PHENOTYPING THE EFFECTS OF NUTRIENT QUALITY ON METABOLIC HEALTH

starring the liver & adipose tissue

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Thesis

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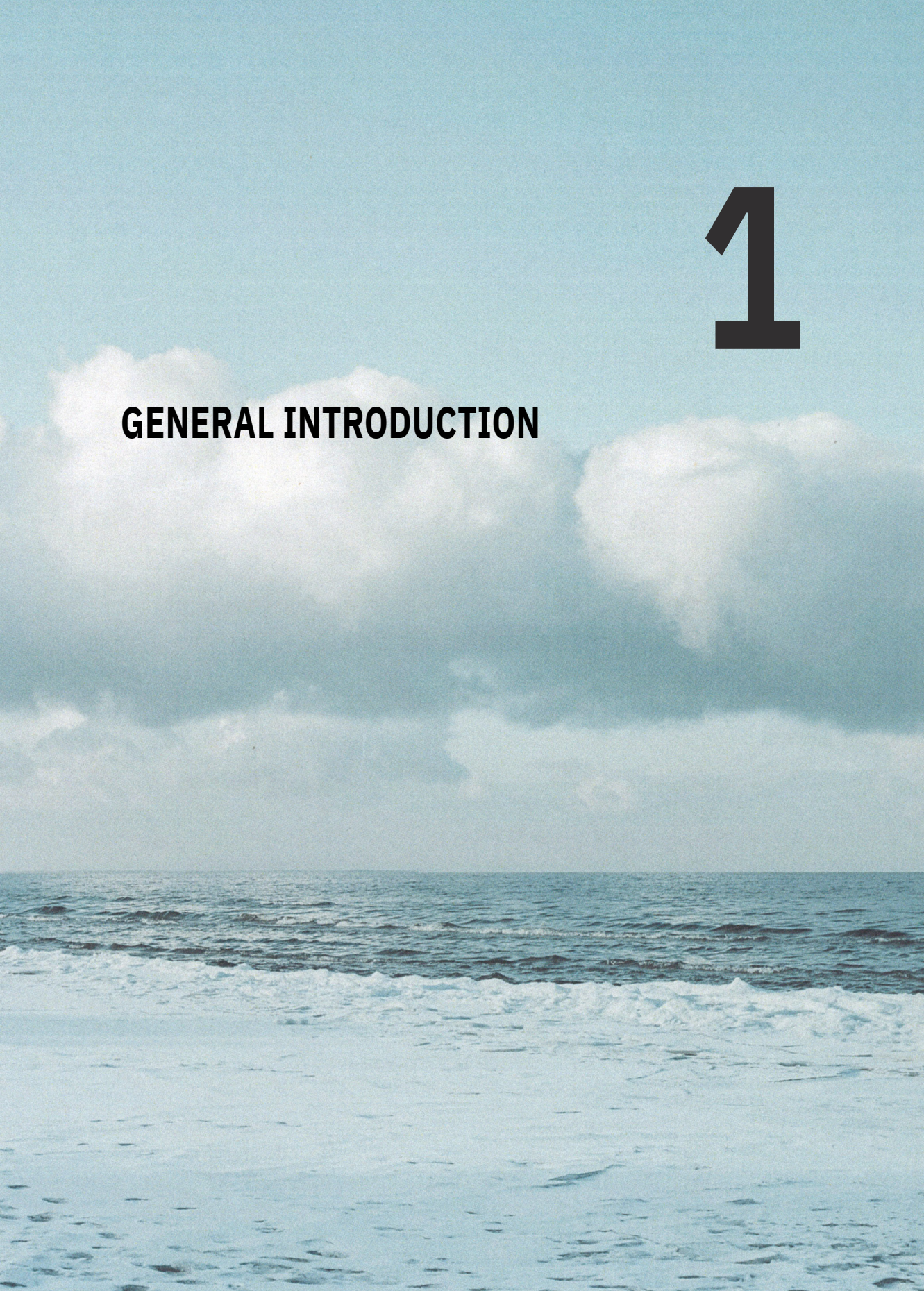

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GENERAL INTRODUCTION

Despite over 4500 years of nutrition research[1], poor diet remains one of the leading risk factors of ill health according to most recent Global Burden of Diseases, Injuries, and Risk Factors Study 2016[2]. While 11% of the world population was undernourished in 2016[3], many of the leading causes of death worldwide that year were related to risk factors associated with obesity, including high systolic blood pressure, insulin resistance, and high total cholesterol levels. These risk factors subsequently play a role in the development of chronic diseases such as non-alcoholic fatty liver disease (NAFLD), cardiovascular disease (CVD), and type 2 diabetes (T2DM)[4]. Consumption of Western-style diets contributes significantly to the development of obesity and its related health-risks. These diets typically include an overconsumption of energy-dense foods high in saturated fats and sugars and a low intake of whole grains, fruit, vegetables, and fish oils. To combat this growing burden of obesity-related pathologies, effective dietary strategies that improve metabolic health are needed more than ever.

LIVER AND ADIPOSE TISSUE; KEY PLAYERS IN METABOLIC HEALTH

Chronic diet-related diseases are the result of perturbations in different metabolic organs and/or their elegant organ interplay. Important metabolic organs in this respect are the liver and the adipose tissue. Both organs are central in energy metabolism and crucial in regulating nutrient fluxes (**Figure 1**). Especially in lipid metabolism, the adipose tissue and liver in concert exert key actions in buffering lipid fluxes, thereby regulating supply to other organs as well as the lipid profile in the blood. Dietary triglycerides, packed in intestinal-derived ApoB48-chylomicrons, are hydrolyzed by the actions of lipoprotein lipases (LPL) allowing the release of non-esterified fatty acids (NEFA) to tissues. This occurs mainly in the adipose tissues but also in other peripheral organs such as skeletal muscle. The actions of LPL can be inhibited by the protein angiopoietin-like 4 (ANGPTL4)[5], which blunts triglyceride clearance and thus raises triglycerides levels in the blood. This mechanism is mainly demonstrated in animal studies. ANGPTL4 is expressed in human liver and adipose tissue but less is known about its function and its contribution to plasma triglycerides in humans.

Fatty acids are mainly stored in the form of triglycerides in adipose tissue. These fatty acids can be readily liberated from the tissue when energy demands increase through the actions of the enzyme hormone sensitive lipase (HSL). In the liver, fatty acids are re-esterified to triglycerides. These triglycerides can either be stored in the liver, assembled into ApoB100-containing very low density lipoproteins (VLDL) and secreted in the circulation, or hydrolyzed again to fatty acids and shuttled to the mitochondria for oxidation. In addition, the liver can produce additional triglycerides from non-lipid precursors, such as carbohydrates, through the process of *de novo* lipogenesis[6]. The triglycerides that are secreted to the circulation in the form of VLDL are gradually

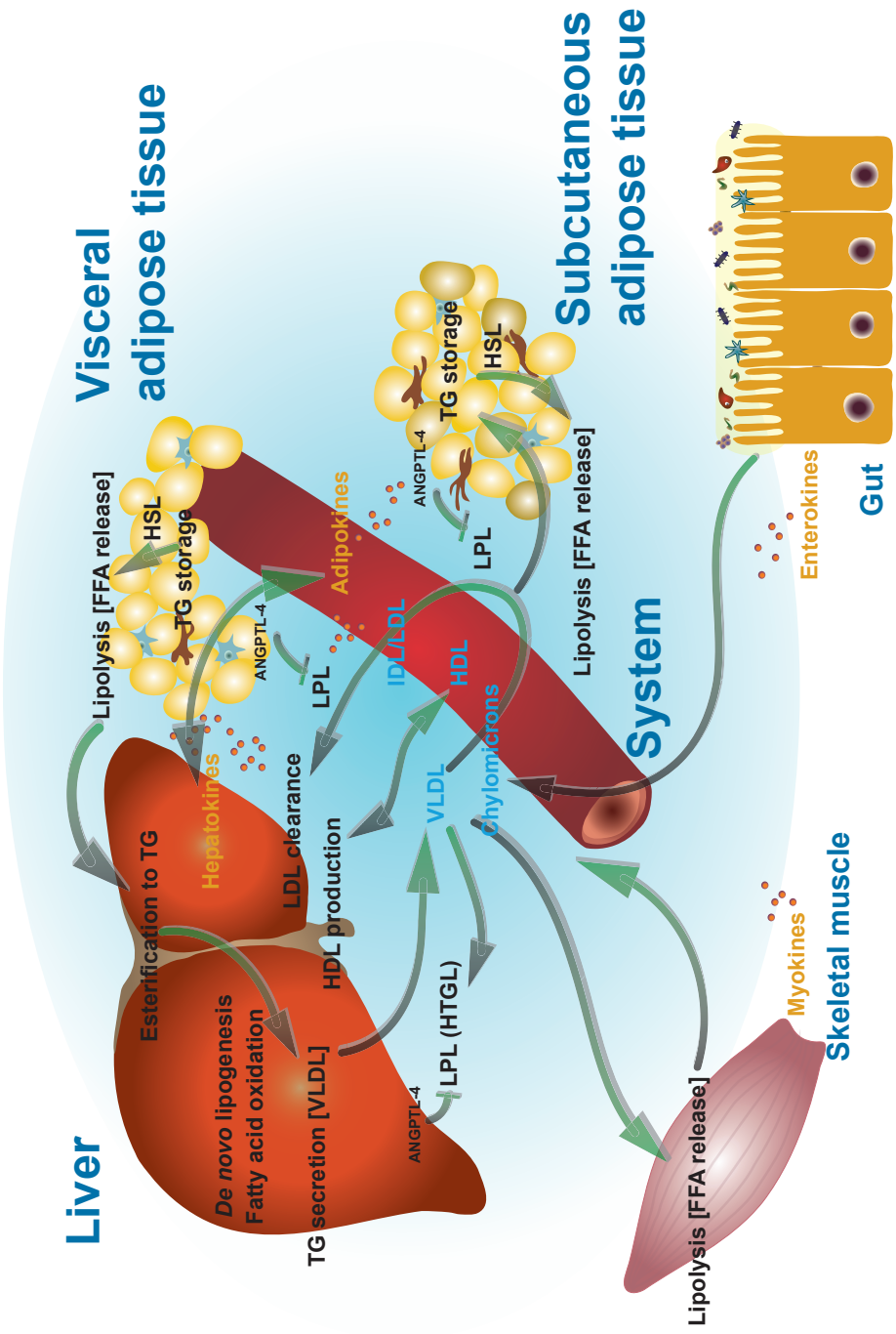

Figure 1. Crosstalk between metabolic organs in lipid metabolism, focused on the liver and adipose tissue

removed from this particle by peripheral tissues such as the adipose tissue. As a result of this triglyceride-clearance process, the particle contains relatively less triglycerides and more cholesteryl esters which lowers its overall density and results in the formation of intermediate density lipoproteins (IDL) and eventually low density lipoproteins (LDL). LDL particles contain a high proportion of cholesteryl esters and are thus responsible for cholesterol transport to tissues. The particles are eventually cleared by the liver. A reversed pathway for cholesterol transport, i.e. from tissues back to the liver, is arranged by the liver through the formation of ApoA1-containing high density lipoproteins (HDL). These small particles take up cholesterol from peripheral tissues and are subsequently transported to and cleared by the liver. In the liver, cholesterol is used for several process including bile acid synthesis. Both the liver and adipose tissue also serve important endocrine functions by secreting hormone-like factors, i.e. hepatokines and adipokines, that are important signaling molecules in the liver-adipose tissue crosstalk. As both organs are sensitive to dietary changes and strongly affected by weight gain, they might be important target organs for nutritional interventions.

Figure 2 visualizes several metabolic disturbances in the liver and adipose tissue and their interaction observed in obesity. These disturbances are critical in the pathogenesis of many diet-related chronic diseases such as T2DM, NAFLD, and CVD.

Adipose tissue

Excess energy intake requires an increase in the lipid storage capacity of both the subcutaneous as well as the visceral adipose tissue. This can be achieved by hyperplasia through the process of adipogenesis as well as by hypertrophy of preexisting adipocytes. In adults, hypertrophy is more dominant than hyperplasia[7] although adipose tissue depot-specific differences and gender-dimorphisms exist. In general, hyperplasia is more present in subcutaneous depots when compared to visceral depots such as omental fat and more dominant in women when compared to men[8]. The adipose tissue expanding processes demand rapid adaptations in extracellular matrix (ECM) remodeling and vascularization. Impaired ECM dynamics, such as an increase in collagen fibers and a decrease in the activity of matrix metalloproteinases, and inadequate angiogenesis limit proper adipocyte growth and oxygen supply[9]. This will result in fibrosis and hypoxia and subsequently in oxidative stress and the presence of mediators that attract immune cells. In addition, adipocyte hypertrophy and subsequent hypoxia is associated with increased cell death, which requires an influx of cells primarily related to innate immunity, predominantly macrophages to phagocytize dead adipocytes. While this action is in essence part of a normal physiological response, adipose tissue macrophages (ATMs) observed in expanding adipose tissue exhibit a

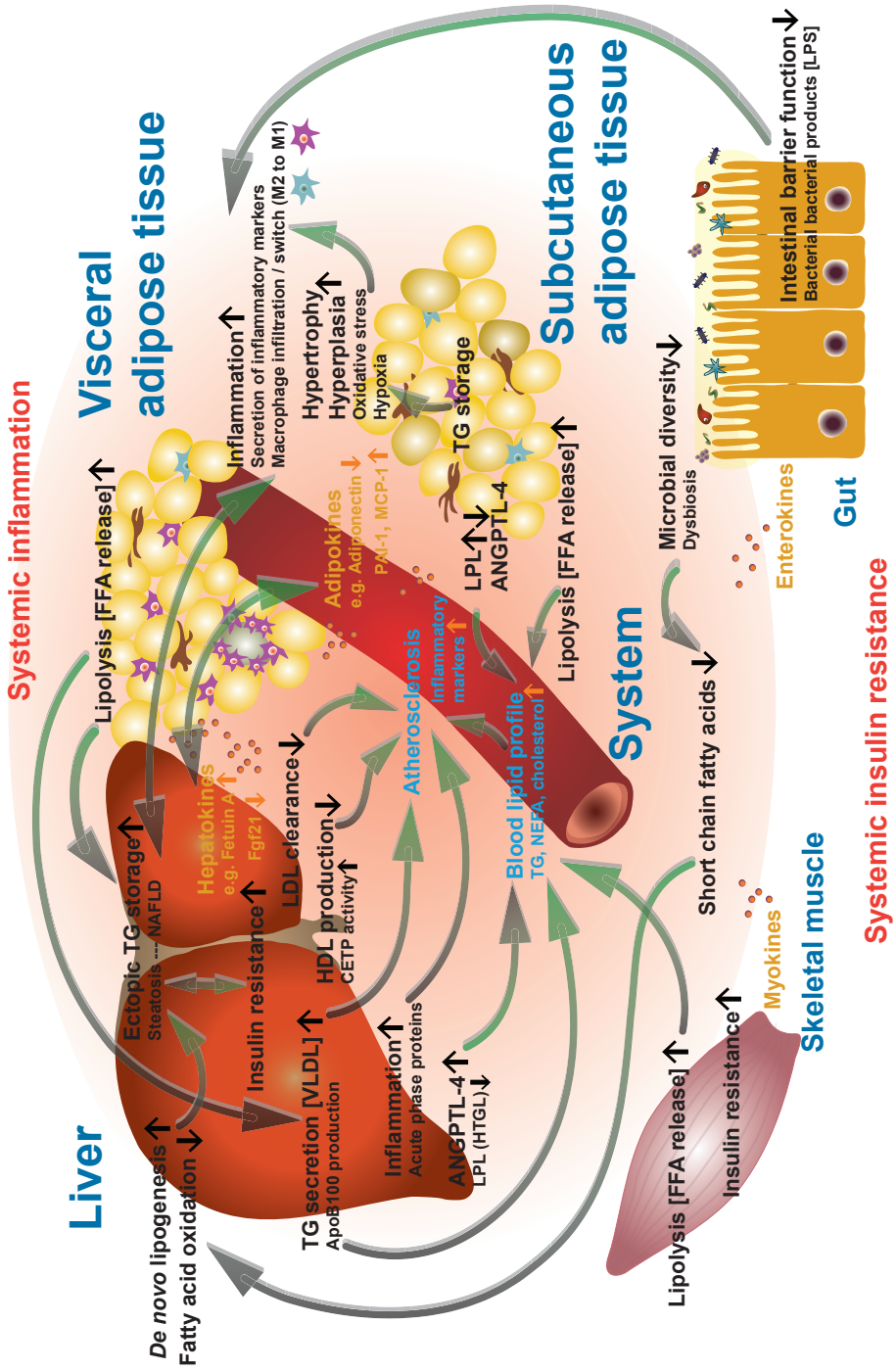

Figure 2. Metabolic dysregulation in metabolic organs, focused on the liver and adipose tissue

certain phenotype that contributes very little to tissue repair. These ATMs, referred to as M1 macrophages, promote inflammation and local insulin resistance by producing pro-inflammatory cytokines and reactive oxygen species[10]. In contrast, the ATMs that aid in tissue repair, called M2 macrophages, secrete a more anti-inflammatory repertoire of cytokines[11]. Rapid adipose tissue expansion is associated with a macrophage phenotype switch from M2 to M1. The M1 ATMs are activated by pro-inflammatory stimuli that arise from improper tissue growth. Pro-inflammatory stimuli can also arise from other sources, for instance from endotoxins such as LPS entering the circulation due to a decrease intestinal barrier function which has been observed in obesity[12,13]. An increase in pro-inflammatory M1 macrophages is mainly observed in the visceral adipose tissue, where ATMs surround dead adipocytes in so-called crown-like structures[11], as depicted in the figure. As a result, the adipose tissue enters a state of chronic low-grade inflammation. Furthermore, obese adipose tissue releases a spectrum of pro-inflammatory adipokines[14], such as macrophages and monocyte chemoattractant protein 1 (MCP-1) and plasminogen activator inhibitor 1 (PAI-1)[15]. In contrast, plasma levels of the anti-inflammatory and insulin-sensitizing adipokine adiponectin have shown to be decreased in obese individuals[16].

Liver

Adipose tissue inflammation and a decrease in insulin sensitivity often underlie an increase in adipose tissue lipolysis[17]. Pro-inflammatory cytokines such as TNF-a and IL-6 have the potential to stimulate lipolysis. As insulin is an important suppressor of lipolysis, a decrease in insulin sensitivity will also increase the release of fatty acids from the adipose tissue. As a result, NEFA levels in the blood as well as the delivery of NEFA to the liver increase. An increase in adipose tissue-derived NEFA is the mechanism that contributes the most to the development of ectopic fat storage in the liver as the liver takes up NEFA from the blood proportional to their concentration[18,19]. Mice studies support a causal relation between plasma NEFA and the pathogenesis of fatty liver[20]. In obese men, NEFA concentrations after a glucose tolerance test are strongly correlated to hepatic lipid content, independent of other measures[21]. It is hypothesized that especially the visceral adipose tissue contributes to an increased NEFA supply to the liver, as this tissue is highly lipolytic and drained by the portal vein thus leading NEFA directly to the liver[22] hence resulting in fatty liver. Other processes that contribute to the development of a fatty liver, referred to as hepatic steatosis, are an increase in *de novo* lipogenesis, thus the production of triglycerides from non-lipid precursors, and a decrease in hepatic beta-oxidation. Hepatic beta-oxidation can decrease as a result of lower levels of gut microbiota-derived short chain fatty acids (SCFA), that have the ability to stimulate hepatic fatty acid oxidation via activation of AMPK. A decrease in microbiota diversity or specific SCFA-producing species might therefore also affect hepatic lipid metabolism. Another efflux route for triglycerides from the liver

is the secretion via VLDL particles. This secretion has been demonstrated to be slightly increased in patients with NAFLD, although not sufficient to compensate for the increase in hepatic lipids[18].

There is a strong association between steatosis and hepatic insulin resistance, which might be caused by cellular stress induced by hepatic NEFA resulting in phosphorylation of insulin receptor substrate proteins[23]. Moreover, steatosis has been described as an independent risk for whole body insulin resistance and the development of T2DM[24]. Different mechanisms have been put forward to explain the link between steatosis and T2DM including inadequate hepatic regulation of blood glucose, amongst others by increased gluconeogenesis[25], and an increase in expression of hepatokines that promote insulin resistance such as fetuin A[26]. High plasma levels fetuin A are described as an independent risk factor for the development of T2DM, which is thought to be caused by the ability of this hepatokine to promote adipose tissue inflammation by acting as a ligand for an important receptor at the crossroads of metabolism and inflammation; Toll-like receptor-4[27]. The arise of insulin resistance will cause a vicious cycle as it further increases triglyceride accumulation in the liver due to increased NEFA supply from the adipose tissue[28,29]. The accumulation of triglycerides in the liver is seen as the "first hit" in the development of NAFLD. The "second hit", leading to steatohepatitis, is multifactorial and includes oxidative stress[30] and inflammation, in which inflammatory mediators released by the adipose might play a role[31].

Blood lipid profile

Obesity-related dysregulation in the adipose tissue and the liver also contributes to a pro-atherogenic blood lipid profile, i.e. increased levels of triglycerides and small dense low density lipoprotein (LDL) particles and a decrease in high density lipoprotein (HDL) levels[32]. Plasma triglycerides levels can rise as a result of increased hepatic VLDL output which, as mentioned previously, slightly increases as a result of increased liver triglycerides. In addition, LPL-mediated clearance of triglyceride-rich VLDL by peripheral tissues including the adipose tissue might be decreased. However, studies show conflicting results on the expression of LPL in adipose tissue of obese subjects, with some studies showing an increased expression[33,34] while others report a decreased expression[35,36]. In addition to effects on plasma triglycerides, obesity can be detrimental to the ratio of plasma LDL and HDL, as increased adiposity causes increased activity of both plasma enzyme cholesteryl ester transfer protein (CETP) and hepatic lipase (HTGL)[37]. CETP exchanges triglycerides for cholesteryl esters, thereby promoting the formation of LDL and lowering the production of HDL. In addition, HTGL increases the lipolysis of LDL which results in the occurrence of smaller, more dense LDL. The liver LDL receptor has a lower affinity for these smaller LDL particles when compared to larger LDL, leading to an accumulation of small dense LDL in the circulation[38].

To summarize, both the liver and adipose tissue are susceptible for metabolic dysregulation and their joint actions through organ-crosstalk trigger a cascade of events that leads to systemic disturbances, such as systemic low-grade inflammation, systemic insulin resistance, and a pro- atherogenic blood lipid profile.

DIETARY STRATEGIES

Diet is an important contributor to obesity and thus a crucial way to potentially reserve the diminished metabolic health associated with it. Different dietary strategies have been described to combat chronic metabolic diseases related to obesity.

Energy restriction / weight loss

An evidently potent and effective dietary strategy to alleviate comorbidities of obesity is limiting the intake of daily calories, i.e. caloric restriction (CR) or energy restriction (ER)[39]. CR is referred to as the limitation of calorie intake in healthy lean individuals applied to extend lifespan while ER is used in the context of limiting energy intake in overweight or obese individuals applied to lose body weight, preferably fat mass. ER is usually targeted around 60-90% of the energy intake of an *ad libitum* diet, in which it is important that an adequate intake of micronutrients, essential amino acids, and essential fatty acids is maintained to prevent malnutrition. The application of ER for a substantial period of time, e.g. 3-12 months, will lead to clinically relevant weight loss regardless which nutrients are emphasized within the diet[40]. Studies show that a reduction of 5% of initial body weight is already associated with improvements in cardio-metabolic risk factors such as increased insulin sensitivity and a more beneficial blood lipid profile[41,42]. In this situation, an increased oxidation of fatty acids and a decrease in NEFA mobilization from the adipose tissue play an important role[43]. Efforts have been made to enlarge metabolic benefits from ER-diets by modulating its macronutrient composition[44]. Popular diets include low-fat, high-protein, or low-carbohydrate ER-diets, whether or not combined into one diet. Evidence from nutritional intervention studies with these diets are conflicting, some showing for instance the potential of low-carbohydrate ER-diets in improving blood lipid profile[45,46] while others large trials found no or only marginal additional effects of emphasizing or reducing certain macronutrients[40,47]. Consequently, the ideal macronutrient composition of weight loss diets is still subject of intense debate.

Nutrient quality

In addition to lowering the energy quantity of the diet, in particular of energy-dense foods, increasing the quality of the diet by consuming more high quality nutrients has also been investigated as strategy to improve metabolic

health in overweight and obese individuals.

Fat

Meta-analyses of large cohort studies demonstrate that replacing saturated fatty acids (SFA) with mono- (MUFA) and/or polyunsaturated fatty acids (PUFA) is associated with reduced CVD-events and mortality[48-50]. Consumption of both MUFA and PUFA is linked to a decrease in adipose tissue inflammation, a favorable plasma lipid profile, and augmented insulin sensitivity[51,52]. Especially the *n-3* PUFAs eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) are highly acclaimed in the context of improving blood lipid profile. Both fatty acids lower plasma triglycerides, which is hypothesized to be caused by a decrease in NEFA availability in the liver, that in turn lowers the hepatic triglycerides-rich VLDL output. The decrease in NEFA is thought to be caused by multiple mechanisms[53]. EPA and DHA have demonstrated in cell and animal studies to reduce NEFA mobilization from the adipose tissue by decreasing local tissue inflammation. Furthermore, both fatty acids might increase hepatic fatty beta-oxidation via the activation of peroxisome proliferator-activated receptor alpha (PPARa).

Protein

An increase in dietary protein at the expense of other macronutrients has been described as a strategy to enhance weight loss, as protein intake is associated with increased satiety and an increase in metabolic rate[54,55]. Many popular weight-loss diets are therefore designed around a high intake of protein[56]. However, the protein-induced increase in satiety and energy expenditure are short-term effects and evidence from long-term human intervention studies is limited[57,58]. There is evidence for differential health effects of protein from animal compared to protein from plant-based sources. Beneficial effects on CVD risk factors have been described to be attributed to plant-based proteins[59]. However, it is difficult to disentangle positive effects from plant protein from effects of other beneficial components in the plant-based food matrix or the simultaneous decrease in detrimental nutrients such as a decrease in SFA from animal sources. On the other hand, there is substantial evidence that the plant-based protein soy exerts beneficial effects on lipid metabolism, most notably a reduction in total and LDL cholesterol[60,61].

Carbohydrates

An increase in intake of low GI- or complex carbohydrates avoids exaggerated glucose responses and might improve overall glucose homeostasis on the long-term[62]. Complex carbohydrates are found in whole grain

products, which are not only a rich source of fiber but also contain more bioactive components such as vitamins, minerals, and phytochemicals when compared to refined products. Dietary fiber might affect metabolic health by its cholesterol-lowering properties[63]. Furthermore, insoluble, fermentable fibers are associated with beneficial effects on the gut microbiota, potentially increasing microbiota diversity and SCFA producers[64,65], although less is known on the effects of these fibers within a whole diet context[66]. Dietary guidelines advocate to limit energy intake from simpler carbohydrates, i.e. sugars such as mono- and disaccharides. However, systematic reviews on the available evidence fail to support strong associations between sugar intake and cardio-metabolic risk factors[67-69]. An exception is the monosaccharide fructose, which is described as detrimental for metabolic health through its potential to increase hepatic lipids by the induction of *de novo* lipogenesis in the liver[70].

Effects on nutrients on the molecular level; nutrigenomics

Understanding how nutrients regulate mechanisms at the molecular level is a prerequisite when investigating the effects of nutrients on metabolic health. The field of study that deals with the molecular-level interaction between nutrition and the genome is referred to as nutrigenomics[71]. Nutrients are dietary signals that are able to influence the expression of genes, via activation of certain transcription factors, and subsequently influence the production of proteins and metabolites[71]. Important examples of these nutrient-transcription factors interactions include the family of peroxisome proliferator-activated receptors (PPARs), activated by fatty acids and important in lipid metabolism[72], the liver X receptor (LXR), activated by oxygenated cholesterol derivatives and important in cholesterol metabolism and glucose homeostasis[73], and sterol regulatory element-binding proteins (SREBPs), activated by insulin and cholesterol and important in cholesterol homeostasis and lipid metabolism[74].

Diet quality

A new development in the nutritional science is the change from studying health effects of single nutrients to investigating effects of whole diets and dietary patterns. Overall diet quality can be improved by an increase in high quality nutrients, a decrease in low quality nutrients, and/or an increase in nutrient diversity. Efforts have been made to develop scoring systems for diet quality, in which two main approaches are used. One possibility is an *a priori* approach in which a diet is scored on how much it is aligned with known healthy dietary patterns and/or national dietary guidelines, such as the Healthy Eating Index (HEI)[75]. Another option is by *a posteriori* looking at which patterns are associated with low risk of disease on the longer term, such as the Mediterranean diet.

Optimal diets for improving metabolic health in overweight and obese individuals

Although general dietary guidelines have been put into practice for over a century, the optimal diet for improving metabolic health in overweight and obese individuals is still under discussion. Options include altering the macronutrient composition of an ER or eucaloric diet, adding specific high quality nutrients to the diet, or a *whole diet approach* that takes into account both macronutrient composition as well as nutrient quality. A whole diet approach within an ER-diet might be a powerful tool to modulate metabolic health. However, not all underlying mechanisms of several high quality nutrients are identified or confirmed in human intervention studies. Furthermore, many intervention studies focus on improvements in only one of a few metabolic parameters. As explained earlier, metabolic health is the result of a complex interplay between metabolic organs such as the liver and adipose tissue. Well-designed randomized controlled trials with multiple study outcomes are needed to investigate the potential of dietary strategies, such as an energy restricted whole diet approach, on metabolic health.

CHALLENGES IN NUTRITION RESEARCH

Measuring the effects dietary strategies on metabolic health is challenging as effects of nutrition on health are usually relatively subtle. Furthermore, classical markers assessed in nutritional intervention studies are typically late disease markers, while these studies are frequently executed in relatively healthy populations. Moreover, measurements are often conducted in the tightly regulated fasted state, which might not be a representable read-out for health status. These challenges call for different approaches in nutrition research to better understand the effect of dietary interventions on metabolic health in overweight and obese individuals.

Sensitive approaches; assessing phenotypic flexibility

Recently, a new definition of health was proposed that emphasizes the ability to adapt to environmental challenges rather than being in a static state of complete physical, mental and social well-being[76]. In a nutritional context this means the physiological adaptations to dietary stressors, such as meals high in fat and/or sugar. These adaptations include for instance the secretion of insulin and other glucoregulatory hormones such as incretins to control postprandial glucose responses. All these actions are executed in order to regain a homeostatic state. Examples of dietary stressors applied in clinical trials include the well-known oral glucose tolerance test (OGTT), consisting of a 75g glucose load used to test the body's capacity to clear glucose from the blood, as well as different high fat load challenges or a mixed meal challenge test, consisting of a combination of fat, protein, and carbohydrates. The mixed meal is gaining popularity as it challenges multiple metabolic

organs and their interplay, requiring adaptation at several levels[77,78]. Read-outs for such a challenge may include multiple measurements such as postprandial excursions of plasma parameters or postprandial changes in vascular parameters. The responses of these plasma parameters reflect the individual's capacity to regain a homeostatic state, referred to as phenotypic flexibility, and might form a more sensitive measure of metabolic health than fasting measurements. Dietary interventions might alter responses to a mixed meal test, for instance by dampening postprandial glucose or insulin excursions, thereby improving phenotypic flexibility.

Comprehensive techniques; the use of ~omics

Recent advances in sensitive high-throughput techniques have increased the possibilities to obtain a detailed comprehensive overview of the effects of nutrition on health by allowing the simultaneous unbiased analysis of hundreds to ten thousands of biological markers in one sample. These measurements may include more early markers rather than late disease markers and may serve as early indicators for changes in homeostatic control and/or disease disposition[79]. Examples of these techniques for large scale data collection in biological specimen are the systematic study of small-molecule metabolites, i.e. metabolomics, or the assessment of whole genome gene expression by technologies such as microarrays and RNA sequencing, i.e. transcriptomics. Both metabolomics and transcriptomics are promising tools in understanding the underlying mechanisms of diet-induced health improvements.

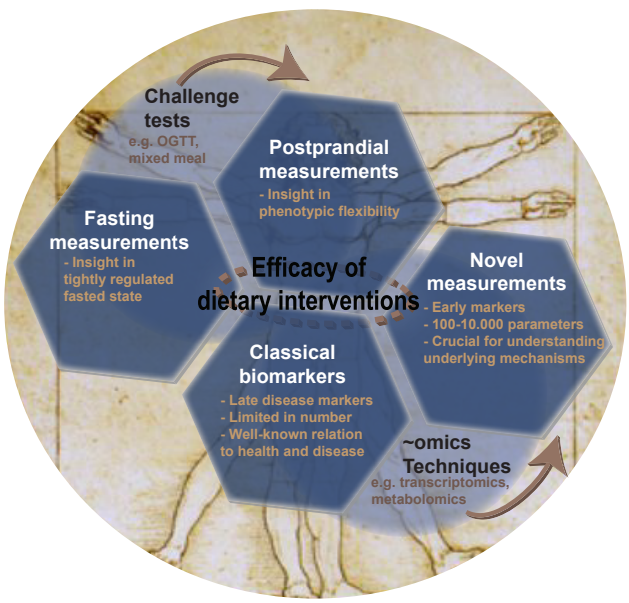

Figure 3. Toolbox of nutritional sciences to determine metabolic health and the efficacy of dietary interventions

Phenotyping the effects of dietary interventions on metabolic health

The application of sensitive approaches and comprehensive techniques are promising additions to the toolbox of nutritional science (**Figure 3**). To accurately phenotype the effects of dietary interventions it is important to combine measurements, e.g. both fasting and postprandial measurements as well as both classical biomarkers as more novel measurements.

AIM AND OUTLINE OF THIS THESIS

The aim of this thesis was to accurately assess the effects of nutrient quality in eucaloric and energy restricted diets on metabolic health, with a focus on the health of liver and adipose tissue. This was done by using a comprehensive phenotyping approach that included the analysis of classical markers, the assessment of phenotypic flexibility, and the employment of novel techniques, such as metabolomics and transcriptomics. In **Chapter 2**, we assessed the effects of nutrient quality within an eucaloric diet by determining the effects of a 12 week whole grain wheat intervention versus a refined wheat intervention on metabolic health, predominantly liver health, in overweight and obese individuals. Outcome measures included several liver-related parameters, such as hepatic lipid content, and liver inflammatory markers, as well as lipid handling capacity after a mixed meal and adipose tissue health. In **Chapter 3**, **4** and **5** we applied comprehensive phenotyping to extensively study the effects of two energy restricted-diets differing in nutrient quality on a myriad of measurements in abdominally obese subjects. The ER-diets both were restricted by 25% of the estimated energy requirement of the participants and were designed in a polarized fashion. One diet resembled a Western-style dietary pattern with low quality nutrients while the other diet was an *a priori* developed whole diet approach that included a range of high quality nutrients such as soy protein, fiber, MUFA and *n-3* PUFA. Effects of the diets upon 12 weeks of intervention were compared to a control group that did not receive any dietary intervention. Outcome measurements, which are described in **Chapter 3**, included fasting plasma markers for insulin sensitivity and lipid metabolism, hepatic lipid content, abdominal body fat distribution, and vascular measurements as well as postprandial responses of several plasma markers and vascular parameters after a mixed meal test. The examination of the potential additional benefits of the high nutrient quality ER-diet was extended by the use of sensitive high-throughput techniques including fasting and postprandial plasma metabolomics, described in **Chapter 4**, and fasting and postprandial adipose tissue transcriptomics, described in **Chapter 5**. Lastly, we gained insight in the expression of two important metabolic regulators in the liver and adipose tissue, LPL and ANGPTL4, and their relation to plasma triglycerides in obese individuals, of which the results are described in **Chapter 6**. An overall discussion of chapters 2 to 6 is presented in **Chapter 7**.

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2

A 12 WEEK WHOLE GRAIN WHEAT INTERVENTION PROTECTS AGAINST HEPATIC FAT; THE GRAANDIOOS STUDY

A RANDOMIZED TRIAL IN OVERWEIGHT SUBJECTS

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ABSTRACT

Background: Whole grain wheat (WGW) is described as nutritionally superior over refined wheat (RW) and thus advocated as the healthy choice, though evidence from intervention studies is often inconsistent. The liver, as central organ in energy metabolism, might be an important target organ for WGW interventions.

Objective: To investigate the potential benefits of WGW consumption compared to RW consumption on liver health and associated parameters.

Design: We performed a double-blind, parallel trial in which 50 overweight 45-70yr old males and postmenopausal females were randomly allocated to a 12wk intervention with either WGW (98g/day) or RW (98g/day) products. Before and after the intervention we assessed intrahepatic triglycerides (IHTG) and fat distribution by ^{1}H-MRS/MRI, fecal microbiota composition, adipose tissue gene expression, and several fasting plasma parameters, as well as postprandial plasma lipids after a mixed meal.

Results: Fasting plasma cholesterol, triglycerides, non-esterified fatty acids, and insulin were not affected by the RW or WGW intervention. We observed a substantial increase of 49.1% in IHTG in the RW when compared to the WGW group (p=0.033). Baseline microbiota composition could not predict the increase in IHTG after RW but gut microbiota diversity decreased in the RW group when compared to the WGW group (p=0.010). In the WGW group, we observed increased postprandial triglyceride levels when compared to the RW group (p=0.020). In addition, the WGW intervention resulted in a trend towards lower fasting levels of the liver acute phase proteins SAA (p=0.057) and CRP (p=0.064) when compared to the RW intervention.

Conclusion: A 12wk RW intervention increases liver fat and might contribute to the development of Non-Alcoholic Fatty Liver Disease, while a 12wk 98g/day WGW intervention prevents a substantial increase in liver fat. Our results show that incorporating feasible doses of WGW in the diet at the expense of RW maintains liver health.

ABBREVIATIONS

^{1}H-MRS	Proton magnetic resonance spectroscopy
ALT	Alanine transaminase
ANGPTL-4	Angiopoietin-like 4
AST	Aspartate transaminase
CD36	Cluster of differentiation 36 (fatty acid translocase)
CD68	Cluster of differentiation 68
CRP	C-reactive protein
GGT	Gamma-glutamyltransferase
HOMA-IR	Homeostatic model assessment of insulin resistance
IHTG	Intrahepatic triglycerides
LOX	Lysyl oxidase
LPL	Lipoprotein lipase
MMP9	Matrix metallopeptidase 9
MRI	Magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NEFA	Non-esterified fatty acids
RW	Refined wheat
RTECs	Ready to eat cereals
SAA	Serum amyloid A
SAT	Subcutaneous adipose tissue
SCFAs	Short chain fatty acids
SLR	Logarithmic ratio of signal intensity
TG	Triglycerides
TGFB1	Transforming growth factor beta 1
TRL	Triglyceride-rich lipoprotein
VAT	Visceral adipose tissue
VLDL	Very low density lipoprotein
WAT	White adipose tissue
WGW	Whole grain wheat

INTRODUCTION

Wheat is an important staple food in many Western countries. Most commonly consumed wheat products are based on refined flour from which the bran and germ of the wheat kernel are excluded. However, whole grain wheat (WGW) products still contain these elements and are described as nutritionally superior over refined wheat (RW) products, as the bran and germ are rich sources of dietary fiber but also of betaine, choline, important minerals and vitamins, components with anti-oxidant properties and other phytochemicals[1, 2]. Consumption of WGW products might therefore have beneficial effects on metabolic health beyond fiber consumption alone.

Observational studies indeed show inverse associations between the consumption of whole grain foods and adiposity as well as risk for chronic diseases including type 2 diabetes and cardiovascular disease[3-7]. However, human intervention studies show conflicting results with regard to the relation between whole grain consumption and health outcomes that could potentially mediate the observed reduction in disease risk, such as improvements in parameters related to insulin sensitivity or lower cholesterol levels[8-11]. Numerous causes could underlie this lack of consistency which include the short duration of interventions, the use of a mixture of grains, or the mere focus on only one or a few metabolic parameters. However, metabolic health is the result of a complex interplay between metabolic organs such as the intestine, adipose tissue, and liver. The liver is important in energy metabolism and is sensitive to dietary changes. Accumulation of triglycerides in the liver, a prerequisite for the development of non-alcoholic fatty liver disease (NAFLD), is now seen as the hepatic manifestation of the metabolic syndrome and can have profound effects on the health of other metabolic organs and several cardio-metabolic risk factors including insulin resistance[12].

We performed a trial to investigate the benefits of whole grain wheat (WGW) in overweight middle-aged subjects. In this randomized controlled, double blind parallel trial we investigated the health effects of 12 weeks of WGW versus refined wheat (RW) consumption in a total of 50 female and male middle aged, overweight participants with mildly elevated total cholesterol levels. In the current manuscript, we describe the effects of the interventions on liver related parameters, including intrahepatic lipid accumulation. We hypothesized that WGW consumption would have favorable effects on liver health and related metabolic organs such as the adipose tissue and gut microbiota composition, when compared to RW consumption.

SUBJECTS AND METHODS

Ethics statement

All subjects gave written informed consent. The study was approved by the Medical Ethics Committee of Wageningen University and registered at ClinicalTrials.gov, identifier: **NCT02385149**. All study procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Study participants

To enable improvements in cardio-metabolic health after WGW intervention, subjects with mildly impaired cardio-metabolic risk profiles were included; middle-aged and overweight males or postmenopausal females (45-70 yrs and BMI between 25-35 kg/m^2) with mildly elevated levels of plasma total cholesterol (>5 mmol/L). Subjects on cholesterol lowering medication were excluded. All subjects indicated that bread and cereals consumption fitted their habitual dietary pattern. Individuals who had an intolerance to gluten, were smoking, or consumed more than 21 glasses of alcohol per week were excluded from participation. Subjects were also excluded if they had a history of medical or surgical events that may significantly affect the study outcome or if they reported weight loss or weight gain of >5 kg in the month prior to pre-study screening. Subjects were stratified among intervention groups based on gender, cholesterol levels, age, and BMI.

Study design

The study was a randomized controlled double blind, parallel trial (**Figure 1**). After a 4 week run-in period with RW products, subjects were randomly assigned to a 12 week intervention of either WGW (98g/day) products (*n*=25) or RW products (*n*=25). Randomization of the participants over the intervention groups was conducted using block randomization using Microsoft Excel by a researcher who was not involved in the study. A run-in period was included in the study to reduce variation of WGW intake in the study population at baseline. Subjects were asked to maintain habitual habits and were not allowed to lose or gain weight. During the complete study subjects were asked to keep a diary and to report all deviations from the protocol. Primary outcome of the study was the change in cardio-metabolic risk factors, including fasting and postprandial plasma levels of cholesterol, triglycerides, non-esterified fatty acids, insulin as well as vascular parameters including pulse wave analyses, flow-mediated dilatation, blood pressure, blood cell activation markers, and other plasma markers of cardio-vascular health. Secondary outcomes included changes in liver and adipose tissue-related health parameters. Sample size was calculated based on previous literature describing the effects of whole grains on total plasma

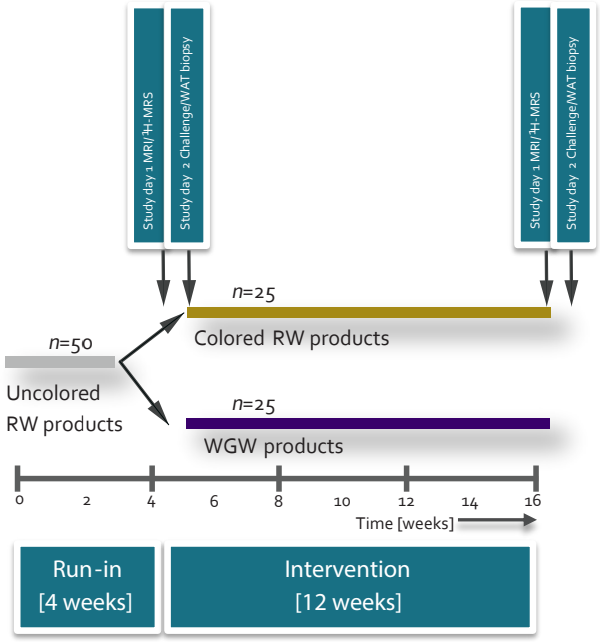

Figure 1. Study design

The study was a randomized controlled double blind, parallel trial. After a 4 week run-in period with refined wheat (RW) products, subjects were randomly assigned to a 12 week intervention of either whole grain wheat (WGW) products or RW products. Abbreviations: MRI: magnetic resonance imaging, ¹H-MRS: proton magnetic resonance spectroscopy, WAT: white adipose tissue.

cholesterol[11, 13-15]. Given a 2 sided test, an alpha of 5% and a power of 80%, and assuming a standard deviation of difference of 0.5 mmol/l, 17 subjects per group were needed to detect a difference of 0.5 mmol/l total cholesterol after 12 weeks between subjects consuming WGW and subjects consuming RW. To allow possible dropouts, we included 25 subjects for each intervention group.

Study procedures

Before and after the 12 week intervention periods, two test days were scheduled. On the first test day body fat distribution and liver fat were assessed in hospital Gelderse Vallei in Ede, The Netherlands. On the second test day, performed at Wageningen University, The Netherlands, an adipose tissue biopsy was conducted as well as the measurement of different plasma parameters before and in response to a mixed meal; the Nutritional PhenFlex challenge. On the day prior to the second test day, subjects consumed a standardized low-fat evening meal, were refrained from alcohol or strenuous exercise and were not allowed to eat or drink anything except

water after 8pm to ensure a 12h fasted state. On the testing day, a cannula was inserted in the antecubital vein for a fasted blood withdraw after which participants underwent the PhenFlex challenge, a 3833kJ shake consisting of 83.5g dextrose (Avebe B.A., Veendam, The Netherlands) which equals 76.3g carbohydrates, 20g of Protifar® protein powder (Sorgente B.V., Houten, the Netherlands) which equals 17.6g of protein, 60.0g of palm olein (Remia C.V., The Netherlands) which equals 60.0g of fat, and 0.5g of aroma (De Lange B.V., Belfeld, The Netherlands). Full details of the Nutritional PhenFlex challenge are described previously[16]. Time points of blood drawing in the postprandial phase included 30, 60, 120, and 240 minutes after consumption.

Intervention products

Subjects received either WGW or RW products during the intervention to replace their habitual intake. WGW products met the requirements of the definition for whole grain cereals and pseudo-cereals as proposed by the consortium of the HEALTHGRAIN EU project[1]. Subjects in both intervention groups consumed each day four slices of bread (in total 100g) and one serving of ready-to-eat-cereals (RTECs) of 33.4g in total adding up to a total of 98g of RW or WGW flour per day. Small buns, currant buns and currant bread were provided for the weekends instead of the slices of bread. RW products in the intervention period were colored using roasted wheat malt and caramelized sugar to match the appearance of WGW products. During the run-in period we used non-colored RW products to suggest a switch to the other treatment arm. Both researchers and participants were blinded with regard to the intervention received, RW or WGW. Products within both intervention arms were similar with respect to energy and macronutrient composition except for fiber content (**Table 1**, analyzed by Triskelion, Zeist, The Netherlands). During the run-in period, subjects were not allowed to eat any whole grain food products including products from other grain sources i.e. brown rice. Similar dietary restrictions were applied during the intervention period, in which subjects in the RW were not allowed to eat whole grain food products and subjects in the WGW were not allowed to eat any additional whole grain food products. All subjects were allowed to complement their daily diet with additional refined grain products depending on energy requirements during the run-in and the intervention periods. Compliance to the two interventions was determined by a weekly recall of empty product packages as well as total plasma alkylresorcinols, a biomarker for whole grain wheat intake. Plasma alkylresorcinols were analyzed using normal-phase LC-MS/MS[17].

Anthropometric assessments

Waist circumference (cm) was determined as the average of two measurements on the location midway between the lowest ribs and the iliac crest. Body weight (kg) was recorded weekly on a calibrated scale to the nearest

Table 1. Nutritional composition of intervention products

	RW Bread	WGW Bread	RW RTEC	WGW RTEC
Per 100g product				
Energy (kJ)	1066	983	1586	1524
Moisture (g)	35.4	38.7	3.4	3.7
Ash (g)	1.3	1.8	1.8	2.5
Carbohydrates (g)	49.3	39.2	78.5	69.7
Fiber [1] (g)	3.3	6.8	3.9	10.8
Protein (g)	9.1	11.3	10.9	11.2
Fat (g)	1.6	2.1	1.5	2.1
Sodium (mg)	0.45	0.46	0.51	0.49
Iron (mg)	0.92	2.1	1.3	3.9
Magnesium (mg)	19	62	31	120
Zinc (mg)	0.67	1.5	0.89	2.4
Folate (µg)	17	28	14	38
Vitamin B1 (mg)	0.06	0.17	<0.1	<0.1
Vitamin B3 (mg)	0.47	0.76	1.1	2.6
Vitamin B6 (mg)	<0.1	<0.1	<0.1	<0.1

[1] Determined using AOAC985.29. Abbreviations: RW: refined wheat, WGW: whole grain wheat, RTEC: ready to eat cereal

0.1kg. Participants who gained or lost >3kg were referred to our research dietician in order to prevent further deviations in body weight.

Intrahepatic triglyceride (IHTG) accumulation

Proton magnetic resonance spectroscopy (^{1}H-MRS) was used to quantify lipid content in liver using a 3T whole body MRI scanner (Siemens, Munich, Germany) at baseline and after 12 weeks of intervention. A single voxel was selected in the right lobe of the liver and fine shimming was performed to optimize the magnetic field homogeneity within the region of interest before acquiring ^{1}H-MRS spectra. Vascular structures and the proximity of subcutaneous fat were avoided in localization of the voxel. In the ^{1}H-MR spectra, the water signal was suppressed using frequency-selective pre-pulses and the spectra was fitted to quantify the lipid peak. A tissue-specific coil was used to optimize data acquisition and signal-to-noise ratio. Analysis of the spectra was performed using jMRUI software v5.2[18].

Body fat distribution

Subcutaneous and visceral adipose tissue as well as their ratio were quantified in the abdominal region on the same day as the IHTG analyses, also using a 3T whole body MRI scanner (Siemens, Munich, Germany). Axial T1-weighted spin echo images were acquired using the body coil with the patient in supine position. Slices were centered at the interspace L4-L5, with the other slices situated above and below to cover the whole abdomen. A breath-hold technique was applied to avoid breathing-induced artefacts. The adipose tissue depots were quantified based on a single slice centered in middle of 9 recorded images the using the semi-automatic software program HippoFat™ [19].

Clinical chemistry

Plasma triglycerides (TG), glucose, insulin, cholesterol, and liver enzymes alanine transaminase (ALT), aspartate transaminase (AST), and glutamyltransferase (GGT) were analyzed by a hospital laboratory (ZGV, Ede, the Netherlands). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated by dividing the product of fasting glucose (mmol/L) and fasting insulin (mU/L) by 22.5. Plasma levels of the ketone body beta-hydroxybutyrate were analyzed by SHO Center for medical diagnostics (Stichting Huisartsenlaboratorium Oost Velp, The Netherlands) using a colorimetric assay. Plasma non-esterified fatty acids (NEFA) were determined using an enzymatic method (INstruchemie, Delfzijl, The Netherlands), while plasma C-reactive protein (CRP) and Serum amyloid A (SAA) concentrations were analyzed on preformatted V-PLEX array (Meso Scale Diagnostics, LLC, Rockville, USA) on a SECTOR Imager 2400 reader (Meso Scale Diagnostics, LLC, Rockville, USA).

