & Fisk ST (eds). (1990). Basic statistical analysis. Prentice Hall, Englewood Cliffs NJ

Standl M., Lattka E., Stach B., Koletzko S., Bauer C.-P., Berg A. von, et al. (2012). FADS1 FADS2 gene cluster, PUFA intake and blood lipids in children: results from the GINIplus and LISAplus studies. PLoS ONE. doi:10.1371/journal.pone.0037780

Steyerberg E. W., Harrell F. E., JR, Borsboom G. J., Eijkemans M. J., Vergouwe Y., Habbema J. D. (2001). Internal validation of predictive models: efficiency of some procedures for logistic regression analysis. J Clin Epidemiol, 54, 774–781.

Storlien L. H., Pan D. A., Kriketos A. D., O'Connor J., Caterson I. D., Cooney G. J., et al. (1996). Skeletal muscle membrane lipids and insulin resistance. Lipids, 31, S261-5.

Storlien L., Oakes N. D., Kelley D. E. (2004). Metabolic flexibility. Proc Nutr Soc. doi:10.1079/PNS2004349

Swallow D. M. (2003). Genetics of lactase persistence and lactose intolerance. Annu Rev Genet. doi:10.1146/annurev.genet.37.110801.143820

Szymanska E., Bouwman J., Strassburg K., Vervoort J., Kangas A. J., Soininen P., et al. (2012). Gender-dependent associations of metabolite profiles and body fat distribution in a healthy population with central obesity: towards metabolomics diagnostics. OMICS. doi:10.1089/omi.2012.0062

Tan G. D., Neville M. J., Liverani E., Humphreys S. M., Currie J. M., Dennis L., et al. (2006). The in vivo effects of the Pro12Ala PPARgamma2 polymorphism on adipose tissue NEFA metabolism: the first use of the Oxford Biobank. Diabetologia. doi:10.1007/s00125-005-0044-z

Tanaka T., Shen J., Abecasis G. R., Kisialiou A., Ordovas J. M., Guralnik J. M., et al. (2009). Genome-wide association study of plasma polyunsaturated fatty acids in the InCHIANTI Study. PLoS Genet. doi:10.1371/journal.pgen.1000338

Taniguchi M. & Okazaki T. (2014). The role of sphingomyelin and sphingomyelin synthases in cell death, proliferation and migration-from cell and animal models to human disorders. Biochim Biophys Acta. doi:10.1016/j.bbalip.2013.12.003

Tein I., Elpeleg O., Ben-Zeev B., Korman S. H., Lossos A., Lev D., et al. (2008). Short-chain acyl-CoA dehydrogenase gene mutation (c.319CT) presents with clinical heterogeneity and is candidate founder mutation in individuals of Ashkenazi Jewish origin. Mol Genet Metab. doi:10.1016/j.ymgme.2007.09.021

ter Veld F., Primassin S., Hoffmann L., Mayatepek E., Spiekerkoetter U. (2009). Corresponding increase in long-chain acyl-CoA and acylcarnitine after exercise in muscle from VLCAD mice. J Lipid Res. doi:10.1194/jlr.M800221-JLR200

Tovar A. R., Ascencio C., Torres N., Gomez E., Bourges H. (1996). Neutral and basic amino acid concentrations in plasma during the day in subjects fed with two model rural and two model urban Mexican diets. Am J Clin Nutr, 63, 335–341.

Truong H., DiBello J. R., Ruiz-Narvaez E., Kraft P., Campos H., Baylin A. (2009). Does genetic variation in the Delta6-desaturase promoter modify the association between alpha-linolenic acid and the prevalence of metabolic syndrome? Am J Clin Nutr. doi:10.3945/ajcn.2008.27107

Trupp M., Zhu H., Wikoff W. R., Baillie R. A., Zeng Z.-B., Karp P. D., et al. (2012). Metabolomics reveals amino acids contribute to variation in response to simvastatin treatment. PLoS ONE. doi:10.1371/journal.pone.0038386

van Maldegem B. T., Duran M., Wanders R. J. A., Niezen-Koning K. E., Hogeveen M., Ijlst L., et al. (2006). Clinical, biochemical, and genetic heterogeneity in short-chain acyl-coenzyme A dehydrogenase deficiency. JAMA. doi:10.1001/jama.296.8.943

van Maldegem B. T., Duran M., Wanders R. J. A., Waterham H. R., Koning T. J. de, Rubio E., et al. (2010). Fasting and fat-loading tests provide pathophysiological insight into short-chain acyl-coenzyme a dehydrogenase deficiency. J Pediatr. doi:10.1016/j.jpeds.2009.07.008

van Maldegem B. T., Wanders R. J. A., Wijburg F. A. (2010). Clinical aspects of short-chain acyl-CoA dehydrogenase deficiency. J Inherit Metab Dis. doi:10.1007/s10545-010-9080-z

van Meer G., Voelker D. R., Feigenson G. W. (2008). Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol. doi:10.1038/nrm2330

van Oostrom A. J. H. H. M., Rabelink T. J., Verseyden C., Sijmonsma T. P., Plokker H. W. M., Jaegere P. P. T. de, et al. (2004). Activation of leukocytes by postprandial lipemia in healthy volunteers. Atherosclerosis. doi:10.1016/j.atherosclerosis.2004.07.004

van Oostrom A. J. H. H. M., Sijmonsma T. P., Rabelink T. J., van Asbeck B. S., Cabezas M. C. (2003a). Postprandial leukocyte increase in healthy subjects. Metab Clin Exp. doi:10.1053/meta.2003.50037

van Oostrom A. J. H. H. M., Sijmonsma T. P., Verseyden C., Jansen E. H. J. M., Koning E. J. P. de, Rabelink T. J., et al. (2003b). Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. J. Lipid Res. doi:10.1194/jlr.M200419-JLR200

Vanagas G. (2004). Receiver operating characteristic curves and comparison of cardiac surgery risk stratification systems. Interact Cardiovasc Thorac Surg. doi:10.1016/j.icvts.2004.01.008

Ventura F. V., Costa C. G., Struys E. A., Ruiter J., Allers P., Ijlst L., et al. (1999). Quantitative acylcarnitine profiling in fibroblasts using U-13C palmitic acid: an improved tool for the diagnosis of fatty acid oxidation defects. Clin Chim Acta, 281, 1–17.

Violante S., Ijlst L., Te Brinke H., Tavares Almeida I. de, Wanders R. J. A., Ventura F. V., et al. (2013). Carnitine palmitoyltransferase 2 and carnitine/acylcarnitine translocase are involved in the mitochondrial synthesis and export of acylcarnitines. FASEB J. doi:10.1096/fj.12-216689

Voight B. F., Scott L. J., Steinthorsdottir V., Morris A. P., Dina C., Welch R. P., et al. (2010). Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet. doi:10.1038/ng.609

Vu-Dac N., Schoonjans K., Kosykh V., Dallongeville J., Fruchart J. C., Staels B., et al. (1995). Fibrates increase human apolipoprotein A-II expression through activation of the peroxisome proliferator-activated receptor. J Clin Invest. doi:10.1172/JCI118118

Vu-Dac N., Schoonjans K., Laine B., Fruchart J. C., Auwerx J., Staels B. (1994). Negative regulation of the human apolipoprotein A-I promoter by fibrates can be attenuated by the interaction of the peroxisome proliferator-activated receptor with its response element. J Biol Chem, 269, 31012–31018.

Wahl S., Krug S., Then C., Kirchhofer A., Kastenmüller G., Brand T., et al. (2013). Comparative analysis of plasma metabolomics response to metabolic challenge tests in healthy subjects and influence of the FTO obesity risk allele. Metabolomics. doi:10.1007/s11306-013-0586-x

Wahren J. & Ekberg K. (2007). Splanchnic regulation of glucose production. Annu Rev Nutr. doi:10.1146/annurev.nutr.27.061406.093806

Wahren J., Felig P., Cerasi E., Luft R. (1972). Splanchnic and peripheral glucose and amino acid metabolism in diabetes mellitus. J Clin Invest. doi:10.1172/JCI106989

Walsh M. C., Brennan L., Malthouse J. P. G., Roche H. M., Gibney M. J. (2006). Effect of acute dietary standardization on the urinary, plasma, and salivary metabolomic profiles of healthy humans. Am J Clin Nutr., 84, 531–539.

Wang-Sattler R., Yu Z., Herder C., Messias A. C., Floegel A., He Y., et al. (2012). Novel biomarkers for pre-diabetes identified by metabolomics. Mol Syst Biol. doi:10.1038/msb.2012.43

Ward L. D. & Kellis M. (2012). HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. doi:10.1093/nar/gkr917

Weickert M. O., Loeffelholz C. V., Roden M., Chandramouli V., Brehm A., Nowotny P., et al. (2007). A Thr94Ala mutation in human liver fatty acid-binding protein contributes to reduced hepatic glycogenolysis and blunted elevation of plasma glucose levels in lipid-exposed subjects. Am J Physiol Endocrinol Metab. doi:10.1152/ajpendo.00337.2007

Westerterp-Plantenga M. S., van Marken Lichtenbelt W. D., Strobbe H., Schrauwen P. (2002). Energy metabolism in humans at a lowered ambient temperature. Eur J Clin Nutr. doi:10.1038/sj.ejcn.1601308

Wikoff W. R., Anfora A. T., Liu J., Schultz P. G., Lesley S. A., Peters E. C., et al. (2009). Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci U.S.A. doi:10.1073/pnas.0812874106

Winnike J. H., Busby M. G., Watkins P. B., O'Connell T. M. (2009). Effects of a prolonged standardized diet on normalizing the human metabolome. Am J Clin Nutr, 90, 1496–1501.

Wishart D. S., Jewison T., Guo C. an, Wilson M., Knox C., Liu Y., et al. (2013). HMDB 3.0--The Human Metabolome Database in 2013. Nucleic Acids Res. doi:10.1093/nar/gks1065

Wold H. & Ed. P. K. (1966). Estimation of principal components and related models by iterative least squares. In: Multivariate Analysis, pp 391–420

Wopereis S., Rubingh C. M., van Erk M. J., Verheij E. R., van Vliet T., Cnubben N. H. P., et al. (2009). Metabolic profiling of the response to an oral glucose tolerance test detects subtle metabolic changes. PLoS ONE. doi:10.1371/journal.pone.0004525

World Health Organization. (2006). Definition and diagnosis of diabetes mellitus and intermediate hyerglycemia. Report of a WHO consultation

Xie L. & Innis S. M. (2008). Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. J Nutr. doi:10.3945/jn.108.096156

Xu F., Tavintharan S., Sum C. F., Woon K., Lim S. C., Ong C. N. (2013). Metabolic signature shift in type 2 diabetes mellitus revealed by mass spectrometry-based metabolomics. J Clin Endocrinol Metab. doi:10.1210/jc.2012-4132

Yano M., Watanabe K., Yamamoto T., Ikeda K., Senokuchi T., Lu M., et al. (2011). Mitochondrial dysfunction and increased reactive oxygen species impair insulin secretion in sphingomyelin synthase 1-null mice. J Biol Chem. doi:10.1074/jbc.M110.179176

Yin J., Zhang Z.-W., Yu W.-J., Liao J.-Y., Luo X.-G., Shen Y.-J. (2010). Stachydrine, a major constituent of the Chinese herb leonurus heterophyllus sweet, ameliorates human umbilical vein endothelial cells injury induced by anoxia-reoxygenation. Am J Chin Med. doi:10.1142/S0192415X10007737

Yu Z., Zhai G., Singmann P., He Y., Xu T., Prehn C., et al. (2012). Human serum metabolic profiles are age dependent. Aging Cell. doi:10.1111/j.1474-9726.2012.00865.x

Zeggini E., Scott L. J., Saxena R., Voight B. F., Marchini J. L., Hu T., et al. (2008). Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet. doi:10.1038/ng.120

Zhao X., Peter A., Fritsche J., Elcnerova M., Fritsche A., Haring H.-U., et al. (2008). Changes of the plasma metabolome during an oral glucose tolerance test: is there more than glucose to look at? Am J Physiol Endocrinol Metab. doi:10.1152/ajpendo.90748.2008

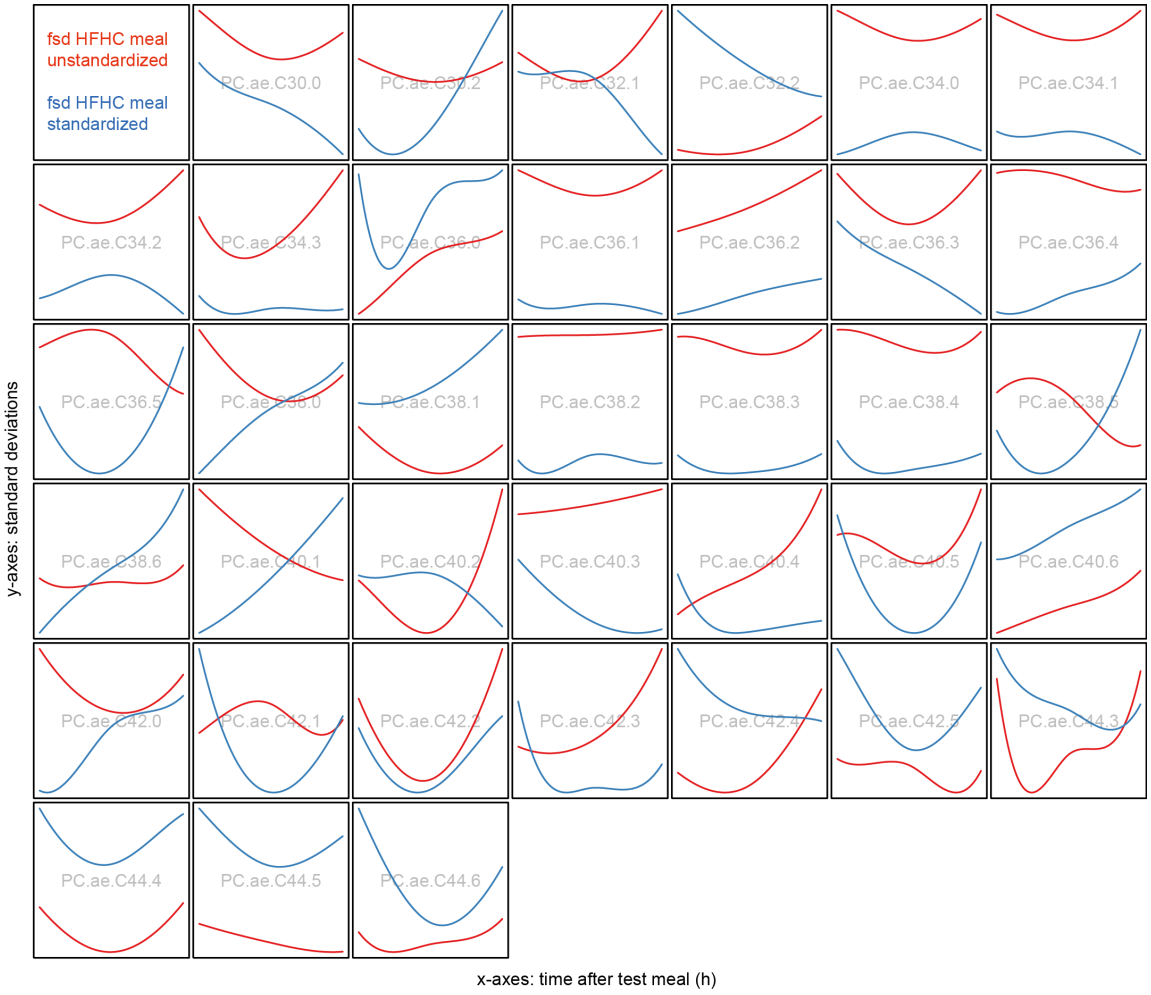
Zhu C., Liang Q.-l., Hu P., Wang Y.-m., Luo G.-a. (2011). Phospholipidomic identification of potential plasma biomarkers associated with type 2 diabetes mellitus and diabetic nephropathy. Talanta. doi:10.1016/j.talanta.2011.05.036

Zietemann V., Kroger J., Enzenbach C., Jansen E., Fritsche A., Weikert C., et al. (2010). Genetic variation of the FADS1 FADS2 gene cluster and n-6 PUFA composition in erythrocyte membranes in the European Prospective Investigation into Cancer and Nutrition-Potsdam study. Br J Nutr. doi:10.1017/S0007114510002916

Zivkovic A. M., Wiest M. M., Nguyen U., Nording M. L., Watkins S. M., German J. B. (2009). Assessing individual metabolic responsiveness to a lipid challenge using a targeted metabolomic approach. Metabolomics. doi:10.1007/s11306-008-0136-0

Zukunft S., Sorgenfrei M., Prehn C., Möller G., Adamski J. (2013). Targeted Metabolomics of Dried Blood Spot Extracts. Chromatographia. doi:10.1007/s10337-013-2429-3

APPENDIX



SUPPLEMENTARY FIGURE 1: Functional standard deviation (fsd) of the 37 PC ae measured with the targeted metabolomic approach after the two HFHC meals

The red lines show the fsd after the HFHC meal without dietary standardization (unstandardized), the blue line shows the fsd after the HFHC with previous three-day dietary standardization (standardized); HFHC, high-fat, high-carbohydrate.

SUPPLEMENTARY TABLE 1: Metabolites measured with the targeted metabolomic approach and assignment to metabolite groups predefined by Biocrates

Metabolite Group	Metabolite	
Acylcarnitines	C0	(Carnitine)
	C10	(Decanoylcarnitine)
	C10.1	(Decenoylcarnitine)
	C10.2	(Decadienylcarnitine)
	C12	(Dodecanoylcarnitine)
	C12.DC	(Dodecanedioylcarnitine)
	C12.1	(Dodecenoylcarnitine)
	C14	(Tetradecanoylcarnitine)
	C14.1	(Tetradecenoylcarnitine)
	C14.1.OH	(Hydroxytetradecenoylcarnitine)
	C14.2	(Tetradecadienylcarnitine)
	C14.2.OH	(Hydroxytetradecadienylcarnitine)
	C16	(Hexadecanoylcarnitine)
	C16.OH	(Hydroxyhexadecanoylcarnitine)
	C16.1	(Hexadecenoylcarnitine)
	C16.1.OH	(Hydroxyhexadecenoylcarnitine)
	C16.2	(Hexadecadienylcarnitine)
	C16.2.OH	(Hydroxyhexadecadienylcarnitine)
	C18	(Octadecanoylcarnitine)
	C18.1	(Octadecenoylcarnitine)
	C18.1.OH	(Hydroxyoctadecenoylcarnitine)
	C18.2	(Octadecadienylcarnitine)
	C2	(Acetylcarnitine)
	C3	(Propionylcarnitine)
	C3.DC..C4.OH.	(Hydroxybutyrylcarnitine)
	C3.OH	(Hydroxypropionylcarnitine)
	C3.1	(Propenoylcarnitine)
	C4	(Butyrylcarnitine)
	C4.1	(Butenylcarnitine)
	C5	(Valerylcarnitine)
	C5.DC..C6.OH.	(Glutarylcarnitine (Hydroxyhexanoylcarnitine))
	C5.M.DC	(Methylglutarylcarnitine)
	C5.OH..C3.DC.M.	(Hydroxyvalerylcarnitine (Methylmalonylcarnitine))
	C5.1	(Tiglylcarnitine)
	C5.1.DC	(Glutaconylcarnitine)
	C6..C4.1.DC.	(Hexanoylcarnitine (Fumarylcarnitine))
	C6.1	(Hexenoylcarnitine)
	C7.DC	(Pimelylcarnitine)
	C8	(Octanoylcarnitine)
	C9	(Nonaylcarnitine)

continued

Metabolite Group	Metabolite	
Amino acids	Ala	(Alanine)
	Arg	(Arginine)
	Asn	(Asparagine)
	Asp	(Aspartate)
	Cit	(Citrulline)
	Gln	(Glutamine)
	Glu	(Glutamate)
	Gly	(Glycine)
	His	(Histidine)
	Ile	(Isoleucine)
	Leu	(Leucine)
	Lys	(Lysine)
	Met	(Methionine)
	Orn	(Ornithine)
	Phe	(Phenylalanine)
	Pro	(Proline)
	Ser	(Serine)
	Thr	(Threonine)
	Trp	(Tryptophan)
	Tyr	(Tyrosine)
	Val	(Valine)
Biogenic amines	Creatinine	
	ADMA	(Asymmetric dimethylarginine)
	Ac.Orn	(Acetylornithine)
	Carnosine	
	DOPA	
	Dopamine	
	Histamine	
	Kynurenine	
	Met.SO	(Methioninesulfoxide)
	Nitro.Tyr	(Nitrotyrosine)
	OH.Pro	(Hydroxyproline)
	PEA	(Phenylethylamine)
	Putrescine	
	SDMA	(Symmetric dimethylarginine)
	Sarcosine	
	Serotonin	
	Spermidine	
	Spermine	