Adipose tissue analyses

Subcutaneous adipose tissue biopsies were taken before and after the intervention, caudally from the umbilicus under local anesthesia (1% lidocaine) using a small liposuction cannula. After rinsing the sample with phosphate-buffered saline to get rid of blood, part of the tissue was snap frozen in liquid nitrogen for RNA isolation, while another part of the tissue was fixed in 4% formaldehyde for 24h after which the sample was dehydrated in 70% ethanol. Ethanol from the fixed tissue particle was cleared from the sample using xylene after which this solvent was replaced by paraffin. Paraffin embedded samples were cut at 10µm and stained for cell morphology using a standard hematoxylin and eosin staining. Adipocyte perimeters were assessed under the microscope by a blinded technician, using the average of 50 cells (10 in 5 selected images). RNA was isolated from the frozen samples isolated using a TRIzol®/chloroform extraction (Thermo Fisher Scientific, Waltham, USA) and purified using the Qiagen Mini column kit according to manufacturer's protocol (Qiagen, Hilden, Germany). RNA yield

was quantified on a NanoDrop ND 1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA) while RNA integrity was measured on an Agilent 2100 BioAnalyzer with RNA 6000 Nanochips (Agilent Technologies, Santa Clara, USA). Samples with RNA integrity (RIN) score of 6 and higher were included, average RIN-score was 7.7. RNA was reversely transcribed to cDNA (Thermo Fisher Scientific, Waltham, USA) after which RT-qPCR was performed to analyze the expression of genes involved in the uptake and hydrolysis of triglyceride-rich lipoproteins, i.e. lipoprotein lipase (LPL), angiopoietin-like 4 (ANGPTL-4), and cluster of differentiation 36/ fatty acid translocase (CD36), as well as genes involved in inflammation and tissue remodeling, i.e. cluster of differentiation 68 (CD68), transforming growth factor beta 1 (TGBF1), matrix metallopeptidase 9 (MMP9), and lysyl oxidase (LOX)[20]. Expression was normalized to the expression of housekeeping gene cyclophilin A, primers sequences of this gene and genes of interest can be found in **Supplemental Table 1**.

Microbiota

Fecal samples were collected and frozen immediately at -80 °C. Samples were mechanically homogenized and genomic DNA was isolated using the AGOWA mag Mini kit (DNA Isolation Kit, AGOWA, Berlin, Germany) according to the manufacturer's instructions. A fragment of the 16S rRNA gene (~270 bp), spanning the V4 hypervariable regions, was PCR amplified using F515/R806 primers within 30 cycles[21]. Purified PCR products were paired-end sequenced on the Illumina MiSeq platform (Illumina, Eindhoven, The Netherlands). The sequence data was processed with Mothur v.1.33.2[22]. All samples were normalized against the sample containing least sequence reads (subsampling method; 16702 reads) and samples were standardized for their sequence reads at 100bp. Taxonomic assignment was performed using the RDP classifier[23] and the Greengenes 16S rRNA gene database[24] up to the level of genera (taxonomy level 6). To exclude genera which were not present in the majority of samples a cut-off for exclusion was made. Genera containing no sequence reads in more than 75% of samples were removed. Some samples were sequenced in duplicate. In those cases duplicate samples were averaged. Microbiome data were imported and analyzed in R v.3.4.0 using the library Phyloseq[25].

In addition, machine learning was used to predict the percentage increase in IHTG from baseline microbiome based on the 35 subjects with both baseline microbiome data and two IHTG measurements. Only OTU's were included which were detected in at least 20% of the 94 stool samples. We also removed OTU's in order to keep correlations of abundances below 85%. After this 832 OTUs remained as candidate predictors. We used gradient boosting (R package xgboost) as well as randomforest (R-package randomForest). For gradient boosting we used 250 iterations, a maximum tree depth of 4, a learning rate of 0.1 and we did not subsample. For RandomForest

we grew 500 trees, randomly sample 1/3 of the variables as candidate at each split and using a terminal node size of 5. We used a 7-fold cross-validation procedure. The dataset was split in 7 sets of 5 test samples. In each test sample IHTG-changes was predicted with an algorithm trained on the remaining 30 samples. In each training sample of 30 subjects we select first the 10 most predictive abundances, and used those to train a final algorithm. The correlation between observed and predicted IHTG-change was used to evaluate the predictive power.

Statistical analysis

Removal of outliers was limited to technical outliers, i.e. end values of beta-hydroxybutyrate of two subjects were below detection limit of the used kit. Furthermore, we excluded end values of plasma acute phase protein of one subject due to reported illness (common cold) which resulted in values that exceeded the reference range 10x. Analysis of changes in fasting parameters between groups was conducted using linear mixed effects modelling with the interaction between treatments (intervention) and time (weeks) as fixed effect. Analysis of changes in postprandial responses was conducted using linear mixed effects modelling with postprandial time points as repeated measures. Dependent variable in this model was the difference (before/after intervention) in response (postprandial value minus fasting value) of a certain parameter. All linear mixed effects models were executed using an autoregressive (AR1) covariance structure and LSD post-hoc testing. Due to its not normally distributed nature, analyses on microbiota composition were conducted using Wilcoxon signed-rank tests for within group and Mann-Whitney U tests for between group analyses. All tests were performed using IBM SPSS Statistics v.22 (IBM Corp, Armonk, NY, USA).

RESULTS

Study logistics

Between 13th of January and the 19th of February 2015, we recruited participants and assessed 136 individuals for eligibility. Sixty-four individuals were excluded because they did not meet our inclusion criteria and 6 withdrew themselves (**Figure 2**). After excluding 16 individuals at random, 50 persons were included in the study and randomly allocated to either the WGW (n=25) or the RW (n=25) intervention group. All subjects completed the intervention study, which ran from the 23rd of February 2015 to the 7th of July 2015. Baseline characteristics of these 50 participants are summarized in **Table 2**. Compliance to the intervention was based on percentages of product packages returned as well as total plasma alkylresorcinol levels (**Supplemental Table 2**). Based on return of packages, compliance ranged between 99.5% and 100% for the different products. The change in

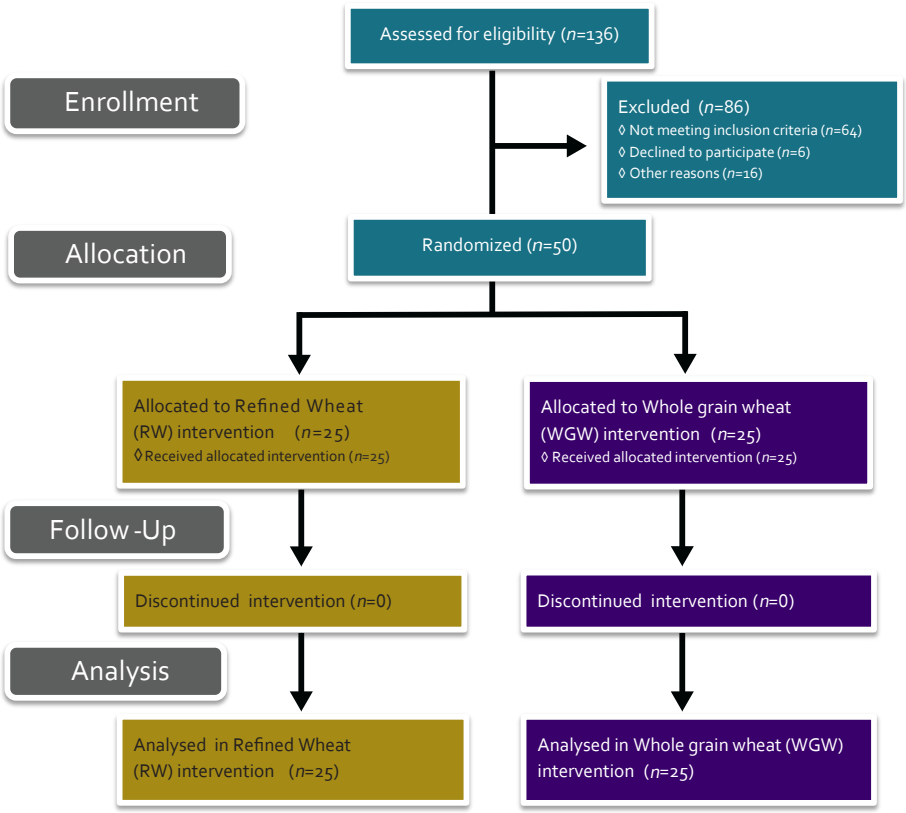

Figure 2. Flow chart of the participants

total alkylresorcinol levels was significantly different between the RW and the WGW group ($p<0.001$). Ninety-six % of participants in both the RW as well as the WGW group could be classified into the right intervention group without prior knowledge on the randomization, solely based on alkylresorcinol levels. At the end of the intervention, 76% of participants in the WGW group believed to have received WGW products versus 64% in the RW group.

Blood lipid profile, glucose homeostasis, or body composition are not affected by RW or WGW

Participants in both intervention groups remained weight stable during the 12 week intervention period (**Table 3**). Fasting plasma lipids (total cholesterol, HDL cholesterol, TG, and NEFA) and fasting markers of glucose

Table 2. Baseline characteristics

	RW group n=25	WGW group n=25
Gender, n males , %	16, 64%	15, 60%
Age, years [1]	61 (19)	61 (22)
Body weight, kg	84.6 ± 9.5	86.2 ± 9.7
BMI, kg/m²	27.6 ± 2.6	28.0 ± 2.1
Waist circumference, cm	102.2 ± 6.0	103.4 ± 5.9
Total cholesterol, mmol/L	5.8 ± 0.9	5.8 ± 0.7
HDL cholesterol, mmol/L	1.3 ± 0.3	1.3 ± 0.4
Glucose, mmol/L	5.4 ± 0.5	5.5 ± 0.7
Insulin, mU/L	8.9 ± 7.8	13.4 ± 27.5
HOMA-IR	2.2 ± 2.0	2.1 ± 1.1
TG, mmol/L	1.6 ± 0.7	1.7 ± 0.8
NEFA, mmol/L	0.46 ± 0.15	0.42 ± 0.14
ALT, U/L	30.4 ± 9.5	34.2 ± 11.9
AST, U/L	19.0 ± 6.3	19.0 ± 4.6
GGT, U/L	23.7 ± 19.9	19.5 ± 12.3

Data are presented as mean ± 1SD, [1] Presented as median (range). Abbreviations: RW: refined wheat, WGW: whole grain wheat, BMI: body mass index, HDL: high density lipoprotein, HOMA-IR: homeostatic model assessment of insulin resistance, TG: triglycerides, NEFA: non-esterified fatty acids, ALT: alanine transaminase, AST: aspartate transaminase, GGT: gamma-glutamyltransferase.

homeostasis (glucose, insulin, and HOMA-IR) were not different between the two groups after 12 weeks intervention. Changes in abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) mass as well as their ratio were also not different between the two groups after 12 week intervention (**Table 3**).

RW negatively affects liver health by increasing IHTG

The 12 week RW intervention resulted in a 49.1% increase in IHTG which was significantly different from the WGW group (p=0.033) (**Table 3**). Levels of IHTG increased significantly in the RW group (p=0.002) but remained stable in the WGW group (p=0.822). When applying the steatosis cut-off value of 5.56%, these results indicate an increase in the number of participants with steatosis from 33 to 44% in the RW group in contrast to a decrease from 35 to 25% in the WGW group[26]. To assess whether the increase in liver fat in the RW group was due to a decrease in hepatic beta-oxidation, fasting plasma beta-hydroxybutyrate was determined. Changes in levels of this ketone body showed a trend towards a difference between the two interventions (p=0.063), but there were no significant within-group differences (RW p=0.179, WGW p=0.190). Fasting plasma levels of liver

Table 3. Change in anthropometrics, fasting plasma parameters, liver health markers, and abdominal body fat distribution upon RW or WGW intervention

	RW GROUP *n*=25		WGW GROUP *n*=25		GROUP COMPARISON
	Baseline	Change after 12wk	Baseline	Change after 12wk	p-value
Anthropometrics and fasting plasma parameters					
Body weight, kg	84.6 ± 9.5	-0.1 ± 1.2	86.2 ± 9.7	-0.5 ± 1.9	0.324
BMI, kg/m²	27.6 ± 2.6	0.0 ± 0.4	28.0 ± 2.1	-0.2 ± 0.6	0.300
Waist circumference, cm	102.2 ± 6.0	0.3 ± 1.7	103.4 ± 5.9	-0.2 ± 1.4	0.302
Total cholesterol, mmol/L	5.8 ± 0.9	-0.1 ± 0.5	5.8 ± 0.7	-0.1 ± 0.6	0.813
HDL cholesterol, mmol/L [1]	1.3 ± 0.3	0.0 ± 0.2	1.3 ± 0.4	0.0 ± 1.2	0.867
Glucose, mmol/L [1]	5.4 ± 0.5	0.0 ± 0.2	5.5 ± 0.7	0.1 ± 0.5	0.371
Insulin, mU/L [1]	8.9 ± 7.8	-0.4 ± 5.0	13.4 ± 27.5	-2.0 ± 31.5	0.769
HOMA-IR [1]	2.2 ± 2.0	0.1 ± 0.6	2.1 ± 1.1	-0.5 ± 7.9	0.860
TG, mmol/L [1]	1.6 ± 0.7	0.1 ± 0.4	1.7 ± 0.8	-0.1 ± 0.5	0.406
NEFA, mmol/L [1]	0.46 ± 0.15	-0.04 ± 0.14	0.42 ± 0.14	-0.03 ± 0.15	0.174
Liver health					
IHTG, % [1]	4.08 ± 3.05	49.1 ± 56.7	5.04 ± 5.98	10.6 ± 49.3	**0.033**
Beta-hydroxybutyrate, mmol/L [1]	0.30 ± 0.21	-0.01 ± 0.35	0.19 ± 0.14	0.08 ± 0.17	0.063
ALT, U/L [1]	30.4 ± 9.5	-0.5 ± 7.4	34.2 ± 11.9	-2.1 ± 7.8	0.490
AST, U/L [1]	19.0 ± 6.3	-0.4 ± 3.9	19.0 ± 4.6	-0.4 ± 4.1	0.788
GGT, U/L [1]	23.7 ± 19.9	-0.1 ± 4.7	19.5 ± 12.3	-0.3 ± 4.5	0.539
SAA, ng/mL [1]	2282 ± 2083	416 ± 1803	9149 ± 19681	-7191 ± 19084	0.057
CRP, ng/mL [1]	2575 ± 2702	-20 ± 1658	5294 ± 8140	-3132 ± 7260	0.064
Abdominal body fat distribution					
SAT, cm²	223.0 ± 61.2	-5.8 ± 24.1	250.3 ± 72.3	-0.4 ± 36.8	0.445
VAT, cm² [1]	136.4 ± 75.1	9.5 ± 20.0	148.5 ± 68.5	1.0 ± 28.1	0.372
VAT/SAT ratio [1]	67.8 ± 46.5	5.2 ± 12.4	66.5 ± 42.3	-1.1 ± 14.4	0.232

Data are presented as mean ± 1SD. Group comparison was performed using linear mixed effects modelling with the interaction between treatments (RW or WGW) and time (weeks) as fixed effect. Abbreviations: RW; refined wheat, WGW: whole grain wheat, BMI: Body mass index, HDL: high-density lipoprotein, HOMA-IR: homeostatic model assessment of insulin resistance, TG: triglycerides, NEFA: non-esterified fatty acids, IHTG: intrahepatic triglycerides, ALT: alanine transaminase, AST: aspartate transaminase, GGT: gamma-glutamyltransferase, SAA: serum amyloid A, CRP: C-reactive protein, SAT: subcutaneous adipose tissue, VAT: visceral adipose tissue. For IHTG: RW *n*=18, WGW *n*=20, for SAT/VAT: RW: *n*=22, WGW: *n*=22. Values denoted with a [1] are log transformed (base 10) for statistical analyses to improve normality.

enzymes ALT, AST, and GGT were unaffected by the interventions. Plasma liver inflammatory markers SAA and CRP showed a trend towards a difference between the groups (p=0.057 for SAA and p=0.064 for CRP), caused by a significant decrease in SAA (p=0.010) and CRP levels (p=0.035) within the WGW group.

RW decreases microbial diversity

To assess a potential effect of the intervention on microbial composition, we assessed the change in microbial diversity within samples (alpha-diversity), using the Shannon index. Changes in the Shannon index differed significantly between the RW and WGW group (p=0.010) as the diversity score decreased after RW intervention (p=0.035) and remained stable after WGW (**Figure 3**). We visualized the dissimilarity in bacterial communities between the samples, before and after the intervention, using a principal coordinates analysis based on the Bray-Curtis distance (**Supplemental Figure 1**). No specific clusters were observed. Several gut bacteria are able to produce short-chain fatty acids (SCFAs) upon the fermentation of dietary fiber. We specifically examined the effect of the interventions on the abundance of two SCFA producing families, *Lachnospiraceae* and *Ruminococcaceae*, as well as changes in commonly observed genera of these families (**Supplemental Table 3**). No significant differences in changes in abundance of these species between the RW and WGW intervention were observed.

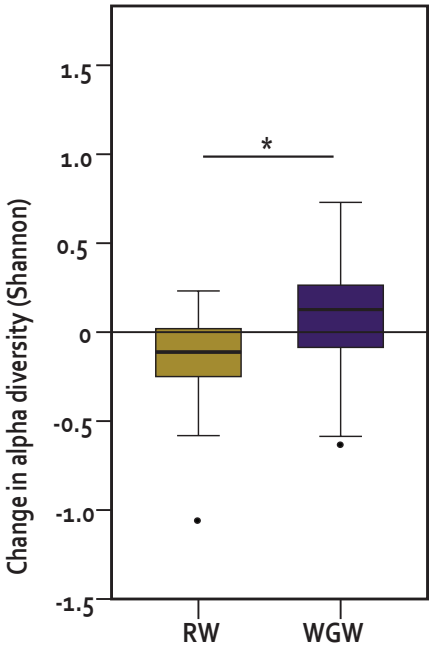

Figure 3. Changes in microbial diversity upon RW and WGW intervention

Diversity was assessed using the Shannon index. Abbreviations: RW: refined wheat, WGW: whole grain wheat. RW *n*=21, WGW *n*=24. Data are presented as a descriptive boxplot, in which the center represents the median, the outlines of the box indicate Q1 and Q3 while the whiskers show the most extreme data points within 1.5 times the IQR, otherwise indicated by a • symbol. The * symbol indicates a significant difference in change in diversity between intervention groups using Mann-Whitney U test for not normally distributed values.

Baseline microbiota is not a predictor for changes in IHTG

Finally, we examined whether baseline gut microbiome could predict changes in IHTG after the intervention. Machine learning algorithms using microbiome abundance could not predict the percentage change in IHTG from microbiome data, either with or without adding gender, age, BMI, bodyweight, fasting glucose or insulin to the set of predictors. In addition, baseline gut microbiome could also not predict the increase in IHTG in RW specifically.

WGW increases postprandial triglycerides

To determine whether the interventions affected lipid handling ability, we analyzed postprandial levels of TG and NEFA after the mixed meal challenge. The change in overall TG response was significantly different between the RW and WGW intervention (p=0.020) due to a significant change in this response within the WGW group (p=0.004), which was mainly caused by a significant increase in TG levels 4h postprandial in the WGW group (**Figure 4**). Changes in postprandial NEFA excursions were not different between the interventions (**Figure 4**).

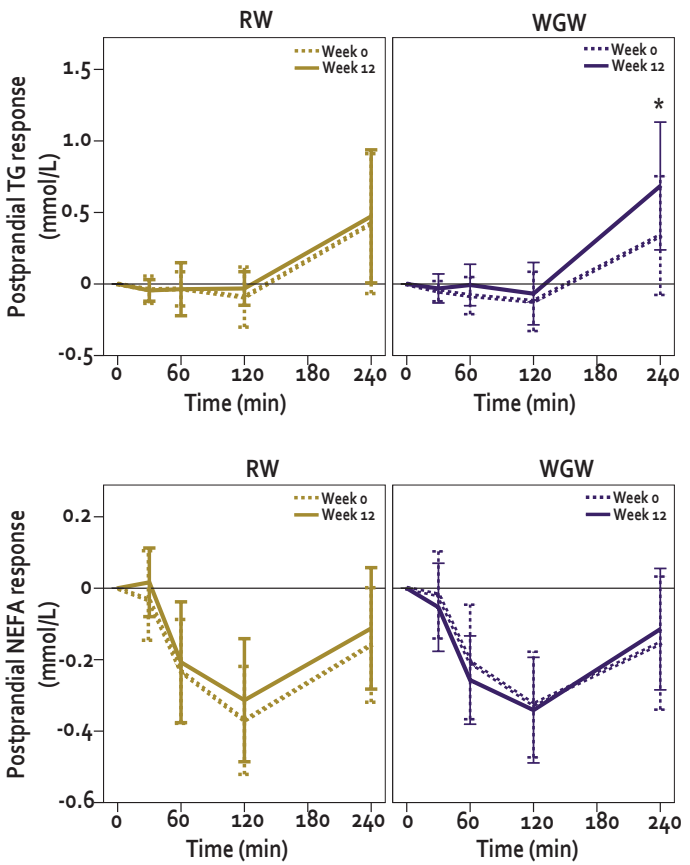

Figure 4. Postprandial response of plasma triglycerides and non-esterified fatty acids

Postprandial response after consumption of a mixed meal (76.3g carbohydrates, 17.6g protein, 60.0g fat). Abbreviations: RW: refined wheat, WGW: whole grain wheat, TG; triglycerides, NEFA: non-esterified fatty acids. Data are presented as mean changes, error bars indicate 1SD. Analyzed using Linear Mixed Models for repeated measures. The change in overall TG curve was significantly different between the RW and WGW intervention due to a significant change in this response within the WGW group. No significant differences in changes in overall NEFA curve were observed. The * symbol indicates a significant difference in postprandial value when comparing baseline (0 weeks) and after the intervention (12 weeks).

Subcutaneous adipose tissue health is not affected by RW or WGW

To determine whether the interventions would affect the capacity of the adipose tissue to take up triglyceride-rich lipoproteins, we determined the expression of genes involved in the uptake and hydrolysis of triglycerides as well as the size of the adipocytes. Both the RW and the WGW intervention did not affect adipocyte size (**Supplemental Figure 2**) or gene expression of LPL, ANGPTL4 or CD36 (**Supplemental Figure 2**). Furthermore, we assessed expression of genes involved in inflammation, fibrosis, and adipose tissue remodeling, i.e. CD68, TGFB1, MMP9, and LOX, but no effects of the interventions were observed (**Supplemental Figure 2**).

DISCUSSION

We investigated the effects of a 12 week intervention with whole grain products compared to refined wheat products on liver and cardio-metabolic health in overweight, middle-aged male and female subjects with slightly elevated plasma cholesterol. The refined wheat intervention increased liver fat while the 98 g/day whole grain wheat intervention had a protective effect against lipid accumulation in the liver.

Intrahepatic triglycerides increased by 49.1% on RW intervention in the absence of weight gain, which increased the number of steatosis patients in this group from 33 to 44%. In contrast, this number decreased from 35 to 25% in the WGW group. Consumption of whole grains has been proposed for the prevention and treatment of NAFLD[27] but to the best of our knowledge this is the first study that assessed the effects of whole grain wheat on liver fat in a randomized controlled trial. The observed prevention of liver fat accumulation in the WGW group as compared to RW may be caused by beneficial effects of WGW on hepatic lipid influx/synthesis or lipid efflux/removal. Hepatic lipid influx is determined by uptake from circulating NEFA and dietary chylomicrons or by newly synthesized TG via *de novo* lipogenesis. As we observed no changes in fasting NEFA or postprandial NEFA upon RW or WGW intervention, there are no indications for differences in NEFA supply to the liver. It is unknown if the chylomicron remnants and *de novo* lipogenesis were affected by our interventions and therefore we cannot completely rule out an effect on the hepatic lipid influx.

Hepatic lipid efflux is determined by the secretion of TG-rich lipoproteins (TRLs) and by removal of lipids via beta-oxidation. Levels of beta-hydroxybutyrate, as a marker for hepatic beta-oxidation, tended to be differentially changed between RW and WGW but the effects within intervention groups were ambiguous. WGW might exert its effects on lipid removal in the liver via numerous mechanisms, but we describe two main routes that have been defined previously; a direct effect of certain phytochemicals or an indirect effect via the fermentation

of cereal fiber into SCFAs by colonic bacteria. Studies in mice show that SCFAs, mainly butyrate, stimulate hepatic beta-oxidation via activation of AMPK[28-30]. This effect has not been studied in humans yet, but there is compelling evidence that SCFAs propionate and butyrate arising from the gut are efficiently taken up by the liver in humans[31]. Furthermore, Vetrani et al. showed that a whole grain wheat-based diet was able to increase plasma propionate in individuals with the metabolic syndrome[32] and a recent trial by Vanegas et al. found an increase in total stool SCFAs after a 6 week whole grain, predominantly wheat, intervention when compared to a refined grain intervention in healthy middle-aged adults[33]. This trial also found an increase in SCFA-producing *Lachnospira* genus. We did not observe significant differences in changes in SCFA-producing families or in any of their related genera but the 12 week RW intervention did result in a loss of microbial diversity when compared to the WGW intervention. Low diversity in fecal microbiota has been found in non-obese NAFLD patients when compared to healthy controls[34]. To further investigate the link between microbiota composition and IHTG we, like several recent trials[35,36], applied an increasing popular concept of predicting metabolic responses using microbiota-based classifiers. In our study, machine learning algorithms using microbiome abundance could not predict the percentage change in IHTG in the whole group and also not the increase in IHTG after RW from microbiome data. It might be that the diet-induced changes in microbiota composition by RW overrule the contribution of baseline composition, if this contribution is indeed present. Another option is that the sample size (n=35) was too small as the gut microbiome is highly variable[37]. Important to note is the threat of overfitting in these models due to the use of large scale data, which might result in non-reproducible findings.

Besides potential effects of SCFAs, specific phytochemicals in plant-based foods and especially grains have the ability to directly affect hepatic lipid metabolism[38]. These compounds have so-called lipotropic effects i.e. the capacity to remove fat from the liver and/or reduce hepatic lipid synthesis. Important lipotropes in WGW are the methyl donors choline and betaine and, to a lesser extent, the sugar alcohol myo-inositol[39]. Choline and myo-inositol are precursors for phosphatidylcholine and phosphatidylinositol, which are both indispensable compounds in VLDL-formation. However, choline can also be oxidized to betaine so sufficient levels of betaine spare choline for proper VLDL formation and thus efficient TG disposal[27]. In line with this, we observed an increase in postprandial TG levels in the WGW group which was significantly different from the RW group. This effect was mainly observed 4h in the postprandial phase when the contribution of VLDL to plasma TG levels is most significant[40]. This might indicate better lipid disposal in the WGW group when compared to the RW group. To summarize, with regard to hepatic lipid metabolism we observed no differences in changes in NEFA supply between intervention but modest clues for differential changes in TRL-secretion between intervention groups.

In addition to evaluating IHTG, we used magnetic resonance to determine abdominal body fat distribution, i.e. SAT and VAT mass and their ratio. An increased VAT/SAT ratio might indicate a diminished capacity of SAT to store lipids and hence a higher storage of fat in VAT. Visceral fat is highly lipolytic and secreted NEFA to the portal vein may easily spill over to the liver, thus contributing to enhanced fat storage in the liver[41-43]. In contrast with the effects of RW on IHTG, we found no differences between groups in changes in abdominal VAT or SAT mass. Furthermore, we found no changes in the expression of genes related to tissue fibrosis and remodeling in SAT upon either RW or WGW intervention.

Inflammation might play a role in the underlying mechanism of the beneficial effects of WGW, as several constituents of WGW including polyphenols, such as ferulic acid, and SCFAs might exert anti-inflammatory effects[44-47]. We observed trends towards lower levels of liver acute phase proteins SAA and CRP after WGW intervention when compared to RW, which might indicate a modest anti-inflammatory effect on the liver. Most whole grain food or WGW intervention studies found no or mixed effects on CRP and systemic inflammatory markers such as Il-1, Il-6, and TNF-a[10,11,32,48-49].

In this study, we have assessed the effects of whole grain wheat, rather than a mixture of whole grains, versus refined wheat on liver health in an at-risk population using advanced magnetic resonance techniques and a dietary challenge to better determine potential changes in lipid handling capacity. None of the participants dropped out and compliance was high, as reflected by increased plasma alkylresorcinols levels in the WGW group. Both interventions were well tolerated with respect to palatability and gastrointestinal effects and participants remained weight stable. Furthermore, all intervention products were provided in a real-life/feasible dose and we included a run-in period with RW products. This run-in period was incorporated to reduce variation in the population at the start, as WGW consumption is already quite high in The Netherlands, which could underlie the finding of detrimental effects of RW on IHTG rather than beneficial effects of WGW. A limitation of our study could be the sample size. As the study was powered to detect changes in plasma cholesterol levels, we might have missed effects of either RW or WGW on parameters related to hepatic lipid metabolism. While the fact that we have performed a range of measurements can be considered a strength, it might have led to false positive findings.

We have demonstrated that a 12 week 98g/day WGW intervention prevents a substantial increase in IHTG, when compared to a 12 week RW intervention. In addition, the RW intervention resulted in less diverse

microbiota. Consumption of WGW on the other hand was associated with stable IHTG levels and a tendency towards decreased acute phase inflammation. Our results showed that refined wheat increases liver fat and might contribute to NAFLD, while incorporating feasible doses of whole grain wheat in a diet maintains liver health. Hepatic effects could be a crucial underlying mechanism in the positive health effects of WGW described in observational studies.

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AUTHORS' CONTRIBUTIONS

DE, MGP, RJV, SW, and LAA designed the study. SS, DE and LAA were responsible for the execution of the trial and data collection. Data analysis was conducted by SS and DE, supported by FPMH. GJEJH was involved in the analysis of the microbiota data. SS wrote the first draft of the manuscript, which was critically reviewed and improved by DE, FPMH, MGP, RJV, SW, and LAA. All authors read and approved the final manuscript.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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SUPPLEMENTAL MATERIAL

Supplemental Table 1. Primer sequences

	FORWARD	REVERSE
LPL	TCATTCCCGGAGTAGCAGAGT	GGCCACAAGTTTTGGCACC
ANGPTL4	CACAGCCTGCAGACACAACTC	GGAGGCCAAACTGGCTTTGC
CD36	CTTTGGCTTAATGAGACTGGGAC	GCAACAAACATCACCACACCA
CD68	GCTACATGGCGGTGGAGTACAA	ATGATGAGAGGCAGCAAGATGG
TGFB1	CAATTCCTGGCGATACCTCAG	GCACAACTCCGGTGACATCAA
MMP9	AGACCTGGGCAGATTCCAAAC	CGGCAAGTCTTCCGAGTAGT
LOX	GCCGACCAAGATATTCCTGGG	GCAGGTCATAGTGGCTAAACTC
CypA	CCCACCGTGTTCTTCGACAT	TCTTTGGGACCTTGTCTGCAA

Abbreviations: LPL: lipoprotein lipase, ANGPTL4: angiopoietin-like 4, CD36: Cluster of differentiation 36 (fatty acid translocase), CD68: cluster of differentiation 68, TGFB1: transforming growth factor beta 1, MMP9: matrix metallopeptidase 9, LOX: lysyl oxidase, CypA: cyclophilin A.

Supplemental Table 2. Compliance to RW and WGW intervention

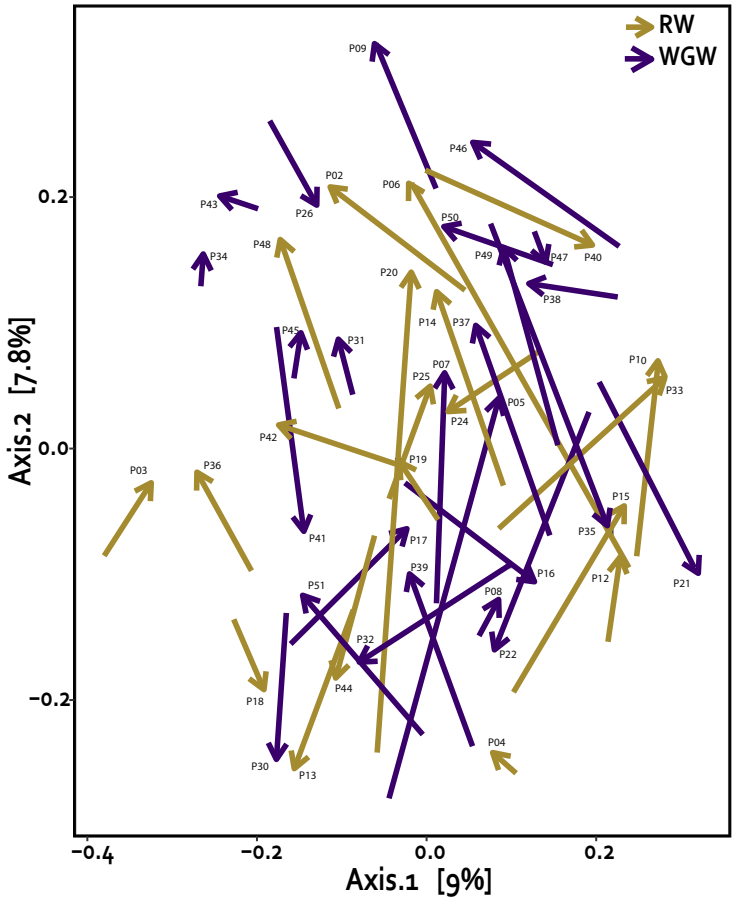
	RW GROUP *n*=25	WGW GROUP *n*=25	p-value
Based on product packages			
Bread, slices			
Regular, currant	99.8% , 99.5%	99.8%, 99.8%	
Bread, buns			
Regular, currant	99.5%, 99.5%	99.6%, 100%	
RTEC	99.7%	99.8%	
Plasma alkylresorcinol, nmol/L			
Baseline value	53.8 ± 13.3	50.2 ± 20.5	0.456
Change	-12 ± 19	159 ± 94	**<0.001**
% of correct classification	96%	96%	

Data on plasma alkylresorcinols are presented as mean ±1SD. P-values calculated using Independent samples t-test for baseline comparisons between RW and WGW as well as for the change in plasma alkylresorcinols levels between the intervention groups Abbreviations: RW: refined wheat, WGW: whole grain wheat, RTEC: ready to eat cereal

Supplemental Table 3. Relative changes in abundance of SCFA-producing families *Lachnospiraceae* and *Ruminococcaceae* and their commonly observed genera upon RW and WGW intervention

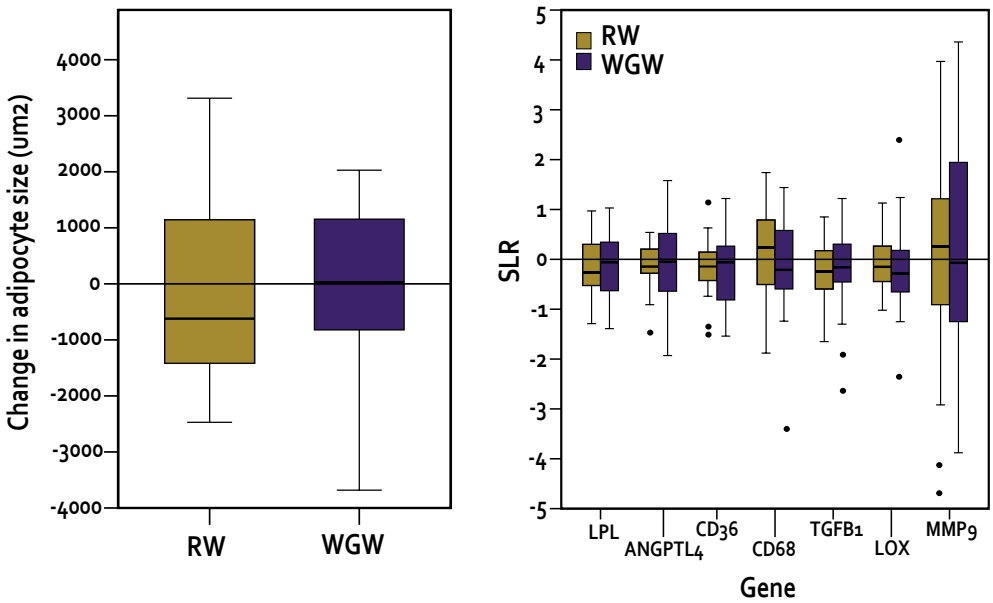
	RW GROUP *n*=21		WGW GROUP *n*=24		Group comparison
	Change in relative abundance after 12wk	p-value	Change in relative abundance after 12wk	p-value	p-value
Family					
Lachnospiraceae	-0.16 (2.10)	0.520	0.01 (2.27)	0.954	0.750
Genus					
Anaerostipes	-0.19 (6.07)	0.414	-0.68 (5.04)	0.407	0.856
Blautia	-0.46 (6.84)	0.079	-0.12 (3.68)	0.241	0.375
Clostridium_XlVa	0.32 (8.36)	0.375	0.30 (4.99)	0.424	0.820
Coprococcus	-0.47 (6.86)	0.048	0.13 (5.32)	0.753	0.258
Dorea	-0.65 (4.21)	0.068	-0.43 (3.27)	0.048	0.534
Lachnospira	0.36 (4.91)	0.332	0.44 (8.69)	0.412	0.774
Roseburia	0.05 (7.40)	0.664	-0.12 (4.46)	0.458	0.585
Ruminococcus 2	-6.65 (5.06)	0.852	-7.06 (5.56)	0.648	0.699
Family					
Ruminococcaceae	-0.28 (3.53)	0.289	-0.23 (2.00)	0.475	0.820
Genus					
Butyricicoccus	-0.56 (7.77)	0.940	0.10 (6.13)	0.530	0.525
Clostridium_IV	-0.22 (5.03)	0.322	-0.37 (4.02)	0.440	1.000
Faecalibacterium	0.17 (8.53)	0.848	-0.02 (3.31)	1.000	0.802
Flavonifractor	-0.19 (4.37)	0.711	0.36 (4.36)	0.149	0.198
Gemmiger	-0.14 (4.91)	0.227	-0.18 (4.90)	0.086	0.961
Oscillibacter	-0.20 (5.48)	0.689	0.34 (3.60)	0.067	0.179
Ruminococcus	-0.37 (11.26)	0.170	-0.20 (9.59)	0.179	0.838
Sporobacter	-0.18 (5.53)	0.455	0.33 (5.70)	0.391	0.255
Subdoligranulum	0.05 (10.08)	0.664	1.04 (9.88)	0.278	0.413

Data are presented as median (range). Abbreviations: RW; refined wheat, WGW: whole grain wheat. Changes within group analyzed using Wilcoxon signed-rank test, changes between groups analyzed using Mann-Whitney U test for not normally distributed values.



Supplemental Figure 1. Dissimilarity in bacterial communities between the samples upon RW or WGW intervention

Abbreviations: RW: refined wheat, WGW: whole grain wheat. Principal coordinates analysis based on the Bray-Curtis distance. RW *n*=21, WGW *n*=24. Each Px represents one individual participant in the study, (participant or 'P') with its number.



Supplemental Figure 2. Changes in adipocyte size and subcutaneous adipose tissue gene expression upon RW or WGW intervention

Left: Changes in adipocyte size. RW *n*=11, WGW *n*=12. Right: Changes in gene expression in subcutaneous adipose tissue. RW *n*=24, WGW *n*=23. Abbreviations: SLR: logarithmic ratio of signal intensity, RW: refined wheat, WGW: whole grain wheat, LPL: lipoprotein lipase, ANGPTL-4: angiopoietin-like 4, CD36: cluster of differentiation 36 (fatty acid translocase), CD68: cluster of differentiation 68, TGFB1: transforming growth factor beta 1, LOX: lysyl oxidase, MMP9: matrix metallopeptidase 9. Data are presented as descriptive boxplots, in which the center represents the median, the outlines of the box indicate Q1 and Q3 while the whiskers show the most extreme data points within 1.5 times the IQR, otherwise indicated by a • symbol..

3

THE EFFECTS OF TWO ENERGY RESTRICTED DIETS DIFFERING IN NUTRIENT QUALITY ON METABOLIC HEALTH IN OBESE MEN AND WOMEN

A RANDOMIZED CONTROLLED TRIAL

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In preparation

ABSTRACT

Objective: We examined the additional effects of nutrient quality on top of energy restriction (ER) by investigating the effects of two ER-diets differing in nutrient quality on cardio-metabolic risk factors in subjects with abdominal obesity.

Research Design and Methods: We performed a parallel-designed 12 week 25% ER dietary intervention study. Participants aged 40-70 with abdominal obesity (BMI >27kg/m^2 or waist circumference >88cm for females, >102cm for males) were randomized over three groups; a Targeted diet (*n*=40, TD) enriched with monounsaturated fatty acids, *n-3* polyunsaturated fatty acids, soy protein, and fiber; a Western-type diet (*n*=40, WD) high in saturated fatty acids and fructose or a control group (*n*=30). Before and after the intervention, fasting HbA1c, glucose, insulin, triglycerides, free fatty acids, total cholesterol, HDL-cholesterol, liver enzymes as well as intra-hepatic lipids, abdominal body fat distribution by MRS/MRI and vascular measurements were assessed. Postprandial responses of plasma glucose, insulin, triglycerides, and free fatty acids and vascular parameters after a mixed meal challenge test were determined.

Results: Participants in the TD lost significantly more weight (-8.4 ± 3.2kg in total) than in the WD (-6.3 ± 3.9kg). Both ER-diets significantly reduced intra-hepatic lipids as well as subcutaneous and visceral adipose tissue mass. Only the TD significantly lowered fasting total cholesterol and triglycerides while both ER-diets reduced fasting glucose, insulin, GGT, HbA1c, and postprandial responses of glucose as well as systolic and diastolic blood pressure.

Conclusions: Enhancing nutrient quality of a 25% ER-diet results in greater weight loss and reductions in total cholesterol and triglycerides, and can be considered as the superior whole diet approach towards improving metabolic health in abdominally obese subjects.

INTRODUCTION

Abdominal obesity is associated with an elevated risk of developing chronic diseases such as non-alcoholic fatty liver disease, cardiovascular disease, and type 2 diabetes[1]. These metabolic complications are a result of perturbations in different metabolic organs such as the liver, adipose tissue, skeletal muscle, and gut. This overall disturbance in metabolic functioning is referred to as a reduction in metabolic health. A well-known dietary strategy to improve metabolic health is by limiting daily energy intake, usually a 10-30% reduction compared to an ad libitum diet. Numerous studies have demonstrated that energy restriction (ER) without malnutrition successfully induces weight loss and ameliorates several cardio-metabolic risk factors in humans, such as reductions in blood pressure, HbA1c and plasma glucose, LDL-cholesterol, and triglycerides[2,3]. Furthermore, weight loss can be induced by any ER-diet regardless which macronutrients are emphasized[4].

However, enhancement of nutrient quality might benefit metabolic health, beyond weight loss. For example, polyunsaturated fatty acids (PUFA) exert beneficial effects on lipid metabolism resulting in a decrease in inflammatory status, a favorable plasma lipid profile, and augmented insulin sensitivity[5,6]. An increased proportion of protein in the diet has beneficial effects on body composition[7]. In addition, there is moderate evidence for beneficial effects of high-protein diets on several cardio-metabolic risk factors, such as a reduction in plasma triglycerides[8]. Multiple studies also report beneficial effects of the plant protein soy on blood lipids and adipose tissue health[9,10]. With regard to carbohydrates, an increased intake of low GI- or complex carbohydrates avoids exaggerated glucose responses and might improve overall glucose homeostasis on the long-term[11]. Furthermore, dietary fiber has cholesterol-lowering properties and favorably affects the gut microbiota[12,13]. Finally, a reduced consumption of fructose, a potent inducer of de novo lipogenesis in the liver, might aid in lowering intrahepatic lipids[14].

Consequently, a *whole diet approach* may be a powerful tool to modify health. However, nutritional effects on static health measurements in the fasted state are often hard to detect due to the tight regulation of homeostasis. The application of a dietary stressor can reveal the capacity of an individual to keep metabolic balance and to return to homeostasis, referred to as phenotypic flexibility. This phenotypic flexibility might form a more sensitive measure of metabolic health in comparison to measurements in the fasting state[15,16]. A mixed meal test, i.e. a high fat/sugar/protein load, as dietary stressor challenges multiple metabolic organs and affects their interplay, requiring adaptation at several levels. Readouts for such as challenge can include excursions of plasma parameters e.g. postprandial glucose and insulin.

We aimed to examine the additional effects of nutrient quality over ER on metabolic health. We investigated the effects of two ER-diets that differed in nutrient quality on risk factors for type 2 diabetes and other cardio-metabolic risk factors, including responses to a mixed meal test, in abdominally obese subjects. We used a whole diet approach with one diet that resembled a Western-type diet (WD) and one diet, referred to as the Targeted diet (TD), that aimed to improve metabolic health through the inclusion of nutrients such as monounsaturated fatty acids, *n-3* PUFA, soy protein, and fiber. Effects of both ER-diets were compared to a control group that did not receive any dietary intervention.

RESEARCH DESIGN AND METHODS

Study design

This study was a parallel-designed randomized 12wk intervention study conducted at Wageningen University, the Netherlands. The study was approved by the Medical Ethics Committee of Wageningen University and registered at ClinicalTrials.gov, identifier: **NCT02194504**. Procedures were in accordance with the Helsinki Declaration of 1975 as revised in 1983. Participants were randomized over three groups; the Western-type (WD, *n*=40) and the Targeted (TD, *n*=40) 25% ER dietary intervention groups and a non-ER control group (*n*=30). The intervention was based on dietary advice and the provision of key food products. Participants in the control group were instructed to maintain their habitual diet. Schofield equations[17] using a body mass corresponding with a BMI of 27kg/m^2 were applied to estimate the basal metabolic rate of each participant. This rate was multiplied by a physical activity level value based on participants' reported activity level to calculate estimated energy requirement (EER). Participants were instructed to maintain their physical activity habits. An energy restriction of 25% was applied to the EER to allocate participants to an energy group of either 6, 7, 8 or 9 MJ/day.

Nutritional composition of the diets and reported dietary intake are listed in **Table 1**. The diets were equally energy-restricted and matched for alcohol, sodium, and total fat intake, but differed in fatty acid composition, amount and sources of protein as well as fiber and fructose content. The composition of the diets formed the basis of meal plans which were communicated to the participants (**Supplemental Table 1**). Participants visited the facility weekly for an individual session with a qualified dietician, after which the key food products were provided for the following week. Adherence to the diet was determined based on the participant's weekly reports. To calculate reported intake of energy and nutrients, the meal plan was used. Additional consumed food items were added to the intake while leftovers from supplied products were collected and subtracted.

Table 1. Nutritional composition of the two experimental diets and reported intake

	WESTERN-TYPE DIET GROUP *n*=39		TARGETED DIET GROUP *n*=34		Differences in reported intake between diets
	Advised	Reported	Advised	Reported	p-value
Energy intake (MJ)	6.95 ± 0.78	7.13 ± 0.82	6.99 ± 0.90	7.07 ± 1.00	0.778
ER (%)	25.8 ± 5.4	23.9 ± 0.82	26.9 ± 5.6	26.2 ± 5.9	0.775
En% Fat	31.5 ± 0.4	31.4 ± 1.6	32.1 ± 0.3	32.5 ± 1.2	**0.001**
En% SFA	15.5 ± 0.4	14.9 ± 1.5	7.9 ± 0.5	8.3 ± 0.6	**<0.001**
En% MUFA	9.0 ± 0.4	9.3 ± 1.0	13.9 ± 0.7	13.4 ± 0.9	**<0.001**
En% PUFA	3.9 ± 0.3	4.1 ± 0.8	7.8 ± 0.2	7.7 ± 0.4	**<0.001**
En% Trans fat	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	**0.015**
ALA (mg)	1057 ± 67	1104 ± 278	1795 ± 35	1815 ± 354	**<0.001**
EPA & DHA (mg)	33 ± 5	103 ± 226	1270 ± 0	1224 ± 55	**<0.001**
Cholesterol (mg)	183 ± 23	185 ± 30	124 ± 19	132 ± 20	**<0.001**
En% Protein	15.4 ± 0.2	16.0 ± 1.4	20.4 ± 0.1	20.6 ± 1.7	**<0.001**
En% Animal-based	9.9 ± 0.3	10.3 ± 1.1	9.8 ± 0.2	10.3 ± 1.9	0.886
En% Plant-based	5.5 ± 0.1	5.7 ± 1.0	10.5 ± 0.1	10.3 ± 0.5	**<0.001**
En% Carbohydrates	50.5 ± 0.2	49.6 ± 2.0	44.0 ± 0.3	43.7 ± 1.5	**<0.001**
En% Mono- and disaccharides	25.3 ± 0.8	24.8 ± 1.9	19.0 ± 0.5	18.8 ± 1.1	**<0.001**
En% Polysaccharides	25.2 ± 0.9	24.8 ± 1.2	25.0 ± 0.4	24.9 ± 1.1	0.905
En% Fructose	7.4 ± 0.1	7.0 ± 0.9	3.5 ± 0.4	3.4 ± 0.4	**<0.001**
En% Fiber	2.2 ± 0.1	2.2 ± 0.2	3.2 ± 0.0	3.1 ± 0.2	**<0.001**
En% Alcohol	0.8 ± 0.3	1.1 ± 0.9	0.8 ± 0.3	1.0 ± 0.7	0.599
Sodium (g)	1.6 ± 0.2	2.3 ± 0.6	1.7 ± 0.3	2.3 ± 0.4	0.659

Data are presented as mean ± 1SD. Values were calculated based on the Dutch Food Composition Database 2011 (NEVO 2011/3.0, RIVM, Bilthoven, The Netherlands). Comparisons between groups were conducted using Independent t-tests. Advised values represent the average intake values across the 6, 7, 8, and 9MJ energy groups. Values are averaged over a 1-week menu cycle. Abbreviations: MJ: Mega joule, ER: Energy restriction, En%: Energy percentage, SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, ALA: alpha linolenic acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid

Participants

The study population consisted of healthy abdominally obese men and women, aged 40-70 at time of recruitment. Abdominal obesity was defined as a BMI >27kg/m^2 or a waist circumference >88cm (women) or >102cm (men).

Exclusion criteria were:

- Earlier diagnosed with diabetes or diagnosed during our screening (OGTT, fasting glucose >7mmol/L, after 2h >11.1mmol/L)
- Daily alcohol intake of >20g (women) or 30g (men)
- Smoking
- Unstable body weight (weight gain or loss of >3kg in the past three months)
- Diagnosed with a long-term medical condition
- Using medication known to interfere with glucose or lipid homeostasis
- Being allergic to fish oil or restricted to a vegetarian dietary regime

All subjects gave written informed consent. The study was powered to detect changes in intrahepatic lipid accumulation. Due to the lack of studies describing the effects of whole diet approaches or a combination of nutrients on intrahepatic lipids, we extrapolated information from single nutrients studies, i.e. fructose, *n-3* and *n-6* PUFA[18-20] on intrahepatic lipids.

Randomization and masking

Randomization of the participants over the intervention groups was conducted using block randomization using Microsoft Excel by a researcher who was not involved in the study. Stratification among the groups was based on BMI, age, and gender. Data analysis was performed blinded.

Procedures

At the start of the study and after 12wks intervention, abdominal body fat distribution and intra-hepatic lipids were determined using a 3T whole body MRI-scanner (Siemens, Munich, Germany) at hospital Gelderse Vallei, Ede, The Netherlands. Intra-hepatic lipid measurements were conducted as described previously[21]. For adipose tissue mass measurements, axial T1-weighted spin echo images were acquired, slices were centered at the interspace L4-L5. A breath-hold technique was applied to avoid breathing-induced artefacts. On a separate study day at the start of the study and after 12wk intervention, measurements on metabolic health were conducted at Wageningen University, The Netherlands. Prior to this day, participants were asked to refrain from alcohol, avoid strenuous exercise, ensure a sufficient night's rest, and consume a low-fat standardized meal before 8pm, after which they had to fast. Fasting blood samples were drawn from the antecubital vein, subsequently fasting vascular measurements were performed after 10 min of rest. Systolic blood pressure, diastolic blood pressure and heart rate were assessed automatically (DINAMAP® PRO100) for 10 minutes with a 3 minute interval. Heart rate corrected augmentation index as measure of arterial stiffness[22] was assessed by pulse wave analysis of the radial artery (SphygmoCor®CP System, ATcor Medical) as described previously[23]. After completing fasting measurements participants consumed a mixed meal within 5min. The mixed meal was a 3833kJ shake consisting of 76.3g carbohydrates, 17.6g protein, and 60g fat[16]. Blood drawings were conducted in the postprandial phase at 30, 60, 120, 180, 240 and 360 minutes, and vascular measurements were performed at 120 and 240 minutes.