continued

Metabolite Group	Metabolite	
Biogenic amines	Taurine	
	alpha.AAA	(Alpha-Aminoadipic acid)
	total.DMA	(Total dimethylarginine)
Phosphatidylcholines acyl-alkyl	PC.ae.C30.0	(Phosphatidylcholine acyl-alkyl C30:0)
	PC.ae.C30.1	(Phosphatidylcholine acyl-alkyl C30:0)
	PC.ae.C30.2	(Phosphatidylcholine acyl-alkyl C30:2)
	PC.ae.C32.1	(Phosphatidylcholine acyl-alkyl C32:1)
	PC.ae.C32.2	(Phosphatidylcholine acyl-alkyl C32:2)
	PC.ae.C34.0	(Phosphatidylcholine acyl-alkyl C34:0)
	PC.ae.C34.1	(Phosphatidylcholine acyl-alkyl C34:1)
	PC.ae.C34.2	(Phosphatidylcholine acyl-alkyl C34:2)
	PC.ae.C34.3	(Phosphatidylcholine acyl-alkyl C34:3)
	PC.ae.C36.0	(Phosphatidylcholine acyl-alkyl C36:0)
	PC.ae.C36.1	(Phosphatidylcholine acyl-alkyl C36:1)
	PC.ae.C36.2	(Phosphatidylcholine acyl-alkyl C36:2)
	PC.ae.C36.3	(Phosphatidylcholine acyl-alkyl C36:3)
	PC.ae.C36.4	(Phosphatidylcholine acyl-alkyl C36:4)
	PC.ae.C36.5	(Phosphatidylcholine acyl-alkyl C36:5)
	PC.ae.C38.0	(Phosphatidylcholine acyl-alkyl C38:0)
	PC.ae.C38.1	(Phosphatidylcholine acyl-alkyl C38:1)
	PC.ae.C38.2	(Phosphatidylcholine acyl-alkyl C38:2)
	PC.ae.C38.3	(Phosphatidylcholine acyl-alkyl C38:3)
	PC.ae.C38.4	(Phosphatidylcholine acyl-alkyl C38:4)
	PC.ae.C38.5	(Phosphatidylcholine acyl-alkyl C38:5)
	PC.ae.C38.6	(Phosphatidylcholine acyl-alkyl C38:6)
	PC.ae.C40.1	(Phosphatidylcholine acyl-alkyl C40:1)
	PC.ae.C40.2	(Phosphatidylcholine acyl-alkyl C40:2)
	PC.ae.C40.3	(Phosphatidylcholine acyl-alkyl C40:3)
	PC.ae.C40.4	(Phosphatidylcholine acyl-alkyl C40:4)
	PC.ae.C40.5	(Phosphatidylcholine acyl-alkyl C40:5)
	PC.ae.C40.6	(Phosphatidylcholine acyl-alkyl C40:6)
	PC.ae.C42.0	(Phosphatidylcholine acyl-alkyl C42:0)
	PC.ae.C42.1	(Phosphatidylcholine acyl-alkyl C42:1)
	PC.ae.C42.2	(Phosphatidylcholine acyl-alkyl C42:2)
	PC.ae.C42.3	(Phosphatidylcholine acyl-alkyl C42:3)
	PC.ae.C42.4	(Phosphatidylcholine acyl-alkyl C42:4)
	PC.ae.C42.5	(Phosphatidylcholine acyl-alkyl C42:5)
	PC.ae.C44.3	(Phosphatidylcholine acyl-alkyl C44:3)
	PC.ae.C44.4	(Phosphatidylcholine acyl-alkyl C44:4)
	PC.ae.C44.5	(Phosphatidylcholine acyl-alkyl C44:5)
	PC.ae.C44.6	(Phosphatidylcholine acyl-alkyl C44:6)

continued

Metabolite Group	Metabolite	
Phosphatidylcholines diacyl	PC.aa.C24.0	(Phosphatidylcholine diacyl C24:0)
	PC.aa.C26.0	(Phosphatidylcholine diacyl C26:0)
	PC.aa.C28.1	(Phosphatidylcholine diacyl C28:1)
	PC.aa.C30.0	(Phosphatidylcholine diacyl C30:0)
	PC.aa.C30.2	(Phosphatidylcholine diacyl C30:2)
	PC.aa.C32.0	(Phosphatidylcholine diacyl C32:0)
	PC.aa.C32.1	(Phosphatidylcholine diacyl C32:1)
	PC.aa.C32.2	(Phosphatidylcholine diacyl C32:2)
	PC.aa.C32.3	(Phosphatidylcholine diacyl C32:3)
	PC.aa.C34.1	(Phosphatidylcholine diacyl C34:1)
	PC.aa.C34.2	(Phosphatidylcholine diacyl C34:2)
	PC.aa.C34.3	(Phosphatidylcholine diacyl C34:3)
	PC.aa.C34.4	(Phosphatidylcholine diacyl C34:4)
	PC.aa.C36.0	(Phosphatidylcholine diacyl C36:0)
	PC.aa.C36.1	(Phosphatidylcholine diacyl C36:1)
	PC.aa.C36.2	(Phosphatidylcholine diacyl C36:2)
	PC.aa.C36.3	(Phosphatidylcholine diacyl C36:3)
	PC.aa.C36.4	(Phosphatidylcholine diacyl C36:4)
	PC.aa.C36.5	(Phosphatidylcholine diacyl C36:5)
	PC.aa.C36.6	(Phosphatidylcholine diacyl C36:6)
	PC.aa.C38.0	(Phosphatidylcholine diacyl C38:0)
	PC.aa.C38.1	(Phosphatidylcholine diacyl C38:1)
	PC.aa.C38.3	(Phosphatidylcholine diacyl C38:3)
	PC.aa.C38.4	(Phosphatidylcholine diacyl C38:4)
	PC.aa.C38.5	(Phosphatidylcholine diacyl C38:5)
	PC.aa.C38.6	(Phosphatidylcholine diacyl C38:6)
	PC.aa.C40.1	(Phosphatidylcholine diacyl C40:1)
	PC.aa.C40.2	(Phosphatidylcholine diacyl C40:2)
	PC.aa.C40.3	(Phosphatidylcholine diacyl C40:3)
	PC.aa.C40.4	(Phosphatidylcholine diacyl C40:4)
	PC.aa.C40.5	(Phosphatidylcholine diacyl C40:5)
	PC.aa.C40.6	(Phosphatidylcholine diacyl C40:6)
	PC.aa.C42.0	(Phosphatidylcholine diacyl C42:0)
	PC.aa.C42.1	(Phosphatidylcholine diacyl C42:1)
	PC.aa.C42.2	(Phosphatidylcholine diacyl C42:2)
	PC.aa.C42.4	(Phosphatidylcholine diacyl C42:4)
	PC.aa.C42.5	(Phosphatidylcholine diacyl C42:5)
	PC.aa.C42.6	(Phosphatidylcholine diacyl C42:6)

continued

Metabolite Group	Metabolite	
Shingolipids	SM..OH..C14.1	(Hydroxysphingomyeline C14:1)
	SM..OH..C16.1	(Hydroxysphingomyeline C16:1)
	SM..OH..C22.1	(Hydroxysphingomyeline C22:1)
	SM..OH..C22.2	(Hydroxysphingomyeline C22:2)
	SM..OH..C24.1	(Hydroxysphingomyeline C24:1)
	SM.C16.0	(Sphingomyeline C16:0)
	SM.C16.1	(Sphingomyeline C16:1)
	SM.C18.0	(Sphingomyeline C18:0)
	SM.C18.1	(Sphingomyeline C18:1)
	SM.C20.2	(Sphingomyeline C20:2)
	SM.C22.3	(Sphingomyeline C22:3)
	SM.C24.0	(Sphingomyeline C24:0)
	SM.C24.1	(Sphingomyeline C24:1)
	SM.C26.0	(Sphingomyeline C26:0)
	SM.C26.1	(Sphingomyeline C26:1)
Lyso phosphatidylcholines	lysoPC.a.C14.0	(lysoPhosphatidylcholine acyl C14:0)
	lysoPC.a.C16.0	(lysoPhosphatidylcholine acyl C16:0)
	lysoPC.a.C16.1	(lysoPhosphatidylcholine acyl C16:1)
	lysoPC.a.C17.0	(lysoPhosphatidylcholine acyl C17:0)
	lysoPC.a.C18.0	(lysoPhosphatidylcholine acyl C18:0)
	lysoPC.a.C18.1	(lysoPhosphatidylcholine acyl C18:1)
	lysoPC.a.C18.2	(lysoPhosphatidylcholine acyl C18:2)
	lysoPC.a.C20.3	(lysoPhosphatidylcholine acyl C20:3)
	lysoPC.a.C20.4	(lysoPhosphatidylcholine acyl C20:4)
	lysoPC.a.C24.0	(lysoPhosphatidylcholine acyl C24:0)
	lysoPC.a.C26.0	(lysoPhosphatidylcholine acyl C26:0)
	lysoPC.a.C26.1	(lysoPhosphatidylcholine acyl C26:1)
	lysoPC.a.C28.0	(lysoPhosphatidylcholine acyl C28:0)
	lysoPC.a.C28.1	(lysoPhosphatidylcholine acyl C28:1)
	H1	(Hexose)

SUPPLEMENTARY TABLE 2: Metabolites measured with the untargeted metabolomic approach and assignment to metabolite groups and subgroups predefined by Metabolon

Metabolite Group	Metabolite Subgroup	Metabolite
Amino acids	alanine and aspartate metabolism	alanine
	alanine and aspartate metabolism	N-acetyl-beta-alanine
	alanine and aspartate metabolism	N-acetylalanine
	butanoate metabolism	2-aminobutyrate
	creatine metabolism	creatine
	creatine metabolism	creatinine
	cysteine, methionine, sam, taurine metabolism	alpha-ketobutyrate
	cysteine, methionine, sam, taurine metabolism	methionine
	cysteine, methionine, sam, taurine metabolism	N-formylmethionine
	cysteine, methionine, sam, taurine metabolism	S-methylcysteine
	glutamate metabolism	glutamine
	glutamate metabolism	pyroglutamine
	glutathione metabolism	5-oxoproline
	glutathione metabolism	glutathione, oxidized (GSSG)
	glycine, serine and threonine metabolism	betaine
	glycine, serine and threonine metabolism	N-acetylthreonine
	glycine, serine and threonine metabolism	threonine
	guanidino and acetamido metabolism	4-acetamidobutanoate
	histidine metabolism	3-methylhistidine
	histidine metabolism	cis-urocanate
	histidine metabolism	histidine
	histidine metabolism	trans-urocanate
	lysine metabolism	glutarylcarnitine (C5)
	lysine metabolism	lysine
	lysine metabolism	N6-acetyllysine
	lysine metabolism	pipecolate
	phenylalanine & tyrosine metabolism	3-(4-hydroxyphenyl)lactate
	phenylalanine & tyrosine metabolism	3-methoxytyrosine
	phenylalanine & tyrosine metabolism	3-phenylpropionate (hydrocinnamate)
	phenylalanine & tyrosine metabolism	4-hydroxyphenylpyruvate
	phenylalanine & tyrosine metabolism	p-cresol sulfate
	phenylalanine & tyrosine metabolism	phenol sulfate
	phenylalanine & tyrosine metabolism	phenylacetylglutamine
	phenylalanine & tyrosine metabolism	phenylalanine
	phenylalanine & tyrosine metabolism	phenyllactate (PLA)
	phenylalanine & tyrosine metabolism	tyrosine

continued

Metabolite Group	Metabolite Subgroup	Metabolite
Amino acids	tryptophan metabolism	3-indoxyl sulfate
	tryptophan metabolism	C-glycosyltryptophan
	tryptophan metabolism	indoleacetate
	tryptophan metabolism	indolelactate
	tryptophan metabolism	indolepropionate
	tryptophan metabolism	kynurenine
	tryptophan metabolism	tryptophan
	tryptophan metabolism	tryptophan betaine
	urea cycle; arginine-, proline-, metabolism	arginine
	urea cycle; arginine-, proline-, metabolism	citrulline
	urea cycle; arginine-, proline-, metabolism	N-acetylornithine
	urea cycle; arginine-, proline-, metabolism	N-methyl proline
	urea cycle; arginine-, proline-, metabolism	proline
	urea cycle; arginine-, proline-, metabolism	trans-4-hydroxyproline
	urea cycle; arginine-, proline-, metabolism	urea
	valine, leucine and isoleucine metabolism	2-hydroxy-3-methylvalerate
	valine, leucine and isoleucine metabolism	2-methylbutyrylcarnitine (C5)
	valine, leucine and isoleucine metabolism	3-hydroxyisobutyrate
	valine, leucine and isoleucine metabolism	3-methyl-2-oxobutyrate
	valine, leucine and isoleucine metabolism	3-methyl-2-oxovalerate
	valine, leucine and isoleucine metabolism	3-methylglutarylcarnitine (C6)
	valine, leucine and isoleucine metabolism	4-methyl-2-oxopentanoate
	valine, leucine and isoleucine metabolism	alpha-hydroxyisocaproate
	valine, leucine and isoleucine metabolism	alpha-hydroxyisovalerate
	valine, leucine and isoleucine metabolism	beta-hydroxyisovalerate
	valine, leucine and isoleucine metabolism	isobutyrylcarnitine
	valine, leucine and isoleucine metabolism	isoleucine
	valine, leucine and isoleucine metabolism	isovalerylcarnitine
	valine, leucine and isoleucine metabolism	leucine
	valine, leucine and isoleucine metabolism	levulinate (4-oxovalerate)
	valine, leucine and isoleucine metabolism	tiglyl carnitine
	valine, leucine and isoleucine metabolism	valine

continued

Metabolite Group	Metabolite Subgroup	Metabolite
Carbohydrates	fructose, mannose, galactose, starch, and sucrose metabolism	methyl-beta-glucopyranoside
	glycolysis, gluconeogenesis, pyruvate metabolism	1,5-anhydroglucitol (1,5-AG)
	glycolysis, gluconeogenesis, pyruvate metabolism	lactate
	glycolysis, gluconeogenesis, pyruvate metabolism	pyruvate
	isobar	Isobar: glucose, fructose, mannose, galactose, allose, altrose, etc.
	isobar	Isobar: glucose, mannose, galactose, gulose
Cofactors and vitamins	hemoglobin and porphyrin metabolism	bilirubin (E,E)
	hemoglobin and porphyrin metabolism	bilirubin (Z,Z)
	hemoglobin and porphyrin metabolism	biliverdin
	hemoglobin and porphyrin metabolism	heme
	hemoglobin and porphyrin metabolism	L-urobilin
	hemoglobin and porphyrin metabolism	urobilinogen
	nicotinate and nicotinamide metabolism	nicotinamide
	nicotinate and nicotinamide metabolism	trigonelline (N'-methylnicotinate)
	pantothenate and coa metabolism	pantothenate
	vitamin b6 metabolism	pyridoxate
Energy	krebs cycle	citrate
	krebs cycle	malate
	krebs cycle	succinylcarnitine
	oxidative phosphorylation	phosphate
Lipids	bile acid metabolism	cholate
	bile acid metabolism	deoxycholate
	bile acid metabolism	glycochenodeoxycholate
	bile acid metabolism	glycocholate
	bile acid metabolism	glycocholenate sulfate
	bile acid metabolism	glycodeoxycholate
	bile acid metabolism	glycolithocholate sulfate
	bile acid metabolism	glycoursodeoxycholate
	bile acid metabolism	taurochenodeoxycholate
	bile acid metabolism	taurocholate
	bile acid metabolism	taurocholenate sulfate
	bile acid metabolism	taurodeoxycholate
	bile acid metabolism	taurolithocholate 3-sulfate

continued

Metabolite Group	Metabolite Subgroup	Metabolite
Lipids	carnitine metabolism	3-dehydrocarnitine
	carnitine metabolism	acetylcarnitine
	carnitine metabolism	carnitine
	carnitine metabolism	cis-4-decenoyl carnitine
	carnitine metabolism	decanoylcarnitine
	carnitine metabolism	deoxycarnitine
	carnitine metabolism	hexanoylcarnitine
	carnitine metabolism	myristoylcarnitine
	carnitine metabolism	octanoylcarnitine
	carnitine metabolism	oleoylcarnitine
	carnitine metabolism	palmitoylcarnitine
	carnitine metabolism	stearoylcarnitine
	endocannabinoid	palmitoyl ethanolamide
	essential fatty acid	dihomo-linolenate (20:3n3 or n6)
	essential fatty acid	docosahexaenoate (DHA; 22:6n3)
	essential fatty acid	docosapentaenoate (n3 DPA; 22:5n3)
	essential fatty acid	docosapentaenoate (n6 DPA; 22:5n6)
	essential fatty acid	eicosapentaenoate (EPA; 20:5n3)
	essential fatty acid	linoleate (18:2n6)
	essential fatty acid	linolenate [alpha or gamma; (18:3n3 or 6)]
	fatty acid metabolism	isovalerate
	fatty acid metabolism (also bcaa metabolism)	butyrylcarnitine
	fatty acid metabolism (also bcaa metabolism)	propionylcarnitine
	fatty acid, amide	linoleamide (18:2n6)
	fatty acid, amide	oleamide
	fatty acid, amide	palmitic amide
	fatty acid, amide	stearamide
	fatty acid, branched	13-methylmyristic acid
	fatty acid, branched	15-methylpalmitate (isobar with 2-methylpalmitate)
	fatty acid, branched	17-methylstearate
	fatty acid, dicarboxylate	3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)
	fatty acid, dicarboxylate	azelate (nonanedioate)
	fatty acid, dicarboxylate	dodecanedioate
	fatty acid, dicarboxylate	hexadecanedioate
	fatty acid, dicarboxylate	octadecanedioate
	fatty acid, dicarboxylate	tetradecanedioate

continued

Metabolite Group	Metabolite Subgroup	Metabolite
Lipids	fatty acid, monohydroxy	2-hydroxydecanoic acid
	fatty acid, monohydroxy	2-hydroxypalmitate
	fatty acid, monohydroxy	2-hydroxystearate
	fatty acid, monohydroxy	3-hydroxydecanoate
	fatty acid, monohydroxy	3-hydroxyoctanoate
	glycerolipid metabolism	choline
	ketone bodies	3-hydroxybutyrate (BHBA)
	long chain fatty acid	10-heptadecenoate (17:1n7)
	long chain fatty acid	10-nonadecenoate (19:1n9)
	long chain fatty acid	adrenate (22:4n6)
	long chain fatty acid	arachidonate (20:4n6)
	long chain fatty acid	dihomo-linoleate (20:2n6)
	long chain fatty acid	docosadienoate (22:2n6)
	long chain fatty acid	eicosenoate (20:1n9 or 11)
	long chain fatty acid	margarate (17:0)
	long chain fatty acid	mead acid (20:3n9)
	long chain fatty acid	myristate (14:0)
	long chain fatty acid	myristoleate (14:1n5)
	long chain fatty acid	nonadecanoate (19:0)
	long chain fatty acid	oleate (18:1n9)
	long chain fatty acid	palmitate (16:0)
	long chain fatty acid	palmitoleate (16:1n7)
	long chain fatty acid	pentadecanoate (15:0)
	long chain fatty acid	stearate (18:0)
	lysolipid	1-arachidonoylglycerophosphocholine
	lysolipid	1-arachidonoylglycerophosphoethanolamine
	lysolipid	1-arachidonoylglycerophosphoinositol
	lysolipid	1-docosahexaenoylglycerophosphocholine
	lysolipid	1-docosapentaenoylglycerophosphocholine
	lysolipid	1-eicosadienoylglycerophosphocholine
	lysolipid	1-eicosatrienoylglycerophosphocholine
	lysolipid	1-heptadecanoylglycerophosphocholine
	lysolipid	1-linoleoylglycerophosphocholine
	lysolipid	1-linoleoylglycerophosphoethanolamine
	lysolipid	1-myristoylglycerophosphocholine
	lysolipid	1-oleoylglycerophosphocholine
	lysolipid	1-oleoylglycerophosphoethanolamine

continued

Metabolite Group	Metabolite Subgroup	Metabolite
Lipids	lysolipid	1-palmitoleoylglycerophosphocholine
	lysolipid	1-palmitoylglycerophosphocholine
	lysolipid	1-palmitoylglycerophosphoethanolamine
	lysolipid	1-palmitoylglycerophosphoinositol
	lysolipid	1-palmitoylplasmenylethanolamine
	lysolipid	1-pentadecanoylglycerophosphocholine
	lysolipid	1-stearoylglycerophosphocholine
	lysolipid	1-stearoylglycerophosphoethanolamine
	lysolipid	1-stearoylglycerophosphoinositol
	lysolipid	2-arachidonoylglycerophosphocholine
	lysolipid	2-arachidonoylglycerophosphoethanolamine
	lysolipid	2-linoleoylglycerophosphocholine
	lysolipid	2-linoleoylglycerophosphoethanolamine
	lysolipid	2-myristoylglycerophosphocholine
	lysolipid	2-oleoylglycerophosphocholine
	lysolipid	2-oleoylglycerophosphoethanolamine
	lysolipid	2-palmitoleoylglycerophosphocholine
	lysolipid	2-palmitoylglycerophosphocholine
	lysolipid	2-palmitoylglycerophosphoethanolamine
	lysolipid	2-stearoylglycerophosphocholine
	medium chain fatty acid	10-undecenoate (11:1n1)
	medium chain fatty acid	5-dodecenoate (12:1n7)
	medium chain fatty acid	caprate (10:0)
	medium chain fatty acid	caproate (6:0)
	medium chain fatty acid	caprylate (8:0)
	medium chain fatty acid	heptanoate (7:0)
	medium chain fatty acid	laurate (12:0)
	medium chain fatty acid	pelargonate (9:0)
	medium chain fatty acid	undecanoate (11:0)
	monoacylglycerol	1-oleoylglycerol (1-monoolein)
	monoacylglycerol	1-palmitoylglycerol (1-monopalmitin)
	monoacylglycerol	1-stearoylglycerol (1-monostearin)
	sterol/steroid	21-hydroxypregnenolone disulfate
	sterol/steroid	4-androsten-3beta,17beta-diol disulfate 1
	sterol/steroid	4-androsten-3beta,17beta-diol disulfate 2
	sterol/steroid	5alpha-androstan-3beta,17beta-diol disulfate
	sterol/steroid	5alpha-pregnan-3beta,20alpha-diol disulfate
	sterol/steroid	7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)