Outcomes

Analysis of the MRS spectra was performed using jMRUI software v5.2, while adipose tissue depots were quantified based on a single slice centered in middle of 9 recorded images using the software program

HippoFatTM[24]. HbA1c was determined in whole blood samples by hospital Gelderse Vallei, Ede, The Netherlands. Plasma glucose, triglycerides and insulin as well as serum total cholesterol, HDL-cholesterol, and liver enzymes were analyzed photometrically (Cobas® 8000, Roche Diagnostic Limited, Switzerland) by a center for medical diagnostics (Stichting Huisartsenlaboratorium Oost, Velp, The Netherlands). Plasma free fatty acids were determined using an enzymatic assay (INstruchemie, Delfzijl, The Netherlands). The homeostatic model assessment of insulin resistance, HOMA-IR, was calculated by dividing the product of fasting glucose (mmol/L) and fasting insulin (mU/L) by 22.5. The quantitative insulin sensitivity check index, QUICKI, was calculated using the equation: 1/[log fasting insulin (mU/L)+log fasting glucose (mg/dL)]. Clinical chemistry was performed on all samples available, in a small number of cases samples were missing (WD *n*=35-39, TD *n*=30-34, control group *n*=23-27 samples). The primary outcome of the study was the combined results of the reported measurements, as a novel and comprehensive readout for metabolic health status.

Statistical analysis

Data analysis was executed on a per-protocol basis. Comparisons between the two ER-groups only were performed using Independent samples t-tests. Analyses of changes in fasting parameters were conducted using a general linear model for univariate analysis with baseline values as covariates (ANCOVA), after which LSD post-hoc testing was applied. Analyses of changes in postprandial responses to the mixed meal test were conducted using linear mixed effects modelling with postprandial time points as repeated measures. Dependent variable in this model was the difference between the response of a certain parameter to the mixed meal test (postprandial value minus fasting value) at each time point between before and after the intervention. Linear mixed effects modeling was executed using an autoregressive covariance structure and LSD post-hoc testing. Analyses were conducted using IBM SPSS Statistics v22 (IBM Corp, Armonk, NY, USA).

RESULTS

A Between July 21 and October 21 2014, we assessed 151 individuals for eligibility of which 41 were excluded from participation (**Figure 1**). The 110 remaining participants were randomly allocated to one of the three intervention groups. Eleven participants dropped out of the study, of which one in the WD, seven in the TD and three in the control group. Due to the phased entry of participants in the study, one drop-out in the TD could be replaced with another individual. Baseline characteristics of the 100 study participants that completed the study are summarized in **Supplemental Table 2**.

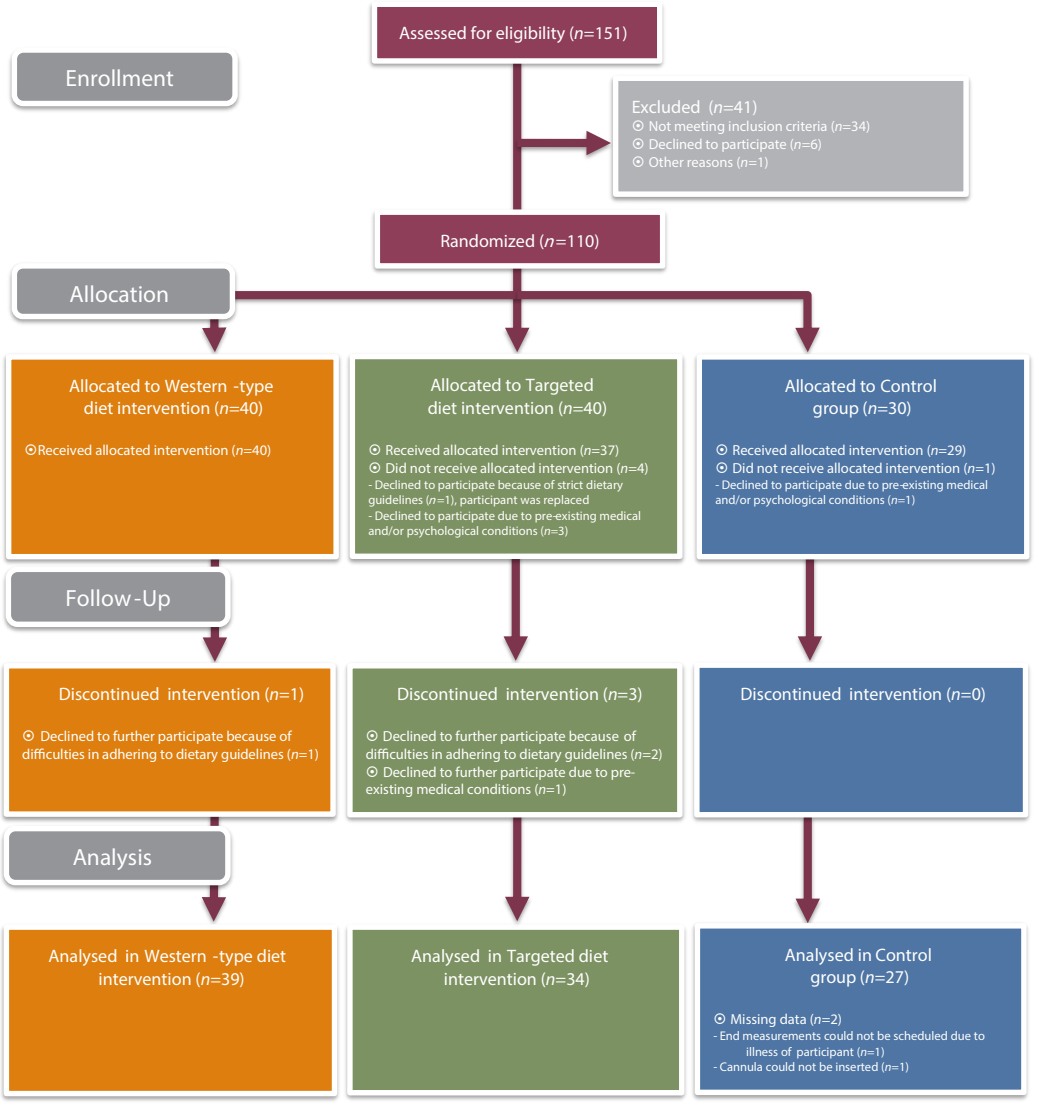

Figure 1. Flow chart of study participants

Weight loss upon the intervention differed significantly between the three groups. Total body weight decreased significantly in both the WD (-6.3±3.9kg) and the TD (-8.4±3.2kg) (**Figure 2**). Although both diets were designed to have an equal ER, total weight loss was significantly higher in the TD when compared to the WD. A detailed comparison between the advised and reported intake values per diet group can be found in **Supplemental Table 3**. Reported intake resembled the advised composition for both diets (**Table 1**). Although weight loss was more pronounced in the TD, there was no significant difference in reported energy intake between diets groups.

To explore the contribution of digestibility of macronutrients to the differences in weight loss, we recalculated advised total energy intake using specific digestibility values derived from the Atwater system[25] (**Supplemental Table 4**). The metabolizable energy of a 7 MJ/day week menu amounted 97.2% in the WD and 96.0% in the TD, accounting for 237g of the difference in weight loss.

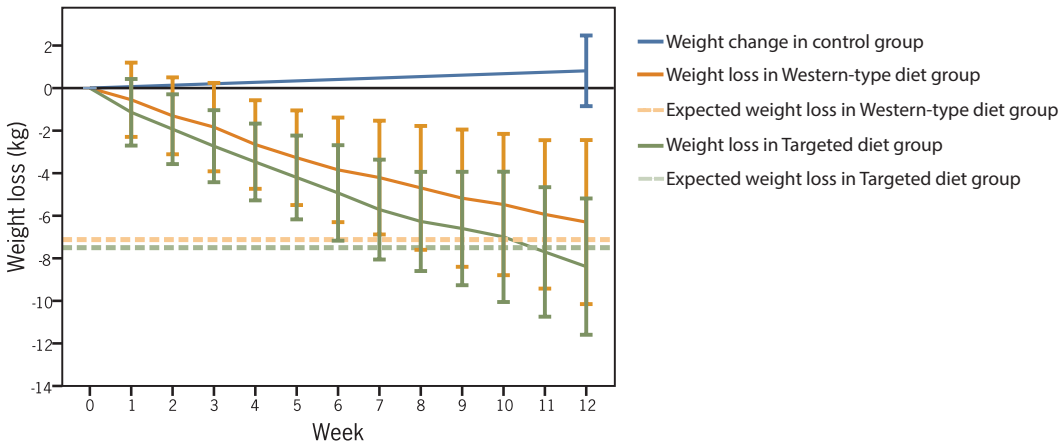

Figure 2. Cumulative weight loss over 12 weeks of dietary intervention

Cumulative weight loss in the three study groups over 12 weeks of intervention, including expected weight loss based on calculated energy restriction. Error bars represent 1SD. * indicates a significant difference ($p<0.05$) in cumulative weight loss between the two ER-groups.

Effects of the 12wk intervention on body composition and fasting plasma parameters are summarized in **Table 2**. Changes in BMI, waist circumference, measures of abdominal fat distribution, and intrahepatic lipids differed significantly between the groups. In line with weight loss, both BMI and waist circumference decreased significant in both ER-groups compared to the control group. In addition, BMI decreased significantly more in the TD compared to WD. Post-hoc analysis showed that intra-hepatic lipids were significantly lowered by both interventions compared to the control. Subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) mass were significantly reduced in both ER-groups compared to the control group. In addition, VAT/SAT ratio was also reduced in both diet groups compared to the control. With regard to blood lipids, changes in plasma triglycerides and serum total cholesterol differed significantly between the groups. Post-hoc analyses showed that total cholesterol and triglycerides decreased significantly in the TD compared to WD and control. Plasma free fatty acids and serum HDL-cholesterol were unaffected in all groups. Fasting plasma glucose, insulin, HbA1c, HOMA-IR, and QUICKI improved significantly in both ER-groups compared to the control group. Serum of GGT

Table 2. Changes in anthropometrics, body fat distribution, fasting plasma parameters and vascular measurements

	BASELINE	**CHANGE** after 12 weeks	Within group **p-value**	Difference between groups **p-value**
Body weight, kg				
Western-type diet group	93.1 ± 13.9	-6.3 ± 3.9[a]	**<0.001**	
Targeted diet group	94.8 ± 13.2	-8.4 ± 3.2[b]	**<0.001**	**<0.001**
Control group	92.1 ± 12.6	0.8 ± 1.7[c]	**0.018**	
BMI, kg/m²				
Western-type diet group	31.2 ± 3.3	-2.1 ± 1.3[a]	**<0.001**	
Targeted diet group	31.8 ± 3.4	-2.8 ± 1.1[b]	**<0.001**	**<0.001**
Control group	30.9 ± 3.9	0.3 ± 0.6[c]	**0.015**	
Waist circumference, cm				
Western-type diet group	108.7 ± 9.8	-3.1 ± 5.3[a]	**<0.001**	
Targeted diet group	109.5 ± 9.8	-4.7 ± 5.7[a]	**<0.001**	**<0.001**
Control group	106.6 ± 8.1	4.1 ± 5.2[b]	**<0.001**	
SAT, cm²*				
Western-type diet group	332.5 ± 92.4	-45.3 ± 32.7[a]	**<0.001**	
Targeted diet group	348.0 ± 106.5	-54.1 ± 55.3[a]	**<0.001**	**<0.001**
Control group	355.6 ± 118.8	1.6 ± 36.9[b]	0.628	
VAT, cm²*				
Western-type diet group	149.1 ± 77.0	-44.1 ± 48.2[a]	**<0.001**	
Targeted diet group	175.7 ± 94.2	-61.5 ± 51.3[a]	**<0.001**	**<0.001**
Control group	115.8 ± 26.1	3.7 ± 17.9[b]	0.551	
VAT/SAT ratio*				
Western-type diet group	50.6 ± 34.8	-9.4 ± 16.9[a]	**0.001**	
Targeted diet group	61.2 ± 54.9	-16.0 ± 28.1[a]	**0.001**	**0.032**
Control group	36.2 ± 16.0	0.8 ± 6.3[b]	0.775	
Intra-hepatic lipids, % of water peak*				
Western-type diet group	7.3 ± 6.5	-3.4 ± 5.1[a]	**<0.001**	
Targeted diet group	7.1 ± 8.7	-3.9 ± 5.7[a]	**0.001**	**<0.001**
Control group	5.9 ± 5.8	1.4 ± 1.9[b]	**0.021**	
Plasma glucose, mmol/L				
Western-type diet group	5.6 ± 0.8	-0.3 ± 0.6[a]	**0.003**	
Targeted diet group	5.7 ± 0.5	-0.3 ± 0.4[a]	**<0.001**	**0.007**
Control group	5.6 ± 0.4	0.0 ± 0.4[b]	0.585	
Plasma insulin, mU/L				
Western-type diet group	14.6 ± 10.3	-3.9 ± 7.5[a]	**0.002**	
Targeted diet group	13.5 ± 7.8	-3.6 ± 5.3[a]	**<0.001**	**0.012**
Control group	12.1 ± 5.3	0.3 ± 6.1[b]	0.782	
HbA1c, mmol/mol				
Western-type diet group	36.3 ± 2.1	-0.8 ± 2.0[a]	**0.013**	
Targeted diet group	37.3 ± 2.9	-1.5 ± 2.0[a]	**<0.001**	**0.014**
Control group	35.1 ± 2.6	0.4 ± 1.3[b]	0.146	
HOMA-IR				
Western-type diet group	3.8 ± 3.1	-1.3 ± 2.3[a]	**0.001**	
Targeted diet group	3.5 ± 2.1	-1.0 ± 1.5[a]	**<0.001**	**0.006**
Control group	3.0 ± 1.4	0.1 ± 1.5[b]	0.824	
QUICKI				
Western-type diet group	0.33 ± 0.04	0.01 ± 0.03[a,b]	**0.022**	
Targeted diet group	0.33 ± 0.03	0.02 ± 0.03[a]	**<0.001**	**0.014**
Control group	0.33 ± 0.02	0.00 ± 0.02[b]	0.845	
Serum total cholesterol, mmol/L				
Western-type diet group	5.7 ± 1.1	-0.2 ± 0.8[a]	0.218	
Targeted diet group	5.5 ± 0.8	-0.5 ± 0.6[b]	**<0.001**	**0.013**
Control group	5.4 ± 1.0	0.0 ± 0.5[a]	0.633	
Serum HDL cholesterol, mmol/L				
Western-type diet group	1.3 ± 0.4	0.0 ± 0.2	0.096	
Targeted diet group	1.4 ± 0.4	-0.1 ± 0.2	0.084	0.878
Control group	1.4 ± 0.4	0.0 ± 0.3	0.439	

	BASELINE	CHANGE after 12 weeks	Within group p-value	Difference between groups p-value
Plasma triglycerides, mmol/L				
Western-type diet group	1.8 ± 0.8	-0.2 ± 0.6[a]	**0.018**	
Targeted diet group	1.6 ± 0.6	-0.4 ± 0.6[b]	**0.001**	**0.026**
Control group	1.7 ± 0.7	-0.1 ± 0.5[a]	0.280	
Plasma free fatty acids, mmol/L				
Western-type diet group	0.41 ± 0.21	-0.02 ± 0.20	0.522	
Targeted diet group	0.57 ± 0.37	-0.04 ± 0.49	0.471	0.389
Control group	0.42 ± 0.18	0.05 ± 0.24	0.291	
Serum ALT, U/L				
Western-type diet group	26.4 ± 10.1	-2.2 ± 9.8	0.164	
Targeted diet group	27.6 ± 11.2	-3.6 ± 11.3	0.071	0.288
Control group	27.2 ± 10.9	0.0 ± 6.4	1.000	
Serum AST, U/L				
Western-type diet group	24.1 ± 13.3	-2.4 ± 14.1	0.302	
Targeted diet group	22.9 ± 6.1	-1.0 ± 5.5	0.282	0.123
Control group	27.2 ± 13.8	-0.4 ± 7.4	0.778	
Serum GGT, U/L				
Western-type diet group	31.2 ± 23.6	-9.2 ± 15.7[a]	**0.001**	
Targeted diet group	25.0 ± 9.8	-7.3 ± 7.4[a]	**<0.001**	**<0.001**
Control group	27.4 ± 18.8	-0.2 ± 3.4[b]	0.781	
Systolic blood pressure, mmHg				
Western-type diet group	125 ± 19	-6 ± 13[a]	**0.005**	
Targeted diet group	131 ± 15	-12 ± 13[a]	**<0.001**	**<0.001**
Control group	127 ± 15	1 ± 9[b]	0.674	
Diastolic blood pressure, mmHg				
Western-type diet group	71 ± 8	-3 ± 7[a]	**0.018**	
Targeted diet group	76 ± 9	-5 ± 7[a]	**<0.001**	**<0.001**
Control group	75 ± 9	2 ± 5[b]	0.076	
Heart rate, BPM				
Western-type diet group	59 ± 10	-2 ± 10	0.158	
Targeted diet group	61 ± 7	-4 ± 7	**<0.001**	0.170
Control group	59 ± 10	1 ± 9	0.733	
Augmentation index, %				
Western-type diet group	23.5 ± 8.9	0.5 ± 3.1	0.413	
Targeted diet group	25.4 ± 7.9	-1.1 ± 3.1	0.060	0.455
Control group	22.2 ± 6.8	-0.8 ± 4.7	0.372	
Central systolic pressure, mmHg				
Western-type diet group	122 ± 19	-5 ± 14	0.074	
Targeted diet group	121 ± 15	-8 ± 9	<0.001	0.390
Control group	116 ± 15	-4 ±13	0.082	

Data are presented as mean ± 1SD. Different letters (a, b, c) indicate significant post hoc differences ($p<0.05$) between diet groups. Changes within group were analyzed using paired t-tests, differences in changes between groups were analyzed using a general linear model for univariate analysis with baseline values as covariates (ANCOVA) with LSD post-hoc testing. For SAT and VAT: Western-type diet group *n*=28, Targeted diet group *n*=26, Control group *n*=16, for IHL: Western-type diet group *n*=34, Targeted diet group *n*=29, Control group *n*=19, for measures from Pulse Wave Analysis (AIX and central SBP): Western-type diet group *n*=37, Targeted diet group *n*=32, Control group *n*=26. For all other values: Western-type diet group *n*=39, Targeted diet group *n*=34, Control group *n*=27. Values denoted with an * are log2 transformed for statistical analyses to improve normality. Abbreviations: BMI: body mass index, SAT: subcutaneous adipose tissue mass, VAT: visceral adipose tissue mass, IHL: intrahepatic lipid, HbA1c: glycosylated hemoglobin, HOMA-IR: homeostatic model assessment of insulin resistance, QUICKI: quantitative insulin sensitivity check index, HDL: high-density lipoprotein, ALT: alanine transaminase, AST: aspartate transaminase, GGT: gamma-glutamyltransferase. BPM: beats per minute

decreased significantly in both diet groups compared to the control group. ALT and AST were unaffected in all groups. Systolic and diastolic blood pressure decreased significantly in both diet groups compared to the control. Changes in heart rate and the outcomes of the pulse wave analysis, AIX and central systolic blood pressure, did not differ between groups.

Effects of the 12wk intervention on responses to the mixed meal test of plasma glucose, insulin, triglycerides, and free fatty acids as well as vascular measurements are summarized in **Table 3**. None of the changes in plasma responses upon intervention were statistically significant different between the three groups. However, the response of plasma glucose to the mixed meal test upon 12wks dietary intervention was significantly reduced within the WD and borderline significant in the TD (p=0.066) while being unaffected in the control group (**Supplemental Figure 1A**). Changes in this response curve were due to significantly lower postprandial glucose upon 12wks of ER in the early postprandial phase, i.e. T=30 for the WD and T=60min for the WD and TD. The overall postprandial response of plasma insulin to the mixed meal test was unaffected by the interventions (**Supplemental Figure 1B**). In addition, the overall postprandial response of plasma triglycerides to the mixed meal test was also unaffected by the interventions, but the response at T=360min was significantly reduced within the WD (**Supplemental Figure 1C**). Responses of plasma free fatty acids showed no changes upon intervention, neither at the overall response nor at separate time points (**Supplemental Figure 1D**). Changes in blood pressure measures and AIX after the mixed meal test were unaffected by the interventions. Changes in postprandial heart rate differed between the three groups and was borderline significantly affected within the TD (p=0.056), caused by a tendency towards a lower response at T=240min (p=0.075) in this group.

Table 3. Responses to a mixed meal test; changes in postprandial glucose, insulin, triglycerides, free fatty acids, and vascular measurements

	WESTERN-TYPE DIET GROUP *n*=39					TARGETED DIET GROUP *n*=34					CONTROL GROUP *n*=27					Group comp.
Plasma glucose, mmol/L	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	
0 min	5.6 ± 0.7		5.3 ± 0.4			5.7 ± 0.5		5.4 ± 0.5			5.6 ± 0.4		5.6 ± 0.5			
30 min	7.7 ± 1.2	2.1 ± 1.0	6.7 ± 1.1	1.4 ± 1.0	**0.019**	8.1 ± 1.4	2.4 ± 1.1	7.3 ± 1.2	1.9 ± 1.0	0.173	7.2 ± 0.9	1.5 ± 0.9	7.1 ± 0.9	1.5 ± 0.9	0.690	
60 min	7.9 ± 2.0	2.3 ± 1.9	6.2 ± 1.7	0.9 ± 1.6	**<0.001**	8.6 ± 2.7	2.9 ± 2.4	7.2 ± 1.8	1.8 ± 1.7	**0.001**	7.4 ± 2.0	1.8 ± 1.9	7.0 ± 2.0	1.4 ± 2.0	0.256	0.239
120 min	5.1 ± 1.4	-0.5 ± 1.3	4.5 ± 1.4	-0.8 ± 1.5	0.260	5.8 ± 1.7	0.1 ± 1.6	4.9 ± 1.2	-0.5 ± 1.3	0.086	5.1 ± 1.5	-0.5 ± 1.5	5.1 ± 1.3	-0.5 ± 1.3	0.940	
180 min	5.0 ± 1.0	-0.6 ± 1.2	4.4 ± 0.7	-0.9 ± 0.8	0.365	4.8 ± 0.8	-0.9 ± 1.0	4.5 ± 0.9	-0.9 ± 1.0	0.929	4.8 ± 1.0	-0.9 ± 1.0	4.9 ± 1.0	-0.7 ± 1.0	0.779	
240 min	4.3 ± 0.6	-1.3 ± 0.8	4.2 ± 0.6	-1.1 ± 0.6	0.410	4.3 ± 0.5	-1.4 ± 0.7	4.2 ± 0.4	-1.2 ± 0.5	0.616	4.3 ± 0.6	-1.4 ± 0.7	4.2 ± 0.6	-1.4 ± 0.7	0.841	
Change in overall response within diet					**<0.001**					**0.066**					**0.858**	
Plasma insulin, mU/L	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	
0 min	14.6 ± 10.3		10.7 ± 5.7			13.5 ± 7.8		9.5 ± 5.5			12.1 ± 5.3		12.4 ± 6.2			
60 min	132.7 ± 105.6	118.1 ± 97.6	103.6 ± 93.1	92.9 ± 89.1	0.095	131.7 ± 101.6	118.2 ± 95.7	102.3 ± 77.6	90.6 ± 73.4	0.056	111.0 ± 45.3	98.9 ± 44.1	104.6 ± 50.1	92.2 ± 47.3	0.484	
120 min	79.8 ± 94.0	65.2 ± 86.0	46.9 ± 56.6	36.6 ± 52.6	0.059	77.8 ± 79.3	64.3 ± 72.9	51.1 ± 45.0	41.0 ± 42.1	0.106	60.5 ± 41.7	48.4 ± 41.8	57.2 ± 43.5	44.8 ± 40.0	0.703	0.432
240 min	14.2 ± 10.4	-0.6 ± 5.6	9.3 ± 8.5	-1.7 ± 5.4	0.984	13.5 ± 13.3	0.0 ± 7.8	6.9 ± 5.3	-2.8 ± 3.5	0.847	10.6 ± 6.4	-1.4 ± 7.6	9.8 ± 5.0	-2.6 ± 4.2	0.903	
Change in overall response within diet					0.266					0.400					0.924	
Plasma triglycerides, mmol/L	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	
0 min	1.8 ± 0.8		1.6 ± 0.5			1.6 ± 0.6		1.2 ± 0.5			1.7 ± 0.7		1.6 ± 0.9			
120 min	1.8 ± 0.8	0.0 ± 0.2	1.6 ± 0.6	0.0 ± 0.2	0.855	1.5 ± 0.5	-0.1 ± 0.2	1.1 ± 0.5	-0.1 ± 0.2	0.944	1.6 ± 0.6	-0.1 ± 0.3	1.5 ± 0.8	-0.1 ± 0.3	0.747	
240 min	2.4 ± 1.1	0.6 ± 0.6	2.1 ± 0.8	0.5 ± 0.6	0.499	2.1 ± 0.8	0.5 ± 0.5	1.6 ± 0.7	0.3 ± 0.4	0.104	2.0 ± 0.8	0.3 ± 0.1	2.1 ± 1.0	0.5 ± 0.6	0.151	0.057
360 min	2.9 ± 1.4	1.0 ± 0.8	2.4 ± 1.0	0.7 ± 0.7	**0.029**	2.5 ± 0.9	0.9 ± 0.6	2.0 ± 0.7	0.8 ± 0.5	0.325	2.7 ± 1.1	0.8 ± 0.7	2.3 ± 1.0	0.7 ± 0.5	0.286	
Change in overall response within diet					0.335					0.513					0.149	
Plasma free fatty acids, mmol/L	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	
0 min	0.41 ± 0.21		0.40 ± 0.19													
120 min	0.14 ± 0.21	-0.27 ± 0.22	0.17 ± 0.29	-0.23 ± 0.30	0.557	0.57 ± 0.37		0.51 ± 0.34			0.42 ± 0.18		0.47 ± 0.33			
240 min	0.29 ± 0.20	-0.13 ± 0.23	0.27 ± 0.15	-0.12 ± 0.22	0.984	0.11 ± 0.10	-0.47 ± 0.30	0.15 ± 0.26	-0.35 ± 0.46	0.179	0.07 ± 0.04	-0.40 ± 0.17	0.10 ± 0.08	-0.36 ± 0.31	0.886	
360 min	0.58 ± 0.37	0.16 ± 0.39	0.54 ± 0.22	0.15 ± 0.21	0.828	0.32 ± 0.29	-0.24 ± 0.20	0.30 ± 0.17	-0.21 ± 0.36	0.723	0.25 ± 0.40	-0.17 ± 0.19	0.25 ± 0.11	-0.22 ± 0.33	0.508	0.561
Change in overall response within diet					0.839	0.58 ± 0.25	0.0 ± 0.25	0.64 ± 0.21	0.13 ± 0.37	0.105	0.56 ± 0.39	0.14 ± 0.29	0.56 ± 0.22	0.11 ± 0.30	0.586	
										0.353					0.909	
Systolic blood pressure, mmHg	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	
0 min	125 ± 19		119 ± 16			131 ± 15		119 ± 17			127 ± 15		128 ± 15			
120 min	122 ± 15	-3 ± 14	114 ± 15	-4 ± 8	0.460	126 ± 17	-5 ± 8	116 ± 16	-3 ± 9	0.299	124 ± 13	-4 ± 10	124 ± 17	-4 ± 10	0.987	
240 min	122 ± 14	-4 ± 11	116 ± 15	-3 ± 9	0.629	123 ± 15	-8 ± 9	114 ± 14	-5 ± 9	0.177	125 ± 12	-2 ± 11	125 ± 14	-3 ± 9	0.808	0.500
Change in overall response within diet					0.295					0.809					0.852	

3

	WESTERN-TYPE DIET GROUP *n*=39					TARGETED DIET GROUP *n*=34					CONTROL GROUP *n*=27					Group comp.
Diastolic blood pressure, mmHg	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	
0 min	71 ± 8		69 ± 9			76 ± 9		70 ± 7			75 ± 9		77 ± 9			
120 min	68 ± 9	-3 ± 5	64 ± 9	-5 ± 4	0.188	72 ± 8	-4 ± 6	65 ± 7	-5 ± 4	0.392	71 ± 9	-4 ± 5	72 ± 8	-5 ± 5	0.453	
240 min	70 ± 9	-2 ± 6	66 ± 8	-3 ± 5	0.316	71 ± 9	-5 ± 6	66 ± 7	-4 ± 4	0.650	72 ± 9	-4 ± 5	73 ± 9	-4 ± 5	0.837	0.734
Change in overall response within diet					0.807					0.311					0.745	
Heart rate, BPM	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	
0 min	59 ± 10		57 ± 9			61 ± 7		57 ± 8			59 ± 10		60 ± 9			
120 min	64 ± 8	5 ± 7	60 ± 8	3 ± 5	0.072	64 ± 8	3 ± 4	60 ± 7	4 ± 5	0.595	63 ± 9	4 ± 5	62 ± 10	2 ± 5	0.182	
240 min	62 ± 9	3 ± 7	59 ± 8	2 ± 5	0.613	65 ± 9	4 ± 5	59 ± 7	2 ± 6	0.075	62 ± 9	3 ± 6	61 ± 9	2 ± 5	0.347	**0.015**
Change in overall response within diet					0.299					0.056					0.754	
Augmentation index, %	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	
0 min	23.5 ± 8.9		23.4 ± 8.4			25.4 ± 7.9		24.2 ± 8.3			22.2 ± 6.8		21.0 ± 8.1			
120 min	18.8 ± 9.6	-4.8 ± 4.1	17.6 ± 9.4	-5.1 ± 4.3	0.072	21.1 ± 7.2	-3.9 ± 3.7	19.5 ± 8.3	-4.4 ± 3.4	0.323	16.9 ± 7.3	-5.4 ± 4.9	17.4 ± 9.4	-3.6 ± 4.8	0.140	
240 min	21.2 ± 9.9	-2.0 ± 4.6	18.8 ± 8.9	-3.8 ± 5.4	0.075	21.9 ± 8.0	-3.3 ± 3.7	20.2 ± 8.0	-4.0 ± 4.8	0.668	19.0 ± 7.2	-3.1 ± 3.3	18.9 ± 6.9	-2.1 ± 4.7	0.420	0.288
Change in overall response within diet					0.252					0.640					0.601	
Central systolic pressure, mmHg	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	**Wk. 0** Mean	Response	**Wk.12** Mean	Response	Change in response (p-value)	
0 min	122 ± 19		117 ± 19			121 ± 15		112 ± 14			116 ± 15		111 ± 12			
120 min	110 ± 13	-12 ± 12	107 ± 17	-10 ± 8	0.992	117 ± 17	-5 ± 10	108 ± 16	-5 ± 7	0.835	111 ± 12	-6 ± 8	106 ± 11	-5 ± 9	0.665	
240 min	111 ± 13	-9 ± 11	109 ± 17	-7 ± 9	0.182	115 ± 14	-6 ± 12	108 ± 14	-5 ± 7	0.503	112 ± 14	-5 ± 8	108 ± 10	-4 ± 9	0.587	0.746
Change in overall response within diet					0.345					0.510					0.926	

Data are presented as mean ± 1SD. Response values are determined as the postprandial change in a parameter on a certain time point compared to its fasting value. Group comparison indicates the change in response between the different diets. All analyses were conducted using linear mixed effects modeling with an autoregressive covariance structure and LSD post-hoc testing.

DISCUSSION

We have investigated the effects of two 25% energy-restricted diets that differed in nutrient quality on risk factors for type 2 diabetes and other cardio-metabolic risk factors, including responses to a mixed meal test, in abdominally obese subjects. The high nutrient quality ER-diet (TD) induced a greater reduction in body weight than the low nutrient quality ER-diet (WD) and caused a significant reduction in serum total cholesterol and plasma triglycerides, which were not observed in the low nutrient quality ER diet or control group. Both ER-diets reduced SAT and VAT mass and induced strong reductions in intra-hepatic lipids. Furthermore, both diets induced significant changes in metabolic parameters that reflect improved glucose homeostasis and insulin sensitivity as well as significant reductions in systolic and diastolic blood pressure.

Weight loss in both diet groups was accompanied by reductions in both SAT and VAT mass in the abdominal region, but the decrease in VAT/SAT ratio indicated a preferential loss of VAT in both WD and TD. Likewise, many other studies report that VAT is preferentially lost during modest weight loss, possibly due to its highly lipolytic nature[26]. Interestingly, participants in the TD lost on average 2.1kg body weight more than participants in the WD. A small inequality in weight loss, 429g, could be expected since actual ER varied due to the classification of participants into energy groups. The rest of the difference could not be solely explained by differences in reported dietary intake or metabolizable energy. The difference in reported intake, which was not significant between the groups, could account for a mere 276g in weight loss while differences in digestibility could account for 237g. Accordingly, 1.2kg of the difference in weight loss remains unexplained. It is possible that certain nutrients included in the TD might have activated specific energy-related molecular pathways, such as beta-oxidation by PUFA or gluconeogenesis due to less carbohydrates in this diet. Furthermore, the larger proportion of protein in the TD might have increased diet-induced thermogenesis. Another potential explanation is better adherence to the dietary guidelines in the TD, whether or not due to increased satiety driven by protein and/or fiber. However this was not reflected by the reported intake. Theoretically, it is possible that participants in the TD increased their physical activity level. This explanation is not very plausible as participants were randomly allocated to one of the three groups and were instructed to maintain their habitual level of physical exercise.

Intra-hepatic lipids were strongly reduced by both dietary interventions, on average by 46% in the WD and 55% in the TD. ER is indeed a strong driver of ectopic fat loss[27]. The reduction in liver fat marks an important clinically relevant study outcome as fatty liver may play a key role in the pathogenesis of type 2 diabetes[28]. We also observed reductions in GGT in both diets, but not in ALT or AST. GGT might be closer related to steatosis

than other liver enzymes, as GGT is one of the predictors in the validated Fatty Liver Index algorithm to predict fatty liver[29], whereas AST and ALT are not. Although an important part of nutrients in the TD were selected based on their ability to decrease liver fat[30], we observed no additional effects of nutrient quality over ER in intra-hepatic lipid or GGT reductions.

Interestingly, the TD diet was the only diet that significantly reduced total cholesterol. As HDL-cholesterol was not affected by either one the interventions, the reduction is likely due to a decrease in LDL-cholesterol. An explanation for this reduction could be the exchange of SFA for MUFA and PUFA in the TD as SFA intake has repeatedly been linked to LDL increase while MUFA and PUFA are thought to decrease LDL[6,31]. Other mechanisms by which TD could have lowered total cholesterol are an increased excretion of bile acids caused by the higher amount of fiber[12] in this diet and/or a specific reduction of LDL-cholesterol caused by soy protein[32]. Fasting triglycerides were also significantly reduced upon the TD diet compared to both the WD and the control group. The *n-3* PUFA in the TD could have contributed to this reduction, as these fatty acids are recognized for their plasma triglyceride lowering properties[6,33]. We conclude that adherence to a high nutrient quality ER-diet is an effective strategy for improving blood lipids such as triglycerides and total cholesterol, both known to be associated with increased CVD risk[34], while this is not demonstrated for a low nutrient quality Western-type ER-diet.

Participants in both ER-groups displayed significant improvements in risk factors for type 2 diabetes. These factors included markers that reflect improved glucose homeostasis, such as lower fasting plasma glucose and insulin, as well as improved insulin sensitivity, including lower values of the surrogate markers HOMA-IR and QUICKI[35] and lower postprandial glucose. In addition, we observed improvements in long-term glycemic control, as reflected by lower HbA1c after both ER-interventions. It has been demonstrated that responses of glucose and insulin after a mixed meal test are reliable and reproducible across ranges of insulin sensitivity and body weight, making the mixed meal test a valuable tool in assessing insulin sensitivity in intervention studies[36,37]. In our study, improvements in postprandial glucose and insulin followed the same pattern as improvements in fasting parameters. As improvements in glucose metabolism were already reflected in fasting parameters, the use of phenotypic flexibility as a sensitive health marker might be of marginal added value in this study. However, post-challenge glucose concentrations are a better proxy for insulin sensitivity than fasting concentrations[38] or surrogate markers. Furthermore, dynamic measures may help to enlarge differences between persons as compared to static measures.

We observed no additional effect of nutrient quality over ER in improvements in risk factors for type 2 diabetes. Inclusion of additional protein or specifically soy protein, unsaturated fatty acids, and/or fiber have been described as dietary strategies to improve glucose metabolism. However, data on the effect of incorporating these nutrients together into an ER-diet are limited. For individual nutrients such as protein, similar findings as in our study were observed. Specifically, multiple trials found that increasing protein quantity or altering protein source within an ER-diet did not have additional benefits on fasting glucose homeostasis when compared to a low or normal protein ER-diet[4,39-41]. However, two studies reported lower postprandial glucose or insulin responses[42,43] after high versus low protein ER-diets, while these reductions were not apparent in the fasted state. The lack of differences in changes in postprandial responses of glucose between our two ER-diets might be due to the fact that the difference in protein En% between the WD and TD was only 4.6% while in the other studies this difference was above 11%. Studies that assess the influence of fatty acid saturation within an ER-diet on insulin sensitivity are also limited. As reviewed by Ros[44], most MUFA-rich isoenergetic diets indeed improve glycemic control whereas MUFA enrichment within ER-diets has no additional beneficial effect on glucose metabolism. The same holds true for PUFA, for which evidence suggests that isoenergetic PUFA-rich and especially linoleic acid-rich diets improve insulin sensitivity[45], while data on PUFA-enriched ER-diets are conflicting[46-48].

Insulin resistance is directly correlated to the severity of essential hypertension[49]. Both ER-interventions effectively reduced branchial systolic and diastolic blood pressure, which is in line with previous research on weight loss diets and blood pressure measures[4]. We observed no additional effects of nutrient quality on blood pressure, despite improvements in other CVD risk markers including cholesterol and triglycerides. For protein there is no conclusive evidence from intervention studies for hypotensive effects[50], but *n-3* PUFA have been described to lower CVD risk partly by lowering blood pressure[51]. Effects of *n-3* PUFA within an ER-diet are again mixed, with some studies finding no effects of *n-3* supplementation in ER[46,48], while Bao et al[52] observed additional benefits of a daily fish meal within a weight loss diet on blood pressure. To summarize, in our study ER is the most dominant factor in improving measures of glucose homeostasis, insulin sensitivity, and blood pressure, whereas nutrient quality beneficially affects blood lipids.

Our study had a large sample size and relatively few drop-outs. The dietary intervention was implemented in a feasible, real-life fashion and the weekly visits caused high compliance, as indicated by reported intake and weight loss. Our findings are generalizable to a broad population, as we included both males and females with

a common metabolic phenotype since, at least in the Netherlands, almost half of the population is overweight. A limitation of our study is that self-reported dietary intake always carries a degree of uncertainty, which may have contributed to the relatively large difference in weight loss between the diets. In addition, many other variables contribute to the complex relation between caloric intake and actual weight loss, including satiety, energy expenditure, metabolic rate, and the amount of metabolizable energy. We tried to take most of these variables into account but still conclude that our findings on weight loss are remarkable and are fuel to the long running debate whether a calorie is a calorie[53].

Our results clearly demonstrate that a 12wk intervention with any 25% ER-diet results in clinically relevant weight loss accompanied by reductions in SAT and VAT, a strong decrease in intra-hepatic lipids, improvements in measures of glucose homeostasis and insulin sensitivity and a reduction in blood pressure. Remarkably, enhancing the nutrient quality of a 25% ER-diet via enrichment with MUFA, *n-3* PUFA, soy protein, and fiber results in more pronounced weight loss and significant reductions in total cholesterol and triglycerides compared to a Western-type ER-diet. In conclusion, an ER-diet with high quality nutrients can be considered as the superior whole diet approach to improve metabolic health in abdominal obesity and may additionally lower CVD risk on the long run.

CONFLICT OF INTEREST

This study was funded by the Division of Human Nutrition and Health, Wageningen University, The Netherlands. We declare no competing interests.

AUTHORS' CONTRIBUTIONS

S.S., M.M., and L.A.A. designed and developed the protocol. E.S. was responsible for the dietary intervention. D.E. led the preparations and execution of the trial. Data was collected and analyzed by D.E. and S.S, H.C.B gave advice on the analyses. S.S. wrote the first draft of the manuscript, which was critically reviewed and improved by M.M. and L.A.A. All authors read and approved the final manuscript.

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SUPPLEMENTAL MATERIAL

Supplemental Table 1. Meal plans of the two experimental diets: Western-type and Targeted diet

	WESTERN-TYPE DIET GROUP	TARGETED DIET GROUP
Breakfast	Refined wheat bread with **butter** and jam Egg 1/wk.	Whole grain wheat bread with **low-fat margarine**, jam or lean cold cooked meats Egg 1/wk.
Snack	**Ginger bread** with **butter** **Apple juice** or **grape juice**	Fruit
Lunch	Refined wheat bread with **butter** and full-fat cheese Crudités with yoghurt-based dressing Semi-skimmed milk	Whole grain wheat bread with **low-fat margarine** and low-fat cheese Crudités with yoghurt-based dressing Semi-skimmed milk
Snack	Fruit **Ready-made bouillon**	Fruit **Soy milk**
Dinner	Vegetables (raw/cooked/steamed/stir-fried) Potatoes 3/wk. Regular pasta 2/wk. White rice 2/wk. Lean meats 7/wk. Pasta/rice: 2 units of sauce Potatoes: gravy from cooking fat (**margarine**)	Vegetables (raw/cooked/steamed/stir-fried) Potatoes 2/wk. Wheat pasta 3/wk. Brown rice 2/wk. Lean meats 3/wk. **Tofu** 2/wk. Lean fish 1/wk. Oily fish 1/wk. Pasta/rice: 1 unit of sauce Potatoes: gravy from cooking fat (**olive oil**)
Snack	Fruit Low-fat fruit yoghurt or custard Unit of alcohol (wine/beer) 1/wk.	**Soy nuts** Low-fat fruit cottage cheese or **soy dessert** Unit of alcohol (wine/beer) 1/wk.
Extra	**Low-sodium salt alternative**	**Low-sodium salt alternative** **1 fish oil capsule** **700mg EPA and DHA***

Table shows the standard meal plan used as a basis for all participants, plans could be tailored to the participants individual preferences (i.e. breakfast cereals instead of bread) as long as these adjustments would still fit the overall energy and macronutrient requirement dictated by the diet. Products in **bold** indicate key food products supplied weekly to the participants. *MusclePharm Corporation, Denver, USA. Fish oil capsules contained 400mg EPA and 300mg DHA according to manufacturers' label.

Supplemental Table 2. Baseline characteristics of the study participants

	WESTERN-TYPE DIET GROUP *n*=39	TARGETED DIET GROUP *n*=34	CONTROL GROUP *n*=27
Gender, n males, %	17, 44%	16, 47%	13, 48%
Age, years[1]	64 (28)	62 (29)	62 (29)
Body weight, kg	93.1 ± 13.9	94.8 ± 13.2	92.1 ± 12.6
BMI, kg/m²	31.2 ± 3.3	31.8 ± 3.4	30.9 ± 3.9
Waist circumference, cm	108.7 ± 9.8	109.5 ± 9.8	106.6 ± 8.1
Plasma glucose, mmol/L	5.6 ± 0.8	5.7 ± 0.5	5.6 ± 0.4
Plasma insulin, mU/L	14.6 ± 10.3	13.5 ± 7.8	12.1 ± 5.3
HbA1c, mmol/mol	36.3 ± 2.1	37.3 ± 2.9	35.1 ± 2.6
HOMA-IR	3.8 ± 3.1	3.5 ± 2.1	3.0 ± 1.4
QUICKI	0.33 ± 0.04	0.33 ± 0.03	0.33 ± 0.02
Serum total cholesterol, mmol/L	5.7 ± 1.1	5.5 ± 0.8	5.4 ± 1.0
Serum HDL cholesterol, mmol/L	1.3 ± 0.4	1.4 ± 0.4	1.4 ± 0.4
Plasma triglycerides, mmol/L	1.8 ± 0.8	1.6 ± 0.6	1.7 ± 0.7
Plasma free fatty acids, mmol/L	0.4 ± 0.2	0.6 ± 0.4	0.4 ± 0.2

Data are presented as mean ± 1SD, except [1] is presented as median (range). Abbreviations: BMI: body mass index, HbA1c: glycosylated hemoglobin, HOMA-IR: homeostatic model assessment of insulin resistance, QUICKI: quantitative insulin sensitivity check index, HDL: high-density lipoprotein

Supplemental Table 3. Comparison between advised and reported intake in the two dietary intervention groups: Western-type and Targeted diet group

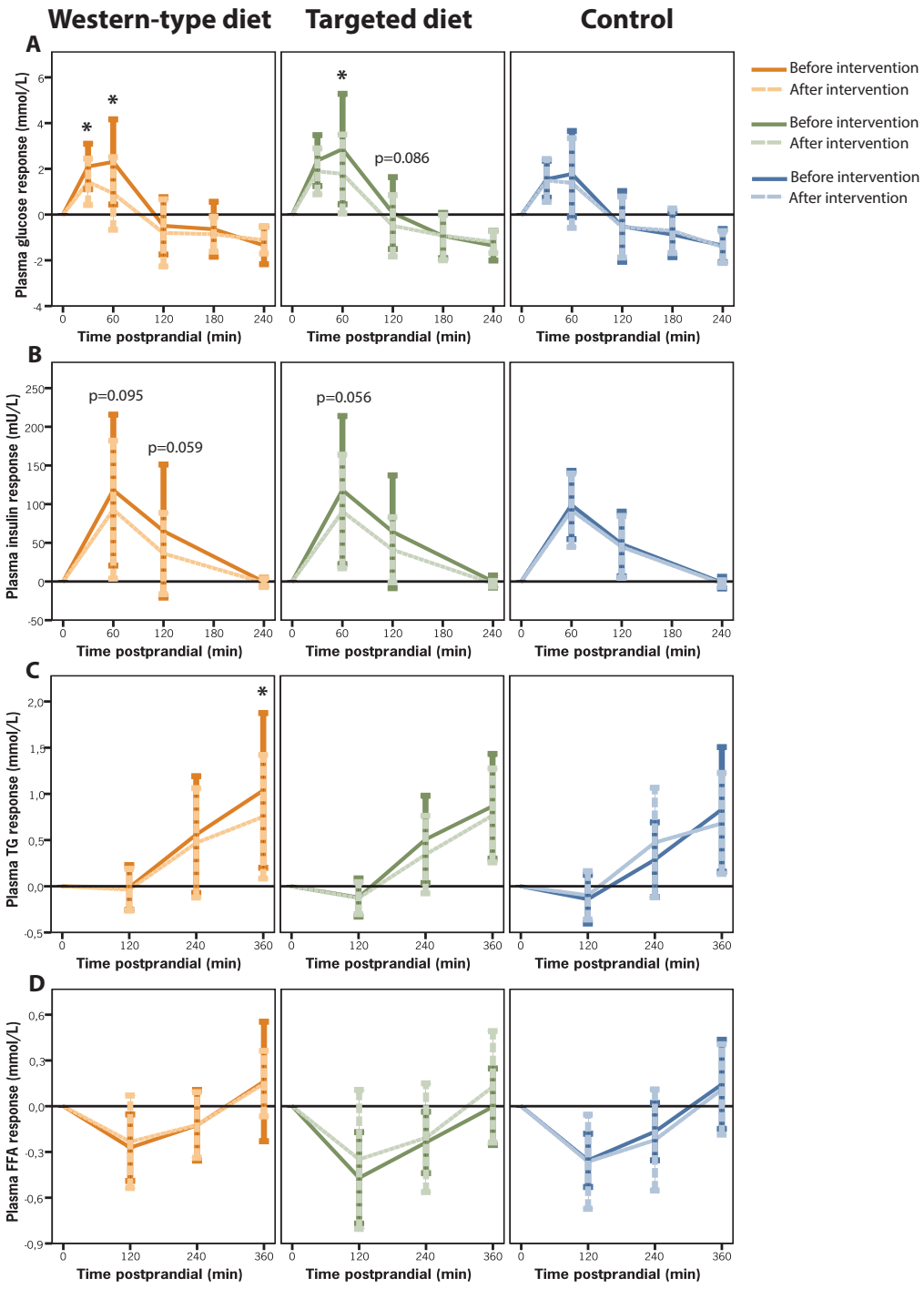
	WESTERN-TYPE DIET GROUP *n*=39			TARGETED DIET GROUP *n*=34		
	Advised	Reported	Difference (p-value)	Advised	Reported	Difference (p-value)
Energy intake (MJ)	6.95 ± 0.78	7.13 ± 0.82	**0.027**	6.99 ± 0.90	7.07 ± 1.00	0.271
ER (%)	25.8 ± 5.4	23.9 ± 0.82	**0.017**	26.9 ± 5.6	26.2 ± 5.9	0.221
En% Fat	31.5 ± 0.4	31.4 ± 1.6	0.616	32.1 ± 0.3	32.5 ± 1.2	**0.015**
En% SFA	15.5 ± 0.4	14.9 ± 1.5	**0.016**	7.9 ± 0.5	8.3 ± 0.6	**0.001**
En% MUFA	9.0 ± 0.4	9.3 ± 1.0	0.113	13.9 ± 0.7	3.4 ± 0.9	**<0.001**
En% PUFA	3.9 ± 0.3	4.1 ± 0.8	0.072	7.8 ± 0.2	7.7 ± 0.4	0.307
En% Trans fat	0.4 ± 0.0	0.2 ± 0.0	**<0.001**	0.1 ± 0.0	0.1 ± 0.0	**<0.001**
ALA (mg)	1057 ± 67	1104 ± 278	0.300	1795 ± 35	1815 ± 354	0.626
EPA & DHA (mg)	33 ± 5	103 ± 226	0.060	1270 ± 0	1224 ± 55	**<0.001**
Cholesterol (mg)	183 ± 23	185 ± 30	0.698	124 ± 19	132 ± 20	**<0.001**
En% Protein	15.4 ± 0.2	16.0 ± 1.4	**0.009**	20.4 ± 0.1	20.6 ± 1.7	0.421
En% Animal-based	9.9 ± 0.3	10.3 ± 1.1	**0.031**	9.8 ± 0.2	10.3 ± 1.9	0.200
En% Plant-based	5.5 ± 0.1	5.7 ± 1.0	0.196	10.5 ± 0.1	10.3 ± 0.5	**0.041**
En% Carbohydrates	50.5 ± 0.2	49.6 ± 2.0	0.138	44.0 ± 0.3	43.7 ± 1.5	0.228
En% Mono- and disaccharides	25.3 ± 0.8	24.8 ± 1.9	0.065	19.0 ± 0.5	18.8 ± 1.1	0.277
En% Polysaccharides	25.2 ± 0.9	24.8 ± 1.2	0.158	25.0 ± 0.4	24.9 ± 1.1	0.562
En% Fructose	7.4 ± 0.1	7.0 ± 0.9	**0.006**	3.5 ± 0.4	3.4 ± 0.4	**0.048**
En% Fiber	2.2 ± 0.1	2.2 ± 0.2	0.158	3.2 ± 0.0	3.1 ± 0.2	**0.028**
En% Alcohol	0.8 ± 0.3	1.1 ± 0.9	0.064	0.8 ± 0.3	1.0 ± 0.7	0.123
Sodium (g)	1.6 ± 0.2	2.3 ± 0.6	**<0.001**	1.7 ± 0.3	2.3 ± 0.4	**<0.001**

Data are presented as mean ± 1SD. Values were calculated based on the Dutch Food Composition Database 2011 (NEVO 2011/3.0, RIVM, Bilthoven, The Netherlands). Advised values represent the average intake values across the 6, 7, 8, and 9MJ energy groups. Values are averaged over a 1-week menu cycle. Abbreviations: MJ: Mega joule, ER: Energy restriction, En%: Energy percentage, SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, ALA: alpha-linolenic acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid

Supplemental Table 4. Adjusted caloric content food & food groups derived from the Atwater system by Merrill & Watt

	PROTEIN kcal	FAT kcal	CHO kcal	REFERENCE GROUP
Apple juice	3.36	8.37	3.92	All fruit juice (except lemon, lime)
Grape juice	3.36	8.37	3.92	All fruit juice (except lemon, lime)
Fruit	3.36	8.37	3.60	All fruits (except lemons, limes)
Bread, whole grain wheat	3.69	8.37	3.87	Average wheat 97-100 and 85-93% extraction
Bread, wheat (refined)	4.05	8.37	4.12	Wheat 70-74% extraction
Butter, unsalted	4.27	8.79	3.87	Butter
Bread topping (marmalade)	4.00	9.00	3.87	Cane or beet sugar (sucrose)
Egg, boiled	4.36	9.02	3.68	Eggs
Pasta, refined	3.91	8.37	4.12	Macaroni, spaghetti
Pasta, whole grain	3.91	8.37	4.12	Macaroni, spaghetti
Margarine 80% fat, >24g SFA	4.27	8.84	3.87	Margarine, vegetable
Margarine light	4.27	8.84	3.87	Margarine, vegetable
Lean meat <5% fat	4.27	9.02	4.11	Meat, fish
Fish, lean 0-2g fat	4.27	9.02	4.11	Meat, fish
Fish oily >10g fat	4.27	9.02	4.11	Meat, fish
Lean cold cooked meats	4.27	9.02	4.11	Meat, fish
Low fat yoghurts w/ fruits	4.27	8.79	3.87	Milk, milk products
Milk, semi-skimmed	4.27	8.79	3.87	Milk, milk products
Full fat cheese	4.27	8.79	3.87	Milk, milk products
Custard, full fat (48+)	4.27	8.37	3.87	Milk, milk products
Cottage cheese w/ fruits	4.27	8.79	3.87	Milk, milk products
Low fat cheese, less sodium (30+)	4.27	8.49	3.87	Milk, milk products
Salad dressing 20% oil w/ yoghurt	4.27	8.84	3.87	Other vegetable fats and oils
Olive oil	4.27	8.84	3.87	Other vegetable fats and oils
Crudities	2.44	8.37	3.57	Other vegetables
Vegetables	2.44	8.37	3.57	Other vegetables
Potatoes, boiled	2.78	8.37	4.03	Potatoes and starchy roots
Rice, brown (unpolished)	3.41	8.37	4.12	Rice, brown
Rice, white (polished)	3.82	8.37	4.16	Rice, white or polished
Gingerbread	3.41	8.37	4.07	Rye flour (light)
Soy dessert	3.47	8.37	4.07	Soybeans
Soy milk	3.47	8.37	4.07	Soybeans
Soy yoghurt	3.47	8.37	4.07	Soybeans
Soy nuts	3.47	8.37	4.07	Soybeans
Tofu	3.47	8.37	4.07	Soybeans

Abbreviations: CHO: carbohydrates, kcal: kilocalories, SFA: saturated fatty acid



Supplemental Figure 1. Postprandial responses to a mixed meal test (MMT)

Curves reflect the changes in postprandial levels compared to the fasting levels before and after the 12wk dietary intervention for plasma glucose (A), insulin (B), triglycerides (Tg) (C), and free fatty acids (FFA) (D). * indicates a significant difference in response upon 12 weeks of ER. Error bars represent 1SD.