continued

Metabolite Group	Metabolite Subgroup	Metabolite
Lipids	sterol/steroid	andro steroid monosulfate 2
	sterol/steroid	androsterone sulfate
	sterol/steroid	corticosterone
	sterol/steroid	cortisol
	sterol/steroid	cortisone
	sterol/steroid	dehydroisoandrosterone sulfate (DHEA-S)
	sterol/steroid	epiandrosterone sulfate
	sterol/steroid	pregn steroid monosulfate
	sterol/steroid	pregnanediol-3-glucuronide
	sterol/steroid	pregnen-diol disulfate
	sterol/steroid	pregnenolone sulfate
Nucleotides	purine metabolism, (hypo)xanthine/inosine containing	hypoxanthine
	purine metabolism, adenine containing	adenosine 5'-monophosphate (AMP)
	purine metabolism, adenine containing	N1-methyladenosine
	purine metabolism, guanine containing	7-methylguanine
	purine metabolism, guanine containing	N6-carbamoylthreonyladenosine
	purine metabolism, urate metabolism	urate
	pyrimidine metabolism, cytidine containing	N4-acetylcytidine
	pyrimidine metabolism, uracil containing	5-methyluridine (ribothymidine)
	pyrimidine metabolism, uracil containing	pseudouridine
	pyrimidine metabolism, uracil containing	uridine
Peptides	dipeptide	phenylalanyltryptophan
	dipeptide	pro-hydroxy-pro
	fibrinogen cleavage peptide	DSGEGDFXAEGGGVR
	gamma-glutamyl	gamma-glutamylglutamine
	gamma-glutamyl	gamma-glutamylisoleucine
	gamma-glutamyl	gamma-glutamylleucine
	gamma-glutamyl	gamma-glutamylmethionine
	gamma-glutamyl	gamma-glutamylphenylalanine
	gamma-glutamyl	gamma-glutamyltyrosine
	gamma-glutamyl	gamma-glutamylvaline
	polypeptide	bradykinin
	polypeptide	bradykinin, des-arg(9)
	polypeptide	bradykinin, hydroxy-pro(3)

continued

Metabolite Group	Metabolite Subgroup	Metabolite
Xenobiotics	benzoate metabolism	3-hydroxyhippurate
	benzoate metabolism	4-hydroxyhippurate
	benzoate metabolism	4-methylcatechol sulfate
	benzoate metabolism	4-vinylphenol sulfate
	benzoate metabolism	catechol sulfate
	benzoate metabolism	hippurate
	chemical	2-ethylhexanoate (isobar with 2-propylpentanoate)
	drug	4-acetaminophen sulfate
	drug	p-acetamidophenylglucuronide
	edta	EDTA
	food component/plant	piperine
	food component/plant	stachydrine
	food component/plant	thymol sulfate
	xanthine metabolism	1-methylxanthine
	xanthine metabolism	3-methylxanthine
	xanthine metabolism	caffeine
	xanthine metabolism	paraxanthine
	xanthine metabolism	theobromine
	xanthine metabolism	theophylline

SUPPLEMENTARY TABLE 3: LD blocks (r2 = 1.0) of lead SNP rs2014355 according to different public data bases Hap Map release 22 and 1,000 Genome Pilot 1: CEU (Utah residents with acestry from northern and western Europe) population, data obtained from SNAP (Broad institute) (Johnson et al. 2008). 1,000 Genome Phase 1: European Population, data obtained from HaploReg (Broad institute) (Ward and Kellis 2012); Chr, chromosome; MAF, minor allele frequency; UTR, untranslated region.

	tag SNP	proxy SNP	Chr	Position	MAF	type of SNP	Nearest Gene(s)
HapMap release 22 CEU data	rs2014355	rs3916	12	119661655 [GRCh36/hg18]	0.25	3'-UTR	ACADS
		rs3999408	12	119651770 [GRCh36/hg18]	0.25	INTRONIC	ACADS
		rs2066938	12	119644998 [GRCh36/hg18]	0.25	INTRONIC	UNC119B
		rs7306541	12	119614429 [GRCh36/hg18]	0.25	INTRONIC	KIAA0152
1,000 Genome Pilot 1 CEU data	rs2014355	rs1799958	12	119660466 [GRCh36/hg18]	0.242	**MISSENCE**	ACADS
		rs3916	12	119661655 [GRCh36/hg18]	0.242	3'-UTR	ACADS
		rs34708625	12	119664402 [GRCh36/hg18]	0.242	DOWNSTREAM	N/A
1,000 Genome Phase 1 European population	rs2014355	rs1799958	12	121176083 [GRCh37/hg19]	0.26	MISSENCE	ACADS
		rs3916	12	121177272 [GRCh37/hg19]	0.26	3'-UTR	ACADS
		rs34708625	12	121180019 [GRCh37/hg19]	0.26	N/A	RP11-173P15.7

SUPPLEMENTARY TABLE 4: Results of the logistic regression model for the top ten percent of metabolites measured with the targeted metabolomics approach in the partial study examining rs2014355 in the ACADS locus

Results are shown for baseline (after 12h overnight fast) and time-resolved metabolite levels during the fasting period and the OGTT; adj. wc., analysis adjusted for waist circumference; OGTT, oral glucose tolerance test; a detailed description of the metabolite abbrevations is given in SUPPLEMENTARY TABLE 1.

	Metabolite	C-index (adj. wc)	Metabolite	C-index (adj. wc)
study day 1	12 h fasting		Time-resolved analysis (fasting)	
	C4	**0.950** (0.969)	C4	**0.999** (0.978)
	SM C16:0	0.837 (0.906)	PC ae C42:0	**0.906** (0.914)
	C5 M DC	0.824 (0.908)	Methionine	0.883 (0.954)
	PC ae C44:5	0.817 (0.898)	PC ae C42:4	0.878 (0.899)
	NEFA	0.808 (0.933)	C16:1 OH	0.877 (0.892)
	PC aa C32:0	0.788 (0.940)	Ornithine	0.875 (0.874)
	lyso PC a C18:1	0.784 (0.847)	PC aa C40:2	0.872 (0.882)
	PC ae C44:6	0.776 (0.929)	PC ae C38:1	0.871 (0.931)
	SM C24:1	0.773 (0.878)	C16:1	0.859 (0.885)
study day 2	12 h fasting		Time-resolved analysis (OGTT)	
	C4	**0.969** (0.970)	C4	**0.964** (0.976)
	Serine	0.866 (0.915)	lysoPC a C20:4	**0.917** (0.926)
	Creatinine	0.860 (0.912)	Glutamine	**0.909** (0.912)
	lyso PC a C28:0	0.775 (0.856)	lyso PC a C16:1	0.895 (0.915)
	lyso PC a C26:0	0.774 (0.861)	Spermidine	0.890 (0.905)
	SM C16:0	0.769 (0.896)	SM OH C24:1	0.867 (0.914)
	C10:2	0.754 (0.874)	Serine	0.865 (0.909)
	SM C24:1	0.745 (0.871)	lyso PC a C16:0	0.856 (0.924)
	lyso PC a C18:0	0.740 (0.901)	C5:1	0.854 (0.878)

SUPPLEMENTARY TABLE 5: Results of the logistic regression model with acylcarnitine metabolite ratios approximating enzyme activity of ß-oxidation enzymes in the partial study examining rs2014355 in the ACADS locus

Results are shown for baseline (after 12h overnight fast) and time-resolved metabolite levels during the fasting period; adj. wc., analysis adjusted for waist circumference; a detailed description of the metabolite abbrevations is given in SUPPLEMENTARY TABLE 1.

	Metabolite ratio	C-index (adj. wc)		Metabolite ratio	C-index (adj. wc.)	
study day 1	12 h fasting			Time-resolved analysis (fasting)		
	C3/C4	**0.967**	(1.000)	**C3/C4**	**0.999**	(1.000)
	C2/C4	**0.913**	(0.889)	**C2/C4**	**0.961**	(0.934)
	C2/C6..C4.1.DC.	0.668	(0.880)	C2/C6..C4.1.DC.	0.839	(0.882)
	C2/C8	0.559	(0.856)	C2/C8	0.746	(0.875)
	C2/C10	0.624	(0.856)	C2/C10	0.749	(0.858)
	C2/C12	0.542	(0.831)	C2/C12	0.675	(0.877)
	C2/C14	0.573	(0.887)	C2/C14	0.738	(0.897)
	C2/C16	0.500	(0.846)	C2/C16	0.758	(0.882)
	C2/C18	0.523	(0.828)	C2/C18	0.590	(0.850)
	C4/C6..C4.1.DC.	**0.969**	(0.988)	**C4/C6..C4.1.DC.**	**1.000**	(0.995)
	C6..C4.1.DC./C8	0.668	(0.856)	C6..C4.1.DC./C8	0.601	(0.813)
	C8/C10	0.605	(0.824)	C8/C10	0.595	(0.803)
	C10/C12	0.508	(0.852)	C10/C12	0.620	(0.842)
	C12/C14	0.505	(0.862)	C12/C14	0.628	(0.862)
	C14/C16	0.622	(0.856)	C14/C16	0.634	(0.821)
	C16/C18	0.502	(0.836)	C16/C18	0.627	(0.819)

SUPPLEMENTARY TABLE 6: LD blocks (r2 = 1.0) of lead SNP rs174547 according to different public data bases. Hap Map release 22 and 1,000 Genome Pilot 1: CEU (Utah residents with acestry from northern and western Europe) population, data obtained from SNAP (Broad institute) (Johnson et al. 2008). 1,000 Genome Phase 1: European Population, data obtained from HaploReg (Broad institute) (Ward and Kellis 2012). Chr, chromosome; MAF, minor allele frequency; UTR, untranslated region.

	tag SNP	proxy SNP	Chr	Position	MAF	type of SNP	Nearest Gene(s)
HapMap release 22 CEU data	rs174547	rs174550	11	61328054 [GRCh36/hg18]	0.367	INTRONIC	FADS1,FADS3
		rs174546	11	61326406 [GRCh36/hg18]	0.367	3'-UTR	FADS1,FADS3
		rs174545	11	61325882 [GRCh36/hg18]	0.367	3'-UTR	FADS1,FADS3
		rs102275	11	61314379 [GRCh36/hg18]	0.367	INTRONIC	C11orf10
		rs174537	11	61309256 [GRCh36/hg18]	0.367	INTRONIC	C11orf9
		rs174536	11	61308503 [GRCh36/hg18]	0.367	INTRONIC	C11orf9
		rs174535	11	61307932 [GRCh36/hg18]	0.367	SYNONYMOUS CODING	C11orf9
		rs1535	11	61354548 [GRCh36/hg18]	0.367	INTRONIC	FADS1,FADS3,FADS2
		rs174574	11	61356918 [GRCh36/hg18]	0.367	INTRONIC	FADS1,FADS3,FADS2
		rs174576	11	61360086 [GRCh36/hg18]	0.367	INTRONIC	FADS1,FADS3,FADS2
		rs174577	11	61361390 [GRCh36/hg18]	0.367	INTRONIC	FADS1,FADS3,FADS2
		rs174578	11	61362075 [GRCh36/hg18]	0.367	INTRONIC	FADS1,FADS3,FADS2
		rs174583	11	61366326 [GRCh36/hg18]	0.367	INTRONIC	FADS1,FADS3,FADS2
1,000 Genome Pilot 1 CEU data	rs174547						
1,000 Genome Phase 1 European population	rs174547	rs174545	11	61569306 [GRCh37/hg19]	0.36	3'-UTR	FADS1
		rs174546	11	61569830 [GRCh37/hg19]	0.36	3'-UTR	FADS1
		rs174547	11	61570783 [GRCh37/hg19]	0.36	INTRONIC	FADS1
		rs174550	11	61571478 [GRCh37/hg19]	0.36	INTRONIC	FADS1
		rs174553	11	61575158 [GRCh37/hg19]	0.36		FADS1
		rs174554	11	61579463 [GRCh37/hg19]	0.36		FADS1
		rs174562	11	61585144 [GRCh37/hg19]	0.36		FADS1

SUPPLEMENTARY TABLE 7: Results of the logistic regression model for the top ten percent of metabolites measured with the targeted metabolomics approach in the partial study examining rs174547 in the FADS1 locus

Results are shown for baseline (after 12h overnight fast) and time-resolved metabolite levels after the OLTT and the OGTT; OLTT, oral lipid tolerance test; OGTT, oral glucose tolerance test; a detailed description of the metabolite abbrevations are given in SUPPLEMENTARY TABLE 1.