4

PLASMA METABOLITE AND LIPOPROTEIN PROFILING REVEALS ADDITIONAL CARDIO-METABOLIC BENEFITS OF NUTRIENT QUALITY WITHIN WEIGHT LOSS DIETS

A RANDOMIZED CONTROLLED TRIAL IN OBESE SUBJECTS

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In preparation

ABSTRACT

Introduction: Energy restriction (ER) is an evidently effective dietary strategy to induce weight loss and lower the risk of chronic diet-related diseases such as cardiovascular disease (CVD). However, the ideal nutrient composition of ER-diets in maximizing risk reduction has been debated.

Objective: We examined additional effects of nutrient quality on top of ER by investigating the effects of two ER-diets differing in nutrient quality on a wide range of plasma metabolites in subjects with abdominal obesity.

Methods: A parallel-designed 12wk 25% ER dietary intervention study was performed previously. Participants aged 40-70 with abdominal obesity (BMI >27kg/m^2 or waist circumference >88cm for females, >102cm for males) were randomized over three groups; a Targeted diet (*n*=40, TD) enriched with soy protein, fiber, monounsaturated fatty acids, and *n-3* polyunsaturated fatty acids, a Western-type diet (*n*=40, WD) high in saturated fatty acids and fructose or a control group (*n*=30). In this study, we analyzed plasma levels of over 150 metabolites including lipoprotein sub-fractions, using a NMR-based metabolomics platform. Profiling was performed in the fasted state as well as 2, 4, and 6h after a mixed meal test (76.3g carbohydrates, 17.6g protein, 60.0g fat).

Results: Both diets improved postprandial suppression of lipolysis. The TD greatly affected VLDL-metabolism by lowering almost all VLDL particles and components, both fasting and in the postprandial phase. Furthermore, this diet promoted an anti-atherogenic lipoprotein profile by a potential shift of small HDL towards larger HDL and by reducing postprandial levels of the atherogenic ApoB-carrying IDL and LDL. The WD was not able to induce significant changes in fasting lipoproteins, although we observed improvements in several postprandial VLDL, IDL, and LDL-related particles.

Conclusion: A high quality 25% ER-diet with soy protein, fiber, MUFA and *n-3* PUFA is a powerful whole diet approach to achieve a favorable blood lipid profile, thereby potentially lowering CVD risk.

ABBREVIATIONS

AAA	Aromatic amino acids
BCAA	Branched chain amino acids
CETP	Cholesteryl ester transfer protein
CVD	Cardiovascular disease
ER	Energy restriction
FA	Fatty acid
FDR	False discovery rate
HDL	High density lipoprotein
IDL	Intermediate density lipoprotein
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
LXR	Liver X receptor
MUFA	Monounsaturated fatty acid
NMR	Nuclear magnetic resonance
PLS-DA	Partial least square discriminant analysis
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
TD	Targeted diet
TG	Triglyceride
VLDL	Very low density lipoprotein
WD	Western-type diet

INTRODUCTION

The increased global burden of obesity stresses the need for dietary strategies that induce weight loss and alleviate its comorbidities, thereby reducing the risk of chronic disease such as cardiovascular disease (CVD). An evidently effective dietary strategy in this context is the restriction of daily caloric intake i.e. energy restriction (ER), usually targeted at ~10 to 40% of the estimated energy requirement of overweight or obese individuals[1]. Studies show that cardio-metabolic risk factors such as blood pressure, dyslipidemia, and measures of insulin sensitivity often already improve after a 5% loss of initial body weight induced by ER[2,3].

The ideal nutrient composition of an ER-diet has been subject of intense debate[4,5]. Numerous trials have put effort into studying the superior ER-diet to augment weight loss or to enlarge health improvements. These approaches include emphasizing a specific macronutrient such as high-protein diets[6,7], aligning the diet to an *a posteriori*-defined pattern that is linked to lower risk of chronic diseases such as the Mediterranean diet[8], or adding specific bioactive compounds such as fish oil or flavanols[9-14]. Although the effectiveness of the diets varies, these studies do acknowledge the promising potential of nutrient quality within ER and pave the way for combining these nutrients into powerful whole diet approaches. However, the effects of nutrition on cardio-metabolic health are usually subtle. Furthermore, classical biomarkers assessed in nutritional intervention studies are typically disease markers, while these studies are often executed in relatively healthy populations. Fortunately, recent advances in sensitive high-throughput techniques have increased the possibilities to obtain a detailed comprehensive overview of the effects of nutrition on health by allowing the unbiased analysis of hundreds to thousands biomarkers in one sample.

The simultaneous analysis of all kinds of circulating small-molecule metabolites, i.e. metabolomics, can provide an overview of the effects of our dietary interventions on physiological processes in different metabolic organs. For instance, levels of glucose, ketone bodies, fatty acids (FA), or branched chain amino acids (BCAA) can serve as a read-out for insulin sensitivity and the functioning of both the liver and peripheral tissues such as the skeletal muscle. In addition, analysis of the levels of lipid-related particles including triglycerides (TG), cholesterol, and lipoprotein levels might reveal the occurrence of particles with high atherogenic potential. Different sub-fractions of lipoproteins, defined by variations in density, composition, and size, are differentially associated with CVD risk[15]. Therefore it is of crucial importance to assess a broad range of lipoproteins and constituents when evaluating the effects of dietary interventions on disease risk. Furthermore, it has long been argued that analyzing fasting lipoprotein profiles has limited value in assessing cardio-metabolic risk[16]. Postprandial levels

are not only more representative for real-life situations but are also of crucial importance in the determination of which potential atherogenic particles are secreted in the circulation[17,18]. In the postprandial phase, complex and tightly regulated processes determine the flux of particles varying in TG and cholesterol content. Maintaining healthy postprandial lipoprotein profiles requires optimal functioning of the liver, for VLDL secretion, remnant particle uptake, and TG to cholesteryl esters exchange, as well as optimal functioning of the peripheral tissues such as the adipose tissue for TG-rich lipoprotein clearance through the actions of lipoprotein lipase (LPL). Responses of lipoproteins to a dietary stressor that contains fat might therefore serve as a more sensitive health marker than fasting measurements, as they possibly reveal early perturbations in this system.

We wanted to demonstrate the power of nutrition on health by developing a high nutrient quality 25% ER-diet that combines multiple nutrients that have well-described beneficial effects on cardio-metabolic risk factors and compared effects of this diet to a Western-type 25% ER-diet (WD). The high quality diet, referred to as Targeted diet (TD), was enriched in soy protein, fiber, MUFA, and *n-3* PUFA. Previously, we have demonstrated that a 12 week intervention with either our TD or WD resulted in clinically relevant weight loss and improvements in cardio-metabolic risk factors in abdominally obese males and females. Interestingly, only the TD was able to reduce plasma TG and serum total cholesterol levels. In this manuscript, we aimed to obtain a more elaborate overview of potential additional effects of the TD over the WD by analyzing changes in a broad spectrum of plasma metabolites using a NMR spectroscopy-based metabolomics platform that covers over 150 metabolites, including sub-fractions of lipoproteins. Plasma profiling was not only conducted in a fasted state but also after consumption of a protein/fat/sugar mixed meal.

METHODS

Study design and participants

The study was approved by the Medical Ethics Committee of Wageningen University and registered at ClinicalTrials.gov, identifier: **NCT02194504**. A detailed description of the study and primary study outcomes can be found elsewhere. In short, 110 healthy participants aged 40-70 years with abdominal obesity (BMI >27kg/m^2 or waist circumference >88cm for females, >102cm for males) were randomized over three groups; a Western-type (WD) or Targeted (TD) energy restriction diet group or a control group. The two diets had an equal energy restriction of 25%. The TD aimed to improve metabolic health via enrichment with soy protein, fiber, MUFA, and *n-3* PUFA. In the WD these components were substituted by SFA, predominantly animal protein, and fructose.

Participants in the control group did not receive intervention and were asked to maintain their habitual dietary habits. The intervention lasted 12 weeks and was based on dietary advice and the provision of key products, e.g. cooking oils and fats, dairy or soy products, snacks. Participants visited our facility on a weekly basis for an individual session with one of the qualified dieticians. Reported intake values of participants in both diets groups resembled advices intake values and are shown in **Table 1**. Before and after 12 weeks of intervention several cardio-metabolic risk factors were assessed, including plasma markers that reflect glucose homeostasis and insulin sensitivity, blood lipids, intrahepatic lipids, body fat distribution, and vascular parameters including blood pressure. Plasma markers were assessed in the fasted state but also in the postprandial phase after consumption of an in-house prepared mixed meal, consisting of 76.3g carbohydrates, 17.6g protein, and 60.0g fat.

Table 1. Nutritional composition of the two experimental diets and reported intake

	WESTERN-TYPE DIET GROUP *n*=39		TARGETED DIET GROUP *n*=34		Differences in reported intake between diets
	Advised	Reported	Advised	Reported	p-value
Energy intake (MJ)	6.95 ± 0.78	7.13 ± 0.82	6.99 ± 0.90	7.07 ± 1.00	0.778
ER (%)	25.8 ± 5.4	23.9 ± 0.82	26.9 ± 5.6	26.2 ± 5.9	0.775
En% Fat	31.5 ± 0.4	31.4 ± 1.6	32.1 ± 0.3	32.5 ± 1.2	**0.001**
En% SFA	15.5 ± 0.4	14.9 ± 1.5	7.9 ± 0.5	8.3 ± 0.6	**<0.001**
En% MUFA	9.0 ± 0.4	9.3 ± 1.0	13.9 ± 0.7	13.4 ± 0.9	**<0.001**
En% PUFA	3.9 ± 0.3	4.1 ± 0.8	7.8 ± 0.2	7.7 ± 0.4	**<0.001**
En% Trans fat	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	**0.015**
ALA (mg)	1057 ± 67	1104 ± 278	1795 ± 35	1815 ± 354	**<0.001**
EPA & DHA (mg)	33 ± 5	103 ± 226	1270 ± 0	1224 ± 55	**<0.001**
Cholesterol (mg)	183 ± 23	185 ± 30	124 ± 19	132 ± 20	**<0.001**
En% Protein	15.4 ± 0.2	16.0 ± 1.4	20.4 ± 0.1	20.6 ± 1.7	**<0.001**
En% Animal-based	9.9 ± 0.3	10.3 ± 1.1	9.8 ± 0.2	10.3 ± 1.9	0.886
En% Plant-based	5.5 ± 0.1	5.7 ± 1.0	10.5 ± 0.1	10.3 ± 0.5	**<0.001**
En% Carbohydrates	50.5 ± 0.2	49.6 ± 2.0	44.0 ± 0.3	43.7 ± 1.5	**<0.001**
En% Mono- and disaccharides	25.3 ± 0.8	24.8 ± 1.9	19.0 ± 0.5	18.8 ± 1.1	**<0.001**
En% Polysaccharides	25.2 ± 0.9	24.8 ± 1.2	25.0 ± 0.4	24.9 ± 1.1	0.905
En% Fructose	7.4 ± 0.1	7.0 ± 0.9	3.5 ± 0.4	3.4 ± 0.4	**<0.001**
En% Fiber	2.2 ± 0.1	2.2 ± 0.2	3.2 ± 0.0	3.1 ± 0.2	**<0.001**
En% Alcohol	0.8 ± 0.3	1.1 ± 0.9	0.8 ± 0.3	1.0 ± 0.7	0.599
Sodium (g)	1.6 ± 0.2	2.3 ± 0.6	1.7 ± 0.3	2.3 ± 0.4	0.659

Data are presented as mean ± 1SD. Values were calculated based on the Dutch Food Composition Database 2011 (NEVO 2011/3.0, RIVM, Bilthoven, The Netherlands). Comparisons between groups were conducted using Independent t-tests. Advised values represent the average intake values across the 6, 7, 8, and 9MJ energy groups. Values are averaged over a 1-week menu cycle. Abbreviations: MJ: Mega joule, ER: Energy restriction, En%: Energy percentage, SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, ALA: alpha linolenic acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid

Metabolomics

For metabolite profiling we included fasting samples (T=0h) as well as three postprandial samples, i.e. 2h, 4h, and 6h after consumption of the mixed meal. We used plasma EDTA samples for the analysis, performed on a high-throughput NMR metabolomics platform (Nightingale Health Ltd., Vantaa, Finland), that has been used in many epidemiological and genetic studies[19,20]. In total, we used 153 metabolites for analysis assessed in 7 subclasses[21], which are summarized in **Table 2.**

Table 2. Overview of metabolites measured

CLASS OF METABOLITES		*n* metabolites
LIPOPROTEINS in different densities:		114
VLDL	Average size (nm)	
Extremely large (might include chylomicrons)	>75.0	
Very large	64.0	
Large	53.6	
Medium	44.5	
Small	36.8	
Very small	31.3	
IDL	28.6	
LDL		
Large	25.5	
Medium	23.0	
Small	18.7	
HDL		
Very large	14.3	
Large	12.1	
Medium	10.9	
Small	8.7	
Measurements include particle concentration and constituents of the lipoprotein: Lipids, phospholipids; Total cholesterol, free cholesterol, and cholesterol esters; Triglycerides		
APOLIPOPROTEIN CLASSES ApoA1, ApoB		2
FATTY ACIDS (INCLUDING RATIOS)		16
PHOSPHOLIPIDS AND CHOLINE DERIVATIVES		4
ENERGY METABOLISM (I.E. GLUCOSE/KETONES)		6
AMINO ACIDS		8
PROTEINS		3
TOTAL		**153**

4

Data analysis and visualization

All values were log2 transformed, analyses were performed on the log2 ratios (difference between baseline and after intervention) of the metabolites. Metabolite concentrations that were too low and subsequently labelled as 0 in the raw data file were removed from the dataset. We performed paired t-tests to assess changes in fasting metabolite levels within each diet group and Analysis of variance (ANOVA) with Hochberg's GT2 post-hoc testing to assesses changes in fasting metabolite levels between diet groups. P-values of the between-group comparisons were corrected for false positives using a false discovery rate (FDR) of 0.05 in the simultaneous analysis of all 153 metabolites. Postprandial metabolite changes were assessed by calculating the response i.e. the concentration of a metabolite in the postprandial phase minus its fasting value. Changes in responses were analyzed using linear mixed effects modelling with postprandial time points as repeated measures. Linear mixed effects modeling was executed using an unstructured covariance structure and LSD post-hoc testing. All analyses were conducted using IBM SPSS Statistics v22 (IBM Corp, Armonk, NY, USA), FDR corrections were performed manually using Microsoft Excel. A Partial Least Squares Discriminant Analysis (PLS-DA) and Sparse Partial Least Squares (SPLS) were performed using R[22] while visualization of the effects of the diets on lipoproteins levels was performed using Cytoscape v.3.4.0[23].

RESULTS

In the previous study, 110 participants were randomly allocated to one of the three intervention groups. In total 100 participants completed the trial, of which 39 in the WD, 34 in the TD, and 27 in the control group. Plasma samples of all 100 participants were available for metabolomics analysis. Baseline characteristics of the participants and effects of the dietary interventions on body composition and several cardio-metabolic risk factors are summarized in **Supplemental Table 1**. Twelve weeks of ER resulted in significant weight loss of -6.3 ± 3.9kg in the WD and -8.4 ± 3.2kg in the TD, which was significantly different from the control group but also significantly different between the two diet groups, despite equal ER. Weight loss in both diet groups was accompanied by a reductions in waist circumference, subcutaneous and visceral adipose tissue mass, and intrahepatic lipids. Both diets improved HbA1c and fasting plasma glucose and insulin. Only the TD reduced fasting plasma triglycerides and serum total cholesterol.

Fasting effects on metabolite profile

In total 153 metabolites were measured of which changes in fasting levels of 56 metabolites were significantly different between the diet groups (**Figure 1**). The vast majority of the significantly changed metabolites were

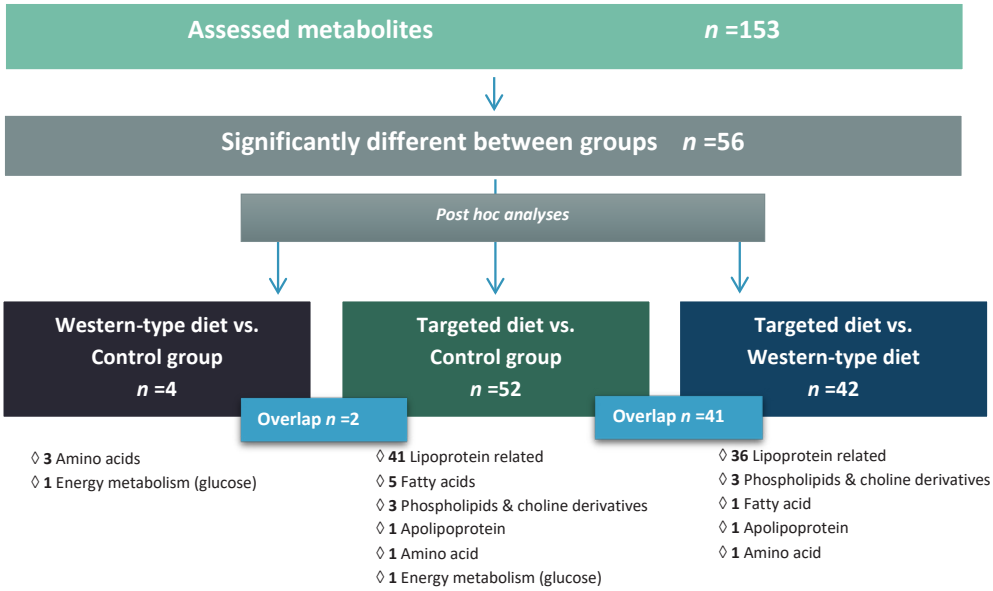

Figure 1. Significantly changed metabolites between Western-type diet group, Targeted diet group, and the control group

lipoproteins. To visualize changes in fasting levels of lipoproteins, we developed a network structure containing all lipoprotein sizes, classes, and constituents (**Figure 2A & 2B**). In the TD, the concentration of VLDL particles in all sizes except small and very small and almost all their constituents were reduced when compared to the control and the WD (**Supplemental Table 2 & 3**). In addition, the TD lowered levels of ApoB, levels of total plasma triglycerides and triglycerides bound to VLDL, and levels of cholesterol, both esterified as well as bound to VLDL and chylomicron remnants. There were no significant effects of the WD on VLDL particle concentrations or composition when compared to the control. With regard to HDL, the TD significantly reduced the concentration of medium and small HDL including lipids, phospholipids, and free cholesterol in these particles when compared to the control and the WD. In contrast, very large HDL and elements of large HDL particles were significantly increased within the TD group, but these changes were not significant when compared to the control. Similar patterns in changes in HDL, i.e. an increase in large particles and a decrease in smaller particles, were observed in the WD but all these changes were not significant when compared to the control. Finally, the TD exerted mild effects in LDL levels, seen as a decrease in phospholipids in medium and small LDL and a decrease in free cholesterol in small LDL when compared to the control and the WD.

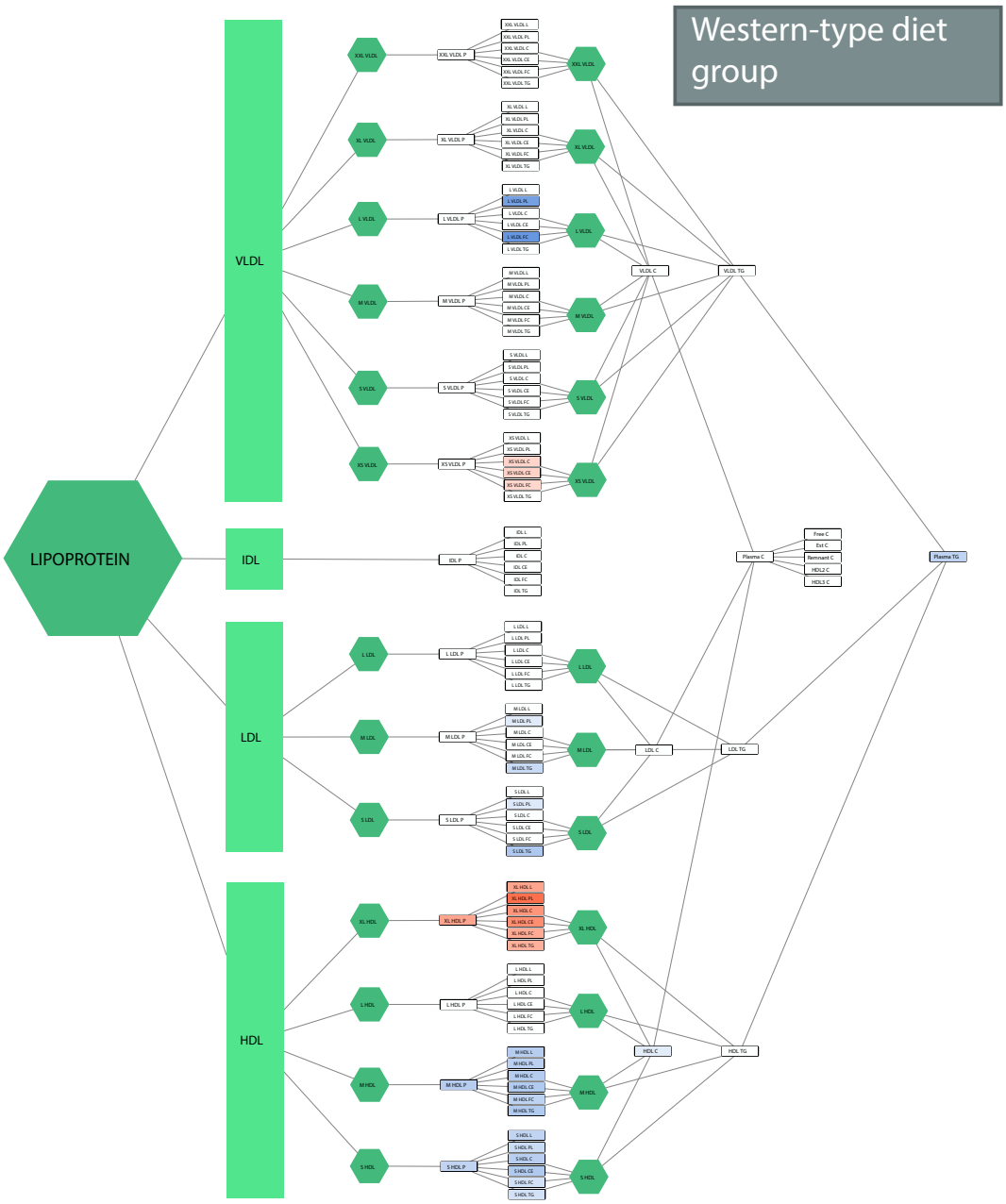

Figure 2A. Changes in fasting lipoprotein profile after 12wks of WD

Colored boxes indicate significantly changed (log2 ratio) particles within the WD as assessed with a paired t-test, where the red color indicates increased levels and the blue color indicates decreased levels. Color intensity is related to the size of the effect, darker color indicates a larger change. No significant differences between the WD and control were observed, as analyzed using ANOVA and Hochberg's GT2 post hoc analyses on changes in metabolites in all three groups. P-values were corrected for false positives using a false discovery rate (FDR) of 0.05 in the simultaneous analysis of all 153 metabolites. Abbreviations: P: particles, L: lipids, PL: phospholipids, C: cholesterol, CE: cholesterol esters, FC: free cholesterol, TG: triglycerides.

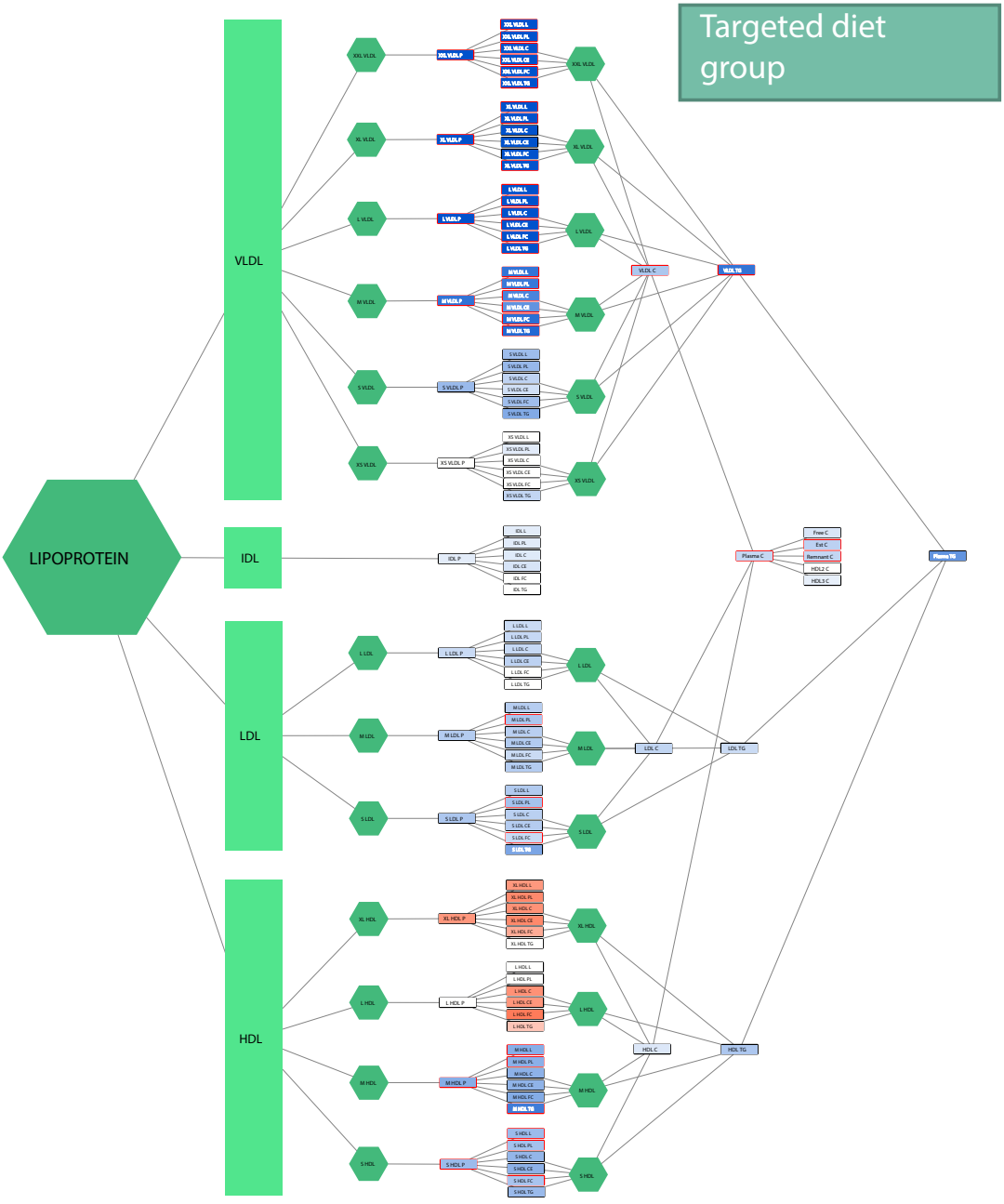

Figure 2B. Changes in fasting lipoprotein profile after 12wks of TD

Colored boxes indicate significantly changed (log2 ratio) particles within the TD as assessed with a paired t-test, where the red color indicates increased levels and the blue color indicates decreased levels. Color intensity is related to the size of the effect, darker color indicates a larger change. Red outline on the boxes indicates a significant difference between TD and the control, as analyzed using ANOVA and Hochberg's GT2 post hoc analyses on changes in metabolites in all three groups. P-values were corrected for false positives using a false discovery rate (FDR) of 0.05 in the simultaneous analysis of all 153 metabolites. Abbreviations: P: particles, L: lipids, PL: phospholipids, C: cholesterol, CE: cholesterol esters, FC: free cholesterol, TG: triglycerides.

In addition to lipoproteins, changes in 5 FA were significantly different between groups. Levels of FA were designated as the main group of metabolites in our dataset that could reflect dietary intake or so-called exposure markers (**Supplemental Table 4**). Changes in total FA, MUFA, SFA as well the ratio of SFA to total FA, and the estimated degree of unsaturation were significantly different between groups. Post hoc analyses showed that these effects were caused by a reduction in levels of total FA, MUFA, and SFA in the TD compared to the control group and an increase in estimated degree of unsaturation in the TD compared to the control. Despite daily supplementation of 400mg EPA and 300mg DHA in the TD, we did not observe an increase in plasma levels of *n-3* FA in this diet group when compared to the WD or the control. Therefore, capsules were analyzed for FA composition at our research facility using gas chromatography. Analysis revealed an average EPA content per capsule of 347mg and an average DHA content of 291mg, thus respectively 87% and 97% of the producer's claimed content.

Changes in total phosphoglycerides, phosphatidylcholine and other cholines as well as total cholines were also significantly different between groups, caused by a reduction in these VLDL-related metabolites in the TD when compared to the WD and the control (**Supplemental Table 5**). As observed and described previously, there was a significant difference in changes in glucose levels between groups, caused by a reduction in glucose in both the WD and the TD when compared to the control (**Supplemental Table 6**). Furthermore, we observed differences in changes in levels of the amino acids tyrosine, phenylalanine, and leucine, caused by a reduction in the WD when compared to the control (**Supplemental Table 7**).

Individual changes in levels of all 56 significantly different metabolites are visualized in the heat map in **Figure 3**. The individual data shows that changes in metabolites were robust within a person over the different lipoprotein particles but not all individuals responded similarly to the diet. Finally, we performed a PLS-DA in order to maximize the explained variance in changes in fasting levels of all 153 metabolites between the intervention groups. Results are visualized in **Figure 4**. The 95% confidence ellipsoids of the different groups overlapped. Furthermore, outcomes of the SPLS analysis showed that the selection of most predictive features for the separation between the groups included 147 metabolites, meaning that we could not select specific metabolites that contributed most to the between-group variability.

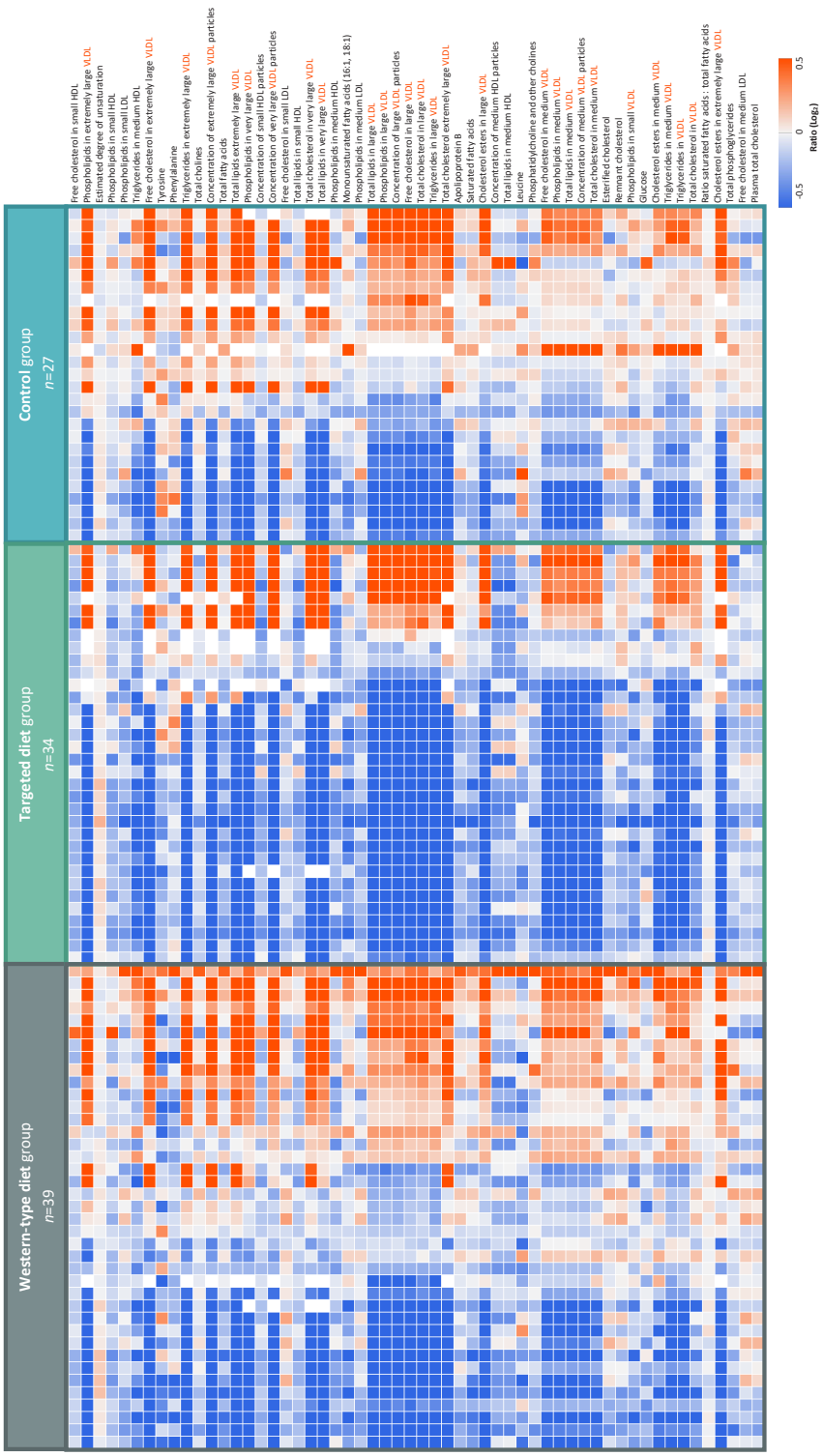

Figure 3. Heat map of the individual changes in the 56 significantly changed metabolites, ranked by p-value (FDR0.05)

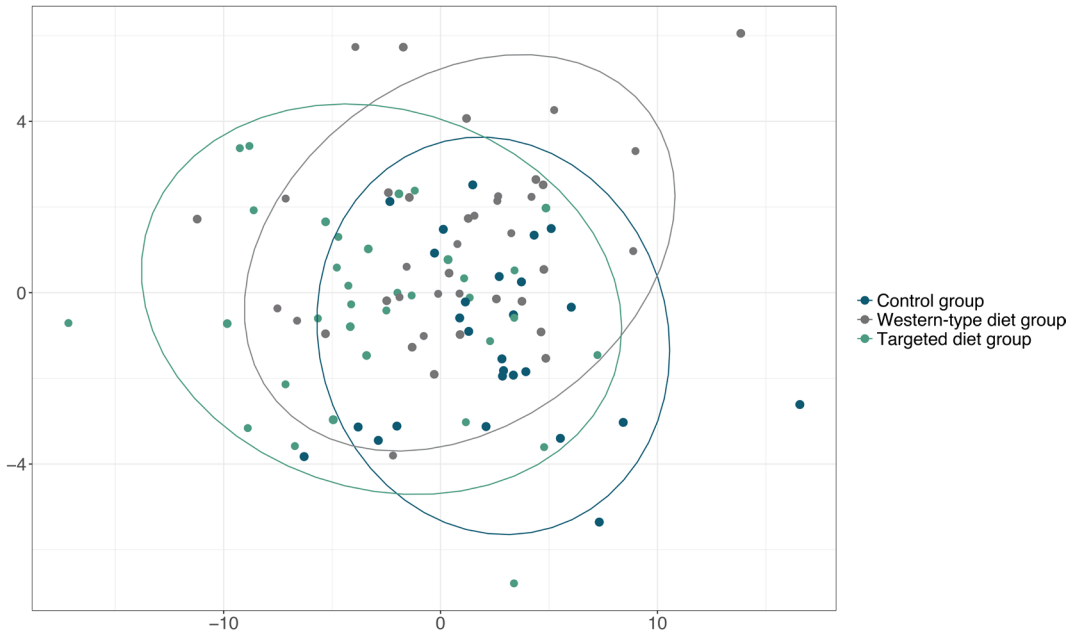

Figure 4. Partial Least Square Discriminant Analysis (PLS-DA) on the changes in metabolite after 12 weeks intervention

Postprandial effects on metabolite profile

We also examined the effects of the dietary interventions on changes in postprandial levels of all metabolites including lipoproteins and their constituents. Analysis was conducted at 2, 4, and 6 hours after consumption of the mixed meal. Our statistical model was designed to examine the changes in the overall response of a certain metabolite, thus whether the shape of the response curve changed after intervention, and to examine changes in specific postprandial time points, thus whether postprandial levels changed after intervention. As expected, consumption of the mixed meal induced an increase in VLDL particles and a decrease in IDL, LDL, and HDL particles (**Supplemental Figure 1**). HDL particles increased again between 4h and 6h. We observed no significant differences in overall response of any of the lipoproteins or their constituents between the different groups. However, there were significant differences in overall response curve within the diet groups (**Supplemental Tables 9 & 10** | **Figure 5A-C**). The TD significantly lowered the overall postprandial response of VLDL particles, mainly in medium and large VLDL components. In contrast, the WD significantly lowered the overall postprandial response curve of TG in IDL and small and large LDL as well as cholesterol in IDL and medium LDL.

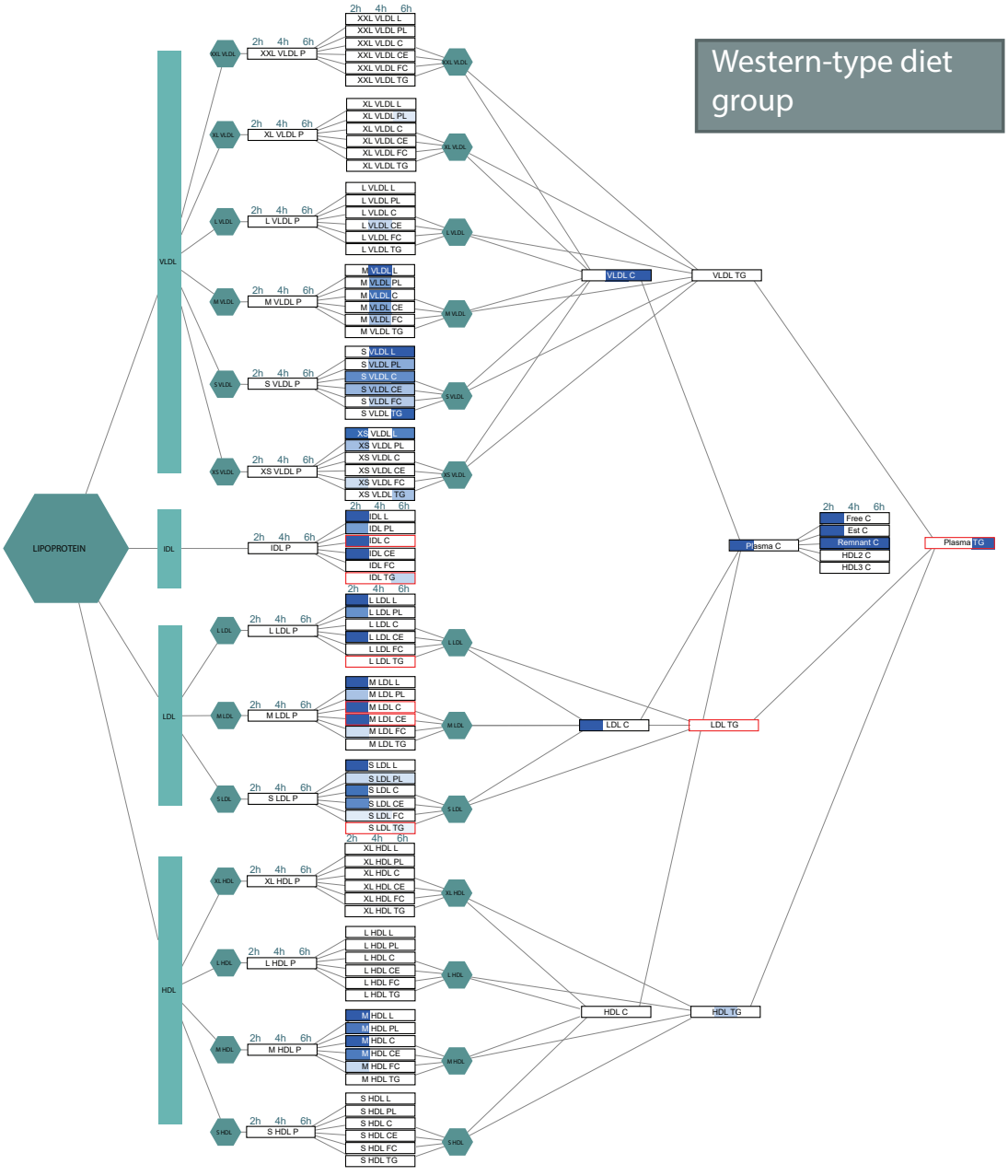

Figure 5A. Changes in postprandial lipoprotein profile after 12wks of WD

Red outline indicates a significant change in overall postprandial response (log2 ratio) within the WD, colored boxes indicate a significant change in response (log2 ratio) on a certain time point within the WD as assessed using Linear Mixed models with LSD post hoc testing for specific time points. Blue color indicates decreased levels and the color intensity is related to the size of the effect, darker color indicates a larger change. Abbreviations: P: particles, L: lipids, PL: phospholipids, C: cholesterol, CE: cholesterol esters, FC: free cholesterol, TG: triglycerides.

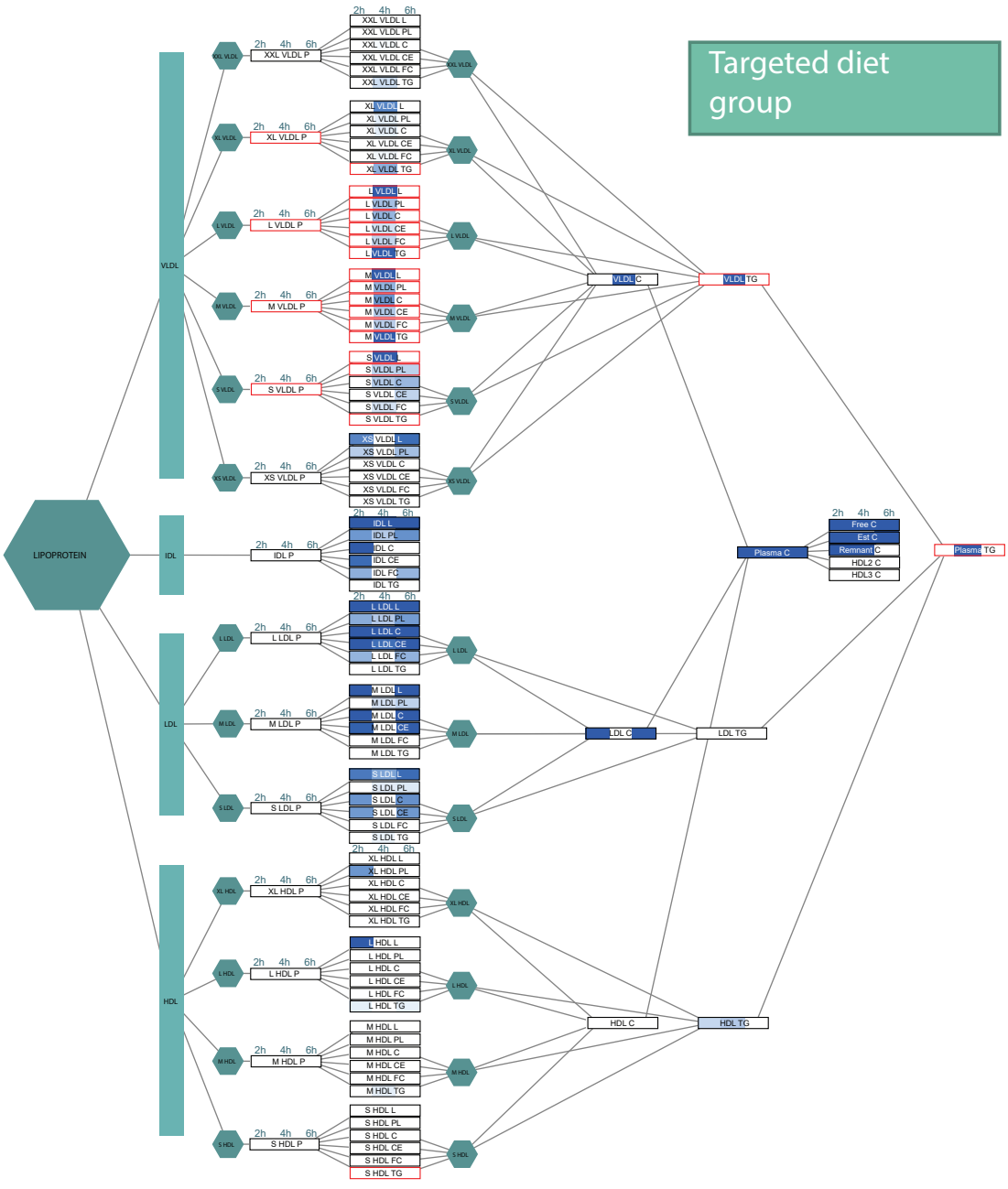

Figure 5B. Changes in postprandial lipoprotein profile after 12wks of TD

Red outline indicates a significant change in overall postprandial response (log2 ratio) within the WD, colored boxes indicate a significant change in response (log2 ratio) on a certain time point Red outline indicates a significant change in overall postprandial response (log2 ratio) within the TD, colored boxes indicate a significant change in response (log2 ratio) on a certain time point within the TD as assessed using Linear Mixed models with LSD post hoc testing for specific time points. Blue color indicates decreased levels and the color intensity is related to the size of the effect, darker color indicates a larger change. Abbreviations: P: particles, L: lipids, PL: phospholipids, C: cholesterol, CE: cholesterol esters, FC: free cholesterol, TG: triglycerides.

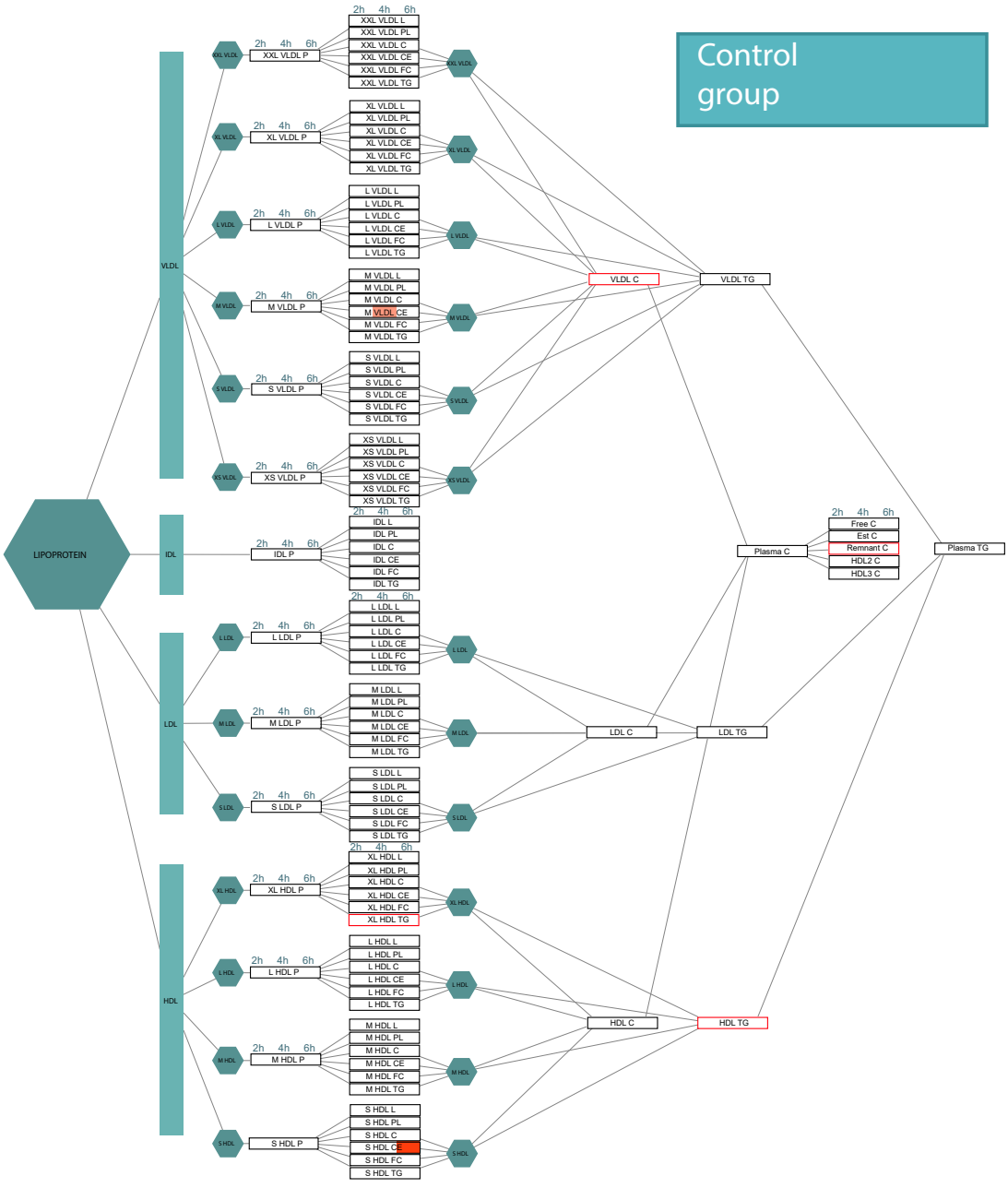

Figure 5C. Changes in postprandial lipoprotein profile after 12wks control group

Red outline indicates a significant change in overall postprandial response (log2 ratio) within the control group, colored boxes indicate a significant change in response (log2 ratio) on a certain time point within the control group as assessed using Linear Mixed models with LSD post hoc testing for specific time points. Blue color indicates decreased levels and the color intensity is related to the size of the effect, darker color indicates a larger change. Abbreviations: P: particles, L: lipids, PL: phospholipids, C: cholesterol, CE: cholesterol esters, FC: free cholesterol, TG: triglycerides.

In addition to effects on overall response curve, both diets reduced levels of several lipoprotein-related particles on specific postprandial time points. Both ER diets lowered postprandial concentrations of VLDL, the TD lowered 35 of the 45 VLDL-related metabolites compared to 21 in the WD. Reductions in VLDL were mainly at 4h when hepatic secretion of VLDL peaks. The TD lowered particles of in all size subclasses whereas the WD only lowered very small to medium VLDL components. The lowering effects of the WD on small VLDL-related particles was observed at almost all postprandial time points and was not restricted to 4h only. Both ER interventions further lowered postprandial concentrations of IDL and LDL, including the VLDL/IDL/LDL-related apolipoprotein ApoB. The WD and TD lowered levels of the same amount of IDL- and LDL-related metabolites but the TD reduced levels at most postprandial time points while the WD mainly reduced levels at 2h only. Effects of both ER-diets on postprandial levels of HDL including its concomitant apolipoprotein ApoA1 were limited. Effects of the TD were diverse, with a reduction in 5 out of the 29 HDL-related metabolites in different sizes and time points. The WD reduced levels of 6 HDL-related metabolites, all in medium HDL and at 2h only.

Changes in postprandial levels of other metabolites were scattered but mainly observed in FA classes, cholines, and plasma proteins (**Supplemental Tables 11-15)**. Concentrations of all FAs decreased after consumption of the mixed meal. Postprandial concentrations of several FAs, mainly PUFAs including linoleic acid, were further decreased upon both the WD and TD with the largest reductions at 2h. Total cholines were reduced within the WD whereas phosphatidylcholine was reduced within both the WD and the TD. As observed and described previously, the overall postprandial curve of glucose was significantly lowered by the WD and borderline significantly lowered by the TD (p=0.085). Postprandial albumin was reduced within the WD at 2h and in the TD at 2, 4, and 6h. Finally, postprandial levels of glycoprotein acetyls, mainly the acute phase protein orosomucoid, were reduced within both the WD and TD at all time points.

DISCUSSION

In this manuscript we evaluated the effects of a 12wk intervention with a high versus a low nutrient quality 25% ER-diet on an array of small-molecule metabolites in plasma, including an extensive panel of lipoproteins and their constituents. The high quality ER-diet enriched in soy protein, fiber, MUFA, and *n-3* PUFA (TD) greatly affected VLDL metabolism by lowering almost all VLDL particles and components, both fasting as well as in the postprandial phase. Furthermore, this diet promoted an anti-atherogenic lipoprotein profile by a potential shift of small HDL towards larger HDL and by reducing postprandial levels of the atherogenic ApoB-carrying IDL and LDL. The low nutrient quality Western-type ER-diet (WD) was not able to induce significant changes in fasting

lipoproteins, although we observed improvements in postprandial levels of several VLDL, IDL, and LDL-related particles.

We observed large reducing effects of the TD on fasting VLDL particles in almost all sizes compared to the WD and the control. The main effects were observed in larger TG-loaded VLDL particles that are positively associated with increased risk of both diabetes type 2 and coronary artery disease[24,25]. The *n-3* FA in the TD might in great part be responsible for the observed reducing effects on fasting VLDL particles. Both EPA and DHA have repeatedly demonstrated to be able to lower plasma TG[26-28]. Suggested mechanisms for this action include the suppression of adipose tissue lipolysis by decreasing adipose tissue inflammation and the stimulation of hepatic beta-oxidation via activation of PPARa, decreasing the availability of FA destined to be incorporated into VLDL[29]. Compared to VLDL the effects of the TD on LDL levels were relatively mild; four LDL-related metabolites were reduced by this diet compared to the control and the WD. These metabolites did include free cholesterol content in the medium and small LDL particles. It is well documented that small LDL particles have a higher atherogenic potential than larger ones[30], furthermore a high cholesterol content in LDL is related to an increased risk of coronary heart disease[31,32]. The contribution of *n-3* to cholesterol levels is ambiguous, as in particular DHA is known for promoting LDL production[33]. However, other nutrients in the TD are known for their total or LDL cholesterol lowering properties, such as dietary fiber[34] and soy protein[35], and might thus be responsible for the beneficial effects on LDL cholesterol. Effects of the TD on HDL were more pronounced and included a decrease in small to medium HDL-related particles. Furthermore, we observed a significant increase in larger HDL particles within the TD but this increase was not significant when compared to the WD or control. This potential shift from small to larger HDL in the TD might beneficially affect cardio-metabolic health as there is an inverse relation between HDL size and CVD risk[36].

Remarkably, we observed no significant effects of the WD on fasting concentrations of any of the lipoproteins or related particles. Previous ER studies showed that weight loss itself exerts beneficial effects on blood lipid profiles, such as a reduction in total and LDL cholesterol[37-39]. It could be that potential favorable effects of ER were counteracted in the WD by the relatively large amount (14.9 En%) of SFA in this diet. SFA such as lauric, myristic, and palmitic acid are known to raise total cholesterol as well as HDL and LDL levels[40]. In addition, dietary fructose, of which the intake was higher in the WD, is able to increase TG and LDL cholesterol levels[41.] It is hypothesized that this is caused by the rapid uptake and potential conversion of fructose to TG in the liver. However, these effects are mainly observed in high-fructose diets (≥ 20 En%)[41] while reported fructose intake

in the WD was 7 En%. To summarize, the high nutrient quality TD but not the lower quality WD beneficially affected fasting lipoproteins by reducing larger TG-rich VLDL and medium to small LDL and -HDL along with a trend towards increasing larger HDL, thereby promoting an anti-atherogenic lipoprotein profile.

We also observed significant effects on postprandial responses of lipoproteins after the mixed meal in both the TD and the WD, whereas effects were virtually absent in the control group. As both ER-diets reduced postprandial levels of multiple lipoprotein particles at multiple time points, it is tempting to state that applying a dietary stressor is indeed a powerful tool to enlarge effects of nutritional interventions. However, it is important to note that we observed no statistical differences in changes in response between the different groups only within groups. Both ER diets reduced postprandial levels of VLDL, IDL, and LDL but the effects were more pronounced in the TD. Reductions in postprandial VLDL levels can be the net result of both a decrease in hepatic production and an increase in clearance by the peripheral tissues. In our study, postprandial levels of VLDL were mainly reduced by both the TD and WD at 4h, when hepatic secretion peaks[42]. This might indicate that both diets mainly affected VLDL production. In contrast to our findings of TD on smaller fasting HDL particles, effects of the interventions on postprandial HDL levels were virtually absent. Postprandial HDL levels are mainly regulated by the activity of cholesteryl ester transfer protein (CETP) that facilitates the transfer of cholesteryl esters from HDL to VLDL and LDL. Vigorous weight loss-inducing therapies such as bariatric surgery are able to reduce CETP activity followed by an increase in HDL levels[43]. A 12wk intervention with 25% ER is probably not potent enough to induce similar effects. To summarize, both ER-diets reduced postprandial levels of the ApoB-carrying atherogenic lipoproteins VLDL, IDL, and LDL but not of the ApoA1-carrying HDL. The TD was more potent in lowering ApoB-lipoproteins, as more of these metabolites were reduced by this diet when compared to the WD.

In our study, the main class of potential biomarkers of intake was represented by FAs, especially EPA and DHA. Interestingly, there was no significant difference in changes in *n-3* levels between the TD and the WD and the control. The supplied capsules did contain EPA and DHA in sufficient amounts and the return of empty pill bottles indicated good compliance. The incorporation of the FAs within an ER-diet might have altered their fate from bioactive signaling molecules into direct fuel for tissues. However, four studies that enriched ER-diets with either fish or fish oil supplements did observe an increase in plasma *n-3* after intervention[9,10,13,44]. Explanations for this discrepancy might be a higher dose used in these studies compared to our study. Moreover, we used a strict FDR-corrected statistical model that assessed the difference in *n-3* between all three groups, while the increase in ratio of *n-3* to total FAs within the TD specifically was significant. Furthermore, the fasting and postprandial

VLDL reductions in the TD gives us reason to believe that the function of these *n-3* exceeded serving as energy substrate in our study.

The TD did affect plasma FA profile in other ways, namely a reduction in total FA and SFA when compared to the control group. In addition, MUFA levels also decreased in the TD despite MUFA-enrichment, mainly oleic acid from olive oil. However, circulating MUFA has only a weak correlation with dietary intake as levels also depend on endogenous synthesis and conversion[45]. The reduction in total FA in the TD might indicate better suppression of adipose tissue lipolysis that could have been caused by improved insulin sensitivity after consuming this diet observed previously. Remarkably, improved insulin sensitivity was also observed in the WD but this did not coincide with lower fasting total FA levels. However, in the postprandial phase, when insulin-mediated suppression of lipolysis is even more relevant, both diets were able to reduce total FAs and several specific FAs, mainly PUFAs including linoleic acid. The release of FAs from adipose tissue into plasma is not proportional to the composition in the tissue but based on their molecular structure[46]. Long-chain unsaturated FAs are more rapidly mobilized than saturated FAs, which might explain why PUFA levels are relatively more affected by improved suppression of lipolysis induced by ER than other FAs.

Other metabolites that were significantly different upon intervention included several amino acids. The WD lowered levels of the aromatic amino acids (AAA) tyrosine and phenylalanine as well as the BCAA leucine. Levels of tyrosine and phenylalanine have been shown to be elevated specifically in obese individuals[47]. Furthermore, insulin resistance is associated with high levels of BCAAs, potentially caused by decreased utilization in the muscle and reduced activity of BCAA-catabolizing enzymes in the adipose tissue[48,49]. Weight loss induced by ER, which subsequently improves insulin sensitivity, can therefore be an effective strategy to lower levels of AAA and BCAA. Interestingly, these effects were observed in the WD only, a possible explanation is the higher dietary contribution of at least phenylalanine and leucine from soy in the TD.

Although we observed significant differences in changes in 56 fasting metabolites between the intervention groups, there were no specific metabolites that contributed most to the between-group variability. In other words, adherence to either the WD or the TD did not result in a distinct metabolic finger print of fasting metabolites that were robustly changed in all individuals, which was also reflected by the visualization of individual changes in the heat map. It is well known that individuals respond differently to dietary interventions, for instance due to their (epi)genetic background and environmental factors. Furthermore, the study was based

on dietary advice, which leads to more free choice and thus variation in the diet. Strengths of our study included the scope of the metabolomics platform, as it covered all lipoprotein sub-fractions and sizes. Moreover, with the application of the mixed meal test we could determine an improvement in the capacity of participants to deal with a dietary stressor, which was reflected by a reduction in the amount of atherogenic ApoB-particles secreted in the circulation.

Our results demonstrate the crucial importance of nutrient quality within ER-diets, as our high nutrient quality TD strongly affected TG-related VLDL metabolism by lowering almost all sizes and constituents of this lipoprotein type. Both ER-diets suppressed postprandial lipolysis and induced an anti-atherogenic shift in lipoproteins but the high nutrient quality diet was more potent in achieving these effects. Based upon these data we conclude that a high quality 25% ER-diet with soy protein, fiber, MUFA and *n-3* PUFA is a more powerful whole diet approach compared to a Western-type 25% ER-diet to achieve a favorable blood lipid profile, which might lower CVD risk on the long run.

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SUPPLEMENTAL MATERIAL

Supplemental Table 1. Baseline characteristics and effects of dietary interventions on body composition and cardio-metabolic risk factors

	BASELINE	CHANGE After 12 weeks	Within group p-value	Difference between groups p-value
BASELINE CHARACTERISTICS				
Age, years				
Western-type diet group	64 (28)			
Targeted diet group	62 (29)			
Control group	62 (29)			
Gender, n males, %				
Western-type diet group	17, 44%			
Targeted diet group	16, 47%			
Control group	13, 48%			
BODY COMPOSITION				
Body weight, kg				
Western-type diet group	93.1 ± 13.9	-6.3 ± 3.9 [A]	**<0.001**	
Targeted diet group	94.8 ± 13.2	-8.4 ± 3.2 [B]	**<0.001**	**<0.001**
Control group	92.1 ± 12.6	0.8 ± 1.7 [C]	**0.018**	
BMI, kg/m²				
Western-type diet group	31.2 ± 3.3	-2.1 ± 1.3 [A]	**<0.001**	
Targeted diet group	31.8 ± 3.4	-2.8 ± 1.1 [B]	**<0.001**	**<0.001**
Control group	30.9 ± 3.9	0.3 ± 0.6 [C]	**0.015**	
Waist circumference, cm				
Western-type diet group	108.7 ± 9.8	-3.1 ± 5.3 [A]	**<0.001**	
Targeted diet group	109.5 ± 9.8	-4.7 ± 5.7 [A]	**<0.001**	**<0.001**
Control group	106.6 ± 8.1	4.1 ± 5.2 [B]	**<0.001**	
Abdominal SAT, cm²*				
Western-type diet group	332.5 ± 92.4	-45.3 ± 32.7 [A]	**<0.001**	
Targeted diet group	348.0 ± 106.5	-54.1 ± 55.3 [A]	**<0.001**	**<0.001**
Control group	355.6 ± 118.8	1.6 ± 36.9 [B]	0.628	
Abdominal VAT, cm²*				
Western-type diet group	149.1 ± 77.0	-44.1 ± 48.2 [A]	**<0.001**	
Targeted diet group	175.7 ± 94.2	-61.5 ± 51.3 [A]	**<0.001**	**<0.001**
Control group	115.8 ± 26.1	3.7 ± 17.9 [B]	0.551	
VAT/SAT ratio*				
Western-type diet group	50.6 ± 34.8	-9.4 ± 16.9 [A]	**0.001**	
Targeted diet group	61.2 ± 54.9	-16.0 ± 28.1 [A]	**0.001**	**0.032**
Control group	36.2 ± 16.0	0.8 ± 6.3 [B]	0.775	
Intrahepatic lipids, % of water peak*				
Western-type diet group	7.3 ± 6.5	-3.4 ± 5.1 [A]	**<0.001**	
Targeted diet group	7.1 ± 8.7	-3.9 ± 5.7 [A]	**0.001**	**<0.001**
Control group	5.9 ± 5.8	1.4 ± 1.9 [B]	**0.021**	
CARDIO-METABOLIC RISK FACTORS				
Plasma glucose, mmol/L				
Western-type diet group	5.6 ± 0.8	-0.3 ± 0.6 [A]	**0.003**	
Targeted diet group	5.7 ± 0.5	-0.3 ± 0.4 [A]	**<0.001**	**0.007**
Control group	5.6 ± 0.4	0.0 ± 0.4 [B]	0.585	
Plasma insulin, mU/L				
Western-type diet group	14.6 ± 10.3	-3.9 ± 7.5 [A]	**0.002**	
Targeted diet group	13.5 ± 7.8	-3.6 ± 5.3 [A]	**<0.001**	**0.012**
Control group	12.1 ± 5.3	0.3 ± 6.1 [B]	0.782	
HbA1c, mmol/mol				
Western-type diet group	36.3 ± 2.1 [a,b]	-0.8 ± 2.0 [A]	**0.013**	
Targeted diet group	37.3 ± 2.9 [a]	-1.5 ± 2.0 [A]	**<0.001**	**0.014**
Control group	35.1 ± 2.6 [b]	0.4 ± 1.3 [B]	0.146	

	BASELINE	**CHANGE** After 12 weeks	Within group **p-value**	Difference between groups **p-value**
Serum total cholesterol, mmol/L				
Western-type diet group	5.7 ± 1.1	-0.2 ± 0.8 [A]	0.218	
Targeted diet group	5.5 ± 0.8	-0.5 ± 0.6 [B]	**<0.001**	**0.013**
Control group	5.4 ± 1.0	0.0 ± 0.5 [A]	0.633	
Serum HDL cholesterol, mmol/L				
Western-type diet group	1.3 ± 0.4	0.0 ± 0.2	0.096	
Targeted diet group	1.4 ± 0.4	-0.1 ± 0.2	0.084	0.878
Control group	1.4 ± 0.4	0.0 ± 0.3	0.439	
Plasma triglycerides, mmol/L				
Western-type diet group	1.8 ± 0.8	-0.2 ± 0.6 [A]	**0.018**	
Targeted diet group	1.6 ± 0.6	-0.4 ± 0.6 [B]	**0.001**	0.026
Control group	1.7 ± 0.7	-0.1 ± 0.5 [A]	0.280	
Plasma free fatty acids, mmol/L				
Western-type diet group	0.41 ± 0.21	-0.02 ± 0.20	0.522	
Targeted diet group	0.57 ± 0.37	-0.04 ± 0.49	0.471	0.389
Control group	0.42 ± 0.18	0.05 ± 0.24	0.291	

Data are presented as mean ± 1SD. For SAT and VAT: Western-type diet group *n*=28, Targeted diet group *n*=26, Control group *n*=16, for IHL: Western-type diet group *n*=34, Targeted diet group *n*=29, Control group *n*=19. For all other values: Western-type diet group *n*=39, Targeted diet group *n*=34, Control group *n*=27. Values denoted with an * are log2 transformed for statistical analyses to improve normality. Changes within group analyzed using paired t-test, changes between groups analyzed using general linear model for univariate analysis with baseline values as covariates (ANCOVA). Post hoc analyses were conducted using LSD, different letters (A, B, C) indicate significant post hoc differences (p<0.05) between diet group. Abbreviations: BMI: body mass index, SAT: subcutaneous adipose tissue mass (assessed by MRI), VAT: visceral adipose tissue mass (assessed by MRI), IHL: intrahepatic lipid (assessed by ^{1}H-MRS), HbA1c: glycosylated hemoglobin, HDL: high-density lipoprotein.

Supplemental Table 2. Changes in fasting lipoproteins upon 12 weeks of intervention

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Concentration of chylomicrons and extremely large VLDL particles, mol/L			
Western-type diet group	-0.171 ± 0.949 [A]	0.273	
Targeted diet group	-0.914 ± 1.338 [B]	**0.001**	**0.001**
Control group	0.100 ± 0.823 [A]	0.549	
Total lipids in chylomicrons and extremely large VLDL, mmol/L			
Western-type diet group	-0.172 ± 0.945 [A]	0.269	
Targeted diet group	-0.909 ± 1.325 [B]	**0.001**	**0.002**
Control group	0.096 ± 0.819 [A]	0.562	
Phospholipids in chylomicrons and extremely large VLDL, mmol/L			
Western-type diet group	-0.200 ± 1.069 [A]	0.257	
Targeted diet group	-1.134 ± 1.573 [B]	**<0.001**	**<0.001**
Control group	0.197 ± 1.009 [A]	0.340	
Total cholesterol in chylomicrons and extremely large VLDL, mmol/L			
Western-type diet group	-0.153 ± 0.906 [A]	0.305	
Targeted diet group	-0.796 ± 1.216 [B]	**0.001**	**0.006**
Control group	-0.006 ± 0.700 [A]	0.966	
Cholesterol esters in chylomicrons and extremely large VLDL, mmol/L			
Western-type diet group	-0.116 ± 0.877 [A]	0.420	
Targeted diet group	-0.703 ± 1.155 [B]	**0.002**	**0.016**
Control group	-0.083 ± 0.604 [A]	0.496	
Free cholesterol in chylomicrons and extremely large VLDL, mmol/L			
Western-type diet group	-0.149 ± 1.158 [A]	0.434	
Targeted diet group	-1.027 ± 1.458 [B]	**0.001**	**0.001**
Control group	0.199 ± 1.031 [A]	0.343	
Triglycerides in chylomicrons and extremely large VLDL, mmol/L			
Western-type diet group	-0.165 ± 0.974 [A]	0.304	
Targeted diet group	-0.942 ± 1.405 [B]	**0.001**	**0.001**
Control group	0.120 ± 0.850 [A]	0.487	
Concentration of very large VLDL particles, mol/L			
Western-type diet group	-0.120 ± 0.838 [A]	0.391	
Targeted diet group	-0.910 ± 1.346 [B]	**0.001**	**0.002**
Control group	-0.067 ± 0.723 [A]	0.653	
Total lipids in very large VLDL, mmol/L			
Western-type diet group	-0.115 ± 0.829 [A]	0.403	
Targeted diet group	-0.887 ± 1.332 [B]	**0.001**	**0.003**
Control group	-0.061 ± 0.724 [A]	0.682	
Phospholipids in very large VLDL, mmol/L			
Western-type diet group	-0.083 ± 1.003 [A]	0.617	
Targeted diet group	-1.102 ± 1.797 [B]	**0.002**	**0.002**
Control group	0.030 ± 0.981 [A]	0.883	
Total cholesterol in very large VLDL, mmol/L			
Western-type diet group	-0.007 ± 0.855	0.960	
Targeted diet group	-0.619 ± 1.324	**0.016**	0.029
Control group	-0.031 ± 0.658	0.821	
Cholesterol esters in very large VLDL, mmol/L			
Western-type diet group	0.016 ± 0.811	0.906	
Targeted diet group	-0.525 ± 1.176	**0.021**	0.041
Control group	-0.054 ± 0.578	0.649	
Free cholesterol in very large VLDL, mmol/L			
Western-type diet group	-0.026 ± 0.937	0.868	
Targeted diet group	-0.754 ± 1.706	0.022	0.027
Control group	0.016 ± 0.810	0.922	
Triglycerides in very large VLDL, mmol/L			
Western-type diet group	-0.119 ± 0.912 [A]	0.433	
Targeted diet group	-1.054 ± 1.702 [B]	**0.002**	**0.003**
Control group	-0.084 ± 0.717 [A]	0.570	

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Concentration of large VLDL particles, mol/L			
Western-type diet group	-0.234 ± 0.723 [A]	0.051	
Targeted diet group	-0.698 ± 0.824 [B]	**<0.001**	**0.004**
Control group	-0.126 ± 0.502 [A]	0.214	
Total lipids in large VLDL, mmol/L			
Western-type diet group	-0.234 ± 0.721 [A]	**0.050**	
Targeted diet group	-0.698 ± 0.829 [B]	**<0.001**	**0.004**
Control group	-0.122 ± 0.506 [A]	0.230	
Phospholipids in large VLDL, mmol/L			
Western-type diet group	-0.271 ± 0.761 [A]	**0.032**	
Targeted diet group	-0.736 ± 0.856 [B]	**<0.001**	**0.004**
Control group	-0.122 ± 0.518 [A]	0.240	
Total cholesterol in large VLDL, mmol/L			
Western-type diet group	-0.190 ± 0.684 [A]	0.092	
Targeted diet group	-0.648 ± 0.836 [B]	**<0.001**	**0.004**
Control group	-0.092 ± 0.507 [A]	0.362	
Cholesterol esters in large VLDL, mmol/L			
Western-type diet group	-0.128 ± 0.593 [A]	0.185	
Targeted diet group	-0.543 ± 0.746 [B]	**<0.001**	**0.007**
Control group	-0.102 ± 0.440 [A]	0.248	
Free cholesterol in large VLDL, mmol/L			
Western-type diet group	-0.299 ± 0.890 [A]	**0.043**	
Targeted diet group	-0.798 ± 0.976 [B]	**<0.001**	**0.004**
Control group	-0.072 ± 0.605 [A]	0.549	
Triglycerides in large VLDL, mmol/L			
Western-type diet group	-0.238 ± 0.766 [A]	0.059	
Targeted diet group	-0.709 ± 0.826 [B]	**<0.001**	**0.005**
Control group	-0.133 ± 0.504 [A]	0.189	
Concentration of medium VLDL particles, mol/L			
Western-type diet group	-0.123 ± 0.455 [A]	0.099	
Targeted diet group	-0.406 ± 0.549 [B]	**<0.001**	**0.013**
Control group	-0.085 ± 0.385 [A]	0.262	
Total lipids in medium VLDL, mmol/L			
Western-type diet group	-0.121 ± 0.449 [A]	0.100	
Targeted diet group	-0.402 ± 0.543 [B]	**<0.001**	**0.013**
Control group	-0.083 ± 0.383 [A]	0.270	
Phospholipids in medium VLDL, mmol/L			
Western-type diet group	-0.123 ± 0.432 [A]	0.083	
Targeted diet group	-0.387 ± 0.515 [B]	**<0.001**	**0.012**
Control group	-0.078 ± 0.363 [A]	0.275	
Total cholesterol in medium VLDL, mmol/L			
Western-type diet group	-0.094 ± 0.413 [A]	0.165	
Targeted diet group	-0.355 ± 0.494 [B]	**<0.001**	**0.013**
Control group	-0.068 ± 0.355 [A]	0.330	
Cholesterol esters in medium VLDL, mmol/L			
Western-type diet group	-0.068 ± 0.385 [A]	0.275	
Targeted diet group	-0.312 ± 0.449 [B]	**<0.001**	**0.015**
Control group	-0.065 ± 0.311 [A]	0.289	
Free cholesterol in medium VLDL, mmol/L			
Western-type diet group	-0.121 ± 0.476 [A]	0.120	
Targeted diet group	-0.417 ± 0.571 [B]	**<0.001**	**0.012**
Control group	-0.068 ± 0.432 [A]	0.421	
Triglycerides in medium VLDL, mmol/L			
Western-type diet group	-0.128 ± 0.509 [A]	0.124	
Targeted diet group	-0.433 ± 0.586 [B]	**<0.001**	**0.015**
Control group	-0.093 ± 0.407 [A]	0.248	
Concentration of small VLDL particles, mol/L			
Western-type diet group	-0.050 ± 0.252	0.226	
Targeted diet group	-0.199 ± 0.295	**<0.001**	0.032
Control group	-0.066 ± 0.196	0.091	

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Total lipids in small VLDL, mmol/L			
Western-type diet group	-0.046 ± 0.242	0.243	
Targeted diet group	-0.191 ± 0.282	**<0.001**	0.028
Control group	-0.061 ± 0.189	0.108	
Phospholipids in small VLDL, mmol/L			
Western-type diet group	-0.064 ± 0.226 [A]	0.086	
Targeted diet group	-0.205 ± 0.243 [B]	**<0.001**	**0.014**
Control group	-0.074 ± 0.165	**0.027**	
Total cholesterol in small VLDL, mmol/L			
Western-type diet group	-0.004 ± 0.205	0.911	
Targeted diet group	-0.127 ± 0.232	**0.003**	0.024
Control group	-0.010 ± 0.169	0.753	
Cholesterol esters in small VLDL, mmol/L			
Western-type diet group	0.025 ± 0.207	0.459	
Targeted diet group	-0.091 ± 0.243	**0.037**	0.040
Control group	0.025 ± 0.175	0.466	
Free cholesterol in small VLDL, mmol/L			
Western-type diet group	-0.045 ± 0.230	0.225	
Targeted diet group	-0.188 ± 0.251	**<0.001**	0.021
Control group	-0.067 ± 0.179	0.063	
Triglycerides in small VLDL, mmol/L			
Western-type diet group	-0.066 ± 0.328	0.216	
Targeted diet group	-0.247 ± 0.384	**0.001**	0.055
Control group	-0.100 ± 0.246	**0.045**	
Concentration of very small VLDL particles, mol/L			
Western-type diet group	0.035 ± 0.159	0.177	
Targeted diet group	-0.030 ± 0.132	0.202	0.115
Control group	0.033 ± 0.133	0.211	
Total lipids in very small VLDL, mmol/L			
Western-type diet group	0.046 ± 0.162	0.083	
Targeted diet group	-0.018 ± 0.135	0.433	0.126
Control group	0.044 ± 0.136	0.108	
Phospholipids in very small VLDL, mmol/L			
Western-type diet group	0.015 ± 0.226	0.689	
Targeted diet group	-0.058 ± 0.142	**0.024**	0.261
Control group	-0.012 ± 0.177	0.732	
Total cholesterol in very small VLDL, mmol/L			
Western-type diet group	0.105 ± 0.183	**0.001**	
Targeted diet group	0.044 ± 0.204	0.219	0.184
Control group	0.126 ± 0.157	**<0.001**	
Cholesterol esters in very small VLDL, mmol/L			
Western-type diet group	0.110 ± 0.186	**0.001**	
Targeted diet group	0.059 ± 0.243	0.170	0.125
Control group	0.166 ± 0.164	**<0.001**	
Free cholesterol in very small VLDL, mmol/L			
Western-type diet group	0.099 ± 0.205	**0.005**	
Targeted diet group	0.013 ± 0.158	0.628	0.138
Control group	0.042 ± 0.189	0.257	
Triglycerides in very small VLDL, mmol/L			
Western-type diet group	-0.026 ± 0.215	0.459	
Targeted diet group	-0.119 ± 0.236	**0.006**	0.174
Control group	-0.064 ± 0.169	0.058	
Concentration of IDL particles, mol/L			
Western-type diet group	0.017 ± 0.206	0.611	
Targeted diet group	-0.057 ± 0.138	**0.021**	0.151
Control group	0.010 ± 0.157	0.742	
Total lipids in IDL, mmol/L			
Western-type diet group	0.020 ± 0.211	0.563	
Targeted diet group	-0.056 ± 0.145	**0.030**	0.156
Control group	0.012 ± 0.164	0.696	

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Phospholipids in IDL, mmol/L			
Western-type diet group	0.015 ± 0.222	0.676	
Targeted diet group	-0.059 ± 0.152	**0.031**	0.247
Control group	-0.007 ± 0.177	0.843	
Total cholesterol in IDL, mmol/L			
Western-type diet group	0.023 ± 0.219	0.513	
Targeted diet group	-0.063 ± 0.168	**0.036**	0.105
Control group	0.025 ± 0.178	0.464	
Cholesterol esters in IDL, mmol/L			
Western-type diet group	0.012 ± 0.208	0.719	
Targeted diet group	-0.079 ± 0.168	**0.009**	0.044
Control group	0.028 ± 0.166	0.383	
Free cholesterol in IDL, mmol/L			
Western-type diet group	0.058 ± 0.273	0.193	
Targeted diet group	-0.020 ± 0.206	0.581	0.391
Control group	0.014 ± 0.234	0.760	
Triglycerides in IDL, mmol/L			
Western-type diet group	0.017 ± 0.199	0.587	
Targeted diet group	-0.016 ± 0.178	0.600	0.571
Control group	-0.028 ± 0.163	0.385	
Concentration of large LDL particles, mol/L			
Western-type diet group	-0.017 ± 0.235	0.662	
Targeted diet group	-0.107 ± 0.150	**<0.001**	0.112
Control group	-0.026 ± 0.179	0.453	
Total lipids in large LDL, mmol/L			
Western-type diet group	-0.013 ± 0.237	0.725	
Targeted diet group	-0.105 ± 0.154	**<0.001**	0.117
Control group	-0.025 ± 0.184	0.490	
Phospholipids in large LDL, mmol/L			
Western-type diet group	-0.025 ± 0.196	0.425	
Targeted diet group	-0.113 ± 0.125	**<0.001**	0.053
Control group	-0.033 ± 0.149	0.265	
Total cholesterol in large LDL, mmol/L			
Western-type diet group	-0.010 ± 0.258	0.804	
Targeted diet group	-0.111 ± 0.177	**0.001**	0.127
Control group	-0.024 ± 0.212	0.569	
Cholesterol esters in large LDL, mmol/L			
Western-type diet group	-0.023 ± 0.268	0.596	
Targeted diet group	-0.131 ± 0.184	**<0.001**	0.102
Control group	-0.031 ± 0.219	0.475	
Free cholesterol in large LDL, mmol/L			
Western-type diet group	0.024 ± 0.240	0.542	
Targeted diet group	-0.060 ± 0.172	0.051	0.232
Control group	-0.005 ± 0.199	0.895	
Triglycerides in large LDL, mmol/L			
Western-type diet group	-0.001 ± 0.233	0.971	
Targeted diet group	-0.039 ± 0.184	0.225	0.735
Control group	-0.017 ± 0.181	0.635	
Concentration of medium LDL particles, mol/L			
Western-type diet group	-0.045 ± 0.259	0.282	
Targeted diet group	-0.149 ± 0.173	**<0.001**	0.106
Control group	-0.060 ± 0.204	0.141	
Total lipids in medium LDL, mmol/L			
Western-type diet group	-0.041 ± 0.254	0.325	
Targeted diet group	-0.145 ± 0.171	**<0.001**	0.098
Control group	-0.056 ± 0.204	0.168	
Phospholipids in medium LDL, mmol/L			
Western-type diet group	-0.059 ± 0.180 [A]	**0.048**	
Targeted diet group	-0.165 ± 0.125 [B]	**<0.001**	**0.003**
Control group	-0.047 ± 0.134 [A]	0.080	

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Total cholesterol in medium LDL, mmol/L			
Western-type diet group	-0.027 ± 0.291	0.560	
Targeted diet group	-0.138 ± 0.206	**<0.001**	0.175
Control group	-0.057 ± 0.256	0.257	
Cholesterol esters in medium LDL, mmol/L			
Western-type diet group	-0.035 ± 0.346	0.526	
Targeted diet group	-0.152 ± 0.245	**0.001**	0.268
Control group	-0.074 ± 0.312	0.227	
Free cholesterol in medium LDL, mmol/L			
Western-type diet group	-0.011 ± 0.173 [A]	0.697	
Targeted diet group	-0.105 ± 0.121 [B]	**<0.001**	**0.018**
Control group	-0.018 ± 0.144	0.529	
Triglycerides in medium LDL, mmol/L			
Western-type diet group	-0.106 ± 0.322	**0.047**	
Targeted diet group	-0.149 ± 0.244	**0.001**	0.783
Control group	-0.114 ± 0.228	**0.015**	
Concentration of small LDL particles, mol/L			
Western-type diet group	-0.053 ± 0.238	0.172	
Targeted diet group	-0.162 ± 0.167	**<0.001**	0.051
Control group	-0.058 ± 0.192	0.127	
Total lipids in small LDL, mmol/L			
Western-type diet group	-0.046 ± 0.234	0.232	
Targeted diet group	-0.155 ± 0.166	**<0.001**	0.049
Control group	-0.054 ± 0.193	0.162	
Phospholipids in small LDL, mmol/L			
Western-type diet group	-0.067 ± 0.166 [A]	**0.016**	
Targeted diet group	-0.178 ± 0.126 [B]	**<0.001**	**0.001**
Control group	-0.048 ± 0.129 [A]	0.064	
Total cholesterol in small LDL, mmol/L			
Western-type diet group	-0.023 ± 0.281	0.616	
Targeted diet group	-0.136 ± 0.207	**0.001**	0.152
Control group	-0.053 ± 0.257	0.298	
Cholesterol esters in small LDL, mmol/L			
Western-type diet group	-0.034 ± 0.343	0.540	
Targeted diet group	-0.147 ± 0.251	**0.002**	0.297
Control group	-0.073 ± 0.326	0.255	
Free cholesterol in small LDL, mmol/L			
Western-type diet group	0.001 ± 0.156 [A]	0.954	
Targeted diet group	-0.109 ± 0.124 [B]	**<0.001**	**0.003**
Control group	-0.010 ± 0.136 [A]	0.715	
Triglycerides in small LDL, mmol/L			
Western-type diet group	-0.158 ± 0.323	**0.004**	
Targeted diet group	-0.260 ± 0.275	**<0.001**	0.128
Control group	-0.122 ± 0.203	**0.004**	
Concentration of very large HDL particles, mol/L			
Western-type diet group	0.220 ± 0.436	**0.004**	
Targeted diet group	0.254 ± 0.466	**0.004**	0.917
Control group	0.267 ± 0.500	**0.013**	
Total lipids in very large HDL, mmol/L			
Western-type diet group	0.225 ± 0.444	**0.004**	
Targeted diet group	0.252 ± 0.472	**0.004**	0.908
Control group	0.278 ± 0.509	**0.012**	
Phospholipids in very large HDL, mmol/L			
Western-type diet group	0.348 ± 0.897	**0.024**	
Targeted diet group	0.288 ± 0.555	**0.005**	0.898
Control group	0.262 ± 0.768	0.101	
Total cholesterol in very large HDL, mmol/L			
Western-type diet group	0.261 ± 0.488	**0.002**	
Targeted diet group	0.266 ± 0.597	**0.015**	0.637
Control group	0.384 ± 0.548	**0.002**	

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Cholesterol esters in very large HDL, mmol/L			
Western-type diet group	0.280 ± 0.502	**0.002**	
Targeted diet group	0.290 ± 0.654	**0.016**	0.564
Control group	0.429 ± 0.575	**0.001**	
Free cholesterol in very large HDL, mmol/L			
Western-type diet group	0.210 ± 0.485	**0.012**	
Targeted diet group	0.205 ± 0.467	**0.017**	0.917
Control group	0.255 ± 0.514	**0.020**	
Triglycerides in very large HDL, mmol/L			
Western-type diet group	0.196 ± 0.535	**0.033**	
Targeted diet group	0.362 ± 1.038	0.053	0.572
Control group	0.195 ± 0.426	**0.031**	
Concentration of large HDL particles, mol/L			
Western-type diet group	-0.034 ± 0.408	0.636	
Targeted diet group	0.114 ± 0.442	0.148	0.245
Control group	-0.050 ± 0.394	0.552	
Total lipids in large HDL, mmol/L			
Western-type diet group	-0.030 ± 0.415	0.678	
Targeted diet group	0.127 ± 0.460	0.122	0.224
Control group	-0.045 ± 0.401	0.593	
Phospholipids in large HDL, mmol/L			
Western-type diet group	-0.093 ± 0.381	0.170	
Targeted diet group	0.025 ± 0.385	0.713	0.430
Control group	-0.066 ± 0.372	0.407	
Total cholesterol in large HDL, mmol/L			
Western-type diet group	0.040 ± 0.485	0.637	
Targeted diet group	0.268 ± 0.630	**0.020**	0.119
Control group	-0.001 ± 0.469	0.991	
Cholesterol esters in large HDL, mmol/L			
Western-type diet group	0.036 ± 0.472	0.663	
Targeted diet group	0.256 ± 0.604	**0.021**	0.123
Control group	-0.003 ± 0.463	0.976	
Free cholesterol in large HDL, mmol/L			
Western-type diet group	0.052 ± 0.549	0.587	
Targeted diet group	0.324 ± 0.777	**0.023**	0.114
Control group	0.007 ± 0.495	0.948	
Triglycerides in large HDL, mmol/L			
Western-type diet group	0.176 ± 0.580	0.090	
Targeted diet group	0.141 ± 0.344	**0.024**	0.082
Control group	-0.088 ± 0.378	0.278	
Concentration of medium HDL particles, mol/L			
Western-type diet group	-0.143 ± 0.199	**<0.001**	
Targeted diet group	-0.239 ± 0.182 [A]	**<0.001**	**0.007**
Control group	-0.083 ± 0.186 [B]	**0.029**	
Total lipids in medium HDL, mmol/L			
Western-type diet group	-0.143 ± 0.202	**<0.001**	
Targeted diet group	-0.236 ± 0.185 [A]	**<0.001**	**0.009**
Control group	-0.083 ± 0.188 [B]	**0.030**	
Phospholipids in medium HDL, mmol/L			
Western-type diet group	-0.134 ± 0.181	**<0.001**	
Targeted diet group	-0.228 ± 0.168 [A]	**<0.001**	**0.003**
Control group	-0.072 ± 0.177 [B]	**0.043**	
Total cholesterol in medium HDL, mmol/L			
Western-type diet group	-0.148 ± 0.228	**<0.001**	
Targeted diet group	-0.225 ± 0.215	**<0.001**	0.049
Control group	-0.087 ± 0.204	**0.035**	
Cholesterol esters in medium HDL, mmol/L			
Western-type diet group	-0.153 ± 0.222	**<0.001**	
Targeted diet group	-0.221 ± 0.214	**<0.001**	0.055
Control group	-0.087 ± 0.201	**0.033**	

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Free cholesterol in medium HDL, mmol/L			
Western-type diet group	-0.128 ± 0.270	**0.005**	
Targeted diet group	-0.242 ± 0.224	**<0.001**	0.036
Control group	-0.088 ± 0.222	**0.048**	
Triglycerides in medium HDL, mmol/L			
Western-type diet group	-0.154 ± 0.312 [A]	**0.004**	
Targeted diet group	-0.378 ± 0.316 [B]	**<0.001**	**0.001**
Control group	-0.097 ± 0.292 [A]	0.095	
Concentration of small HDL particles, mol/L			
Western-type diet group	-0.121 ± 0.127 [A]	**<0.001**	
Targeted diet group	-0.196 ± 0.140 [B]	**<0.001**	**0.002**
Control group	-0.087 ± 0.093 [A]	**<0.001**	
Total lipids in small HDL, mmol/L			
Western-type diet group	-0.121 ± 0.126 [A]	**<0.001**	
Targeted diet group	-0.196 ± 0.140 [B]	**<0.001**	**0.003**
Control group	-0.089 ± 0.092 [A]	**<0.001**	
Phospholipids in small HDL, mmol/L			
Western-type diet group	-0.103 ± 0.156	**<0.001**	
Targeted diet group	-0.181 ± 0.132 [A]	**<0.001**	**0.001**
Control group	-0.041 ± 0.108 [B]	**0.061**	
Total cholesterol in small HDL, mmol/L			
Western-type diet group	-0.139 ± 0.173	**<0.001**	
Targeted diet group	-0.212 ± 0.203	**<0.001**	0.207
Control group	-0.154 ± 0.152	**<0.001**	
Cholesterol esters in small HDL, mmol/L			
Western-type diet group	-0.155 ± 0.218	**<0.001**	
Targeted diet group	-0.225 ± 0.248	**<0.001**	0.428
Control group	-0.192 ± 0.213	**<0.001**	
Free cholesterol in small HDL, mmol/L			
Western-type diet group	-0.085 ± 0.129 [A]	**<0.001**	
Targeted diet group	-0.171 ± 0.118 [B]	**<0.001**	**<0.001**
Control group	-0.053 ± 0.086 [A]	**0.004**	
Triglycerides in small HDL, mmol/L			
Western-type diet group	-0.086 ± 0.235	**0.027**	
Targeted diet group	-0.199 ± 0.282	**<0.001**	0.059
Control group	-0.061 ± 0.203	0.128	
Mean diameter for VLDL particles, nm			
Western-type diet group	-0.015 ± 0.036	**0.015**	
Targeted diet group	-0.031 ± 0.040	**<0.001**	0.028
Control group	-0.007 ± 0.030	0.265	
Mean diameter for LDL particles, nm			
Western-type diet group	0.004 ± 0.004	**<0.001**	
Targeted diet group	0.005 ± 0.005	**<0.001**	0.105
Control group	0.003 ± 0.003	**<0.001**	
Mean diameter for HDL particles, nm			
Western-type diet group	0.008 ± 0.021	**0.025**	
Targeted diet group	0.015 ± 0.021	**<0.001**	0.151
Control group	0.006 ± 0.017	0.084	
Plasma total cholesterol, mmol/L			
Western-type diet group	-0.028 ± 0.183 [A]	0.354	
Targeted diet group	-0.115 ± 0.108 [B]	**<0.001**	**0.018**
Control group	-0.023 ± 0.126 [A]	0.354	
Total cholesterol in VLDL, mmol/L			
Western-type diet group	-0.027 ± 0.241	0.482	
Targeted diet group	-0.164 ± 0.298 [A]	**0.003**	**0.016**
Control group	0.014 ± 0.202 [B]	0.729	
Remnant cholesterol (non-HDL, non-LDL -cholesterol), mmol/L			
Western-type diet group	-0.011 ± 0.200	0.732	
Targeted diet group	-0.119 ± 0.205 [A]	**0.002**	**0.014**
Control group	0.015 ± 0.156 [B]	0.620	

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Total cholesterol in LDL, mmol/L			
Western-type diet group	-0.018 ± 0.272	0.683	
Targeted diet group	-0.123 ± 0.190	**0.001**	0.146
Control group	-0.039 ± 0.232	0.395	
Total cholesterol in HDL, mmol/L			
Western-type diet group	-0.054 ± 0.163	0.044	
Targeted diet group	-0.070 ± 0.170	**0.022**	0.785
Control group	-0.040 ± 0.172	0.233	
Total cholesterol in HDL2, mmol/L			
Western-type diet group	-0.075 ± 0.244	0.064	
Targeted diet group	-0.078 ± 0.249	0.076	0.924
Control group	-0.054 ± 0.277	0.323	
Total cholesterol in HDL3, mmol/L			
Western-type diet group	-0.016 ± 0.085	0.251	
Targeted diet group	-0.049 ± 0.087	**0.003**	0.230
Control group	-0.025 ± 0.071	0.082	
Esterified cholesterol, mmol/L			
Western-type diet group	-0.038 ± 0.194 [A]	0.226	
Targeted diet group	-0.133 ± 0.116 [B]	**<0.001**	**0.013**
Control group	-0.028 ± 0.140 [A]	0.315	
Free cholesterol, mmol/L			
Western-type diet group	-0.005 ± 0.162	0.861	
Targeted diet group	-0.076 ± 0.094	**<0.001**	0.045
Control group	-0.014 ± 0.105	0.508	
Plasma total triglycerides, mmol/L			
Western-type diet group	-0.125 ± 0.358	**0.036**	
Targeted diet group	-0.310 ± 0.418	**<0.001**	0.026
Control group	-0.075 ± 0.283	0.181	
Triglycerides in VLDL, mmol/L			
Western-type diet group	-0.136 ± 0.464	0.076	
Targeted diet group	-0.401 ± 0.539 [A]	**<0.001**	**0.015**
Control group	-0.078 ± 0.373 [B]	0.289	
Triglycerides in LDL, mmol/L			
Western-type diet group	-0.057 ± 0.259	0.176	
Targeted diet group	-0.105 ± 0.202	**0.005**	0.610
Control group	-0.061 ± 0.186	0.102	
Triglycerides in HDL, mmol/L			
Western-type diet group	-0.054 ± 0.229	0.145	
Targeted diet group	-0.162 ± 0.243	**<0.001**	0.103
Control group	-0.059 ± 0.226	0.189	

Supplemental Table 3. Changes in fasting apolipoproteins upon 12 weeks of intervention

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
ApoA1, g/L			
Western-type diet group	-0.050 ± 0.091	**0.001**	
Targeted diet group	-0.084 ± 0.066	**<0.001**	0.031
Control group	-0.027 ± 0.093	0.143	
ApoB, g/L			
Western-type diet group	-0.041 ± 0.165 [A]	0.129	
Targeted diet group	-0.134 ± 0.161 [B]	**<0.001**	**0.015**
Control group	-0.030 ± 0.131 [A]	0.252	

Supplemental Table 4. Changes in fasting fatty acids upon 12 weeks of intervention

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Total fatty acids, mmol/L			
Western-type diet group	-0.087 ± 0.200	**0.010**	
Targeted diet group	-0.182 ± 0.179 [A]	**<0.001**	**0.006**
Control group	-0.036 ± 0.139 [B]	0.191	
Estimated degree of unsaturation			
Western-type diet group	0.014 ± 0.058	0.145	
Targeted diet group	0.037 ± 0.052 [A]	**<0.001**	**0.017**
Control group	-0.001 ± 0.045 [B]	0.907	
DHA (22:6), mmol/L			
Western-type diet group	-0.036 ± 0.372	0.553	
Targeted diet group	-0.093 ± 0.306	0.085	0.560
Control group	0.000 ± 0.338	0.997	
>> Ratio DHA : Total fatty acids			
Western-type diet group	0.052 ± 0.313	0.310	
Targeted diet group	0.089 ± 0.244	**0.042**	0.751
Control group	0.036 ± 0.292	0.532	
LA (18:2), mmol/L			
Western-type diet group	-0.080 ± 0.197	**0.015**	
Targeted diet group	-0.115 ± 0.156	**<0.001**	0.073
Control group	-0.015 ± 0.134	0.568	
>> Ratio LA : Total fatty acids			
Western-type diet group	0.007 ± 0.109	0.692	
Targeted diet group	0.067 ± 0.108	**0.001**	0.040
Control group	0.021 ± 0.080	0.182	
Omega-3 fatty acids, mmol/L			
Western-type diet group	-0.102 ± 0.327	0.058	
Targeted diet group	-0.096 ± 0.273	**0.048**	0.487
Control group	-0.021 ± 0.252	0.668	
>> Ratio Omega-3 : Total fatty acids			
Western-type diet group	-0.015 ± 0.283	0.746	
Targeted diet group	0.085 ± 0.236	**0.043**	0.235
Control group	0.015 ± 0.223	0.731	
Omega-6 fatty acids, mmol/L			
Western-type diet group	-0.068 ± 0.175	**0.020**	
Targeted diet group	-0.124 ± 0.134	**<0.001**	0.028
Control group	-0.020 ± 0.124	0.412	

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
>> Ratio Omega-6 : Total fatty acids			
Western-type diet group	0.019 ± 0.087	0.180	
Targeted diet group	0.058 ± 0.095	**0.001**	0.093
Control group	0.016 ± 0.071	0.253	
PUFA, mmol/L			
Western-type diet group	-0.073 ± 0.177	**0.014**	
Targeted diet group	-0.123 ± 0.135	**<0.001**	0.034
Control group	-0.020 ± 0.126	0.409	
>> Ratio PUFA : Total fatty acids			
Western-type diet group	0.015 ± 0.089	0.305	
Targeted diet group	0.059 ± 0.091	**0.001**	0.056
Control group	0.016 ± 0.070	0.259	
MUFA (16:1, 18:1), mmol/L			
Western-type diet group	-0.081 ± 0.270 [A]	0.067	
Targeted diet group	-0.239 ± 0.268 [B]	**<0.001**	**0.011**
Control group	-0.065 ± 0.205 [A]	0.109	
>> Ratio MUFA : Total fatty acids			
Western-type diet group	0.006 ± 0.109	0.736	
Targeted diet group	-0.057 ± 0.113	**0.006**	0.046
Control group	-0.029 ± 0.098	0.129	
SFA, mmol/L			
Western-type diet group	-0.101 ± 0.205	**0.004**	
Targeted diet group	-0.201 ± 0.184 [A]	**<0.001**	**0.001**
Control group	-0.026 ± 0.141 [B]	0.342	
>> Ratio SFA : Total fatty acids			
Western-type diet group	-0.014 ± 0.043	**0.046**	
Targeted diet group	-0.019 ± 0.042 [A]	**0.011**	**0.016**
Control group	0.010 ± 0.034 [B]	0.150	

Supplemental Table 5. Changes in fasting phospholipids and choline derivatives upon 12 weeks of intervention

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Total phosphoglycerides, mmol/L			
Western-type diet group	-0.056 ± 0.175 [A]	0.053	
Targeted diet group	-0.145 ± 0.116 [B]	**<0.001**	**0.002**
Control group	-0.004 ± 0.150 [A]	0.889	
Phosphatidylcholine and other cholines, mmol/L			
Western-type diet group	-0.049 ± 0.173 [A]	0.085	
Targeted diet group	-0.166 ± 0.135 [B]	**<0.001**	**<0.001**
Control group	-0.024 ± 0.131 [A]	0.353	
Sphingomyelins, mmol/L			
Western-type diet group	0.036 ± 0.150	0.146	
Targeted diet group	-0.047 ± 0.134	**0.050**	0.028
Control group	0.027 ± 0.123	0.267	
Total cholines, mmol/L			
Western-type diet group	-0.024 ± 0.141 [A]	0.290	
Targeted diet group	-0.104 ± 0.092 [B]	**<0.001**	**0.003**
Control group	-0.011 ± 0.100 [A]	0.560	