	Metabolite	C-index	Metabolite	C-index
study day 1	12 h fasting		Time-resolved analysis (OLTT)	
	C9	0.520	SM C20:2	**0.903**
	PC aa C42:5	0.515	PC aa C36:1	0.896
	PC aa C40:3	0.509	lyso PC a C20:4	0.883
	total dimethylarginine	0.505	threonine	0.881
	C16:2 OH	0.504	leucine	0.875
	PC aa C40:6	0.504	PC aa C36:3	0.853
	PC aa C42:2	0.503	PC ae C36:3	0.849
	proline	0.503	tyrosine	0.847
	C14:2 OH	0.503	PC aa C34:1	0.843
study day 2	12 h fasting		Time-resolved analysis (OGTT)	
	C6:1	0.513	lyso PC a C20:4	**0.900**
	lyso PC a C28:1	0.510	PC aa C38:4	0.869
	PC ae C34:0	0.509	C5 OH C3 DC.M.	0.857
	alpha-aminoadipic acid	0.508	PC aa C36:4	0.852
	C3 OH	0.508	C10:1	0.838
	lyso PC a C24:0	0.506	C12	0.833
	C18:1	0.505	PC aa C36:5	0.832
	SM OH C16:1	0.504	lyso PC a C26:1	0.827
	C10:2	0.504	C5:1	0.822

SUPPLEMENTARY TABLE 8: Metabolite ratios calculated for studying the gene variant rs174547 in the *FADS1* locus

	Numerator	Denominator
	lysoPC.a.C16:0	lysoPC.a.C20:4
	lysoPC.a.C18:0	lysoPC.a.C20:4
	lysoPC.a.C18:1	lysoPC.a.C20:4
	lysoPC.a.C18:2	lysoPC.a.C20:4
	lysoPC.a.C20:4	lysoPC.a.C16:0
	lysoPC.a.C20:4	lysoPC.a.C18:1
	lysoPC.a.C20:4	lysoPC.a.C18:2
	lysoPC.a.C20:4	lysoPC.a.C20:3
	PC.aa.C32:2	PC.aa.C38:4
	PC.aa.C34:1	PC.aa.C36:4
	PC.aa.C34:1	PC.aa.C38:4
	PC.aa.C34:2	PC.aa.C36:4
	PC.aa.C34:2	PC.aa.C38:4
	PC.aa.C34:2	PC.aa.C38:5
	PC.aa.C34:4	PC.aa.C34:3
	PC.aa.C36:1	PC.aa.C36:4
	PC.aa.C36:1	PC.aa.C38:4
	PC.aa.C36:2	lysoPC.a.C20:4
	PC.aa.C36:2	PC.aa.C36:4
	PC.aa.C36:2	PC.aa.C38:4
	PC.aa.C36:2	PC.aa.C38:5
	PC.aa.C36:3	lysoPC.a.C20:4
	PC.aa.C36:3	PC.aa.C36:4
	PC.aa.C36:3	PC.aa.C38:4
	PC.aa.C36:3	PC.aa.C38:5
	PC.aa.C36:4	PC.aa.C34:3
	PC.aa.C36:4	PC.aa.C34:1
	PC.aa.C36:4	PC.aa.C34:2
	PC.aa.C36:4	PC.aa.C36:2
	PC.aa.C36:4	PC.aa.C36:3
	PC.aa.C36:5	PC.aa.C.34:3
	PC.aa.C36:5	PC.aa.C.34:4
	PC.aa.C36:5	PC.aa.C.36:4
	PC.aa.C38:3	PC.aa.C38:4
	PC.aa.C38:4	PC.aa.C34:3
	PC.aa.C38:4	PC.aa.C34:2

continued

Numerator	Denominator
PC.aa.C38:4	PC.aa.C36:1
PC.aa.C38:4	PC.aa.C36:2
PC.aa.C38:4	PC.aa.C36:3
PC.aa.C38:4	PC.aa.C38:3
PC.aa.C38:5	PC.aa.C.34:3
PC.aa.C38:5	PC.aa.C.34:4
PC.aa.C38:5	PC.aa.C.36:4
PC.aa.C40:4	PC.aa.C34:2
PC.aa.C40:4	PC.aa.C34:3
PC.aa.C40:4	PC.aa.C36:3
PC.aa.C40:5	PC.aa.C.34:4
PC.aa.C40:5	PC.aa.C.36:4
PC.aa.C40:5	PC.aa.C34:2
PC.aa.C40:5	PC.aa.C34:3
PC.aa.C40:5	PC.aa.C36:3
PC.aa.C40:6	PC.aa.C.34:3
PC.aa.C40:6	PC.aa.C.34:4
PC.aa.C40:6	PC.aa.C.36:4
PC.ae.C36:2	PC.ae.C38:4
PC.ae.C36:3	lysoPC.a.C20:4
PC.ae.C36:3	PC.aa.C36:4
PC.ae.C36:3	PC.aa.C38:4
PC.ae.C36:3	PC.ae.C36:4
PC.ae.C36:3	PC.ae.C36:5
PC.ae.C36:3	PC.ae.C38:4
PC.ae.C36:3	PC.ae.C38:5
PC.ae.C36:4	PC.ae.C34:2
PC.ae.C36:4	PC.ae.C34:3
PC.ae.C36:4	PC.ae.C36:3
PC.ae.C36:5	PC.ae.C.34:3
PC.ae.C36:5	PC.ae.C.34:4
PC.ae.C36:5	PC.ae.C.36:4
PC.ae.C36:5	PC.ae.C36:3
PC.ae.C38:3	PC.aa.C38:4
PC.ae.C38:3	PC.ae.C38:4
PC.ae.C38:4	PC.ae.C34:2
PC.ae.C38:4	PC.ae.C34:3

continued

Numerator	Denominator
PC.ae.C38:4	PC.ae.C36:3
PC.ae.C38:4	PC.ae.C36:3
PC.ae.C38:4	PC.ae.C38:3
PC.ae.C38:5	PC.ae.C.34:3
PC.ae.C38:5	PC.ae.C.34:4
PC.ae.C38:5	PC.ae.C.36:4
PC.ae.C38:5	PC.ae.C36:3
PC.ae.C40:4	PC.ae.C34:2
PC.ae.C40:4	PC.ae.C34:3
PC.ae.C40:4	PC.ae.C36:3
PC.ae.C40:5	PC.ae.C.34:3
PC.ae.C40:5	PC.ae.C.34:4
PC.ae.C40:5	PC.ae.C.36:4
PC.ae.C40:5	PC.ae.C34:2
PC.ae.C40:5	PC.ae.C36:3
PC.ae.C40:6	PC.ae.C.34:3
PC.ae.C40:6	PC.ae.C.34:4
PC.ae.C40:6	PC.ae.C.36:4
SM.C16:0	SM.C16:1
SM.C18:0	SM.C18:1
SM.C24:1	PC.aa.C38:4

SUPPLEMENTARY TABLE 9: Highly distinctive metabolite ratios (metabolites measured with the targeted metabolomics approach) for the respective allele of rs174547 (in the FADS1 locus) calculated with logistic regression Results are shown for baseline (after 12h overnight fast) and time-resolved metabolite levels during the OLTT and the OGTT; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test; a detailed description of the metabolite abbrevations are given in SUPPLEMENTARY TABLE 1.

	Metabolite	C-index	Metabolite	C-index
study day 1	12 h fasting		Time-resolved analysis (OLTT)	
	lysoPC.a.C16.0 / lysoPC.a.C20.4	0.99358974	lysoPC.a.C16.0 / lysoPC.a.C20.4	1
	lysoPC.a.C20.4 / lysoPC.a.C16.0	0.99358974	lysoPC.a.C20.4 / lysoPC.a.C16.0	0.995
	PC.aa.C36.3 / PC.aa.C36.4	0.96620192	PC.aa.C38.4 / PC.aa.C34.2	0.98607372
	PC.aa.C36.4 / PC.aa.C36.3	0.96589744	PC.aa.C34.2 / PC.aa.C38.4	0.97950321
	PC.ae.C36.3 / lysoPC.a.C20.4	0.96262821	PC.aa.C36.4 / PC.aa.C34.2	0.97934295
	PC.aa.C36.4 / PC.aa.C34.2	0.96201923	PC.aa.C34.2 / PC.aa.C36.4	0.97862179
	PC.aa.C38.3 / PC.aa.C38.4	0.96057692	PC.ae.C38.3 / PC.ae.C38.4	0.97102564
	lysoPC.a.C18.0 / lysoPC.a.C20.4	0.95950321	PC.aa.C36.1 / PC.aa.C36.4	0.97032051
	PC.aa.C34.2 / PC.aa.C36.4	0.95940705	PC.ae.C36.3 / PC.ae.C38.4	0.96418269
	PC.aa.C38.4 / PC.aa.C38.3	0.95767628	lysoPC.a.C18.1 / lysoPC.a.C20.4	0.96240385
	PC.aa.C34.1 / PC.aa.C36.4	0.95475962	PC.ae.C38.4 / PC.ae.C36.3	0.96224359
	PC.aa.C36.3 / lysoPC.a.C20.4	0.954375	PC.aa.C38.4 / PC.aa.C36.2	0.96179487
	PC.aa.C36.4 / PC.aa.C34.1	0.95285256	PC.ae.C38.4 / PC.ae.C38.3	0.96129808
	PC.aa.C36.3 / PC.aa.C38.4	0.95224359	PC.aa.C38.4 / PC.aa.C36.1	0.95831731
	PC.ae.C38.5 / PC.ae.C36.3	0.95094551	lysoPC.a.C20.4 / lysoPC.a.C18.1	0.95684295
	PC.aa.C38.4 / PC.aa.C36.3	0.95052885	PC.aa.C34.1 / PC.aa.C38.4	0.94913462
	PC.ae.C40.5 / PC.ae.C36.3	0.9500641	PC.aa.C36.1 / PC.aa.C38.4	0.94884615
	lysoPC.a.C20.4 / lysoPC.a.C18.2	0.94987179	PC.aa.C36.4 / PC.aa.C36.3	0.94878205
	PC.ae.C36.3 / PC.ae.C36.5	0.94875	PC.aa.C36.2 / PC.aa.C38.4	0.94834936
	PC.ae.C36.3 / PC.ae.C38.5	0.94870192	PC.aa.C36.3 / PC.aa.C36.4	0.94738782
	PC.aa.C34.2 / PC.aa.C38.5	0.94854167	PC.aa.C36.3 / lysoPC.a.C20.4	0.94719551
	PC.ae.C38.4 / PC.ae.C36.3	0.9481891	PC.aa.C38.3 / PC.aa.C38.4	0.94613782
	lysoPC.a.C20.4 / lysoPC.a.C20.3	0.94722756	PC.ae.C36.3 / lysoPC.a.C20.4	0.94512821
	PC.ae.C36.5 / PC.ae.C36.3	0.94682692	PC.aa.C36.2 / lysoPC.a.C20.4	0.94129808
	lysoPC.a.C18.1 / lysoPC.a.C20.4	0.94536859	PC.ae.C36.3 / PC.aa.C36.4	0.94028846
	PC.ae.C36.3 / PC.ae.C38.4	0.94453526	PC.ae.C40.5 / PC.ae.C36.3	0.94009615
	lysoPC.a.C18.2 / lysoPC.a.C20.4	0.94421474	PC.aa.C38.4 / PC.aa.C38.3	0.93849359
	PC.ae.C36.3 / PC.aa.C36.4	0.94410256	PC.aa.C36.3 / PC.aa.C38.4	0.93679487
	lysoPC.a.C20.4 / lysoPC.a.C18.1	0.94310897	PC.ae.C38.4 / PC.ae.C34.2	0.93605769
	PC.ae.C38.4 / PC.ae.C34.2	0.94163462	PC.aa.C38.4 / PC.aa.C36.3	0.93386218
	PC.aa.C34.2 / PC.aa.C38.4	0.9380609	PC.ae.C40.4 / PC.ae.C36.3	0.93134615