Supplemental Table 6. Changes in fasting metabolites related to energy metabolism upon 12 weeks of intervention

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Glucose, mmol/L			
Western-type diet group	-0.105 ± 0.184 [A]	**0.001**	
Targeted diet group	-0.115 ± 0.130 [A]	**<0.001**	**0.006**
Control group	0.004 ± 0.132 [B]	0.889	
Lactate, mmol/L			
Western-type diet group	-0.156 ± 0.864	0.268	
Targeted diet group	-0.116 ± 0.539	0.217	0.969
Control group	-0.124 ± 0.625	0.312	
Citrate, mmol/L			
Western-type diet group	0.375 ± 0.463	**<0.001**	
Targeted diet group	0.440 ± 0.455	**<0.001**	0.666
Control group	0.336 ± 0.461	**0.001**	
Acetate, mmol/L			
Western-type diet group	0.176 ± 0.418	**0.012**	
Targeted diet group	0.170 ± 0.406	**0.020**	0.996
Control group	0.167 ± 0.399	**0.039**	
Acetoacetate, mmol/L			
Western-type diet group	0.270 ± 0.870	0.060	
Targeted diet group	0.552 ± 1.142	0.008	0.060
Control group	-0.047 ± 0.848	0.774	
3-Hydroxybutyrate, mmol/L			
Western-type diet group	-0.045 ± 0.781	0.722	
Targeted diet group	0.128 ± 0.992	0.458	0.606
Control group	0.119 ± 0.607	0.319	

Supplemental Table 7. Changes in fasting amino acids upon 12 weeks of intervention

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Alanine, mmol/L			
Western-type diet group	-0.099 ± 0.441	0.168	
Targeted diet group	-0.156 ± 0.273	**0.002**	0.786
Control group	-0.135 ± 0.303	0.029	
Glutamine, mmol/L			
Western-type diet group	0.144 ± 0.288	**0.004**	
Targeted diet group	0.078 ± 0.209	**0.036**	0.048
Control group	-0.001 ± 0.144	0.983	
Histidine, mmol/L			
Western-type diet group	-0.148 ± 0.362	**0.015**	
Targeted diet group	-0.107 ± 0.284	**0.035**	0.625
Control group	-0.186 ± 0.278	**0.002**	
Phenylalanine, mmol/L			
Western-type diet group	-0.177 ± 0.212 [A]	**<0.001**	
Targeted diet group	-0.053 ± 0.141 [B]	**0.034**	**<0.001**
Control group	-0.020 ± 0.183 [B]	0.581	
Tyrosine, mmol/L			
Western-type diet group	-0.173 ± 0.284 [A]	**<0.001**	
Targeted diet group	-0.112 ± 0.178 [A]	**0.001**	**<0.001**
Control group	0.045 ± 0.194 [B]	0.243	

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Leucine (BCAA), mmol/L			
Western-type diet group	-0.164 ± 0.279 [A]	**0.001**	
Targeted diet group	-0.123 ± 0.164	**<0.001**	**0.010**
Control group	0.012 ± 0.223 [B]	0.779	
Isoleucine (BCAA), mmol/L			
Western-type diet group	-0.138 ± 0.419	**0.047**	
Targeted diet group	-0.209 ± 0.376	**0.003**	0.051
Control group	0.022 ± 0.259	0.655	
Valine (BCAA), mmol/L			
Western-type diet group	-0.135 ± 0.318	**0.012**	
Targeted diet group	-0.124 ± 0.178	**<0.001**	0.208
Control group	-0.027 ± 0.242	0.569	

Supplemental Table 8. Changes in fasting proteins upon 12 weeks of intervention

	CHANGE AFTER 12 WKS	p-value	p-value FDR0.05, significant values are indicated in bold
	Log_2 ratio	Within group	Between groups
Creatinine, mmol/L			
Western-type diet group	0.031 ± 0.241	0.424	
Targeted diet group	-0.039 ± 0.156	0.152	0.212
Control group	0.028 ± 0.104	0.175	
Albumin, signal area			
Western-type diet group	0.004 ± 0.188	0.906	
Targeted diet group	0.002 ± 0.093	0.920	0.370
Control group	-0.040 ± 0.066	0.004	
Glycoprotein acetyls, mainly orosomucoid, mmol/L			
Western-type diet group	-0.019 ± 0.183	0.516	
Targeted diet group	-0.053 ± 0.132	**0.026**	0.341
Control group	0.003 ± 0.105	0.897	

Supplemental Table 9. Changes in postprandial lipoproteins upon 12 weeks of intervention

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Concentration of chylomicrons and extremely large VLDL particles, mmol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	0.981	0.000 ± 0.000	0.000 ± 0.000	0.914	0.000 ± 0.000	0.000 ± 0.000	0.591
T=4h	0.000 ± 0.000	0.000 ± 0.000	0.396	0.000 ± 0.000	0.000 ± 0.000	**0.050**	0.000 ± 0.000	0.000 ± 0.000	0.425
T=6h	0.000 ± 0.000	0.000 ± 0.000	0.131	0.000 ± 0.000	0.000 ± 0.000	0.854	0.000 ± 0.000	0.000 ± 0.000	0.169
Change in overall response			0.521			0.128			0.270
Total lipids in chylomicrons and extremely large VLDL, mmol/L									
T=2h	0.003 ± 0.024	0.003 ± 0.010	0.995	0.002 ± 0.013	0.003 ± 0.008	0.916	-0.001 ± 0.013	0.001 ± 0.011	0.603
T=4h	0.019 ± 0.017	0.016 ± 0.018	0.432	0.021 ± 0.016	0.016 ± 0.014	**0.045**	0.015 ± 0.015	0.018 ± 0.020	0.432
T=6h	0.034 ± 0.023	0.029 ± 0.019	0.085	0.033 ± 0.019	0.033 ± 0.020	0.872	0.035 ± 0.023	0.026 ± 0.019	0.177
Change in overall response			0.502			0.095			0.287
Phospholipids in chylomicrons and extremely large VLDL, mmol/L									
T=2h	0.000 ± 0.003	0.000 ± 0.001	0.935	0.000 ± 0.002	0.000 ± 0.001	0.894	0.000 ± 0.002	0.000 ± 0.001	0.599
T=4h	0.002 ± 0.002	0.002 ± 0.002	0.470	0.003 ± 0.002	0.002 ± 0.002	**0.050**	0.002 ± 0.002	0.002 ± 0.002	0.436
T=6h	0.004 ± 0.003	0.004 ± 0.002	0.066	0.004 ± 0.002	0.004 ± 0.002	0.850	0.004 ± 0.003	0.003 ± 0.002	0.154
Change in overall response			0.401			0.101			0.254
Total cholesterol in chylomicrons and extremely large VLDL, mmol/L									
T=2h	0.001 ± 0.004	0.001 ± 0.002	0.580	0.001 ± 0.002	0.001 ± 0.001	0.966	0.000 ± 0.002	0.000 ± 0.002	0.719
T=4h	0.003 ± 0.002	0.002 ± 0.003	0.164	0.003 ± 0.002	0.002 ± 0.002	**0.030**	0.002 ± 0.002	0.002 ± 0.003	0.424
T=6h	0.005 ± 0.003	0.004 ± 0.003	0.106	0.005 ± 0.003	0.004 ± 0.003	0.799	0.005 ± 0.003	0.003 ± 0.003	0.246
Change in overall response			0.851			0.072			0.321
Cholesterol esters in chylomicrons and extremely large VLDL, mmol/L									
T=2h	0.001 ± 0.002	0.000 ± 0.001	0.295	0.000 ± 0.001	0.000 ± 0.001	0.903	0.000 ± 0.001	0.000 ± 0.001	0.864
T=4h	0.001 ± 0.001	0.001 ± 0.001	0.067	0.001 ± 0.001	0.001 ± 0.001	**0.023**	0.001 ± 0.001	0.001 ± 0.001	0.467
T=6h	0.002 ± 0.001	0.002 ± 0.001	0.239	0.002 ± 0.001	0.002 ± 0.001	0.765	0.002 ± 0.001	0.001 ± 0.001	0.261
Change in overall response			0.780			0.075			0.366
Free cholesterol in chylomicrons and extremely large VLDL, mmol/L									
T=2h	0.000 ± 0.002	0.000 ± 0.001	0.955	0.000 ± 0.001	0.000 ± 0.001	0.840	0.000 ± 0.001	0.000 ± 0.001	0.590
T=4h	0.002 ± 0.001	0.001 ± 0.001	0.346	0.002 ± 0.001	0.001 ± 0.001	0.052	0.001 ± 0.001	0.001 ± 0.002	0.407
T=6h	0.003 ± 0.002	0.002 ± 0.002	0.056	0.003 ± 0.001	0.003 ± 0.002	0.890	0.003 ± 0.002	0.002 ± 0.001	0.198
Change in overall response			0.496			0.090			0.272
Triglycerides in chylomicrons and extremely large VLDL, mmol/L									
T=2h	0.002 ± 0.017	0.002 ± 0.007	0.914	0.002 ± 0.010	0.002 ± 0.006	0.911	-0.001 ± 0.009	0.000 ± 0.008	0.585
T=4h	0.014 ± 0.013	0.012 ± 0.013	0.499	0.016 ± 0.012	0.012 ± 0.011	**0.049**	0.011 ± 0.011	0.013 ± 0.015	0.438
T=6h	0.025 ± 0.017	0.022 ± 0.015	0.090	0.025 ± 0.014	0.024 ± 0.015	0.894	0.026 ± 0.017	0.020 ± 0.014	0.169
Change in overall response			0.444			0.102			0.283
Concentration of very large VLDL particles, mol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	0.932	0.000 ± 0.000	0.000 ± 0.000	0.784	0.000 ± 0.000	0.000 ± 0.000	0.465
T=4h	0.000 ± 0.000	0.000 ± 0.000	0.245	0.000 ± 0.000	0.000 ± 0.000	**0.014**	0.000 ± 0.000	0.000 ± 0.000	0.296
T=6h	0.000 ± 0.000	0.000 ± 0.000	0.076	0.000 ± 0.000	0.000 ± 0.000	0.837	0.000 ± 0.000	0.000 ± 0.000	0.320
Change in overall response			0.596			**0.045**			0.327

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Total lipids in very large VLDL, mmol/L									
T=2h	0.006 ± 0.066	0.004 ± 0.024	0.918	0.001 ± 0.030	0.002 ± 0.018	0.813	-0.005 ± 0.032	0.000 ± 0.024	0.477
T=4h	0.044 ± 0.039	0.036 ± 0.041	0.254	0.048 ± 0.035	0.034 ± 0.033	**0.021**	0.031 ± 0.038	0.040 ± 0.047	0.302
T=6h	0.077 ± 0.054	0.064 ± 0.045	0.072	0.078 ± 0.043	0.075 ± 0.046	0.864	0.078 ± 0.055	0.060 ± 0.043	0.314
Change in overall response			0.636			0.051			0.323
Phospholipids in very large VLDL, mmol/L									
T=2h	0.001 ± 0.010	0.000 ± 0.004	0.890	0.000 ± 0.005	0.000 ± 0.003	0.950	-0.001 ± 0.005	0.000 ± 0.004	0.515
T=4h	0.007 ± 0.007	0.006 ± 0.007	0.273	0.008 ± 0.006	0.006 ± 0.006	**0.021**	0.005 ± 0.006	0.007 ± 0.008	0.300
T=6h	0.013 ± 0.009	0.011 ± 0.008	**0.050**	0.013 ± 0.007	0.013 ± 0.008	0.806	0.013 ± 0.009	0.010 ± 0.007	0.270
Change in overall response			0.548			0.065			0.283
Total cholesterol in very large VLDL, mmol/L									
T=2h	0.002 ± 0.012	0.001 ± 0.005	0.734	0.001 ± 0.006	0.001 ± 0.004	0.906	-0.001 ± 0.007	0.000 ± 0.005	0.583
T=4h	0.009 ± 0.007	0.007 ± 0.008	0.168	0.009 ± 0.007	0.007 ± 0.006	**0.046**	0.006 ± 0.007	0.008 ± 0.009	0.330
T=6h	0.014 ± 0.010	0.012 ± 0.008	0.085	0.014 ± 0.008	0.014 ± 0.009	0.893	0.014 ± 0.010	0.011 ± 0.008	0.262
Change in overall response			0.746			0.085			0.272
Cholesterol esters in very large VLDL, mmol/L									
T=2h	0.001 ± 0.007	0.001 ± 0.003	0.686	0.001 ± 0.003	0.001 ± 0.002	0.834	0.000 ± 0.004	0.000 ± 0.003	0.568
T=4h	0.004 ± 0.003	0.003 ± 0.004	0.119	0.005 ± 0.003	0.003 ± 0.003	0.058	0.003 ± 0.004	0.004 ± 0.004	0.320
T=6h	0.007 ± 0.005	0.006 ± 0.004	0.118	0.007 ± 0.004	0.007 ± 0.005	0.922	0.007 ± 0.005	0.005 ± 0.004	0.288
Change in overall response			0.793			0.082			0.278
Free cholesterol in very large VLDL, mmol/L									
T=2h	0.001 ± 0.006	0.000 ± 0.002	0.790	0.000 ± 0.003	0.000 ± 0.002	0.989	0.000 ± 0.003	0.000 ± 0.002	0.605
T=4h	0.004 ± 0.004	0.003 ± 0.004	0.229	0.005 ± 0.003	0.003 ± 0.003	**0.037**	0.003 ± 0.003	0.004 ± 0.004	0.343
T=6h	0.007 ± 0.005	0.006 ± 0.004	0.060	0.007 ± 0.004	0.007 ± 0.004	0.865	0.007 ± 0.005	0.006 ± 0.004	0.234
Change in overall response			0.632			0.091			0.263
Triglycerides in very large VLDL, mmol/L									
T=2h	0.003 ± 0.043	0.003 ± 0.015	0.979	0.000 ± 0.019	0.001 ± 0.011	0.749	-0.004 ± 0.021	0.000 ± 0.015	0.438
T=4h	0.028 ± 0.025	0.023 ± 0.026	0.289	0.031 ± 0.023	0.022 ± 0.021	**0.017**	0.020 ± 0.025	0.026 ± 0.031	0.295
T=6h	0.049 ± 0.035	0.041 ± 0.030	0.078	0.050 ± 0.028	0.049 ± 0.029	0.875	0.050 ± 0.036	0.039 ± 0.028	0.345
Change in overall response			0.622			**0.043**			0.350
Concentration of large VLDL particles, mol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	0.768	0.000 ± 0.000	0.000 ± 0.000	0.601	0.000 ± 0.000	0.000 ± 0.000	0.279
T=4h	0.000 ± 0.000	0.000 ± 0.000	0.128	0.000 ± 0.000	0.000 ± 0.000	**0.008**	0.000 ± 0.000	0.000 ± 0.000	0.182
T=6h	0.000 ± 0.000	0.000 ± 0.000	0.093	0.000 ± 0.000	0.000 ± 0.000	0.971	0.000 ± 0.000	0.000 ± 0.000	0.573
Change in overall response			0.832			**0.025**			0.371
Total lipids in large VLDL, mmol/L									
T=2h	0.013 ± 0.218	0.002 ± 0.072	0.757	-0.018 ± 0.083	-0.012 ± 0.053	0.622	-0.031 ± 0.090	-0.010 ± 0.060	0.286
T=4h	0.109 ± 0.112	0.079 ± 0.109	0.122	0.108 ± 0.092	0.069 ± 0.094	**0.008**	0.061 ± 0.107	0.092 ± 0.128	0.182
T=6h	0.179 ± 0.148	0.148 ± 0.119	0.091	0.191 ± 0.111	0.190 ± 0.126	0.966	0.181 ± 0.147	0.149 ± 0.118	0.565
Change in overall response			0.833			**0.024**			0.367

Supplemental Table 9. *Continued (VLDL)*

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Phospholipids in large VLDL, mmol/L									
T=2h	0.002 ± 0.039	0.000 ± 0.013	0.741	-0.004 ± 0.015	-0.003 ± 0.009	0.746	-0.006 ± 0.016	-0.002 ± 0.011	0.305
T=4h	0.019 ± 0.020	0.014 ± 0.020	0.121	0.019 ± 0.017	0.012 ± 0.017	**0.006**	0.011 ± 0.019	0.016 ± 0.023	0.169
T=6h	0.032 ± 0.027	0.026 ± 0.021	0.079	0.034 ± 0.020	0.034 ± 0.022	0.913	0.033 ± 0.026	0.027 ± 0.021	0.550
Change in overall response			0.827			**0.025**			0.339
Total cholesterol in large VLDL, mmol/L									
T=2h	0.004 ± 0.047	0.000 ± 0.016	0.602	-0.003 ± 0.018	-0.002 ± 0.012	0.748	-0.006 ± 0.020	-0.002 ± 0.013	0.373
T=4h	0.024 ± 0.023	0.016 ± 0.024	0.061	0.024 ± 0.020	0.015 ± 0.020	**0.008**	0.013 ± 0.023	0.020 ± 0.027	0.170
T=6h	0.038 ± 0.031	0.031 ± 0.025	0.076	0.041 ± 0.024	0.040 ± 0.027	0.859	0.038 ± 0.031	0.031 ± 0.026	0.508
Change in overall response			0.858			**0.025**			0.299
Cholesterol esters in large VLDL, mmol/L									
T=2h	0.002 ± 0.022	-0.001 ± 0.008	0.441	-0.001 ± 0.009	-0.001 ± 0.005	0.713	-0.003 ± 0.010	-0.001 ± 0.006	0.408
T=4h	0.010 ± 0.011	0.006 ± 0.011	**0.022**	0.010 ± 0.009	0.006 ± 0.009	**0.011**	0.005 ± 0.010	0.008 ± 0.012	0.148
T=6h	0.015 ± 0.013	0.012 ± 0.010	0.098	0.017 ± 0.011	0.017 ± 0.013	0.909	0.015 ± 0.014	0.013 ± 0.012	0.595
Change in overall response			0.775			**0.021**			0.277
Free cholesterol in large VLDL, mmol/L									
T=2h	0.001 ± 0.025	0.000 ± 0.009	0.767	-0.002 ± 0.010	-0.001 ± 0.006	0.785	-0.003 ± 0.011	-0.001 ± 0.007	0.341
T=4h	0.014 ± 0.013	0.010 ± 0.013	0.138	0.014 ± 0.011	0.009 ± 0.011	**0.007**	0.008 ± 0.013	0.012 ± 0.015	0.198
T=6h	0.023 ± 0.018	0.019 ± 0.015	0.067	0.024 ± 0.013	0.023 ± 0.015	0.823	0.023 ± 0.018	0.019 ± 0.014	0.449
Change in overall response			0.769			**0.032**			0.317
Triglycerides in large VLDL, mmol/L									
T=2h	0.007 ± 0.133	0.002 ± 0.043	0.820	-0.012 ± 0.050	-0.007 ± 0.032	0.546	-0.019 ± 0.054	-0.005 ± 0.036	0.253
T=4h	0.066 ± 0.068	0.049 ± 0.066	0.157	0.065 ± 0.056	0.042 ± 0.057	**0.009**	0.037 ± 0.065	0.056 ± 0.078	0.188
T=6h	0.109 ± 0.090	0.091 ± 0.073	0.101	0.116 ± 0.068	0.116 ± 0.077	0.971	0.110 ± 0.090	0.091 ± 0.072	0.584
Change in overall response			0.819			**0.025**			0.390
Concentration of medium VLDL particles, mol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	0.551	0.000 ± 0.000	0.000 ± 0.000	0.607	0.000 ± 0.000	0.000 ± 0.000	0.213
T=4h	0.000 ± 0.000	0.000 ± 0.000	**0.037**	0.000 ± 0.000	0.000 ± 0.000	**0.015**	0.000 ± 0.000	0.000 ± 0.000	0.071
T=6h	0.000 ± 0.000	0.000 ± 0.000	0.099	0.000 ± 0.000	0.000 ± 0.000	0.993	0.000 ± 0.000	0.000 ± 0.000	0.940
Change in overall response			0.842			**0.014**			0.420
Total lipids in medium VLDL, mmol/L									
T=2h	0.019 ± 0.295	-0.011 ± 0.096	0.529	-0.036 ± 0.105	-0.029 ± 0.072	0.654	-0.049 ± 0.110	-0.021 ± 0.065	0.224
T=4h	0.116 ± 0.152	0.063 ± 0.120	**0.031**	0.100 ± 0.106	0.056 ± 0.118	**0.013**	0.037 ± 0.119	0.085 ± 0.136	0.069
T=6h	0.149 ± 0.171	0.111 ± 0.129	0.092	0.179 ± 0.141	0.177 ± 0.158	0.950	0.137 ± 0.167	0.128 ± 0.135	0.954
Change in overall response			0.839			**0.014**			0.395
Phospholipids in medium VLDL, mmol/L									
T=2h	0.003 ± 0.055	-0.003 ± 0.018	0.490	-0.007 ± 0.019	-0.006 ± 0.013	0.795	-0.009 ± 0.020	-0.004 ± 0.012	0.257
T=4h	0.021 ± 0.029	0.011 ± 0.022	**0.028**	0.019 ± 0.020	0.010 ± 0.021	**0.009**	0.007 ± 0.022	0.016 ± 0.024	0.071
T=6h	0.028 ± 0.032	0.020 ± 0.023	0.059	0.033 ± 0.025	0.032 ± 0.028	0.807	0.026 ± 0.030	0.024 ± 0.025	0.984
Change in overall response			0.888			**0.014**			0.340

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Total cholesterol in medium VLDL, mmol/L									
T=2h	0.003 ± 0.068	-0.010 ± 0.025	0.284	-0.010 ± 0.025	-0.011 ± 0.018	0.786	-0.014 ± 0.027	-0.009 ± 0.016	0.378
T=4h	0.024 ± 0.036	0.008 ± 0.028	**0.004**	0.020 ± 0.025	0.008 ± 0.027	**0.007**	0.005 ± 0.029	0.016 ± 0.030	0.051
T=6h	0.029 ± 0.041	0.018 ± 0.028	0.075	0.038 ± 0.033	0.034 ± 0.036	0.566	0.028 ± 0.038	0.025 ± 0.031	0.848
Change in overall response			0.660			**0.019**			0.154
Cholesterol esters in medium VLDL, mmol/L									
T=2h	0.001 ± 0.034	-0.008 ± 0.014	0.146	-0.005 ± 0.014	-0.007 ± 0.010	0.463	-0.008 ± 0.015	-0.006 ± 0.009	0.531
T=4h	0.009 ± 0.019	0.000 ± 0.014	**0.001**	0.008 ± 0.013	0.002 ± 0.013	**0.008**	0.000 ± 0.016	0.006 ± 0.014	**0.045**
T=6h	0.010 ± 0.021	0.004 ± 0.014	0.091	0.016 ± 0.017	0.012 ± 0.018	0.337	0.010 ± 0.019	0.009 ± 0.015	0.712
Change in overall response			0.344			**0.042**			0.064
Free cholesterol in medium VLDL, mmol/L									
T=2h	0.002 ± 0.035	-0.002 ± 0.012	0.502	-0.005 ± 0.013	-0.004 ± 0.009	0.778	-0.006 ± 0.013	-0.003 ± 0.008	0.245
T=4h	0.014 ± 0.019	0.008 ± 0.015	**0.029**	0.012 ± 0.013	0.007 ± 0.014	**0.010**	0.005 ± 0.015	0.011 ± 0.016	0.087
T=6h	0.019 ± 0.021	0.014 ± 0.016	0.058	0.022 ± 0.017	0.022 ± 0.019	0.852	0.018 ± 0.020	0.016 ± 0.016	0.954
Change in overall response			0.883			**0.014**			0.372
Triglycerides in medium VLDL, mmol/L									
T=2h	0.014 ± 0.173	0.001 ± 0.054	0.666	-0.019 ± 0.062	-0.012 ± 0.043	0.431	-0.026 ± 0.063	-0.008 ± 0.039	0.169
T=4h	0.071 ± 0.090	0.044 ± 0.070	0.072	0.061 ± 0.062	0.038 ± 0.071	**0.023**	0.026 ± 0.070	0.053 ± 0.083	0.084
T=6h	0.092 ± 0.101	0.072 ± 0.078	0.124	0.108 ± 0.085	0.111 ± 0.095	0.844	0.084 ± 0.100	0.079 ± 0.080	0.880
Change in overall response			0.851			**0.014**			0.531
Concentration of small VLDL particles, mol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	0.246	0.000 ± 0.000	0.000 ± 0.000	0.765	0.000 ± 0.000	0.000 ± 0.000	0.439
T=4h	0.000 ± 0.000	0.000 ± 0.000	**0.017**	0.000 ± 0.000	0.000 ± 0.000	**0.027**	0.000 ± 0.000	0.000 ± 0.000	0.072
T=6h	0.000 ± 0.000	0.000 ± 0.000	**0.010**	0.000 ± 0.000	0.000 ± 0.000	0.244	0.000 ± 0.000	0.000 ± 0.000	0.665
Change in overall response			0.890			**0.016**			0.200
Total lipids in small VLDL, mmol/L									
T=2h	0.001 ± 0.146	-0.029 ± 0.052	0.209	-0.033 ± 0.051	-0.038 ± 0.034	0.643	-0.030 ± 0.046	-0.024 ± 0.035	0.543
T=4h	0.032 ± 0.097	-0.003 ± 0.048	**0.012**	0.017 ± 0.051	-0.006 ± 0.054	**0.023**	-0.009 ± 0.049	0.015 ± 0.047	0.078
T=6h	0.031 ± 0.089	-0.004 ± 0.051	**0.009**	0.040 ± 0.068	0.021 ± 0.068	0.190	0.014 ± 0.062	0.022 ± 0.056	0.677
Change in overall response			0.920			**0.019**			0.164
Phospholipids in small VLDL, mmol/L									
T=2h	-0.004 ± 0.030	-0.011 ± 0.011	0.177	-0.012 ± 0.012	-0.013 ± 0.007	0.733	-0.010 ± 0.010	-0.009 ± 0.008	0.630
T=4h	0.003 ± 0.022	-0.004 ± 0.011	**0.026**	0.000 ± 0.012	-0.005 ± 0.011	**0.040**	-0.005 ± 0.011	-0.001 ± 0.011	0.100
T=6h	0.008 ± 0.020	-0.001 ± 0.011	**0.002**	0.008 ± 0.013	0.003 ± 0.012	**0.049**	0.004 ± 0.012	0.006 ± 0.012	0.910
Change in overall response			0.581			**0.040**			0.134
Total cholesterol in small VLDL, mmol/L									
T=2h	0.004 ± 0.037	-0.009 ± 0.019	**0.040**	-0.003 ± 0.015	-0.009 ± 0.012	0.069	-0.003 ± 0.015	-0.005 ± 0.016	0.550
T=4h	0.002 ± 0.030	-0.011 ± 0.015	**0.007**	-0.003 ± 0.014	-0.009 ± 0.015	**0.026**	-0.007 ± 0.018	-0.001 ± 0.014	0.241
T=6h	-0.011 ± 0.031	-0.022 ± 0.018	**0.007**	-0.009 ± 0.021	-0.017 ± 0.017	**0.023**	-0.014 ± 0.019	-0.012 ± 0.015	0.906
Change in overall response			0.914			0.590			0.150

Supplemental Table 9. *Continued (VLDL)*

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Cholesterol esters in small VLDL, mmol/L									
T=2h	0.006 ± 0.020	-0.002 ± 0.015	**0.023**	0.004 ± 0.011	-0.001 ± 0.010	0.068	0.004 ± 0.011	0.001 ± 0.013	0.355
T=4h	0.001 ± 0.019	-0.008 ± 0.012	**0.025**	-0.002 ± 0.010	-0.005 ± 0.012	0.194	-0.003 ± 0.013	-0.001 ± 0.012	0.428
T=6h	-0.014 ± 0.021	-0.020 ± 0.014	**0.015**	-0.012 ± 0.014	-0.017 ± 0.011	**0.032**	-0.015 ± 0.014	-0.014 ± 0.010	0.980
Change in overall response			0.779			0.378			0.285
Free cholesterol in small VLDL, mmol/L									
T=2h	-0.002 ± 0.019	-0.007 ± 0.008	0.134	-0.007 ± 0.007	-0.008 ± 0.004	0.453	-0.006 ± 0.006	-0.006 ± 0.005	0.705
T=4h	0.002 ± 0.014	-0.003 ± 0.007	**0.013**	0.000 ± 0.007	-0.004 ± 0.007	**0.028**	-0.003 ± 0.007	-0.001 ± 0.007	0.145
T=6h	0.003 ± 0.012	-0.003 ± 0.007	**0.005**	0.003 ± 0.009	0.000 ± 0.008	0.074	0.001 ± 0.008	0.002 ± 0.008	0.821
Change in overall response			0.873			0.057			0.191
Triglycerides in small VLDL, mmol/L									
T=2h	0.002 ± 0.082	-0.008 ± 0.029	0.450	-0.018 ± 0.030	-0.016 ± 0.021	0.751	-0.018 ± 0.026	-0.010 ± 0.019	0.153
T=4h	0.027 ± 0.053	0.012 ± 0.031	0.074	0.020 ± 0.031	0.008 ± 0.035	0.051	0.003 ± 0.029	0.017 ± 0.033	0.076
T=6h	0.034 ± 0.048	0.019 ± 0.034	**0.019**	0.040 ± 0.040	0.035 ± 0.045	0.553	0.024 ± 0.043	0.028 ± 0.036	0.693
Change in overall response			0.788			0.015			0.483
Concentration of very small VLDL particles, mol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	**0.030**	0.000 ± 0.000	0.000 ± 0.000	**0.021**	0.000 ± 0.000	0.000 ± 0.000	0.227
T=4h	0.000 ± 0.000	0.000 ± 0.000	**0.046**	0.000 ± 0.000	0.000 ± 0.000	0.098	0.000 ± 0.000	0.000 ± 0.000	0.856
T=6h	0.000 ± 0.000	0.000 ± 0.000	**0.009**	0.000 ± 0.000	0.000 ± 0.000	**0.004**	0.000 ± 0.000	0.000 ± 0.000	0.191
Change in overall response			0.967			0.097			0.507
Total lipids in very small VLDL, mmol/L									
T=2h	-0.012 ± 0.039	-0.030 ± 0.039	**0.031**	-0.009 ± 0.031	-0.023 ± 0.023	**0.031**	-0.005 ± 0.024	-0.014 ± 0.033	0.192
T=4h	-0.024 ± 0.046	-0.039 ± 0.028	0.061	-0.024 ± 0.029	-0.030 ± 0.033	0.243	-0.015 ± 0.031	-0.018 ± 0.035	0.699
T=6h	-0.041 ± 0.050	-0.055 ± 0.039	**0.019**	-0.045 ± 0.038	-0.058 ± 0.037	**0.012**	-0.032 ± 0.041	-0.037 ± 0.031	0.216
Change in overall response			0.896			0.111			0.677
Phospholipids in very small VLDL, mmol/L									
T=2h	-0.007 ± 0.016	-0.014 ± 0.014	**0.017**	-0.006 ± 0.012	-0.011 ± 0.006	**0.012**	-0.005 ± 0.008	-0.007 ± 0.009	0.278
T=4h	-0.013 ± 0.018	-0.017 ± 0.010	0.164	-0.013 ± 0.011	-0.016 ± 0.011	0.115	-0.011 ± 0.012	-0.011 ± 0.013	0.979
T=6h	-0.021 ± 0.020	-0.025 ± 0.013	0.217	-0.021 ± 0.015	-0.027 ± 0.014	**0.015**	-0.019 ± 0.015	-0.017 ± 0.011	0.932
Change in overall response			0.227			0.139			0.684
Total cholesterol in very small VLDL, mmol/L									
T=2h	-0.001 ± 0.026	-0.007 ± 0.028	0.233	0.006 ± 0.024	0.000 ± 0.023	0.272	0.008 ± 0.020	0.000 ± 0.026	0.168
T=4h	-0.012 ± 0.032	-0.019 ± 0.023	0.265	-0.010 ± 0.022	-0.009 ± 0.028	0.837	-0.001 ± 0.020	-0.006 ± 0.025	0.318
T=6h	-0.027 ± 0.030	-0.031 ± 0.028	0.242	-0.030 ± 0.026	-0.033 ± 0.028	0.401	-0.018 ± 0.029	-0.025 ± 0.021	0.167
Change in overall response			0.861			0.201			0.876
Cholesterol esters in very small VLDL, mmol/L									
T=2h	-0.001 ± 0.021	-0.003 ± 0.022	0.549	0.006 ± 0.020	0.001 ± 0.020	0.377	0.007 ± 0.016	0.001 ± 0.021	0.213
T=4h	-0.004 ± 0.025	-0.008 ± 0.018	0.471	-0.002 ± 0.017	-0.002 ± 0.022	0.974	0.004 ± 0.014	0.001 ± 0.017	0.372
T=6h	-0.009 ± 0.019	-0.012 ± 0.019	0.205	-0.013 ± 0.020	-0.014 ± 0.022	0.518	-0.002 ± 0.019	-0.010 ± 0.016	0.086
Change in overall response			0.937			0.355			0.620

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Free cholesterol in very small VLDL, mmol/L									
T=2h	0.000 ± 0.008	-0.004 ± 0.008	**0.016**	0.000 ± 0.007	-0.002 ± 0.005	0.123	0.001 ± 0.006	-0.001 ± 0.007	0.195
T=4h	-0.007 ± 0.010	-0.011 ± 0.008	0.076	-0.009 ± 0.008	-0.008 ± 0.009	0.481	-0.005 ± 0.008	-0.007 ± 0.010	0.362
T=6h	-0.018 ± 0.013	-0.019 ± 0.011	0.470	-0.018 ± 0.011	-0.019 ± 0.010	0.270	-0.016 ± 0.012	-0.015 ± 0.008	0.722
Change in overall response			0.112			0.125			0.889
Triglycerides in very small VLDL, mmol/L									
T=2h	-0.005 ± 0.018	-0.009 ± 0.010	0.201	-0.009 ± 0.009	-0.011 ± 0.007	0.458	-0.008 ± 0.007	-0.007 ± 0.009	0.592
T=4h	0.001 ± 0.016	-0.003 ± 0.009	0.109	-0.001 ± 0.009	-0.005 ± 0.010	0.082	-0.003 ± 0.008	-0.001 ± 0.010	0.315
T=6h	0.007 ± 0.014	0.001 ± 0.009	**0.002**	0.006 ± 0.011	0.002 ± 0.011	0.077	0.004 ± 0.010	0.005 ± 0.010	0.944
Change in overall response			0.239			0.133			0.472

Supplemental Table 9. *Continued (IDL, LDL, HDL)*

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Concentration of IDL particles, mol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	**0.047**	0.000 ± 0.000	000 ± 0.000	**0.003**	0.000 ± 0.000	0.000 ± 0.000	0.276
T=4h	0.000 ± 0.000	0.000 ± 0.000	0.239	0.000 ± 0.000	0.000 ± 0.000	**0.020**	0.000 ± 0.000	0.000 ± 0.000	0.932
T=6h	0.000 ± 0.000	0.000 ± 0.000	0.370	0.000 ± 0.000	0.000 ± 0.000	**0.007**	0.000 ± 0.000	0.000 ± 0.000	0.666
Change in overall response			0.189			0.203			0.620
Total lipids in IDL, mmol/L									
T=2h	-0.026 ± 0.103	-0.068 ± 0.096	**0.048**	-0.005 ± 0.071	-0.045 ± 0.043	**0.004**	-0.013 ± 0.052	-0.029 ± 0.055	0.268
T=4h	-0.062 ± 0.113	-0.084 ± 0.055	0.247	-0.047 ± 0.060	-0.068 ± 0.064	**0.031**	-0.044 ± 0.075	-0.044 ± 0.065	0.957
T=6h	-0.113 ± 0.121	-0.127 ± 0.071	0.452	-0.105 ± 0.073	-0.135 ± 0.077	**0.010**	-0.096 ± 0.086	-0.092 ± 0.060	0.700
Change in overall response			0.141			0.200			0.640
Phospholipids in IDL, mmol/L									
T=2h	-0.006 ± 0.027	-0.017 ± 0.024	**0.049**	0.001 ± 0.019	-0.011 ± 0.010	**0.002**	-0.001 ± 0.014	-0.006 ± 0.014	0.225
T=4h	-0.017 ± 0.029	-0.022 ± 0.016	0.395	-0.013 ± 0.016	-0.019 ± 0.016	**0.022**	-0.013 ± 0.021	-0.012 ± 0.020	0.862
T=6h	-0.031 ± 0.032	-0.035 ± 0.020	0.451	-0.029 ± 0.020	-0.040 ± 0.022	**0.004**	-0.030 ± 0.025	-0.026 ± 0.018	0.846
Change in overall response			0.226			0.125			0.484
Total cholesterol in IDL, mmol/L									
T=2h	-0.011 ± 0.070	-0.040 ± 0.064	**0.041**	0.004 ± 0.049	-0.022 ± 0.036	**0.010**	-0.005 ± 0.037	-0.015 ± 0.042	0.314
T=4h	-0.038 ± 0.077	-0.056 ± 0.040	0.204	-0.027 ± 0.042	-0.040 ± 0.048	0.097	-0.026 ± 0.052	-0.027 ± 0.045	0.995
T=6h	-0.084 ± 0.085	-0.090 ± 0.049	0.664	-0.076 ± 0.052	-0.091 ± 0.054	0.057	-0.068 ± 0.058	-0.068 ± 0.042	0.604
Change in overall response			**0.048**			0.297			0.710
Cholesterol esters in IDL, mmol/L									
T=2h	-0.008 ± 0.050	-0.029 ± 0.046	0.035	0.002 ± 0.035	-0.015 ± 0.028	**0.023**	-0.006 ± 0.026	-0.012 ± 0.032	0.401
T=4h	-0.023 ± 0.053	-0.037 ± 0.027	0.151	-0.016 ± 0.029	-0.025 ± 0.035	0.115	-0.016 ± 0.036	-0.016 ± 0.029	0.926
T=6h	-0.052 ± 0.059	-0.057 ± 0.033	0.581	-0.046 ± 0.037	-0.055 ± 0.038	0.143	-0.040 ± 0.038	-0.042 ± 0.028	0.416
Change in overall response			0.068			0.452			0.602
Free cholesterol in IDL, mmol/L									
T=2h	-0.003 ± 0.025	-0.012 ± 0.020	0.090	0.003 ± 0.016	-0.006 ± 0.009	**0.004**	0.001 ± 0.013	-0.003 ± 0.012	0.221
T=4h	-0.015 ± 0.025	-0.018 ± 0.015	0.419	-0.012 ± 0.014	-0.015 ± 0.015	0.129	-0.010 ± 0.017	-0.011 ± 0.017	0.838
T=6h	-0.031 ± 0.028	-0.033 ± 0.018	0.794	-0.029 ± 0.017	-0.036 ± 0.019	**0.023**	-0.028 ± 0.022	-0.026 ± 0.016	0.965
Change in overall response			0.156			0.123			0.710
Triglycerides in IDL, mmol/L									
T=2h	-0.009 ± 0.012	-0.011 ± 0.012	0.293	-0.009 ± 0.010	-0.012 ± 0.008	0.175	-0.007 ± 0.008	-0.008 ± 0.011	0.628
T=4h	-0.006 ± 0.014	-0.007 ± 0.008	0.712	-0.006 ± 0.008	-0.009 ± 0.010	0.197	-0.005 ± 0.008	-0.005 ± 0.011	0.956
T=6h	0.002 ± 0.014	-0.002 ± 0.010	**0.018**	0.000 ± 0.010	-0.005 ± 0.012	0.060	0.002 ± 0.009	0.002 ± 0.011	0.709
Change in overall response			**0.048**			0.238			0.817
Concentration of large LDL particles, mol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	**0.031**	0.000 ± 0.000	0.000 ± 0.000	**0.008**	0.000 ± 0.000	0.000 ± 0.000	0.491
T=4h	0.000 ± 0.000	0.000 ± 0.000	0.237	0.000 ± 0.000	0.000 ± 0.000	**0.027**	0.000 ± 0.000	0.000 ± 0.000	0.638
T=6h	0.000 ± 0.000	0.000 ± 0.000	0.293	0.000 ± 0.000	0.000 ± 0.000	**0.008**	0.000 ± 0.000	0.000 ± 0.000	0.785
Change in overall response			0.181			0.230			0.555

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Total lipids in large LDL, mmol/L									
T=2h	-0.032 ± 0.120	-0.087 ± 0.119	**0.029**	-0.017 ± 0.089	-0.064 ± 0.041	**0.007**	-0.026 ± 0.064	-0.038 ± 0.060	0.491
T=4h	-0.072 ± 0.133	-0.099 ± 0.069	0.230	-0.057 ± 0.072	-0.085 ± 0.070	**0.029**	-0.063 ± 0.095	-0.054 ± 0.087	0.657
T=6h	-0.142 ± 0.139	-0.164 ± 0.085	0.331	-0.134 ± 0.090	-0.176 ± 0.087	**0.008**	-0.137 ± 0.099	-0.116 ± 0.076	0.770
Change in overall response			0.141			0.235			0.570
Phospholipids in large LDL, mmol/L									
T=2h	-0.008 ± 0.025	-0.020 ± 0.025	0.016	-0.006 ± 0.018	-0.015 ± 0.009	**0.010**	-0.008 ± 0.012	-0.010 ± 0.011	0.543
T=4h	-0.016 ± 0.028	-0.022 ± 0.015	0.183	-0.013 ± 0.015	-0.019 ± 0.015	**0.030**	-0.015 ± 0.021	-0.012 ± 0.018	0.506
T=6h	-0.030 ± 0.029	-0.035 ± 0.018	0.280	-0.027 ± 0.019	-0.037 ± 0.018	**0.007**	-0.029 ± 0.020	-0.024 ± 0.016	0.650
Change in overall response			0.097			0.232			0.472
Total cholesterol in large LDL, mmol/L									
T=2h	-0.016 ± 0.087	-0.057 ± 0.084	**0.024**	-0.004 ± 0.064	-0.038 ± 0.031	**0.006**	-0.013 ± 0.047	-0.021 ± 0.047	0.514
T=4h	-0.050 ± 0.097	-0.072 ± 0.052	0.205	-0.039 ± 0.053	-0.058 ± 0.050	**0.030**	-0.044 ± 0.071	-0.037 ± 0.066	0.672
T=6h	-0.116 ± 0.105	-0.129 ± 0.064	0.441	-0.107 ± 0.067	-0.136 ± 0.064	**0.011**	-0.110 ± 0.075	-0.095 ± 0.059	0.732
Change in overall response			0.067			0.273			0.590
Cholesterol esters in large LDL, mmol/L									
T=2h	-0.010 ± 0.064	-0.042 ± 0.064	**0.018**	-0.003 ± 0.047	-0.029 ± 0.023	**0.005**	-0.010 ± 0.035	-0.015 ± 0.035	0.573
T=4h	-0.034 ± 0.072	-0.051 ± 0.038	0.168	-0.026 ± 0.039	-0.042 ± 0.036	**0.016**	-0.032 ± 0.054	-0.025 ± 0.048	0.586
T=6h	-0.083 ± 0.078	-0.094 ± 0.047	0.363	-0.076 ± 0.049	-0.098 ± 0.045	**0.007**	-0.080 ± 0.054	-0.068 ± 0.043	0.688
Change in overall response			0.068			0.337			0.545
Free cholesterol in large LDL, mmol/L									
T=2h	-0.006 ± 0.025	-0.015 ± 0.021	0.067	-0.001 ± 0.017	-0.010 ± 0.008	**0.010**	-0.003 ± 0.013	-0.006 ± 0.012	0.384
T=4h	-0.016 ± 0.025	-0.020 ± 0.015	0.368	-0.013 ± 0.015	-0.016 ± 0.015	0.173	-0.012 ± 0.018	-0.012 ± 0.019	0.952
T=6h	-0.033 ± 0.028	-0.035 ± 0.018	0.746	-0.031 ± 0.018	-0.038 ± 0.019	**0.028**	-0.030 ± 0.022	-0.027 ± 0.017	0.835
Change in overall response			0.116			0.153			0.703
Triglycerides in large LDL, mmol/L									
T=2h	-0.008 ± 0.012	-0.010 ± 0.011	0.516	-0.007 ± 0.009	-0.010 ± 0.008	0.127	-0.006 ± 0.008	-0.007 ± 0.009	0.430
T=4h	-0.006 ± 0.012	-0.006 ± 0.007	0.797	-0.006 ± 0.007	-0.008 ± 0.010	0.239	-0.004 ± 0.007	-0.005 ± 0.010	0.860
T=6h	0.003 ± 0.014	0.000 ± 0.009	0.075	0.001 ± 0.010	-0.003 ± 0.012	0.097	0.003 ± 0.007	0.002 ± 0.009	0.660
Change in overall response			**0.021**			0.247			0.799
Concentration of medium LDL particles, mol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	**0.020**	0.000 ± 0.000	0.000 ± 0.000	**0.023**	0.000 ± 0.000	0.000 ± 0.000	0.719
T=4h	0.000 ± 0.000	0.000 ± 0.000	0.255	0.000 ± 0.000	0.000 ± 0.000	0.061	0.000 ± 0.000	0.000 ± 0.000	0.518
T=6h	0.000 ± 0.000	0.000 ± 0.000	0.326	0.000 ± 0.000	0.000 ± 0.000	**0.023**	0.000 ± 0.000	0.000 ± 0.000	0.328
Change in overall response			0.124			0.388			0.423
Total lipids in medium LDL, mmol/L									
T=2h	-0.016 ± 0.070	-0.051 ± 0.073	**0.018**	-0.013 ± 0.057	-0.039 ± 0.024	**0.024**	-0.017 ± 0.042	-0.021 ± 0.038	0.733
T=4h	-0.044 ± 0.081	-0.061 ± 0.046	0.237	-0.039 ± 0.048	-0.056 ± 0.041	0.069	-0.045 ± 0.061	-0.036 ± 0.058	0.531
T=6h	-0.098 ± 0.082	-0.111 ± 0.053	0.337	-0.092 ± 0.058	-0.116 ± 0.051	**0.024**	-0.100 ± 0.061	-0.079 ± 0.049	0.346
Change in overall response			0.108			0.368			0.457

Supplemental Table 9. *Continued (IDL, LDL, HDL)*

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Phospholipids in medium LDL, mmol/L									
T=2h	-0.009 ± 0.016	-0.016 ± 0.015	**0.031**	-0.010 ± 0.010	-0.014 ± 0.005	0.102	-0.011 ± 0.007	-0.010 ± 0.006	0.715
T=4h	-0.007 ± 0.016	-0.011 ± 0.007	0.074	-0.006 ± 0.008	-0.009 ± 0.007	**0.041**	-0.009 ± 0.011	-0.006 ± 0.009	0.219
T=6h	-0.009 ± 0.015	-0.014 ± 0.009	0.063	-0.008 ± 0.010	-0.013 ± 0.007	**0.007**	-0.009 ± 0.009	-0.008 ± 0.007	0.899
Change in overall response			0.648			0.248			0.347
Total cholesterol in medium LDL, mmol/L									
T=2h	-0.003 ± 0.053	-0.030 ± 0.052	**0.014**	0.001 ± 0.044	-0.020 ± 0.018	**0.018**	-0.003 ± 0.033	-0.007 ± 0.033	0.666
T=4h	-0.033 ± 0.063	-0.047 ± 0.039	0.247	-0.029 ± 0.039	-0.041 ± 0.032	0.094	-0.033 ± 0.048	-0.027 ± 0.050	0.631
T=6h	-0.090 ± 0.068	-0.097 ± 0.044	0.546	-0.084 ± 0.047	-0.102 ± 0.042	**0.032**	-0.090 ± 0.051	-0.073 ± 0.042	0.314
Change in overall response			**0.049**			0.343			0.437
Cholesterol esters in medium LDL, mmol/L									
T=2h	0.001 ± 0.045	-0.022 ± 0.043	**0.012**	0.005 ± 0.037	-0.014 ± 0.016	**0.013**	0.002 ± 0.028	-0.002 ± 0.027	0.608
T=4h	-0.027 ± 0.054	-0.038 ± 0.034	0.272	-0.024 ± 0.034	-0.035 ± 0.027	0.071	-0.027 ± 0.041	-0.022 ± 0.042	0.625
T=6h	-0.077 ± 0.058	-0.082 ± 0.038	0.607	-0.071 ± 0.040	-0.087 ± 0.036	**0.027**	-0.078 ± 0.044	-0.062 ± 0.036	0.252
Change in overall response			**0.043**			0.356			0.330
Free cholesterol in medium LDL, mmol/L									
T=2h	-0.004 ± 0.009	-0.008 ± 0.009	**0.042**	-0.004 ± 0.007	-0.006 ± 0.004	0.152	-0.004 ± 0.006	-0.005 ± 0.006	0.968
T=4h	-0.006 ± 0.010	-0.009 ± 0.006	0.157	-0.005 ± 0.006	-0.006 ± 0.006	0.512	-0.006 ± 0.007	-0.005 ± 0.008	0.665
T=6h	-0.013 ± 0.010	-0.014 ± 0.007	0.319	-0.013 ± 0.007	-0.015 ± 0.007	0.089	-0.012 ± 0.008	-0.011 ± 0.007	0.869
Change in overall response			0.131			0.288			0.881
Triglycerides in medium LDL, mmol/L									
T=2h	-0.004 ± 0.007	-0.005 ± 0.008	0.472	-0.004 ± 0.006	-0.006 ± 0.006	0.146	-0.003 ± 0.005	-0.004 ± 0.005	0.620
T=4h	-0.004 ± 0.007	-0.003 ± 0.004	0.520	-0.003 ± 0.004	-0.005 ± 0.007	0.192	-0.003 ± 0.005	-0.003 ± 0.005	0.720
T=6h	0.001 ± 0.007	0.000 ± 0.005	0.274	0.000 ± 0.006	-0.002 ± 0.008	0.222	0.000 ± 0.005	0.001 ± 0.005	0.312
Change in overall response			0.140			0.848			0.451
Concentration of small LDL particles, mol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	**0.019**	0.000 ± 0.000	0.000 ± 0.000	**0.019**	0.000 ± 0.000	0.000 ± 0.000	0.801
T=4h	0.000 ± 0.000	0.000 ± 0.000	0.182	0.000 ± 0.000	0.000 ± 0.000	**0.032**	0.000 ± 0.000	0.000 ± 0.000	0.474
T=6h	0.000 ± 0.000	0.000 ± 0.000	0.181	0.000 ± 0.000	0.000 ± 0.000	**0.013**	0.000 ± 0.000	0.000 ± 0.000	0.354
Change in overall response			0.233			0.457			0.472
Total lipids in small LDL, mmol/L									
T=2h	-0.008 ± 0.040	-0.029 ± 0.046	**0.018**	-0.007 ± 0.033	-0.022 ± 0.014	**0.021**	-0.010 ± 0.026	-0.012 ± 0.022	0.803
T=4h	-0.022 ± 0.046	-0.034 ± 0.026	0.171	-0.020 ± 0.027	-0.030 ± 0.023	**0.046**	-0.025 ± 0.036	-0.019 ± 0.032	0.508
T=6h	-0.053 ± 0.046	-0.062 ± 0.030	0.208	-0.050 ± 0.032	-0.064 ± 0.028	**0.015**	-0.056 ± 0.034	-0.044 ± 0.027	0.360
Change in overall response			0.187			0.410			0.512
Phospholipids in small LDL, mmol/L									
T=2h	-0.004 ± 0.010	-0.009 ± 0.010	**0.041**	-0.006 ± 0.006	-0.007 ± 0.003	0.127	-0.006 ± 0.005	-0.006 ± 0.003	0.674
T=4h	-0.002 ± 0.010	-0.006 ± 0.004	**0.041**	-0.002 ± 0.005	-0.005 ± 0.004	**0.018**	-0.005 ± 0.007	-0.002 ± 0.004	0.170
T=6h	-0.003 ± 0.010	-0.007 ± 0.005	**0.029**	-0.003 ± 0.005	-0.006 ± 0.004	**0.005**	-0.004 ± 0.006	-0.004 ± 0.004	0.870
Change in overall response			0.719			0.365			0.067

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Total cholesterol in small LDL, mmol/L									
T=2h	-0.002 ± 0.030	-0.018 ± 0.032	**0.017**	0.001 ± 0.026	-0.012 ± 0.010	**0.017**	-0.002 ± 0.021	-0.004 ± 0.020	0.690
T=4h	-0.020 ± 0.035	-0.028 ± 0.024	0.245	-0.018 ± 0.023	-0.024 ± 0.019	0.121	-0.020 ± 0.029	-0.017 ± 0.030	0.696
T=6h	-0.053 ± 0.038	-0.058 ± 0.026	0.533	-0.050 ± 0.027	-0.060 ± 0.025	**0.034**	-0.055 ± 0.031	-0.044 ± 0.025	0.290
Change in overall response			0.073			0.311			0.470
Cholesterol esters in small LDL, mmol/L									
T=2h	0.000 ± 0.027	-0.013 ± 0.027	**0.018**	0.003 ± 0.023	-0.009 ± 0.009	**0.012**	0.001 ± 0.018	-0.002 ± 0.016	0.606
T=4h	-0.017 ± 0.031	-0.023 ± 0.021	0.327	-0.015 ± 0.020	-0.021 ± 0.016	0.079	-0.017 ± 0.025	-0.014 ± 0.025	0.704
T=6h	-0.046 ± 0.034	-0.049 ± 0.023	0.653	-0.043 ± 0.023	-0.052 ± 0.022	**0.030**	-0.047 ± 0.026	-0.037 ± 0.022	0.241
Change in overall response			0.070			0.332			0.346
Free cholesterol in small LDL, mmol/L									
T=2h	-0.002 ± 0.005	-0.004 ± 0.006	**0.037**	-0.002 ± 0.004	-0.003 ± 0.003	0.362	-0.003 ± 0.004	-0.002 ± 0.004	0.874
T=4h	-0.003 ± 0.005	-0.005 ± 0.003	**0.044**	-0.003 ± 0.003	-0.003 ± 0.003	0.810	-0.003 ± 0.004	-0.003 ± 0.005	0.686
T=6h	-0.007 ± 0.006	-0.009 ± 0.004	0.147	-0.007 ± 0.004	-0.008 ± 0.004	0.228	-0.008 ± 0.005	-0.007 ± 0.004	0.894
Change in overall response			0.230			0.285			0.941
Triglycerides in small LDL, mmol/L									
T=2h	-0.002 ± 0.004	-0.003 ± 0.005	0.238	-0.002 ± 0.003	-0.003 ± 0.003	0.123	-0.002 ± 0.003	-0.002 ± 0.003	0.971
T=4h	0.000 ± 0.005	0.000 ± 0.003	0.611	0.000 ± 0.003	-0.002 ± 0.004	**0.016**	-0.001 ± 0.003	0.000 ± 0.003	0.283
T=6h	0.004 ± 0.005	0.002 ± 0.004	**0.013**	0.003 ± 0.003	0.002 ± 0.004	0.083	0.003 ± 0.003	0.003 ± 0.003	0.772
Change in overall response			**0.020**			0.332			0.375
Concentration of very large HDL particles, mol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	0.519	0.000 ± 0.000	0.000 ± 0.000	0.338	0.000 ± 0.000	0.000 ± 0.000	0.883
T=4h	0.000 ± 0.000	0.000 ± 0.000	0.409	0.000 ± 0.000	0.000 ± 0.000	0.861	0.000 ± 0.000	0.000 ± 0.000	0.769
T=6h	0.000 ± 0.000	0.000 ± 0.000	0.829	0.000 ± 0.000	0.000 ± 0.000	0.506	0.000 ± 0.000	0.000 ± 0.000	0.100
Change in overall response			0.659			0.368			0.146
Total lipids in very large HDL, mmol/L									
T=2h	-0.008 ± 0.157	0.011 ± 0.077	0.500	0.043 ± 0.085	0.027 ± 0.047	0.376	0.006 ± 0.079	0.009 ± 0.074	0.881
T=4h	0.017 ± 0.129	0.037 ± 0.055	0.405	0.047 ± 0.055	0.045 ± 0.053	0.915	0.036 ± 0.035	0.032 ± 0.091	0.762
T=6h	0.077 ± 0.132	0.083 ± 0.058	0.831	0.081 ± 0.082	0.090 ± 0.063	0.483	0.088 ± 0.054	0.054 ± 0.063	0.096
Change in overall response			0.657			0.379			0.150
Phospholipids in very large HDL, mmol/L									
T=2h	-0.017 ± 0.083	-0.015 ± 0.037	0.882	0.003 ± 0.034	-0.009 ± 0.014	**0.041**	-0.010 ± 0.039	-0.010 ± 0.035	0.961
T=4h	-0.009 ± 0.058	-0.001 ± 0.018	0.400	0.003 ± 0.016	-0.001 ± 0.016	0.190	0.001 ± 0.016	0.000 ± 0.038	0.892
T=6h	0.018 ± 0.061	0.027 ± 0.023	0.562	0.026 ± 0.023	0.028 ± 0.022	0.686	0.029 ± 0.014	0.020 ± 0.028	0.351
Change in overall response			0.797			0.126			0.282
Total cholesterol in very large HDL, mmol/L									
T=2h	0.008 ± 0.079	0.024 ± 0.047	0.258	0.038 ± 0.054	0.035 ± 0.037	0.810	0.015 ± 0.042	0.018 ± 0.043	0.830
T=4h	0.023 ± 0.076	0.034 ± 0.040	0.449	0.039 ± 0.041	0.043 ± 0.042	0.687	0.032 ± 0.025	0.029 ± 0.056	0.707
T=6h	0.052 ± 0.075	0.050 ± 0.039	0.864	0.048 ± 0.061	0.056 ± 0.045	0.476	0.052 ± 0.045	0.030 ± 0.038	0.067
Change in overall response			0.477			0.613			0.143

Supplemental Table 9. *Continued (IDL, LDL, HDL)*

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Cholesterol esters in very large HDL, mmol/L									
T=2h	0.011 ± 0.060	0.024 ± 0.039	0.219	0.034 ± 0.043	0.032 ± 0.031	0.878	0.016 ± 0.034	0.018 ± 0.035	0.851
T=4h	0.021 ± 0.060	0.029 ± 0.033	0.477	0.032 ± 0.034	0.036 ± 0.036	0.610	0.027 ± 0.021	0.024 ± 0.045	0.657
T=6h	0.041 ± 0.057	0.038 ± 0.031	0.789	0.036 ± 0.050	0.042 ± 0.037	0.485	0.039 ± 0.037	0.022 ± 0.031	0.064
Change in overall response			0.440			0.631			0.157
Free cholesterol in very large HDL, mmol/L									
T=2h	-0.003 ± 0.020	0.000 ± 0.010	0.429	0.004 ± 0.011	0.002 ± 0.006	0.530	-0.001 ± 0.010	0.000 ± 0.009	0.771
T=4h	0.002 ± 0.017	0.005 ± 0.008	0.377	0.007 ± 0.007	0.007 ± 0.007	0.848	0.004 ± 0.005	0.005 ± 0.011	0.983
T=6h	0.012 ± 0.018	0.012 ± 0.008	0.909	0.012 ± 0.011	0.014 ± 0.009	0.491	0.013 ± 0.009	0.008 ± 0.008	0.103
Change in overall response			0.575			0.499			0.130
Triglycerides in very large HDL, mmol/L									
T=2h	0.000 ± 0.007	0.001 ± 0.004	0.374	0.003 ± 0.004	0.002 ± 0.003	0.232	0.000 ± 0.005	0.001 ± 0.003	0.380
T=4h	0.003 ± 0.009	0.003 ± 0.004	0.833	0.005 ± 0.003	0.004 ± 0.003	0.115	0.003 ± 0.003	0.004 ± 0.005	0.829
T=6h	0.007 ± 0.007	0.005 ± 0.004	0.215	0.007 ± 0.006	0.006 ± 0.004	0.367	0.007 ± 0.005	0.004 ± 0.004	0.070
Change in overall response			0.277			0.940			0.041
Concentration of large HDL particles, mol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	0.739	0.000 ± 0.000	0.000 ± 0.000	**0.039**	0.000 ± 0.000	0.000 ± 0.000	0.369
T=4h	0.000 ± 0.000	0.000 ± 0.000	0.632	0.000 ± 0.000	0.000 ± 0.000	0.804	0.000 ± 0.000	0.000 ± 0.000	0.560
T=6h	0.000 ± 0.000	0.000 ± 0.000	0.927	0.000 ± 0.000	0.000 ± 0.000	0.322	0.000 ± 0.000	0.000 ± 0.000	0.333
Change in overall response			0.604			0.146			0.769
Total lipids in large HDL, mmol/L									
T=2h	-0.067 ± 0.215	-0.079 ± 0.113	0.748	-0.034 ± 0.089	-0.062 ± 0.045	**0.043**	-0.039 ± 0.095	-0.067 ± 0.140	0.397
T=4h	-0.075 ± 0.119	-0.063 ± 0.059	0.621	-0.062 ± 0.060	-0.065 ± 0.063	0.807	-0.043 ± 0.070	-0.062 ± 0.141	0.557
T=6h	0.006 ± 0.133	0.019 ± 0.094	0.944	0.020 ± 0.075	0.008 ± 0.077	0.340	0.041 ± 0.060	0.002 ± 0.119	0.336
Change in overall response			0.606			0.147			0.788
Phospholipids in large HDL, mmol/L									
T=2h	-0.034 ± 0.090	-0.041 ± 0.053	0.678	-0.021 ± 0.042	-0.034 ± 0.023	0.055	-0.019 ± 0.040	-0.036 ± 0.070	0.269
T=4h	-0.040 ± 0.051	-0.035 ± 0.030	0.618	-0.037 ± 0.033	-0.038 ± 0.033	0.896	-0.026 ± 0.040	-0.037 ± 0.069	0.527
T=6h	0.000 ± 0.060	0.004 ± 0.052	0.862	0.003 ± 0.039	-0.005 ± 0.042	0.298	0.015 ± 0.033	-0.004 ± 0.058	0.346
Change in overall response			0.572			0.134			0.725
Total cholesterol in large HDL, mmol/L									
T=2h	-0.032 ± 0.120	-0.037 ± 0.056	0.821	-0.014 ± 0.046	-0.027 ± 0.022	0.075	-0.020 ± 0.052	-0.028 ± 0.064	0.629
T=4h	-0.032 ± 0.065	-0.025 ± 0.028	0.554	-0.022 ± 0.026	-0.023 ± 0.029	0.835	-0.014 ± 0.027	-0.022 ± 0.066	0.575
T=6h	0.008 ± 0.071	0.017 ± 0.041	0.922	0.017 ± 0.036	0.014 ± 0.036	0.510	0.025 ± 0.026	0.008 ± 0.056	0.321
Change in overall response			0.631			0.198			0.652
Cholesterol esters in large HDL, mmol/L									
T=2h	-0.025 ± 0.090	-0.029 ± 0.043	0.836	-0.011 ± 0.035	-0.021 ± 0.017	0.075	-0.016 ± 0.039	-0.023 ± 0.050	0.583
T=4h	-0.024 ± 0.049	-0.018 ± 0.022	0.531	-0.016 ± 0.020	-0.017 ± 0.022	0.842	-0.011 ± 0.021	-0.017 ± 0.052	0.568
T=6h	0.008 ± 0.054	0.015 ± 0.032	0.971	0.015 ± 0.028	0.013 ± 0.027	0.470	0.021 ± 0.021	0.007 ± 0.044	0.288
Change in overall response			0.569			0.180			0.563

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Free cholesterol in large HDL, mmol/L									
T=2h	-0.007 ± 0.029	-0.008 ± 0.013	0.778	-0.002 ± 0.011	-0.005 ± 0.005	0.075	-0.004 ± 0.013	-0.005 ± 0.014	0.805
T=4h	-0.008 ± 0.016	-0.006 ± 0.006	0.634	-0.006 ± 0.006	-0.006 ± 0.007	0.827	-0.004 ± 0.006	-0.005 ± 0.014	0.618
T=6h	-0.001 ± 0.017	0.002 ± 0.009	0.735	0.002 ± 0.008	0.001 ± 0.009	0.653	0.004 ± 0.006	0.001 ± 0.012	0.462
Change in overall response			0.817			0.255			0.876
Triglycerides in large HDL, mmol/L									
T=2h	-0.001 ± 0.008	-0.002 ± 0.006	0.630	0.000 ± 0.005	-0.002 ± 0.002	**0.005**	0.000 ± 0.004	-0.002 ± 0.009	0.169
T=4h	-0.002 ± 0.007	-0.003 ± 0.004	0.561	-0.003 ± 0.005	-0.004 ± 0.003	0.366	-0.002 ± 0.007	-0.003 ± 0.008	0.838
T=6h	-0.001 ± 0.009	-0.002 ± 0.007	0.457	0.000 ± 0.005	-0.001 ± 0.005	**0.047**	0.001 ± 0.006	-0.002 ± 0.007	0.281
Change in overall response			0.961			0.292			0.341
Concentration of medium HDL particles, mol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	**0.023**	0.000 ± 0.000	0.000 ± 0.000	0.653	0.000 ± 0.000	0.000 ± 0.000	0.771
T=4h	0.000 ± 0.000	0.000 ± 0.000	0.195	0.000 ± 0.000	0.000 ± 0.000	0.478	0.000 ± 0.000	0.000 ± 0.000	0.737
T=6h	0.000 ± 0.000	0.000 ± 0.000	0.096	0.000 ± 0.000	0.000 ± 0.000	0.153	0.000 ± 0.000	0.000 ± 0.000	0.955
Change in overall response			0.450			0.425			0.566
Total lipids in medium HDL, mmol/L									
T=2h	-0.080 ± 0.082	-0.118 ± 0.083	0.021	-0.101 ± 0.080	-0.108 ± 0.049	0.656	-0.088 ± 0.054	-0.094 ± 0.100	0.768
T=4h	-0.071 ± 0.106	-0.093 ± 0.073	0.201	-0.085 ± 0.078	-0.096 ± 0.070	0.504	-0.091 ± 0.059	-0.085 ± 0.095	0.764
T=6h	0.003 ± 0.103	-0.020 ± 0.085	0.099	0.003 ± 0.088	-0.018 ± 0.071	0.161	0.002 ± 0.074	-0.005 ± 0.085	0.939
Change in overall response			0.421			0.416			0.602
Phospholipids in medium HDL, mmol/L									
T=2h	-0.038 ± 0.035	-0.053 ± 0.037	**0.030**	-0.047 ± 0.034	-0.049 ± 0.021	0.727	-0.040 ± 0.024	-0.043 ± 0.045	0.750
T=4h	-0.031 ± 0.045	-0.040 ± 0.032	0.210	-0.038 ± 0.033	-0.041 ± 0.031	0.634	-0.038 ± 0.027	-0.037 ± 0.044	0.863
T=6h	0.007 ± 0.044	-0.003 ± 0.038	0.092	0.006 ± 0.039	-0.003 ± 0.034	0.175	0.006 ± 0.034	0.002 ± 0.040	0.878
Change in overall response			0.545			0.353			0.711
Total cholesterol in medium HDL, mmol/L									
T=2h	-0.038 ± 0.040	-0.058 ± 0.043	**0.014**	-0.047 ± 0.041	-0.051 ± 0.028	0.657	-0.042 ± 0.026	-0.045 ± 0.051	0.798
T=4h	-0.038 ± 0.052	-0.049 ± 0.037	0.227	-0.045 ± 0.041	-0.050 ± 0.036	0.534	-0.047 ± 0.027	-0.045 ± 0.047	0.764
T=6h	-0.006 ± 0.051	-0.018 ± 0.044	0.121	-0.007 ± 0.045	-0.017 ± 0.035	0.190	-0.007 ± 0.035	-0.011 ± 0.040	0.879
Change in overall response			0.300			0.429			0.626
Cholesterol esters in medium HDL, mmol/L									
T=2h	-0.029 ± 0.031	-0.044 ± 0.034	**0.016**	-0.036 ± 0.032	-0.039 ± 0.022	0.672	-0.032 ± 0.021	-0.034 ± 0.040	0.853
T=4h	-0.027 ± 0.040	-0.036 ± 0.029	0.241	-0.032 ± 0.032	-0.037 ± 0.027	0.453	-0.036 ± 0.021	-0.033 ± 0.036	0.686
T=6h	-0.002 ± 0.039	-0.012 ± 0.034	0.116	-0.003 ± 0.034	-0.011 ± 0.026	0.169	-0.003 ± 0.025	-0.006 ± 0.031	0.862
Change in overall response			0.301			0.453			0.531
Free cholesterol in medium HDL, mmol/L									
T=2h	-0.009 ± 0.009	-0.014 ± 0.010	**0.012**	-0.011 ± 0.009	-0.012 ± 0.006	0.602	-0.009 ± 0.006	-0.011 ± 0.011	0.605
T=4h	-0.010 ± 0.012	-0.013 ± 0.009	0.197	-0.012 ± 0.009	-0.013 ± 0.009	0.867	-0.012 ± 0.007	-0.012 ± 0.011	0.949
T=6h	-0.004 ± 0.012	-0.006 ± 0.011	0.156	-0.004 ± 0.012	-0.006 ± 0.010	0.280	-0.004 ± 0.010	-0.004 ± 0.010	0.867
Change in overall response			0.350			0.397			0.794

Supplemental Table 9. *Continued (IDL, LDL, HDL)*

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Triglycerides in medium HDL, mmol/L									
T=2h	-0.004 ± 0.012	-0.007 ± 0.005	0.118	-0.007 ± 0.006	-0.008 ± 0.003	0.376	-0.006 ± 0.006	-0.006 ± 0.005	0.712
T=4h	-0.002 ± 0.010	-0.004 ± 0.006	0.074	-0.003 ± 0.005	-0.005 ± 0.005	**0.042**	-0.005 ± 0.006	-0.003 ± 0.006	0.262
T=6h	0.003 ± 0.010	0.001 ± 0.006	0.115	0.005 ± 0.006	0.003 ± 0.006	0.079	0.003 ± 0.008	0.003 ± 0.006	0.494
Change in overall response			0.759			0.539			0.126
Concentration of small HDL particles, mol/L									
T=2h	0.000 ± 0.000	0.000 ± 0.000	0.230	0.000 ± 0.000	0.000 ± 0.000	0.692	0.000 ± 0.000	0.000 ± 0.000	0.675
T=4h	0.000 ± 0.000	0.000 ± 0.000	0.458	0.000 ± 0.000	0.000 ± 0.000	0.863	0.000 ± 0.000	0.000 ± 0.000	0.410
T=6h	0.000 ± 0.000	0.000 ± 0.000	0.285	0.000 ± 0.000	0.000 ± 0.000	0.561	0.000 ± 0.000	0.000 ± 0.000	0.161
Change in overall response			0.903			0.520			0.561
Total lipids in small HDL, mmol/L									
T=2h	-0.057 ± 0.105	-0.079 ± 0.066	0.218	-0.094 ± 0.082	-0.087 ± 0.053	0.691	-0.071 ± 0.053	-0.065 ± 0.061	0.640
T=4h	-0.053 ± 0.114	-0.068 ± 0.066	0.462	-0.077 ± 0.071	-0.079 ± 0.067	0.909	-0.082 ± 0.052	-0.069 ± 0.059	0.417
T=6h	-0.036 ± 0.101	-0.052 ± 0.062	0.298	-0.041 ± 0.080	-0.054 ± 0.066	0.581	-0.051 ± 0.060	-0.031 ± 0.055	0.154
Change in overall response			0.891			0.536			0.570
Phospholipids in small HDL, mmol/L									
T=2h	-0.032 ± 0.062	-0.040 ± 0.019	0.436	-0.049 ± 0.036	-0.041 ± 0.014	0.235	-0.037 ± 0.029	-0.037 ± 0.030	0.959
T=4h	-0.016 ± 0.060	-0.023 ± 0.022	0.448	-0.026 ± 0.027	-0.025 ± 0.021	0.872	-0.027 ± 0.020	-0.023 ± 0.032	0.597
T=6h	0.024 ± 0.058	0.010 ± 0.026	0.113	0.016 ± 0.026	0.012 ± 0.022	0.370	0.017 ± 0.024	0.013 ± 0.028	0.804
Change in overall response			0.344			0.144			0.541
Total cholesterol in small HDL, mmol/L									
T=2h	-0.025 ± 0.052	-0.038 ± 0.049	0.106	-0.042 ± 0.054	-0.042 ± 0.042	0.961	-0.032 ± 0.033	-0.027 ± 0.041	0.531
T=4h	-0.040 ± 0.061	-0.046 ± 0.052	0.611	-0.052 ± 0.050	-0.053 ± 0.048	0.915	-0.055 ± 0.040	-0.046 ± 0.041	0.445
T=6h	-0.066 ± 0.052	-0.067 ± 0.048	0.899	-0.063 ± 0.063	-0.072 ± 0.051	0.724	-0.073 ± 0.041	-0.049 ± 0.040	0.051
Change in overall response			0.302			0.908			0.366
Cholesterol esters in small HDL, mmol/L									
T=2h	-0.018 ± 0.048	-0.030 ± 0.046	0.099	-0.032 ± 0.050	-0.035 ± 0.041	0.812	-0.024 ± 0.031	-0.020 ± 0.039	0.587
T=4h	-0.035 ± 0.054	-0.040 ± 0.048	0.673	-0.045 ± 0.045	-0.048 ± 0.045	0.823	-0.048 ± 0.038	-0.041 ± 0.038	0.462
T=6h	-0.066 ± 0.046	-0.064 ± 0.045	0.845	-0.061 ± 0.059	-0.070 ± 0.049	0.711	-0.071 ± 0.038	-0.048 ± 0.038	**0.038**
Change in overall response			0.195			0.956			0.295
Free cholesterol in small HDL, mmol/L									
T=2h	-0.007 ± 0.010	-0.008 ± 0.004	0.417	-0.010 ± 0.007	-0.008 ± 0.003	0.081	-0.008 ± 0.005	-0.007 ± 0.005	0.426
T=4h	-0.004 ± 0.011	-0.006 ± 0.004	0.378	-0.007 ± 0.006	-0.006 ± 0.005	0.335	-0.007 ± 0.004	-0.006 ± 0.006	0.535
T=6h	0.000 ± 0.010	-0.003 ± 0.005	0.094	-0.002 ± 0.005	-0.002 ± 0.004	0.758	-0.002 ± 0.005	-0.002 ± 0.005	0.794
Change in overall response			0.411			0.084			0.915
Triglycerides in small HDL, mmol/L									
T=2h	-0.001 ± 0.011	-0.002 ± 0.004	0.649	-0.003 ± 0.004	-0.003 ± 0.003	0.938	-0.002 ± 0.004	-0.002 ± 0.002	0.942
T=4h	0.002 ± 0.006	0.001 ± 0.004	0.264	0.001 ± 0.003	0.000 ± 0.005	0.051	0.000 ± 0.004	0.001 ± 0.004	0.182
T=6h	0.005 ± 0.006	0.004 ± 0.005	0.070	0.006 ± 0.004	0.006 ± 0.006	0.851	0.005 ± 0.005	0.005 ± 0.005	0.506
Change in overall response			0.765			**0.048**			0.358

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Mean diameter for VLDL particles, nm									
T=2h	0.104 ± 1.175	0.092 ± 0.388	0.954	-0.051 ± 0.643	-0.019 ± 0.426	0.739	-0.060 ± 0.540	-0.028 ± 0.388	0.785
T=4h	0.579 ± 0.572	0.512 ± 0.545	0.480	0.681 ± 0.630	0.514 ± 0.678	0.082	0.368 ± 0.562	0.488 ± 0.717	0.365
T=6h	0.850 ± 0.701	0.844 ± 0.656	0.666	1.108 ± 0.788	1.353 ± 0.875	0.127	0.802 ± 0.664	0.786 ± 0.577	0.714
Change in overall response			0.936			**0.014**			0.802
Mean diameter for LDL particles, nm									
T=2h	-0.011 ± 0.051	-0.005 ± 0.044	0.448	0.008 ± 0.045	0.009 ± 0.038	0.886	0.008 ± 0.037	0.000 ± 0.030	0.281
T=4h	-0.013 ± 0.053	-0.005 ± 0.033	0.472	0.000 ± 0.042	0.010 ± 0.044	0.262	0.013 ± 0.030	0.002 ± 0.041	0.277
T=6h	0.008 ± 0.043	0.023 ± 0.043	0.099	0.011 ± 0.047	0.030 ± 0.047	0.189	0.032 ± 0.039	0.014 ± 0.039	0.186
Change in overall response			0.700			0.285			0.824
Mean diameter for HDL particles, nm									
T=2h	-0.021 ± 0.178	-0.018 ± 0.089	0.933	0.032 ± 0.092	0.014 ± 0.047	0.306	-0.009 ± 0.090	-0.016 ± 0.101	0.785
T=4h	-0.011 ± 0.129	0.005 ± 0.054	0.490	0.017 ± 0.051	0.021 ± 0.044	0.734	0.006 ± 0.050	0.002 ± 0.110	0.842
T=6h	0.062 ± 0.140	0.078 ± 0.070	0.725	0.071 ± 0.074	0.081 ± 0.057	0.377	0.073 ± 0.047	0.043 ± 0.084	0.262
Change in overall response			0.826			0.253			0.293
Plasma total cholesterol, mmol/L									
T=2h	-0.098 ± 0.350	-0.278 ± 0.382	**0.026**	-0.071 ± 0.246	-0.197 ± 0.114	**0.004**	-0.116 ± 0.191	-0.142 ± 0.127	0.562
T=4h	-0.172 ± 0.362	-0.285 ± 0.176	0.076	-0.151 ± 0.181	-0.232 ± 0.185	**0.013**	-0.189 ± 0.271	-0.151 ± 0.158	0.532
T=6h	-0.308 ± 0.386	-0.380 ± 0.213	0.211	-0.268 ± 0.222	-0.365 ± 0.200	**0.005**	-0.277 ± 0.228	-0.268 ± 0.142	0.679
Change in overall response			0.068			0.380			0.481
Total cholesterol in VLDL, mmol/L									
T=2h	0.012 ± 0.164	-0.025 ± 0.071	0.176	-0.008 ± 0.068	-0.020 ± 0.052	0.339	-0.016 ± 0.070	-0.016 ± 0.057	0.997
T=4h	0.050 ± 0.087	0.003 ± 0.065	**0.003**	0.043 ± 0.057	0.014 ± 0.063	**0.012**	0.017 ± 0.067	0.039 ± 0.056	0.151
T=6h	0.048 ± 0.102	0.011 ± 0.059	**0.030**	0.058 ± 0.078	0.043 ± 0.073	0.329	0.053 ± 0.069	0.033 ± 0.064	0.223
Change in overall response			0.669			0.194			**0.013**
Remnant cholesterol (non-HDL, non-LDL -cholesterol), mmol/L									
T=2h	0.001 ± 0.186	-0.065 ± 0.125	**0.046**	-0.004 ± 0.095	-0.042 ± 0.080	**0.047**	-0.021 ± 0.087	-0.031 ± 0.092	0.678
T=4h	0.012 ± 0.136	-0.053 ± 0.081	**0.012**	0.015 ± 0.076	-0.025 ± 0.085	**0.010**	-0.009 ± 0.104	0.012 ± 0.068	0.366
T=6h	-0.035 ± 0.155	-0.078 ± 0.082	**0.046**	-0.017 ± 0.103	-0.048 ± 0.086	0.137	-0.015 ± 0.085	-0.035 ± 0.070	0.126
Change in overall response			0.686			0.852			**0.013**
Total cholesterol in LDL, mmol/L									
T=2h	-0.021 ± 0.168	-0.104 ± 0.168	**0.019**	-0.003 ± 0.132	-0.070 ± 0.058	**0.010**	-0.017 ± 0.101	-0.032 ± 0.098	0.588
T=4h	-0.103 ± 0.193	-0.146 ± 0.114	0.224	-0.086 ± 0.114	-0.124 ± 0.100	0.057	-0.097 ± 0.147	-0.082 ± 0.146	0.662
T=6h	-0.258 ± 0.210	-0.284 ± 0.134	0.487	-0.241 ± 0.140	-0.298 ± 0.130	**0.018**	-0.255 ± 0.157	-0.211 ± 0.126	0.478
Change in overall response			0.061			0.295			0.524
Total cholesterol in HDL, mmol/L									
T=2h	-0.078 ± 0.172	-0.108 ± 0.130	0.340	-0.064 ± 0.091	-0.085 ± 0.057	0.174	-0.078 ± 0.086	-0.079 ± 0.142	0.961
T=4h	-0.081 ± 0.088	-0.086 ± 0.074	0.739	-0.080 ± 0.069	-0.083 ± 0.077	0.830	-0.083 ± 0.066	-0.081 ± 0.139	0.954
T=6h	-0.014 ± 0.091	-0.018 ± 0.087	0.550	-0.010 ± 0.083	-0.019 ± 0.086	0.521	-0.008 ± 0.059	-0.022 ± 0.116	0.777
Change in overall response			0.700			0.421			0.790

Supplemental Table 9. *Continued (miscellaneous)*

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Total cholesterol in HDL2, mmol/L									
T=2h	-0.041 ± 0.142	-0.065 ± 0.106	0.372	-0.024 ± 0.070	-0.047 ± 0.047	0.058	-0.039 ± 0.076	-0.044 ± 0.136	0.870
T=4h	-0.045 ± 0.076	-0.050 ± 0.054	0.759	-0.044 ± 0.049	-0.053 ± 0.058	0.411	-0.051 ± 0.055	-0.048 ± 0.134	0.923
T=6h	0.012 ± 0.081	0.007 ± 0.063	0.440	0.015 ± 0.061	0.007 ± 0.066	0.392	0.013 ± 0.040	-0.005 ± 0.108	0.677
Change in overall response			0.498			0.401			0.214
Total cholesterol in HDL3, mmol/L									
T=2h	-0.037 ± 0.034	-0.043 ± 0.028	0.235	-0.040 ± 0.026	-0.039 ± 0.017	0.735	-0.039 ± 0.017	-0.035 ± 0.015	0.404
T=4h	-0.035 ± 0.025	-0.037 ± 0.026	0.765	-0.036 ± 0.026	-0.030 ± 0.028	0.356	-0.032 ± 0.022	-0.033 ± 0.022	0.869
T=6h	-0.027 ± 0.027	-0.025 ± 0.032	0.737	-0.025 ± 0.034	-0.025 ± 0.029	0.979	-0.020 ± 0.028	-0.018 ± 0.020	0.917
Change in overall response			0.331			0.626			0.748
Esterified cholesterol, mmol/L									
T=2h	-0.062 ± 0.242	-0.184 ± 0.276	**0.033**	-0.034 ± 0.175	-0.128 ± 0.083	**0.003**	-0.072 ± 0.134	-0.091 ± 0.097	0.572
T=4h	-0.131 ± 0.254	-0.208 ± 0.138	0.095	-0.112 ± 0.135	-0.171 ± 0.138	**0.012**	-0.171 ± 0.263	-0.114 ± 0.121	0.335
T=6h	-0.250 ± 0.284	-0.288 ± 0.169	0.367	-0.216 ± 0.171	-0.288 ± 0.148	**0.006**	-0.230 ± 0.169	-0.218 ± 0.103	0.988
Change in overall response			0.052			0.350			0.302
Free cholesterol, mmol/L									
T=2h	-0.036 ± 0.112	-0.087 ± 0.114	**0.036**	-0.037 ± 0.073	-0.069 ± 0.036	**0.018**	-0.044 ± 0.063	-0.051 ± 0.041	0.596
T=4h	-0.042 ± 0.115	-0.077 ± 0.052	0.061	-0.039 ± 0.051	-0.061 ± 0.055	**0.041**	-0.018 ± 0.196	-0.037 ± 0.048	0.645
T=6h	-0.058 ± 0.113	-0.083 ± 0.067	0.171	-0.051 ± 0.059	-0.077 ± 0.056	**0.014**	-0.047 ± 0.065	-0.050 ± 0.050	0.522
Change in overall response			0.169			0.576			0.975
Plasma total triglycerides, mmol/L									
T=2h	-0.003 ± 0.474	-0.046 ± 0.175	0.580	-0.087 ± 0.162	-0.087 ± 0.118	0.978	-0.100 ± 0.181	-0.059 ± 0.099	0.240
T=4h	0.193 ± 0.266	0.120 ± 0.218	0.078	0.177 ± 0.184	0.088 ± 0.200	**0.005**	0.077 ± 0.209	0.152 ± 0.234	0.112
T=6h	0.337 ± 0.304	0.254 ± 0.230	**0.028**	0.365 ± 0.224	0.342 ± 0.253	0.676	0.316 ± 0.284	0.282 ± 0.235	0.874
Change in overall response			0.746			**0.018**			0.357
Triglycerides in VLDL, mmol/L									
T=2h	0.022 ± 0.458	-0.008 ± 0.149	0.682	-0.058 ± 0.161	-0.044 ± 0.110	0.570	-0.076 ± 0.172	-0.030 ± 0.100	0.189
T=4h	0.207 ± 0.242	0.138 ± 0.202	0.086	0.191 ± 0.176	0.117 ± 0.191	**0.009**	0.093 ± 0.198	0.164 ± 0.232	0.109
T=6h	0.317 ± 0.287	0.245 ± 0.222	0.053	0.344 ± 0.220	0.337 ± 0.252	0.903	0.297 ± 0.282	0.263 ± 0.226	0.873
Change in overall response			0.827			**0.017**			0.429
Triglycerides in LDL, mmol/L									
T=2h	-0.014 ± 0.021	-0.018 ± 0.023	0.407	-0.013 ± 0.018	-0.020 ± 0.016	0.123	-0.011 ± 0.016	-0.013 ± 0.016	0.555
T=4h	-0.010 ± 0.023	-0.008 ± 0.013	0.811	-0.009 ± 0.014	-0.014 ± 0.020	0.132	-0.008 ± 0.015	-0.007 ± 0.017	0.786
T=6h	0.008 ± 0.024	0.002 ± 0.017	0.071	0.004 ± 0.017	-0.003 ± 0.022	0.113	0.005 ± 0.013	0.007 ± 0.017	0.845
Change in overall response			**0.035**			**0.693**			0.685
Triglycerides in HDL, mmol/L									
T=2h	-0.003 ± 0.022	-0.009 ± 0.013	0.112	-0.006 ± 0.011	0.011 ± 0.007	**0.015**	-0.006 ± 0.011	-0.008 ± 0.014	0.757
T=4h	0.002 ± 0.019	-0.003 ± 0.013	**0.023**	0.000 ± 0.010	-0.006 ± 0.011	**0.002**	-0.003 ± 0.014	0.000 ± 0.016	0.334
T=6h	0.011 ± 0.018	0.008 ± 0.014	0.070	0.017 ± 0.013	0.013 ± 0.015	0.182	0.012 ± 0.017	0.011 ± 0.018	0.874
Change in overall response			0.952			0.355			**0.019**

Supplemental Table 10. Changes in postprandial apolipoproteins upon 12 weeks of intervention

		WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
		Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
ApoA1, g/L	T=2h	-0.044 ± 0.074	-0.071 ± 0.092	0.119	-0.043 ± 0.056	-0.058 ± 0.033	0.102	-0.054 ± 0.054	-0.051 ± 0.085	0.906
	T=4h	-0.025 ± 0.066	-0.042 ± 0.052	0.154	-0.027 ± 0.050	-0.040 ± 0.044	0.160	-0.039 ± 0.045	-0.031 ± 0.084	0.694
	T=6h	0.027 ± 0.068	0.008 ± 0.053	0.061	0.027 ± 0.045	0.016 ± 0.041	0.144	0.029 ± 0.031	0.010 ± 0.068	0.368
Change in overall response				0.725			0.958			**0.026**
ApoB, g/L	T=2h	-0.011 ± 0.093	-0.040 ± 0.062	0.080	-0.019 ± 0.035	-0.036 ± 0.026	**0.014**	-0.026 ± 0.038	-0.024 ± 0.034	0.843
	T=4h	0.003 ± 0.067	-0.022 ± 0.036	**0.024**	0.005 ± 0.032	-0.018 ± 0.031	**<0.001**	-0.014 ± 0.051	0.002 ± 0.037	0.190
	T=6h	-0.005 ± 0.070	-0.028 ± 0.036	0.070	0.004 ± 0.041	-0.013 ± 0.032	**0.035**	-0.007 ± 0.043	-0.003 ± 0.037	0.830
Change in overall response				0.919			0.190			**0.023**

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Supplemental Table 11. Changes in postprandial fatty acids upon 12 weeks of intervention

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Total fatty acids, mmol/L									
T=2h	-0.258 ± 1.352	-0.683 ± 0.958	0.113	-0.427 ± 0.604	-0.665 ± 0.419	**0.046**	-0.466 ± 0.640	-0.486 ± 0.485	0.895
T=4h	0.349 ± 0.992	-0.033 ± 0.803	**0.020**	0.328 ± 0.665	-0.026 ± 0.674	**0.005**	0.032 ± 0.773	0.240 ± 0.728	0.287
T=6h	1.149 ± 1.130	0.722 ± 0.746	0.011	1.170 ± 0.661	0.878 ± 0.694	0.052	1.191 ± 0.802	0.847 ± 0.799	0.123
Change in overall response			0.813			0.452			0.021
Estimated degree of unsaturation									
T=2h	0.007 ± 0.038	0.007 ± 0.018	0.985	0.012 ± 0.026	0.017 ± 0.018	0.266	-0.001 ± 0.026	0.015 ± 0.019	**0.017**
T=4h	-0.019 ± 0.028	-0.018 ± 0.025	0.793	-0.021 ± 0.021	-0.018 ± 0.026	0.352	-0.014 ± 0.026	-0.017 ± 0.031	0.838
T=6h	-0.054 ± 0.034	-0.049 ± 0.028	0.118	-0.052 ± 0.029	-0.065 ± 0.027	0.074	-0.059 ± 0.027	-0.045 ± 0.032	0.099
Change in overall response			0.510			**0.038**			**0.011**
DHA (22:6), mmol/L									
T=2h	-0.008 ± 0.032	-0.015 ± 0.021	0.291	-0.006 ± 0.015	-0.013 ± 0.015	0.094	0.002 ± 0.019	-0.012 ± 0.023	**0.029**
T=4h	-0.005 ± 0.029	-0.013 ± 0.021	0.174	-0.010 ± 0.016	-0.011 ± 0.018	0.780	-0.001 ± 0.017	-0.003 ± 0.025	0.832
T=6h	-0.009 ± 0.035	-0.009 ± 0.024	0.958	-0.004 ± 0.026	-0.003 ± 0.018	0.775	0.002 ± 0.020	-0.005 ± 0.023	0.621
Change in overall response			0.245			0.180			0.070
LA (18:2), mmol/L									
T=2h	-0.006 ± 0.259	-0.164 ± 0.283	**0.011**	-0.031 ± 0.141	-0.138 ± 0.105	**<0.001**	-0.055 ± 0.140	-0.073 ± 0.134	0.620
T=4h	-0.001 ± 0.229	-0.098 ± 0.196	**0.026**	-0.024 ± 0.138	-0.102 ± 0.177	**0.032**	-0.071 ± 0.172	-0.030 ± 0.134	0.302
T=6h	0.066 ± 0.227	-0.042 ± 0.169	**0.018**	0.034 ± 0.187	-0.061 ± 0.167	**0.023**	0.021 ± 0.154	-0.030 ± 0.151	0.142
Change in overall response			0.442			0.508			0.097
Omega-3 fatty acids, mmol/L									
T=2h	-0.028 ± 0.078	-0.036 ± 0.043	0.585	-0.011 ± 0.031	-0.024 ± 0.029	0.128	-0.007 ± 0.043	-0.016 ± 0.049	0.532
T=4h	-0.030 ± 0.073	-0.033 ± 0.044	0.908	-0.015 ± 0.039	-0.020 ± 0.041	0.565	-0.006 ± 0.032	-0.012 ± 0.056	0.537
T=6h	-0.045 ± 0.081	-0.043 ± 0.043	0.922	-0.034 ± 0.049	-0.036 ± 0.037	0.984	-0.020 ± 0.039	-0.040 ± 0.046	0.095
Change in overall response			0.644			0.489			0.582
Omega-6 fatty acids, mmol/L									
T=2h	-0.020 ± 0.301	-0.182 ± 0.320	**0.021**	-0.045 ± 0.180	-0.152 ± 0.114	**0.002**	-0.087 ± 0.166	-0.097 ± 0.126	0.777
T=4h	0.018 ± 0.259	-0.101 ± 0.199	**0.011**	-0.006 ± 0.171	-0.096 ± 0.162	**0.014**	-0.068 ± 0.200	-0.024 ± 0.138	0.311
T=6h	0.083 ± 0.269	-0.034 ± 0.171	**0.017**	0.069 ± 0.181	-0.039 ± 0.162	**0.006**	0.058 ± 0.152	-0.002 ± 0.150	0.074
Change in overall response			0.733			0.608			**0.024**
PUFA, mmol/L									
T=2h	-0.047 ± 0.340	-0.218 ± 0.347	**0.028**	-0.056 ± 0.195	-0.176 ± 0.120	**0.002**	-0.093 ± 0.186	-0.113 ± 0.153	0.681
T=4h	-0.013 ± 0.288	-0.133 ± 0.207	**0.019**	-0.021 ± 0.168	-0.116 ± 0.175	**0.011**	-0.074 ± 0.206	-0.036 ± 0.156	0.441
T=6h	0.038 ± 0.299	-0.077 ± 0.179	**0.036**	0.035 ± 0.198	-0.075 ± 0.177	**0.011**	0.038 ± 0.156	-0.042 ± 0.165	**0.039**
Change in overall response			0.691			0.563			**0.021**
MUFA (16:1, 18:1), mmol/L									
T=2h	-0.140 ± 0.538	-0.264 ± 0.338	0.221	-0.250 ± 0.240	-0.303 ± 0.202	0.254	-0.247 ± 0.277	-0.228 ± 0.168	0.740
T=4h	0.176 ± 0.422	0.061 ± 0.378	0.093	0.163 ± 0.285	0.036 ± 0.313	**0.017**	0.025 ± 0.302	0.138 ± 0.336	0.150
T=6h	0.618 ± 0.493	0.482 ± 0.348	**0.039**	0.707 ± 0.252	0.640 ± 0.362	0.296	0.648 ± 0.364	0.551 ± 0.412	0.407
Change in overall response			0.864			0.312			**0.031**

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
SFA, mmol/L									
T=2h	-0.071 ± 0.518	-0.202 ± 0.324	0.192	-0.121 ± 0.244	-0.185 ± 0.160	0.191	-0.126 ± 0.248	-0.147 ± 0.214	0.739
T=4h	0.186 ± 0.349	0.041 ± 0.294	**0.012**	0.185 ± 0.270	0.054 ± 0.240	**0.008**	0.081 ± 0.316	0.137 ± 0.294	0.463
T=6h	0.493 ± 0.434	0.317 ± 0.323	**0.004**	0.428 ± 0.322	0.314 ± 0.252	0.066	0.504 ± 0.352	0.337 ± 0.295	0.118
Change in overall response			0.677			0.241			0.089

Supplemental Table 12. Changes in postprandial phospholipids and choline derivatives upon 12 weeks of intervention

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Total phosphoglycerides, mmol/L									
T=2h	-0.042 ± 0.113	-0.100 ± 0.164	0.057	-0.035 ± 0.095	-0.075 ± 0.075	**0.018**	-0.031 ± 0.099	-0.058 ± 0.149	0.451
T=4h	0.003 ± 0.125	-0.053 ± 0.107	**0.032**	-0.014 ± 0.102	-0.031 ± 0.083	0.346	-0.001 ± 0.118	-0.014 ± 0.147	0.620
T=6h	0.096 ± 0.145	0.049 ± 0.107	0.115	0.082 ± 0.095	0.054 ± 0.087	0.076	0.128 ± 0.087	0.046 ± 0.132	0.051
Change in overall response			0.796			0.273			**0.037**
Phosphatidylcholine and other cholines, mmol/L									
T=2h	0.014 ± 0.145	-0.103 ± 0.198	**0.005**	-0.010 ± 0.113	-0.028 ± 0.088	0.411	-0.013 ± 0.096	-0.019 ± 0.146	0.835
T=4h	0.025 ± 0.145	-0.053 ± 0.122	**0.007**	-0.005 ± 0.104	-0.003 ± 0.122	0.947	0.065 ± 0.361	0.017 ± 0.138	0.485
T=6h	0.045 ± 0.138	-0.032 ± 0.125	**0.016**	0.009 ± 0.103	-0.032 ± 0.104	**0.009**	0.030 ± 0.095	-0.015 ± 0.122	0.160
Change in overall response			0.408			**0.022**			0.382
Sphingomyelins, mmol/L									
T=2h	-0.033 ± 0.047	-0.052 ± 0.057	0.057	-0.035 ± 0.028	-0.031 ± 0.030	0.595	-0.029 ± 0.041	-0.034 ± 0.029	0.624
T=4h	-0.026 ± 0.050	-0.039 ± 0.038	0.118	-0.022 ± 0.031	-0.018 ± 0.037	0.634	-0.016 ± 0.058	-0.020 ± 0.036	0.766
T=6h	-0.037 ± 0.039	-0.030 ± 0.048	0.576	-0.028 ± 0.036	-0.014 ± 0.039	0.189	-0.021 ± 0.038	-0.012 ± 0.027	0.439
Change in overall response			**0.008**			0.596			0.482
Total cholines, mmol/L									
T=2h	-0.026 ± 0.137	-0.112 ± 0.190	**0.024**	-0.023 ± 0.107	-0.058 ± 0.064	0.059	-0.041 ± 0.094	-0.041 ± 0.131	0.983
T=4h	-0.018 ± 0.144	-0.079 ± 0.105	**0.024**	-0.032 ± 0.087	-0.044 ± 0.088	0.469	0.000 ± 0.228	-0.016 ± 0.123	0.702
T=6h	0.017 ± 0.148	-0.026 ± 0.119	0.106	0.014 ± 0.099	-0.014 ± 0.095	0.100	0.029 ± 0.085	-0.002 ± 0.115	0.274
Change in overall response			0.235			0.228			0.308

Supplemental Table 13. Changes in postprandial metabolites related to energy metabolism upon 12 weeks of intervention

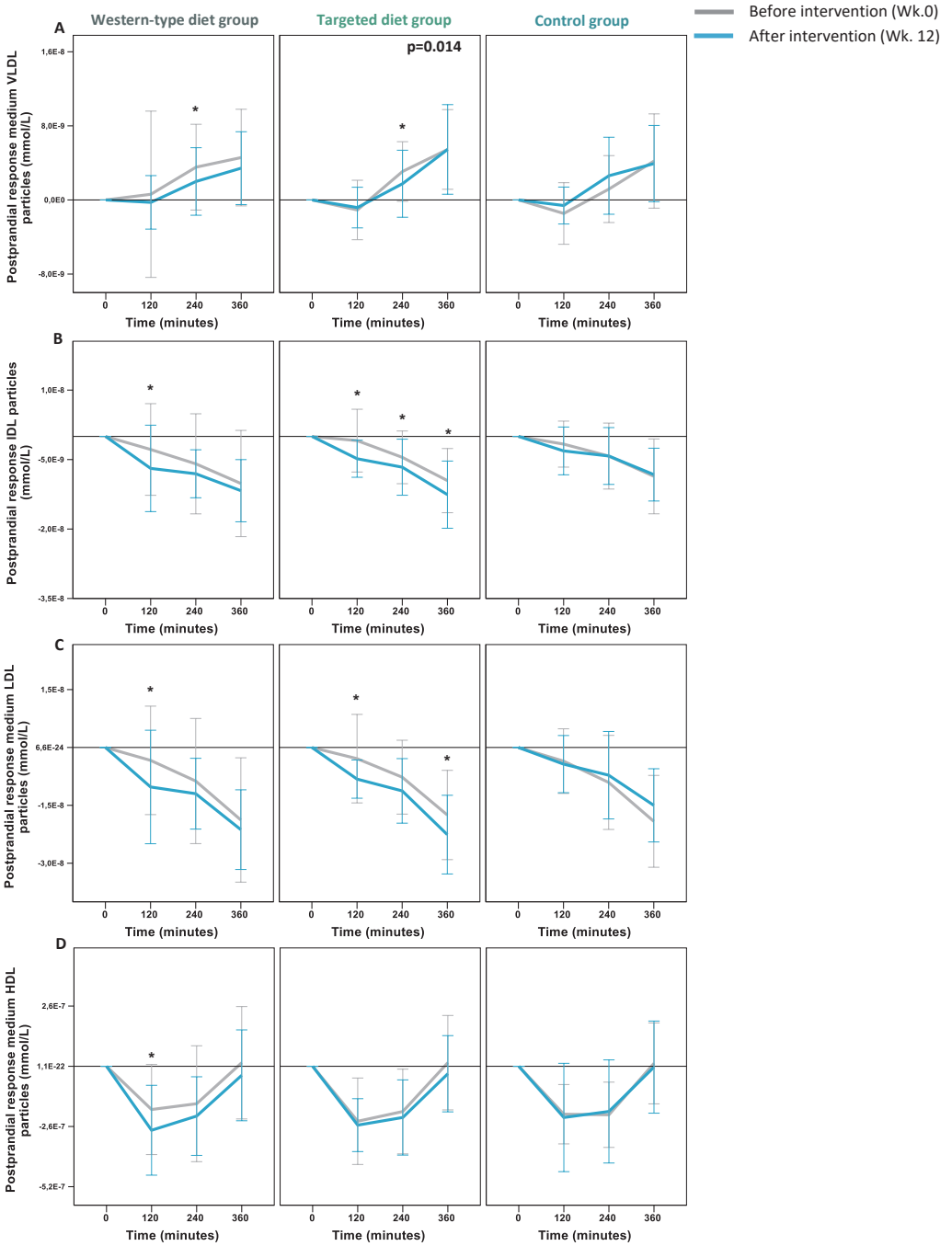
	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Glucose, mmol/L									
T=2h	-0.422 ± 1.055	-0.725 ± 1.230	0.072	0.059 ± 1.351	-0.421 ± 1.088	0.059	-0.300 ± 1.371	-0.463 ± 1.099	0.551
T=4h	-1.109 ± 0.667	-0.934 ± 0.472	0.082	-1.118 ± 0.554	-0.980 ± 0.382	0.173	-1.085 ± 0.684	-1.190 ± 0.594	0.399
T=6h	-0.645 ± 0.682	-0.376 ± 0.392	**0.017**	-0.583 ± 0.271	-0.451 ± 0.318	**0.040**	-0.474 ± 0.446	-0.573 ± 0.490	0.328
Change in overall response			**0.019**			0.085			0.965
Lactate, mmol/L									
T=2h	-0.004 ± 0.428	0.025 ± 0.391	0.749	0.068 ± 0.234	0.052 ± 0.178	0.755	0.072 ± 0.257	0.082 ± 0.258	0.868
T=4h	-0.181 ± 0.361	-0.167 ± 0.366	0.932	-0.085 ± 0.211	-0.076 ± 0.231	0.843	-0.099 ± 0.240	-0.107 ± 0.226	0.960
T=6h	-0.299 ± 0.366	-0.228 ± 0.368	0.557	-0.239 ± 0.255	-0.192 ± 0.190	0.371	-0.230 ± 0.214	-0.176 ± 0.178	0.802
Change in overall response			0.390			0.256			0.933
Citrate, mmol/L									
T=2h	0.008 ± 0.028	0.010 ± 0.017	0.574	0.013 ± 0.026	0.006 ± 0.021	0.145	0.011 ± 0.029	0.006 ± 0.021	0.419
T=4h	-0.002 ± 0.022	-0.002 ± 0.019	0.994	0.001 ± 0.018	-0.003 ± 0.023	0.321	0.003 ± 0.025	-0.003 ± 0.020	0.317
T=6h	0.009 ± 0.022	0.011 ± 0.019	0.728	0.007 ± 0.018	0.011 ± 0.020	0.311	0.013 ± 0.026	0.009 ± 0.022	0.416
Change in overall response			0.842			**0.025**			0.988
Acetate, mmol/L									
T=2h	-0.015 ± 0.009	-0.016 ± 0.016	0.713	-0.013 ± 0.011	-0.016 ± 0.014	0.215	-0.014 ± 0.011	-0.019 ± 0.013	0.079
T=4h	-0.012 ± 0.009	-0.015 ± 0.016	0.306	-0.010 ± 0.013	-0.012 ± 0.015	0.627	-0.012 ± 0.011	-0.017 ± 0.015	**0.028**
T=6h	-0.008 ± 0.010	-0.012 ± 0.015	0.184	-0.008 ± 0.011	-0.009 ± 0.014	0.680	-0.010 ± 0.010	-0.012 ± 0.012	0.235
Change in overall response			0.084			0.462			0.066
Acetoacetate, mmol/L									
T=2h	-0.005 ± 0.014	-0.010 ± 0.018	0.185	-0.008 ± 0.019	-0.018 ± 0.025	**0.047**	-0.006 ± 0.012	-0.008 ± 0.019	0.483
T=4h	0.000 ± 0.019	-0.005 ± 0.026	0.281	0.000 ± 0.022	-0.015 ± 0.032	**0.015**	-0.001 ± 0.016	-0.003 ± 0.021	0.682
T=6h	0.025 ± 0.030	0.028 ± 0.030	0.607	0.044 ± 0.033	0.037 ± 0.030	0.319	0.030 ± 0.030	0.028 ± 0.041	0.955
Change in overall response			0.308			0.097			0.985
3-Hydroxybutyrate, mmol/L									
T=2h	-0.055 ± 0.053	-0.062 ± 0.075	0.536	-0.059 ± 0.078	-0.104 ± 0.127	0.068	-0.046 ± 0.035	-0.060 ± 0.049	0.081
T=4h	-0.008 ± 0.070	0.007 ± 0.111	0.285	0.008 ± 0.090	-0.019 ± 0.114	0.200	0.007 ± 0.064	0.000 ± 0.076	0.771
T=6h	0.215 ± 0.174	0.244 ± 0.164	0.241	0.291 ± 0.164	0.323 ± 0.203	0.402	0.271 ± 0.155	0.249 ± 0.155	0.376
Change in overall response			0.076			0.172			0.514