continued

	Metabolite	C-index	Metabolite	C-index
study day 1	12 h fasting		Time-resolved analysis (OLTT)	
	PC.aa.C38.4 / PC.aa.C34.2	0.93798077	PC.aa.C34.2 / PC.aa.C38.5	0.9305609
	PC.aa.C36.3 / PC.aa.C38.5	0.93778846	lysoPC.a.C18.0 / lysoPC.a.C20.4	0.92921474
	PC.aa.C38.4 / PC.aa.C36.1	0.9369391	PC.aa.C38.5 / PC.aa.C34.3	0.92919872
	PC.aa.C36.1 / PC.aa.C38.4	0.93552885	PC.aa.C34.1 / PC.aa.C36.4	0.92900641
	PC.ae.C36.3 / PC.aa.C38.4	0.93317308	PC.aa.C36.4 / PC.aa.C34.1	0.92823718
	PC.aa.C36.1 / PC.aa.C36.4	0.93283654	PC.aa.C36.5 / PC.aa.C34.3	0.92793269
	PC.aa.C36.2 / lysoPC.a.C20.4	0.93262821	PC.ae.C36.5 / PC.ae.C36.3	0.92709936
	PC.aa.C36.5 / PC.aa.C34.3	0.92525641	PC.ae.C36.3 / PC.ae.C36.5	0.92564103
	PC.aa.C36.2 / PC.aa.C38.4	0.92024038	lysoPC.a.C18.2 / lysoPC.a.C20.4	0.92310897
	PC.ae.C40.4 / PC.ae.C36.3	0.91985577	lysoPC.a.C20.4 / lysoPC.a.C18.2	0.92145833
	PC.aa.C38.4 / PC.aa.C36.2	0.91798077	PC.ae.C36.3 / PC.ae.C38.5	0.92108974
	PC.aa.C36.2 / PC.aa.C38.5	0.91464744	PC.aa.C36.4 / PC.aa.C36.2	0.91932692
	PC.aa.C34.1 / PC.aa.C38.4	0.90774038	PC.ae.C38.5 / PC.ae.C36.3	0.91600962
	PC.ae.C40.5 / PC.ae.C34.2	0.90314103	PC.aa.C36.2 / PC.aa.C36.4	0.91378205
	PC.aa.C36.4 / PC.aa.C36.2	0.90192308	PC.aa.C36.3 / PC.aa.C38.5	0.91238782
	PC.aa.C36.2 / PC.aa.C36.4	0.90144231	PC.aa.C38.4 / PC.aa.C34.3	0.90866987
	PC.aa.C36.4 / PC.aa.C34.3	0.90116987	lysoPC.a.C20.4 / lysoPC.a.C20.3	0.90375
	SM.C18.0 / SM.C18.1	0.90099359	PC.aa.C40.5 / PC.aa.C34.3	0.90248397

	Metabolite	C-index	Metabolite	C-index
study day 2	12 h fasting		Time-resolved analysis (OGTT)	
	lysoPC.a.C18.2 / lysoPC.a.C20.4	1	lysoPC.a.C18.2 / lysoPC.a.C20.4	1
	lysoPC.a.C20.4 / lysoPC.a.C18.2	1	lysoPC.a.C20.4 / lysoPC.a.C18.2	1
	PC.aa.C34.2 / PC.aa.C36.4	1	PC.aa.C34.2 / PC.aa.C38.4	1
	PC.aa.C34.2 / PC.aa.C38.4	1	PC.aa.C36.2 / PC.aa.C38.4	1
	PC.aa.C34.2 / PC.aa.C38.5	1	PC.aa.C38.4 / PC.aa.C36.2	1
	PC.aa.C36.2 / PC.aa.C38.4	1	PC.aa.C36.4 / PC.aa.C34.2	0.99980769
	PC.aa.C36.4 / PC.aa.C34.2	1	PC.aa.C34.2 / PC.aa.C36.4	0.99977273
	PC.aa.C38.4 / PC.aa.C34.2	1	PC.aa.C38.4 / PC.aa.C34.2	0.99807692
	PC.aa.C38.4 / PC.aa.C36.2	1	PC.aa.C34.2 / PC.aa.C38.5	0.99272727
	PC.aa.C38.5 / PC.aa.C34.3	0.97961538	PC.aa.C38.5 / PC.aa.C34.3	0.9923951

continued

	Metabolite	C-index	Metabolite	C-index
study day 2	12 h fasting		Time-resolved analysis (OGTT)	
	lysoPC.a.C20.4 / lysoPC.a.C16.0	0.97692308	PC.aa.C36.4 / PC.aa.C36.3	0.97
	lysoPC.a.C16.0 / lysoPC.a.C20.4	0.97552448	PC.aa.C36.3 / PC.aa.C38.5	0.96466783
	PC.aa.C36.3 / PC.aa.C36.4	0.97447552	lysoPC.a.C20.4 / lysoPC.a.C18.1	0.96391608
	PC.aa.C36.4 / PC.aa.C36.3	0.97444056	PC.aa.C36.2 / PC.aa.C36.4	0.96283217
	PC.aa.C36.3 / PC.aa.C38.5	0.97370629	PC.aa.C36.3 / PC.aa.C36.4	0.96117133
	PC.ae.C36.3 / PC.aa.C36.4	0.97325175	PC.aa.C36.4 / PC.aa.C36.2	0.95994755
	PC.aa.C36.2 / PC.aa.C36.4	0.97173077	PC.aa.C38.4 / PC.aa.C36.3	0.95961538
	PC.aa.C38.4 / PC.aa.C36.3	0.96965035	PC.aa.C36.3 / PC.aa.C38.4	0.95729021
	PC.aa.C36.5 / PC.aa.C34.3	0.96837413	PC.aa.C36.5 / PC.aa.C34.3	0.95659091
	PC.aa.C36.4 / PC.aa.C36.2	0.96776224	lysoPC.a.C18.1 / lysoPC.a.C20.4	0.95592657
	PC.aa.C36.3 / PC.aa.C38.4	0.96732517	PC.ae.C38.4 / PC.ae.C34.2	0.95472028
	PC.ae.C36.5 / PC.ae.C36.3	0.96407343	PC.aa.C40.5 / PC.aa.C34.3	0.94734266
	PC.ae.C36.3 / PC.ae.C36.5	0.96256993	lysoPC.a.C20.4 / lysoPC.a.C16.0	0.94713287
	PC.aa.C38.4 / PC.aa.C38.3	0.95994755	lysoPC.a.C16.0 / lysoPC.a.C20.4	0.94541958
	PC.ae.C38.4 / PC.ae.C34.2	0.95984266	lysoPC.a.C18.0 / lysoPC.a.C20.4	0.93996503
	PC.ae.C36.3 / PC.aa.C38.4	0.95975524	PC.ae.C36.3 / PC.aa.C36.4	0.93952797
	lysoPC.a.C18.0 / lysoPC.a.C20.4	0.9593007	PC.aa.C38.4 / PC.aa.C36.1	0.9377972
	PC.aa.C38.3 / PC.aa.C38.4	0.95893357	PC.aa.C36.1 / PC.aa.C38.4	0.93730769
	PC.ae.C38.4 / PC.ae.C36.3	0.95715035	PC.ae.C36.5 / PC.ae.C36.3	0.93309441
	PC.ae.C36.3 / PC.ae.C38.4	0.95708042	PC.aa.C34.1 / PC.aa.C38.4	0.9320979
	PC.aa.C36.2 / PC.aa.C38.5	0.95676573	PC.aa.C34.4 / PC.aa.C34.3	0.93188811
	lysoPC.a.C18.1 / lysoPC.a.C20.4	0.95302448	PC.aa.C36.5 / PC.aa.C34.4	0.92933566
	PC.aa.C38.4 / PC.aa.C36.1	0.95152098	PC.aa.C36.2 / PC.aa.C38.5	0.92844406
	PC.aa.C36.1 / PC.aa.C38.4	0.95106643	SM.C24.1 / PC.aa.C38.4	0.92793706
	lysoPC.a.C20.4 / lysoPC.a.C18.1	0.95043706	PC.ae.C36.3 / PC.aa.C38.4	0.92697552
	SM.C18.0 / SM.C18.1	0.95013986	PC.ae.C36.3 / PC.ae.C38.4	0.92631119
	PC.aa.C36.4 / PC.aa.C34.3	0.94980769	PC.aa.C36.4 / PC.aa.C34.3	0.92503497
	PC.aa.C38.4 / PC.aa.C34.3	0.94564685	PC.aa.C34.1 / PC.aa.C36.4	0.9248951
	PC.aa.C34.1 / PC.aa.C38.4	0.94188811	PC.aa.C38.4 / PC.aa.C38.3	0.92479021
	PC.ae.C38.5 / PC.ae.C36.3	0.9413986	PC.ae.C38.4 / PC.ae.C36.3	0.92451049
	PC.aa.C40.5 / PC.aa.C34.3	0.93923077	PC.aa.C36.4 / PC.aa.C34.1	0.92388112
	PC.aa.C36.4 / PC.aa.C34.1	0.93881119	PC.aa.C36.1 / PC.aa.C36.4	0.92368881
	PC.aa.C34.1 / PC.aa.C36.4	0.93622378	PC.ae.C36.3 / PC.ae.C36.5	0.9220979

continued

	Metabolite	C-index	Metabolite	C-index
study day 2	12 h fasting		Time-resolved analysis (OGTT)	
	SM.C24.1 / PC.aa.C38.4	0.93393357	PC.aa.C38.3 / PC.aa.C38.4	0.91891608
	PC.ae.C36.3 / PC.ae.C38.5	0.93361888	PC.aa.C40.5 / PC.aa.C36.3	0.91758741
	PC.aa.C40.5 / PC.aa.C36.3	0.93328671	PC.ae.C40.5 / PC.ae.C34.2	0.91638112
	PC.ae.C40.5 / PC.ae.C34.2	0.93314685	SM.C18.0 / SM.C18.1	0.91636364
	PC.aa.C32.2 / PC.aa.C38.4	0.92882867	PC.aa.C38.4 / PC.aa.C34.3	0.91576923
	PC.aa.C34.4 / PC.aa.C34.3	0.92769231	PC.aa.C40.5 / PC.aa.C34.2	0.91195804
	lysoPC.a.C20.4 / lysoPC.a.C20.3	0.92756993	PC.ae.C38.5 / PC.ae.C36.3	0.9063986
	PC.aa.C36.5 / PC.aa.C34.4	0.92678322	PC.ae.C36.3 / PC.ae.C38.5	0.90342657
	PC.aa.C36.5 / PC.aa.C34.4	0.92678322	PC.ae.C38.3 / PC.aa.C38.4	0.90227273
	PC.aa.C36.2 / lysoPC.a.C20.4	0.92335664		
	PC.aa.C36.2 / lysoPC.a.C20.4	0.92335664		
	PC.aa.C40.5 / PC.aa.C34.2	0.91968531		
	PC.aa.C40.5 / PC.aa.C34.2	0.91968531		
	PC.ae.C36.3 / PC.ae.C36.4	0.9186014		
	PC.ae.C36.3 / PC.ae.C36.4	0.9186014		
	PC.aa.C36.3 / lysoPC.a.C20.4	0.91844406		
	PC.aa.C36.3 / lysoPC.a.C20.4	0.91844406		
	PC.ae.C36.4 / PC.ae.C36.3	0.91818182		
	PC.ae.C36.4 / PC.ae.C36.3	0.91818182		
	PC.ae.C40.5 / PC.ae.C36.3	0.91337413		
	PC.ae.C40.5 / PC.ae.C36.3	0.91337413		
	PC.aa.C36.1 / PC.aa.C36.4	0.91202797		
	PC.aa.C36.1 / PC.aa.C36.4	0.91202797		
	PC.ae.C36.3 / lysoPC.a.C20.4	0.90513986		
	PC.ae.C36.3 / lysoPC.a.C20.4	0.90513986		

SUPPLEMENTARY TEXT 1: Detailed description of the non-targeted metabolomic analysis

Non-targeted metabolomics profiles were measured using a previously described method of Metabolon Inc. (Durham, USA) (Evans et al. 2009; Boudonck et al. 2009). Plasma samples (100 µl) were pipetted into a 96 deep well plate. In addition to samples from this study, a pooled human reference plasma sample was extracted in 6 independent times per 96 well plate. These samples served as technical replicates throughout the data set to assess process variability. Besides the reference plasma samples, 100 µL of water was extracted in 5 independent times per 96 well plate to serve as process blanks.

Proteins were precipitated and metabolites were extracted from the samples with methanol containing 4 recovery standards, which allowed the monitoring of extraction efficiency. After centrifugation, the supernatant was split into four aliquots. The first two aliquots were used for LC/MS analysis in positive and negative electrospray ionization mode. Two further aliquots on a second 96 well plate were kept in reserve.

The samples were dried under nitrogen on a TurboVap 96 (Biotage). For LC/MS pos. ion mode samples were reconstituted with 50 µl of 0.1% formic acid and for neg. ion mode with 50 µl of 6.5 mM ammonium bicarbonate pH 8.0. Both reconstitution solvents contained internal standards that were used to monitor instrument performance and as retention index markers. LC/MS analysis was performed on a LTQ XL mass spectrometer (Thermo Fisher Scientific GmbH, Dreieich, Germany) equipped with a Waters Acquity UPLC system (Waters GmbH, Eschborn, Germany). Two separate columns (2.1 x 100 mm Waters BEH C18 1.7 µm particle) were used for acidic (solvent A: 0.1% formic acid in H2O, solvent B: 0.1% formic acid in methanol) and basic (A: 6.5 mM ammonium bicarbonate pH 8.0, B: 6.5 mM ammonium bicarbonate in 95% methanol) mobile phase conditions, optimized for positive and negative electrospray ionization, respectively. After injection of the sample extracts the columns were developed in a gradient of 99.5% A to 98% B in 11 min runtime at 350 µl/min flow rate. The eluent flow was directly connected to the ESI source of the LTQ XL mass spectrometer. The MS interface capillary was maintained at 350 °C, with a sheath gas flow of 35 (arbitrary units) and aux gas flow of 20 (arbitrary units) for both positive and negative injections. The spray voltage for the positive ion injection was 4.23 kV, and it was 4.0 kV for the negative ion injection. The instrument scanned was 80-1000 m/z and alternated between MS and MS/MS scans with dynamic exclusion technique which enables a wide range of metabolome coverage. The scan speed was approximately six scans per second (three MS and three MS/MS scans). The MS scan had an ion-trap target of 2 × 104 (arbitrary units) and an ion-trap fill time cutoff of 200 ms. The MS/MS scan had an ion-trap target of 8 × 103 (arbitrary units) and an ion-trap fill time cutoff of 100 ms. MS/MS normalized collision

energy was set to 30, activation Q 0.25, and activation time 30 ms, with a 3 m/z isolation window. Metabolites were identified by Metabolon from the LC/MS data by automated multiparametric comparison with a proprietary library, containing retention times, m/z ratios, and related adduct/fragment spectra. 265 known metabolites were identified in this study, as well as 136 compounds with unknown chemical structure, indicated by a X followed by a number as compound identifier. For each identified metabolite the raw area counts were normalized to the median value of the run day to correct for inter-day variation of the measurements.

PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS

Brennauer T, Kondofersky I, Ehlers K, Römisch-Margl W, Stückler F, Krumsiek J, Bangert A, Artati A, Prehn C, Adamski J, Kastenmüller G, Fuchs C, Theis FJ, Laumen H, Hauner H. Effect of dietary standardization on the plasma metabolomic response to a defined meal challenge in healthy individuals. (in preparation)

Brennauer T, Kondofersky I, Ehlers K, Römisch-Margl W, Stückler F, Krumsiek J, Bangert A, Artati A, Prehn C, Adamski J, Kastenmüller G, Fuchs C, Theis FJ, Laumen H, Hauner H. Comparative analysis of postprandial plasma metabolic changes to a fast-food meal and a healthy breakfast (in preparation).

Brennauer T, Kondofersky I, Skurk T, Ehlers K, Stückler F, Heier M, Meisinger C, Peters A, Prehn C, Römisch-Margl W, Adamski J, Kastenmüller G, Suhre K, Illig T, Fuchs C, Theis FJ, Grallert H, Laumen H, Hauner H. Characterization of the rs2014355 variant in the acetyl-CoA dehydrogenase short chain (*ACADS*) locus by time-resolved metabolomics in the anabolic and catabolic state (in preparation).

Ehlers K, Brand T, Bangert A, Hauner H, Laumen H. (2014). Postprandial activation of metabolic and inflammatory signalling pathways in human peripheral mononuclear cells. Br J Nutr, 28:1-9.

Wahl S, Krug S, Then C, Kirchhofer A, Kastenmüller G, Brand T, Skurk T, Claussnitzer M, Huth C, Heier M, Meisinger C, Peters A, Thorand B, Gieger C, Prehn C, Römisch-Margl W, Adamski J, Suhre K, Illig T, Grallert H, Laumen H, Seissler J, Hauner H. (2014). Comparative analysis of plasma metabolomics response to metabolic challenge tests in healthy subjects and influence of the FTO obesity risk allele. Metabolomics. DOI 10.1007/s11306-013-0586-x.

PRESENTATIONS

Update in the Field of Metabolic Syndrome, Obesity, Diabetes and Atherosclerosis; School of Medicine; University of Banja Luka; Bosnien and Herzegovina, 3rd July 2011

Talk on “Metabolomic response of subjects with gene variants in lipid metabolism to defined nutritional challenges.”

Summer School in Clinical Pharmacology and Nutritional Medicine; School of Medicine; University of Podgorica; Montenegro, 12rd September 2011

Talk on “Metabolomic response of subjects with gene variants in lipid metabolism to defined nutritional challenges.”

Systems Biology of Metabotypes (SysMBo) Abschlußkolloquium, 12rd December 2012

Talk on “Gene variants – functional human studies.”

POSTER-PRESENTATIONS

Wahl S, Krug S, Then C, Brand T, Ehlers K, Claussnitzer M, Skurk T, Lechner A, Wichmann H, Huth C, Meisinger C, Prehn C, Stückler F, Kastenmüller G, Adamski J, Suhre K, Illig T, Grallert H, Laumen H, Seissler J, Hauner H. Metabolic characterization of common variants of the FTO and TCF7L2 loci by nutritional challenge tests. Abstract/Poster P13.09. European Human Genetics Conference, Nürnberg, Germany 23-26 June 2012.

Ehlers K, Brand T, Bangert A, Hastreiter L, Skurk T, Adamski J, Halama A, Möller G, Suhre K, Römisch-Margl W, Kastenmüller G, Hauner H, Laumen H. Postprandial inflammatory and metabolic activation of PBMC: a subject of inter-individual differences. Abstract/Poster 87. EMBO/EMBL Symposium Diabetes and Obesity, Heidelberg, Germany, 13-16 September 2012.

Ehlers K, Brand T, Hastreiter L, Bangert A, Hauner H, Laumen H. Inflammatory and metabolic activation of PBMC: a subject of inter-individual differences. Abstract/Poster P30. 28. Jahrestagung der Deutschen Adipositas-Gesellschaft, Stuttgart, Germany, 4-6 October 2012.

Wahl S, Then C, Krug S, Brand T, Ehlers K, Claussnitzer M, Skurk T, Lechner A, Wichmann H.-E., Heier M, Huth C, Thorand B, Meisinger C, Prehn C, Stückler F, Kasternmüller G, Adamski J, Suhre K, Illig T, Grallert H, Laumen H, Hauner H, Seissler J. TCF7L2 and FTO-What can we learn from metabolic challenge tests. Abstract/Poster P93. 28. Jahrestagung der Deutschen Adipositas-Gesellschaft, Stuttgart, Germany 4-6 October 2012.

Brand T, Kondofersky I, Ehlers K, Römisch-Margl W, Stückler F, Krumsiek J, Bangert A, Artati A, Prehn C, Adamski J, Kastenmüller G, Fuchs C, Theis FJ, Laumen H, Hauner H. Time-resolved studies of postprandial metabolism: improvement by dietary standardization? Abstract/Poster 10/6. 10th NuGOweek Nutrigenomics & more, Freising-Weihenstephan, Germany, 9-12 September 2013.