Supplemental Table 14. Changes in postprandial amino acids upon 12 weeks of intervention

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Alanine, mmol/L									
T=2h	0.014 ± 0.072	0.027 ± 0.049	0.298	0.010 ± 0.048	0.017 ± 0.034	0.365	0.010 ± 0.045	0.024 ± 0.067	0.217
T=4h	-0.019 ± 0.054	-0.005 ± 0.051	0.205	-0.029 ± 0.048	-0.008 ± 0.038	**0.011**	-0.023 ± 0.054	-0.003 ± 0.070	0.112
T=6h	-0.047 ± 0.049	-0.051 ± 0.044	0.585	-0.062 ± 0.051	-0.050 ± 0.035	0.174	-0.056 ± 0.050	-0.044 ± 0.067	0.455
Change in overall response			**0.001**			**0.037**			0.148
Glutamine, mmol/L									
T=2h	-0.006 ± 0.062	-0.012 ± 0.043	0.600	-0.008 ± 0.050	-0.005 ± 0.049	0.751	-0.009 ± 0.048	-0.002 ± 0.043	0.494
T=4h	0.009 ± 0.057	0.002 ± 0.037	0.389	-0.003 ± 0.040	0.000 ± 0.045	0.744	-0.001 ± 0.051	0.005 ± 0.046	0.597
T=6h	0.016 ± 0.058	-0.006 ± 0.043	**0.041**	0.014 ± 0.037	-0.003 ± 0.047	0.231	0.021 ± 0.039	0.014 ± 0.032	0.534
Change in overall response			0.149			0.129			0.425
Histidine, mmol/L									
T=2h	-0.002 ± 0.016	-0.004 ± 0.008	0.396	-0.003 ± 0.010	-0.002 ± 0.012	0.613	-0.003 ± 0.009	-0.004 ± 0.010	0.673
T=4h	0.000 ± 0.010	0.000 ± 0.010	0.901	0.001 ± 0.009	-0.002 ± 0.011	0.182	-0.001 ± 0.010	-0.002 ± 0.011	0.488
T=6h	-0.002 ± 0.013	-0.003 ± 0.008	0.954	0.000 ± 0.010	-0.004 ± 0.012	0.213	-0.007 ± 0.011	-0.001 ± 0.012	0.145
Change in overall response			0.425			0.107			**0.004**
Phenylalanine, mmol/L									
T=2h	-0.003 ± 0.006	-0.001 ± 0.006	0.128	-0.002 ± 0.007	-0.003 ± 0.006	0.646	0.000 ± 0.007	-0.001 ± 0.006	0.756
T=4h	-0.004 ± 0.006	-0.003 ± 0.005	0.151	-0.003 ± 0.005	-0.004 ± 0.004	0.447	-0.004 ± 0.006	-0.003 ± 0.006	0.829
T=6h	-0.005 ± 0.006	-0.003 ± 0.005	0.091	-0.004 ± 0.006	-0.006 ± 0.006	0.182	-0.003 ± 0.006	-0.004 ± 0.004	0.544
Change in overall response			0.958			0.797			0.522
Tyrosine, mmol/L									
T=2h	-0.003 ± 0.010	0.001 ± 0.010	**0.027**	0.001 ± 0.009	-0.001 ± 0.008	0.333	0.000 ± 0.010	-0.001 ± 0.010	0.684
T=4h	-0.003 ± 0.011	0.003 ± 0.010	**0.005**	0.001 ± 0.010	0.001 ± 0.009	0.917	-0.001 ± 0.012	-0.001 ± 0.013	0.850
T=6h	-0.004 ± 0.011	-0.001 ± 0.009	0.157	-0.006 ± 0.009	-0.005 ± 0.011	0.605	-0.006 ± 0.008	-0.007 ± 0.009	0.616
Change in overall response			0.376			0.502			0.737
Leucine (BCAA), mmol/L									
T=2h	-0.004 ± 0.014	-0.001 ± 0.010	0.255	-0.001 ± 0.013	-0.002 ± 0.011	0.632	0.001 ± 0.012	-0.001 ± 0.012	0.470
T=4h	0.000 ± 0.010	0.005 ± 0.010	**0.021**	0.004 ± 0.012	0.001 ± 0.008	0.248	0.002 ± 0.010	0.002 ± 0.015	0.936
T=6h	0.010 ± 0.015	0.011 ± 0.011	0.854	0.010 ± 0.010	0.009 ± 0.010	0.968	0.012 ± 0.011	0.008 ± 0.010	0.133
Change in overall response			0.179			0.629			0.293
Isoleucine (BCAA), mmol/L									
T=2h	-0.002 ± 0.018	0.001 ± 0.012	0.271	0.003 ± 0.013	0.000 ± 0.011	0.310	0.003 ± 0.014	0.001 ± 0.010	0.450
T=4h	0.002 ± 0.012	0.007 ± 0.012	0.058	0.008 ± 0.016	0.004 ± 0.009	0.106	0.005 ± 0.013	0.006 ± 0.015	0.669
T=6h	0.016 ± 0.020	0.017 ± 0.013	0.726	0.020 ± 0.014	0.019 ± 0.015	0.688	0.021 ± 0.014	0.016 ± 0.011	0.128
Change in overall response			0.109			0.498			0.215
Valine (BCAA), mmol/L									
T=2h	-0.003 ± 0.030	-0.002 ± 0.021	0.904	0.003 ± 0.028	0.000 ± 0.022	0.504	0.009 ± 0.022	0.002 ± 0.025	0.326
T=4h	-0.005 ± 0.023	0.005 ± 0.020	**0.037**	0.006 ± 0.022	0.002 ± 0.017	0.394	0.004 ± 0.021	0.004 ± 0.030	0.910
T=6h	0.003 ± 0.030	0.007 ± 0.019	0.718	0.004 ± 0.019	0.007 ± 0.017	0.518	0.007 ± 0.019	0.008 ± 0.020	0.660
Change in overall response			0.159			0.432			0.679

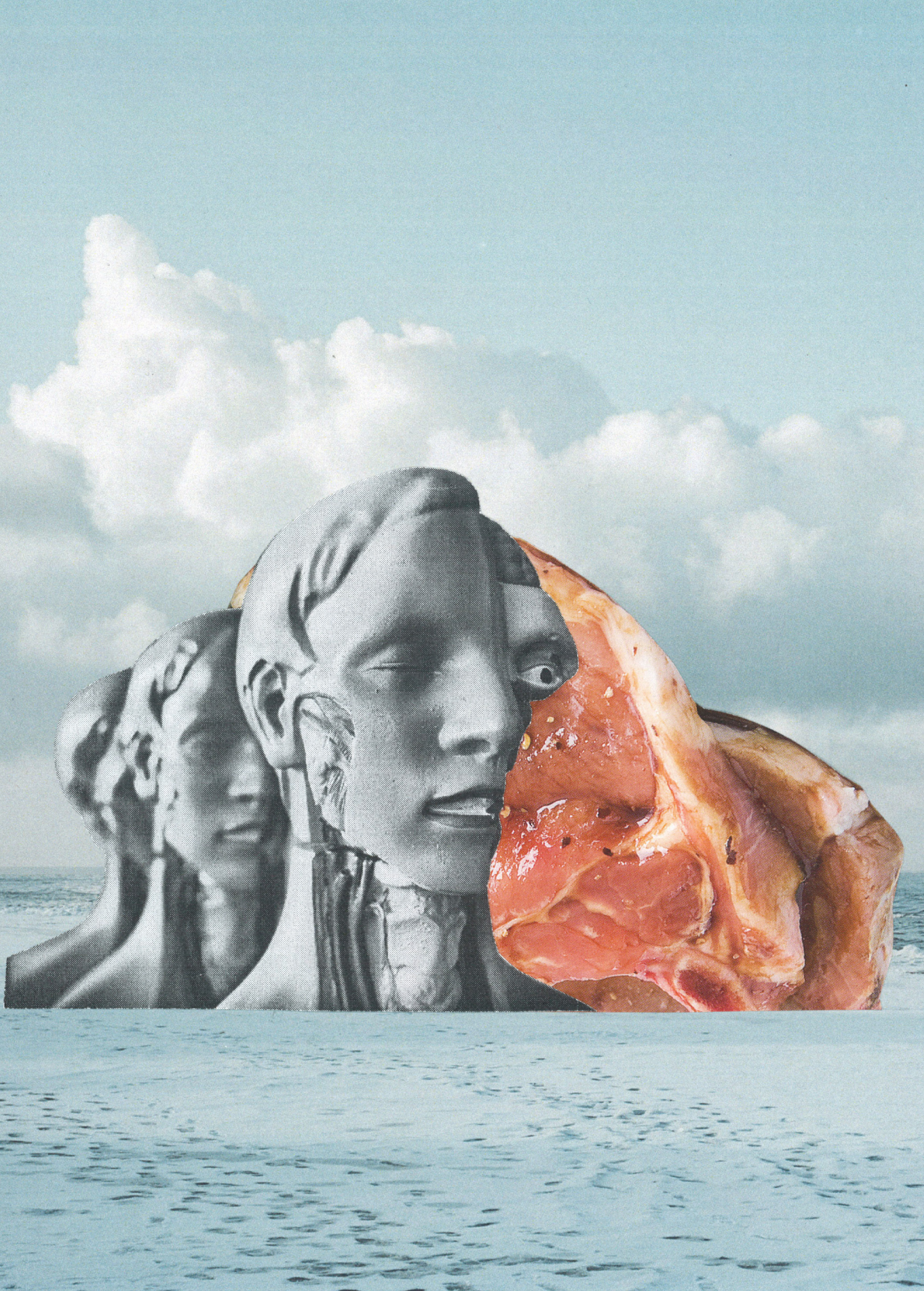
Supplemental Table 15. Changes in postprandial proteins upon 12 weeks of intervention

	WESTERN-TYPE DIET GROUP			TARGETED DIET GROUP			CONTROL GROUP		
	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value	Response baseline Log_2 ratio	Response after 12 weeks Log_2 ratio	p-value
Creatinine, mmol/L									
T=2h	-0.001 ± 0.011	-0.002 ± 0.005	0.710	-0.002 ± 0.006	-0.002 ± 0.005	0.952	-0.001 ± 0.006	-0.002 ± 0.007	0.775
T=4h	-0.002 ± 0.009	-0.004 ± 0.005	0.212	0.005 ± 0.005	-0.003 ± 0.005	0.266	-0.002 ± 0.007	-0.004 ± 0.006	0.210
T=6h	-0.001 ± 0.010	-0.004 ± 0.005	0.156	-0.003 ± 0.006	-0.001 ± 0.004	0.274	-0.001 ± 0.006	-0.004 ± 0.004	0.065
Change in overall response			0.426			0.330			0.417
Albumin, signal area									
T=2h	-0.001 ± 0.009	-0.004 ± 0.004	**0.049**	-0.003 ± 0.006	-0.006 ± 0.003	**0.030**	-0.003 ± 0.004	-0.003 ± 0.004	0.493
T=4h	-0.002 ± 0.010	-0.005 ± 0.004	0.104	-0.003 ± 0.005	-0.005 ± 0.004	**0.015**	-0.004 ± 0.004	-0.003 ± 0.003	0.141
T=6h	-0.003 ± 0.009	-0.006 ± 0.004	0.070	-0.004 ± 0.005	-0.007 ± 0.003	**0.003**	-0.004 ± 0.003	-0.004 ± 0.003	0.895
Change in overall response			0.982			0.519			**0.043**
Glycoprotein acetyls, mainly orosomucoid, mmol/L									
T=2h	-0.003 ± 0.147	-0.049 ± 0.065	0.074	-0.036 ± 0.099	-0.068 ± 0.044	**0.056**	-0.026 ± 0.095	-0.046 ± 0.064	0.338
T=4h	0.030 ± 0.128	-0.023 ± 0.057	**0.011**	0.010 ± 0.069	-0.036 ± 0.053	**<0.001**	-0.018 ± 0.065	0.000 ± 0.063	0.221
T=6h	0.047 ± 0.129	-0.004 ± 0.066	**0.017**	0.036 ± 0.070	0.002 ± 0.062	**0.039**	0.023 ± 0.072	-0.001 ± 0.062	0.248
Change in overall response			0.865			0.467			**0.006**



Supplemental Figure 1. Examples of changes in postprandial responses of lipoproteins

Example of postprandial responses (minus fasting value) of medium VLDL (A), IDL (B), medium LDL (C) and medium HDL particles (D). * indicates a significant difference ($p<0.05$) in response on a specific time point between before and after the intervention.



5

A HIGH QUALITY NUTRIENT ER-DIET INDUCES A DISTINCT ADIPOSE TISSUE GENE EXPRESSION PATTERN WHEN COMPARED TO A LOW QUALITY WESTERN-TYPE ER-DIET

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ABSTRACT

Background: White adipose tissue (WAT) is an important metabolic organ of which dysregulation can accelerate the onset of metabolic diseases. Energy restricted (ER) diets, whether or not enhanced in nutrient quality, might be important modulators of WAT health and functioning which can be assessed using sensitive techniques such as transcriptomics. In this manuscript we investigate the effects of two ER-diets differing in nutrient quality on changes in fasting and postprandial whole genome gene expression in WAT of abdominally obese individuals.

Methods: A parallel-designed, randomized trial was performed previously in which 110 abdominally obese males and females (BMI >27 kg/m^2 or waist circumference >88cm females, >102cm males) were allocated to either a high nutrient quality 25% ER-diet, Targeted diet (TD), rich in soy protein, fiber, monounsaturated fatty acids, and *n-3* polyunsaturated fatty acids, a low nutrient quality Western-type 25% ER-diet (WD), rich in saturated fatty acids and fructose, or a control group not receiving dietary intervention. SAT biopsies were taken before and after the 12wk intervention, after an overnight fast as well as 4h after consumption of a mixed meal (76.3g carbohydrates, 17.6g protein, 60.0g fat). Samples of 82 participants (29 TD, 31 WD, 21 control) were used for micro array analysis using human whole-genome Affymetrix arrays.

Results & Conclusion: Both diets induced clinically relevant weight loss of -8.5 ± 3.1kg in the TD and -6.1 ± 4.1kg in the WD. In the fasted state, the TD induced a distinct adipose tissue gene expression pattern when compared to the WD, characterized by a greater attenuation in lipid, cholesterol, and energy metabolism and a reduction in insulin-related PI3/Akt signaling. Although weight loss was significantly larger in the TD than in the WD, changes in the expression of genes involved in these pathways were in almost all cases not correlated to weight loss, suggesting a role for nutrient quality in the observed differential effects between the diets. In the postprandial phase, the WD showed a decreased adaptive response to the mixed meal, reflected by a blunted response of lipid anabolic processes such as lipid and cholesterol synthesis. We conclude that a high nutrient quality ER-diet enhances the benefits of weight loss and might improve adipose tissue health on the long run.

ABBREVIATIONS

CR	Calorie restriction
DHA	Docosahexaenoic acid
EGF	Epidermal growth factor
EPA	Eicosapentaenoic acid
ER	Energy restriction
FDR	False discovery rate
FGF	Fibroblast growth factor
GSEA	Gene set enrichment analysis
HOMA-IR	Homeostatic model assessment of insulin resistance
IGF	Insulin-like growth factor
IRS	Insulin receptor substrate
MMT	Mixed meal test
mTOR	Mechanistic target of rapamycin
MUFA	Monounsaturated fatty acid
PI3/Akt	Phosphoinositide 3-kinase / protein kinase B
PPAR	Peroxisome proliferator-activated receptor
PUFA	Polyunsaturated fatty acid
SAT	Subcutaneous adipose tissue
SFA	Saturated fatty acid
SREBP	Sterol regulatory element-binding protein
TCA	Tricarboxylic acid
TD	Targeted diet
UPP	Ubiquitin proteasome pathway
WAT	White adipose tissue
WD	Western-type diet

INTRODUCTION

White adipose tissue (WAT) is an important endocrine organ involved in metabolic processes such as buffering lipid fluxes by storing, synthesizing, desaturating, oxidizing, and releasing fatty acids[1]. Furthermore, WAT is involved in systemic processes such as whole body insulin sensitivity and inflammation through the production of adipokines and inflammatory mediators[2]. Obesity is characterized by a dysregulation of WAT, which include impairments in lipid metabolism, local tissue insulin resistance, adipocyte hypertrophy, inadequate vascularization and thus decreased oxygenation, and tissue inflammation[3]. These disturbances can accelerate the onset of metabolic diseases such as type 2 diabetes[4-6]. Therefore, WAT is an important target organ of dietary interventions that are designed to induce weight loss and improve cardio-metabolic risk factors. An evidently effective dietary intervention in this context is a restriction of daily energy intake by ~10-40%, i.e. energy restriction (ER), which results in loss of body weight and can alleviate WAT dysfunctioning for instance by lowering inflammation[7].

Although ER can induce weight loss regardless which macronutrients are emphasized within the diet, different nutrients might exert distinct effects on WAT. For instance, dietary fatty acids can serve as ligands for transcriptional regulators expressed in the adipose tissue, such as the family of peroxisome proliferator-activated receptors (PPARs) which are involved in various pathways related to lipid and glucose metabolism[8,9]. However, this action has mainly been described for polyunsaturated fatty acids (PUFA). Activation of PPARs by saturated fatty acids (SFA) is suggested to be minimal or even absent[10], elegantly demonstrating differential effects of certain nutrients on WAT metabolism.

As WAT is sensitive and responsive to dietary changes, analysis of changes in gene expression in this tissue might be a valuable tool to assess the effects of nutritional interventions. Although taking biopsies might be an invasive procedure, WAT transcriptomics has indeed been applied more often to assess the effectiveness of experimental diets, either eucaloric or energy restricted[11]. Furthermore, there is growing interest in optimizing the nutrient composition of these diets to further enhance cardio-metabolic benefits, potentially via improvements of WAT functioning. Popular dietary strategies studied on WAT transcriptomics include modulating the macronutrient composition of ER-diets, for instance by reducing energy percentage from fat[12] or increasing protein content[13,14]. Another strategy is increasing the quality of nutrients, for instance by modulating the glycemic index of the diet[15]. Combining these strategies into one whole diet approach can be a powerful tool to increase cardio-metabolic health through enhanced WAT health and functioning. These improvements, for instance a decrease

in inflammation, can be studied in the fasted state. However, as WAT plays a crucial role in buffering fatty acids fluxes in the postprandial period1, it might be more interesting to study potential improvements in these processes after consumption of a test meal. The application of a so-called mixed meal test, high in fat, sugar and protein, is becoming increasingly popular as a nutritional challenge test[16]. Readouts for such a challenge can range from analyzing postprandial plasma markers to analyzing postprandial gene expression patterns in tissues such as WAT. These measurements reflect the capacity of an individual to adapt to the challenge and regain a homeostatic state.

In this manuscript we investigate the effects of two ER-diets differing in nutrient quality on changes in whole genome gene expression in subcutaneous white adipose tissue (SAT). We designed a high nutrient quality ER-diet, rich in soy protein, fiber, MUFA, and *n-3* PUFA, and compared the effects of this diet to a Western-type ER-diet and a control group that did not receive any dietary intervention. We analyzed the effects of the diets on gene expression profiles in the fasted state but also in the postprandial phase, after consumption of a mixed meal. This allowed us to investigate the effects of interventions on the capacity of SAT to adapt to this dietary stressor.

METHODS

The study was approved by the Medical Ethics Committee of Wageningen University and registered at ClinicalTrials.gov, identifier: **NCT02194504**. A detailed description of the study and primary study outcomes can be found elsewhere. In short, 110 healthy participants aged 40-70 years with abdominal obesity (BMI >27kg/m^2 or waist circumference >88cm for females, >102cm for males) were randomized over three groups; a Western-type (WD) or Targeted (TD) energy restriction diet group or a control group. The two diets had an equal energy restriction of 25%. The TD aimed to improve metabolic health via enrichment with soy protein, fiber, MUFA, and *n-3* PUFA. In the WD these components were substituted by SFA, predominantly animal protein, and fructose. Participants in the control group did not receive intervention and were asked to maintain their habitual dietary habits. The intervention lasted 12 weeks and was based on dietary advice and the provision of key products, e.g. cooking oils and fats, dairy or soy products, snacks. Participants visited our facility on a weekly basis for an individual session with one of the qualified dieticians. Reported intake values of participants in both diets groups resembled advices intake values and are shown in **Table 1**. Before and after 12 weeks of intervention several cardio-metabolic risk factors were assessed, including plasma markers that reflect glucose homeostasis and insulin sensitivity, blood lipids, intrahepatic lipids, and abdominal body fat distribution. Plasma markers were

assessed in the fasted state but also in the postprandial phase after consumption of an in-house prepared mixed meal, consisting of 76.3g carbohydrates, 17.6g protein, and 60.0g fat.

Table 1. Nutritional composition of the two experimental diets and reported intake

	WESTERN-TYPE DIET GROUP *n*=39		TARGETED DIET GROUP *n*=34		Differences in reported intake between diets
	Advised	Reported	Advised	Reported	p-value
Energy intake (MJ)	6.95 ± 0.78	7.13 ± 0.82	6.99 ± 0.90	7.07 ± 1.00	0.778
ER (%)	25.8 ± 5.4	23.9 ± 0.82	26.9 ± 5.6	26.2 ± 5.9	0.775
En% Fat	31.5 ± 0.4	31.4 ± 1.6	32.1 ± 0.3	32.5 ± 1.2	**0.001**
En% SFA	15.5 ± 0.4	14.9 ± 1.5	7.9 ± 0.5	8.3 ± 0.6	**<0.001**
En% MUFA	9.0 ± 0.4	9.3 ± 1.0	13.9 ± 0.7	13.4 ± 0.9	**<0.001**
En% PUFA	3.9 ± 0.3	4.1 ± 0.8	7.8 ± 0.2	7.7 ± 0.4	**<0.001**
En% Trans fat	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	**0.015**
ALA (mg)	1057 ± 67	1104 ± 278	1795 ± 35	1815 ± 354	**<0.001**
EPA & DHA (mg)	33 ± 5	103 ± 226	1270 ± 0	1224 ± 55	**<0.001**
Cholesterol (mg)	183 ± 23	185 ± 30	124 ± 19	132 ± 20	**<0.001**
En% Protein	15.4 ± 0.2	16.0 ± 1.4	20.4 ± 0.1	20.6 ± 1.7	**<0.001**
En% Animal-based	9.9 ± 0.3	10.3 ± 1.1	9.8 ± 0.2	10.3 ± 1.9	0.886
En% Plant-based	5.5 ± 0.1	5.7 ± 1.0	10.5 ± 0.1	10.3 ± 0.5	**<0.001**
En% Carbohydrates	50.5 ± 0.2	49.6 ± 2.0	44.0 ± 0.3	43.7 ± 1.5	**<0.001**
En% Mono- and disaccharides	25.3 ± 0.8	24.8 ± 1.9	19.0 ± 0.5	18.8 ± 1.1	**<0.001**
En% Polysaccharides	25.2 ± 0.9	24.8 ± 1.2	25.0 ± 0.4	24.9 ± 1.1	0.905
En% Fructose	7.4 ± 0.1	7.0 ± 0.9	3.5 ± 0.4	3.4 ± 0.4	**<0.001**
En% Fiber	2.2 ± 0.1	2.2 ± 0.2	3.2 ± 0.0	3.1 ± 0.2	**<0.001**
En% Alcohol	0.8 ± 0.3	1.1 ± 0.9	0.8 ± 0.3	1.0 ± 0.7	0.599
Sodium (g)	1.6 ± 0.2	2.3 ± 0.6	1.7 ± 0.3	2.3 ± 0.4	0.659

Data are presented as mean ± 1SD. Values were calculated based on the Dutch Food Composition Database 2011 (NEVO 2011/3.0, RIVM, Bilthoven, The Netherlands). Comparisons between groups were conducted using Independent t-tests. Advised values represent the average intake values across the 6, 7, 8, and 9MJ energy groups. Values are averaged over a 1-week menu cycle. Abbreviations: MJ: Mega joule, ER: Energy restriction, En%: Energy percentage, SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, ALA: alpha linolenic acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid

Adipose tissue biopsies

Before and after the intervention, subcutaneous adipose tissue biopsies were taken caudally from the umbilicus under local anesthesia (1% lidocaine) using a small liposuction cannula. Sampling was conducted after an overnight fast, both before consumption of the mixed meal (T=0h) as well as four hours after (T=4h). Samples were rinsed with phosphate-buffered saline to eliminate blood and immediately frozen in liquid nitrogen and stored at -80 °C. Biopsies were taken by trained nurses on sagittal different locations on T=0h compared to T=4h to prevent analysis of acute inflammation and wound healing mediators.

RNA isolation and microarray processing

RNA was isolated from frozen samples using a TRIzol®/chloroform extraction (Thermo Fisher Scientific, Waltham, USA) and purified using the Qiagen Mini column kit according to manufacturers' protocol (Qiagen, Hilden,

Germany). RNA yield was quantified on a NanoDrop ND 1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA) while RNA integrity was measured on an Agilent 2100 BioAnalyzer with RNA 6000 Nanochips (Agilent Technologies, Santa Clara, USA). Samples with RNA integrity (RIN) score of 6.2 and higher were included, average RIN-score was 8.0. Total RNA was labelled using a one-cycle cDNA labeling kit (MessageAmp II-Biotin Enhanced Kit; Ambion) and hybridized to Genechip Human Gene 2.1 ST arrays (Affymetrix, Santa Clara, CA). Sample labelling, hybridization to chips and image scanning was performed according to the manufacturers' instructions. Raw CEL files were normalized using the Robust Multi-array Average algorithm[17], as implemented in the affyPLM R-package[18]. A custom annotation was used based on reorganized oligonucleotide probes, which combines all individual probes for a gene (MBNI Brainarray CDF file (ENTREZG v21)).

Microarray analysis

Significant differences in expression were assessed using LIMMA[19]. For all comparisons genes were defined as significantly different when the P-value was < 0.05. Analyses of changes in fasting gene expression were conducted by comparing the change (12wk-0 wk) in gene expression between the three different groups (F-test and t-tests) as well as by analysing the change within an intervention group (paired t-test). Analyses of changes in postprandial gene expression were conducted by comparing the change (12wk-0wk) in response (4hr-0hr) between the three different groups (F-test and t-tests) as well as by analysing the change in response within an intervention group (paired t-test). To take into account the paired design of our study, subjects were treated as random effects using the "duplicateCorrelation" function.

Pathway analysis

We performed pre-ranked gene set enrichment analysis (GSEA; http://www.broad.mit.edu/gsea)[20]. Briefly, genes were ranked based on the t-statistic and analyzed for positive and negative enrichment in predefined gene sets. Gene sets were derived from Biocarta, KEGG, Reactome and Wikipathways databases. Gene sets more or less enriched at FDR Q-value <0.25 were considered as statistically significant, these sets were subsequently visualized and clustered using the Enrichment Map[21] feature of Cytoscape 3.4.0.

RESULTS

In the previous study, 110 participants were randomly allocated to one of the three intervention groups. In total 100 participants completed the trial, of which 39 in the WD, 34 in the TD, and 27 in the control group. Adipose tissue biopsies from 90 participants of the 100 that completed the trial were collected, some samples

were missing or not collected due anticoagulant use, lidocaine allergy or a low yield of fatty tissue. Furthermore, samples of 2 participants were excluded because of low quality RNA. The samples of 81 participants out of the 88 with good quality RNA were randomly selected for microarray of which 31 in the Western-type diet group, 29 in the Targeted diet group, and 21 in the control group. The *n*=81 subgroup was a representative sample of the total study population, baseline characteristics and changes in important metabolic parameters after intervention of this group are summarized in **Supplemental Table 1**. Mean age of the participants was 60-61 years, 42-45% of the participants were male. Twelve weeks of ER resulted in significant weight loss of -6.1 ± 4.1kg in the WD and -8.5 ± 3.1kg in the TD, which was significantly different from the control group but also significantly different between the two diet groups despite equal ER. Weight loss in both diet groups was accompanied by a reductions in waist circumference, subcutaneous and visceral adipose tissue mass, and intrahepatic lipids. Both diets improved fasting plasma glucose and insulin. Only the TD reduced fasting plasma triglycerides and serum total cholesterol.

Effects of the dietary interventions on fasting gene expression

Figure 1A shows a flow chart of the number of genes differentially changed between the three intervention groups. Upon intervention, there were 1643 genes of which the change in expression was significantly different between the three groups. Between the control group and the TD 932 genes were differentially changed, compared to 692 genes between the control group and the WD. Between the two ER-diets there were 838 genes differentially changed. To better understand the biological pathways affected by the two ER-diets, Gene set enrichment analysis (GSEA) was performed. **Figure 1B** shows a flow chart of the number of pathways differentially enriched between the three intervention groups.

745 pathways were significant differentially enriched between the TD and the WD. To determine the robust effect of the intervention, all pathways differentially enriched between the two ER-diets at baseline were excluded as well as all pathways that were not significantly enriched within either the TD or the WD or both, resulting in 128 remaining gene sets. To identify overlap in genes between the different gene sets, this 128 gene sets that were left were clustered using Cytoscape EnrichmentMap, (**Supplemental Figure 1**). A summary of the findings from this clustering analysis is presented in **Table 2**. Three main clusters that could be distinguished were metabolic pathways, mitochondrial energy production, and phosphoinositide 3-kinase / protein kinase B (PI3/Akt) signaling. The metabolic pathways contained gene sets related to lipid metabolism, including fatty acid and triglyceride synthesis, fatty acid oxidation, and PPARa targets, cholesterol biosynthesis, and carbohydrate

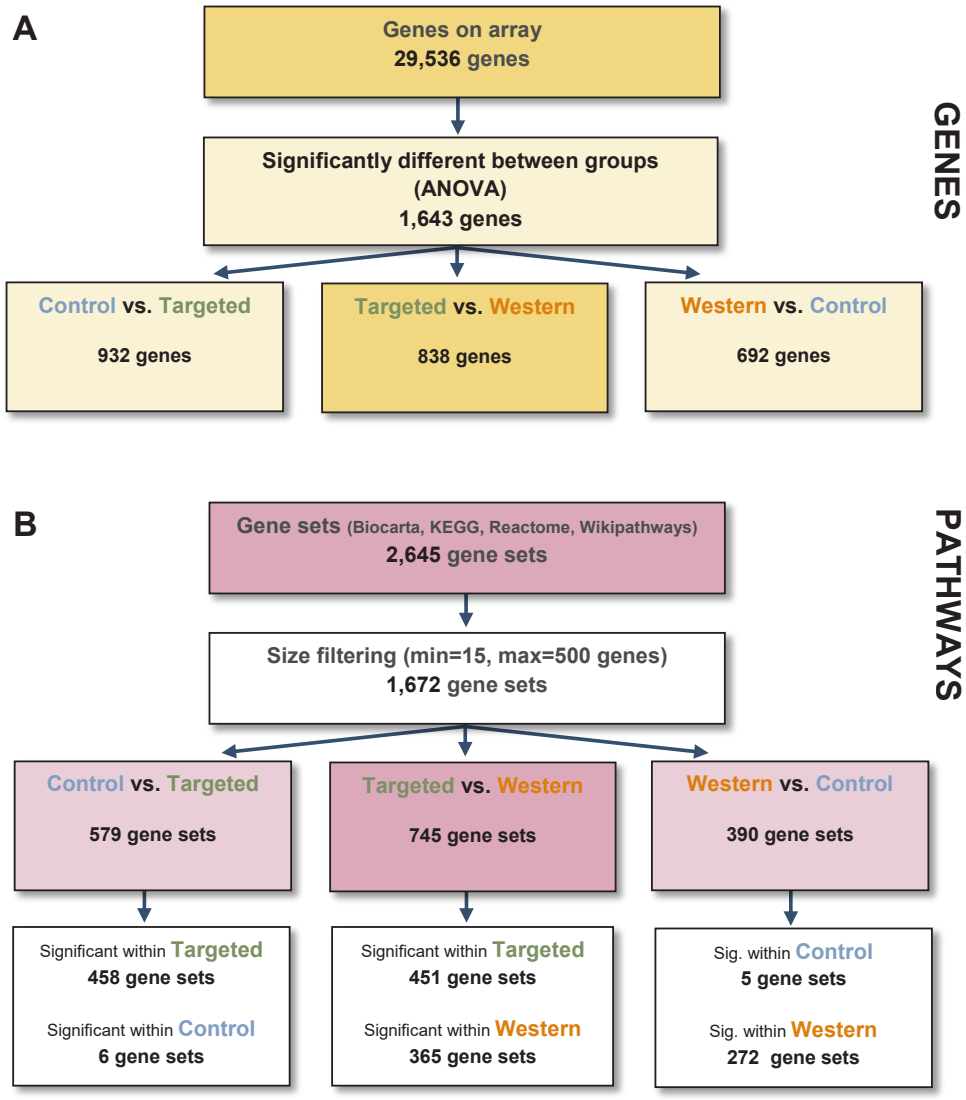


Figure 1. Effects of dietary interventions on fasting gene expression

Flow charts shows the number of genes (A) differentially changed between the interventions as well as the number of pathways (B) indicated by gene sets differentially changed between the interventions.

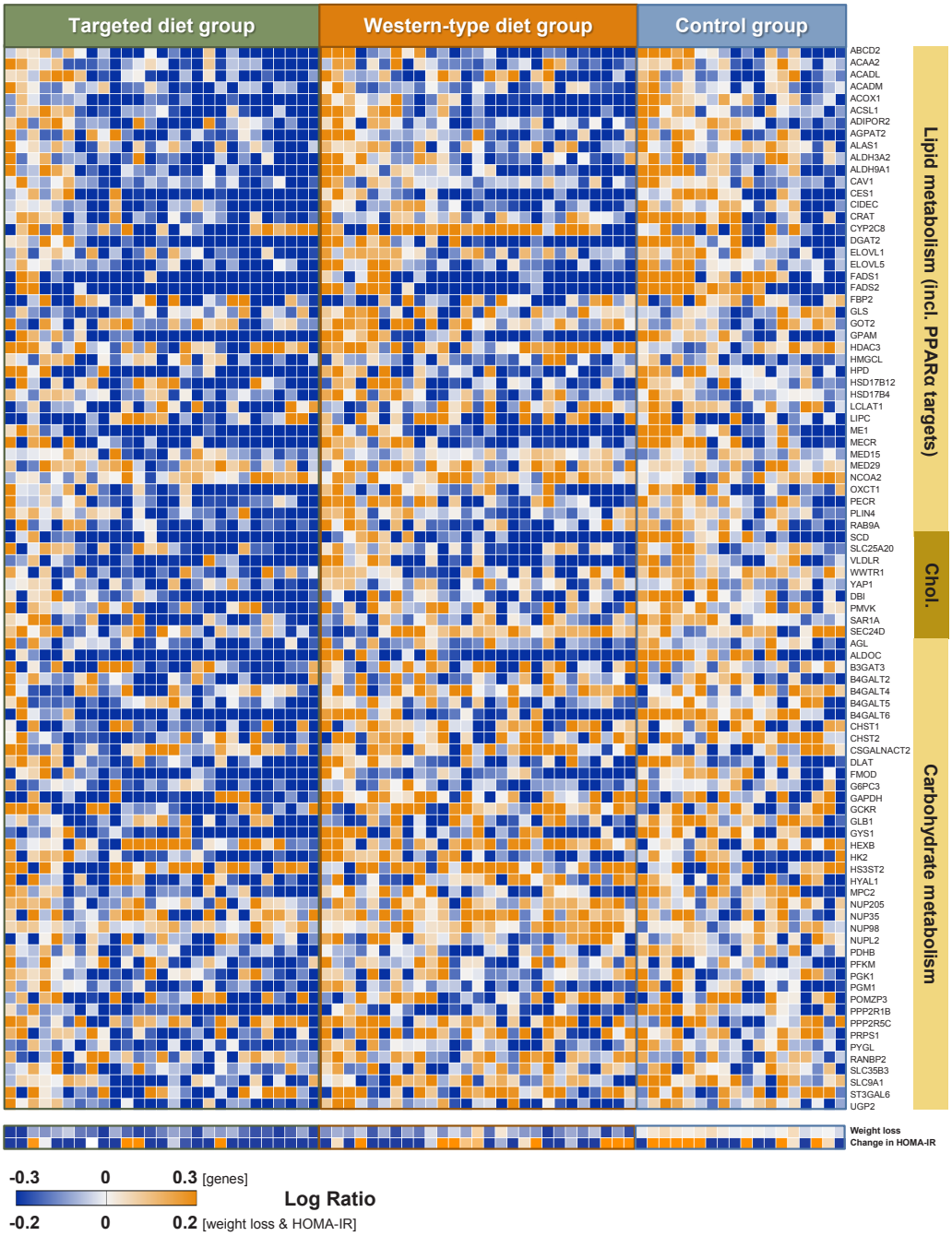
Table 2. Pathways differentially enriched between the two ER-diets using GSEA

	EFFECT WITHIN TARGETED DIET GROUP	EFFECT WITHIN WESTERN-TYPE DIET GROUP
1. METABOLIC PATHWAYS		
Lipid metabolism, including fatty acid and triglyceride synthesis	↓↓	↓
Fatty acid beta-oxidation	↓↓	↓
PPARa targets	↓↓	↓
Cholesterol biosynthesis	↓↓	↓
Carbohydrate metabolism, including glycolysis and gluconeogenesis	↓↓	↓
2. MITOCHONDRIAL ENERGY PRODUCTION		
Oxidative phosphorylation	↓↓	↓
Electron transport chain	↓	-
TCA cycle	↓↓	↓
3. PI3/AKT SIGNALING		
PI3/Akt signaling	↓	-
Stimulation of PI3/Akt by EGF, IGF, and FGF	↓	-
Downstream target of PI3/Akt mTOR and IRS signaling	↓	-

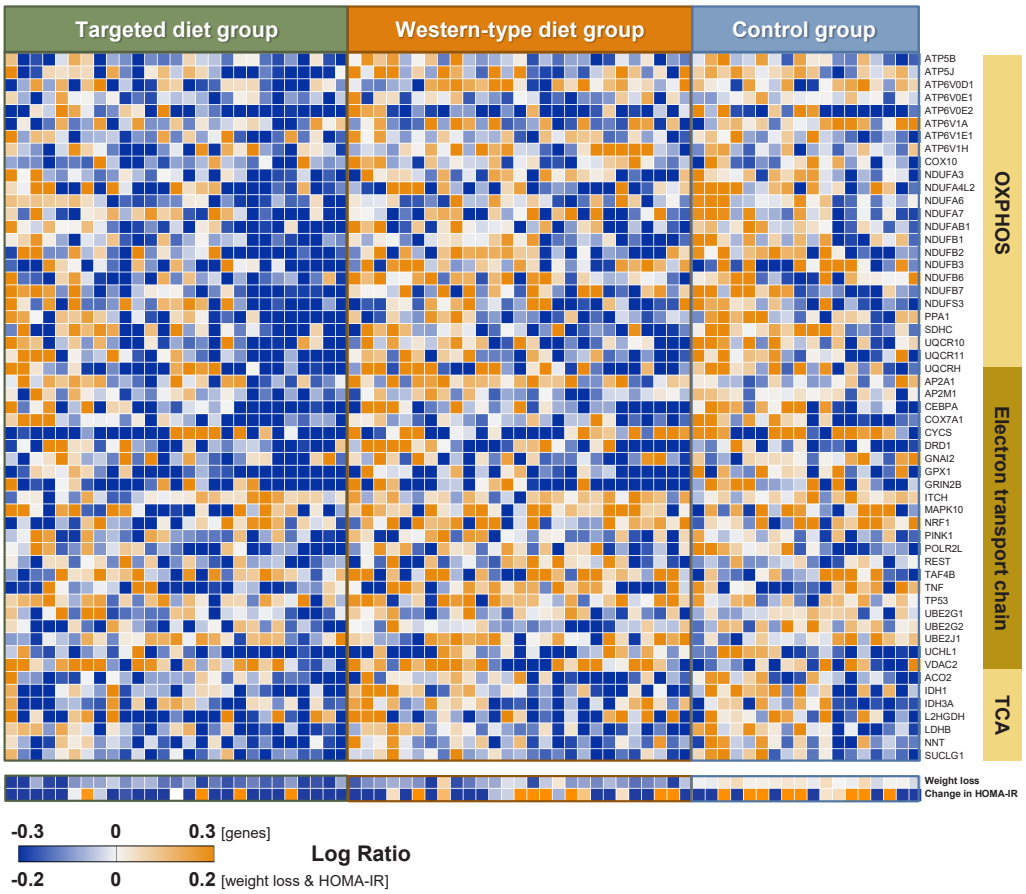
Gene sets differentially affected by the Targeted and Western-type diet using a False Discovery Rate Q-value <0.25. Abbreviations: PPARa: peroxisome proliferator-activated receptor alpha, TCA: tricarboxylic acid, PI3/Akt: phosphoinositide 3-kinase / protein kinase B, EGF: epidermal growth factor, IGF: insulin-like growth factor, FGF: fibroblast growth factor, mTOR: mechanistic target of rapamycin, IRS: insulin receptor substrate

metabolism. Mitochondrial energy production consisted of oxidative phosphorylation, the electron transport chain, and the tricarboxylic acid (TCA) cycle. The PI3/Akt signaling cluster included the stimulation of this pathway by the growth factors epidermal growth factor (EGF), insulin-like growth factor (IGF), and fibroblast growth factors (FGF), as well as two cytosolic downstream targets of PI3/Akt namely mechanistic target of rapamycin (mTOR) and insulin receptor substrate (IRS) signaling. All pathways were significantly more decreased by the TD than the WD. Genes that contributed to the enrichment in the 128 gene sets were extracted, genes that were not significantly changed within the either the TD or WD were removed. Individual changes in the remaining 172 genes were visualized by the heatmaps in **Figure 2A-C**. Weight loss and changes in HOMA-IR, as measure of insulin resistance, were included in the figures to visualize the relation between these parameters and changes in gene expression. In addition, correlation analyses between these parameters were performed (**Supplemental Table 2**). Of the 172 genes, weight loss was correlated to the change in 2 genes in the TD and 5 in the WD while the change in HOMA-IR was correlated to the change in 3 genes in the TD and 1 gene in the WD. No significant correlations were observed in the control group. Between the control and the TD, 579 gene sets

A. Metabolic pathways



B. Mitochondrial energy production



C. PI3/Akt signaling

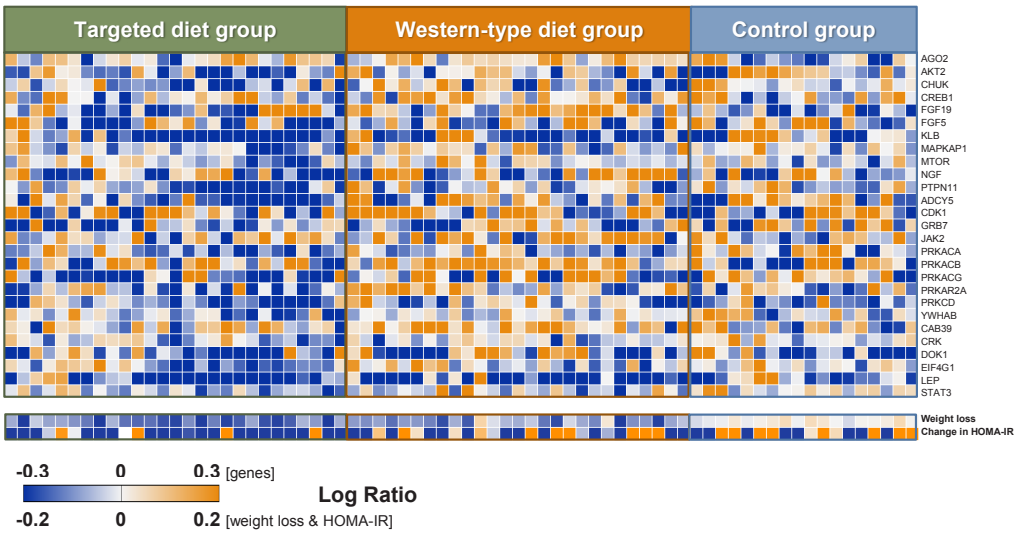


Figure 2. Heatmaps of individual changes in genes from differentially enriched pathways between the Targeted and Western-type diet

Individual changes in gene expression, clustered per diet, of genes contributing to differential enrichment in the three clusters between the Targeted and Western-type diet. A: genes related to metabolic pathways, B: genes related to mitochondrial energy production, C: PI3/Akt signaling.

were differentially enriched compared to 390 gene sets between the control and WD. A summary of the effects of the two diets compared to the control can be found in **Supplemental Table 3**.

Effects of the dietary interventions on postprandial gene expression

Figure 3A shows a flowchart of the number of genes of which the response to the MMT changed after the interventions. Upon intervention, there were 1118 genes of which the change in response to the MMT was significantly different between the three groups. Between the control group and the TD the change in response of 571 genes was significantly different, compared to the change in response of 487 genes between the control group and the WD. Between the two ER-diets the response of 611 genes was significantly different. Figure 3B shows a flow chart of the number of pathways of which the response to the MMT was significantly different between the three intervention groups. Between the control and the TD, the response of 79 gene sets was significantly different compared to the response of two gene sets between the control and WD. Between the two ER-diets the response of 420 gene sets was significantly different. In line with the analysis on fasting gene expression, all pathways of which the response was different between the two ER-diets at baseline as well as all pathways of which the response was not significantly changed within the TD, WD, or both were excluded. This was done to determine the robust effect of the intervention on the change in pathways response between the two ER-diets. The 18 gene sets that were left using this approach included metabolic pathways, such as lipid metabolism and cholesterol biosynthesis, the TCA cycle, and the synthesis and release of neurotransmitters including the appetite-regulating hormone ghrelin (**Supplemental Table 4**).

As lipid metabolism, cholesterol biosynthesis, and the TCA cycle were also affected by the two ER-diets in the fasted state, the effects of the interventions on both the fasting and postprandial regulation of these pathways were summarized and visualized in **Figure 4A-C**. All three pathways showed a comparable pattern. In the fasted state, the pathways were decreased upon both interventions but significantly more in the TD when compared to the WD. In the postprandial, the initial increase in all three pathways upon consumption of the MMT was significantly attenuated or abolished in the WD. The TD induced significant attenuation of the increase in cholesterol biosynthesis.

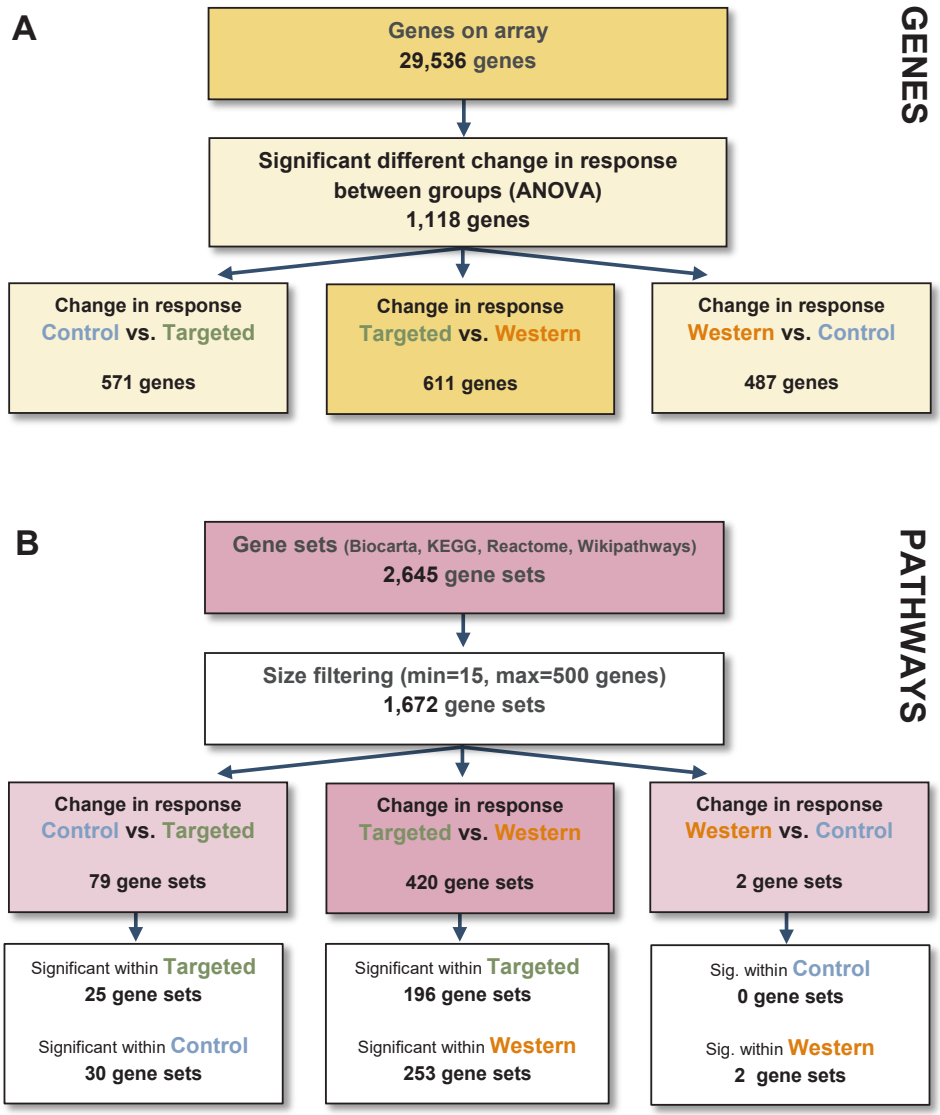


Figure 3. Effects of dietary interventions on postprandial gene expression

Flow charts shows the number of genes (A) differentially changed between the interventions as well as the number of pathways (B) indicated by gene sets differentially changed between the interventions

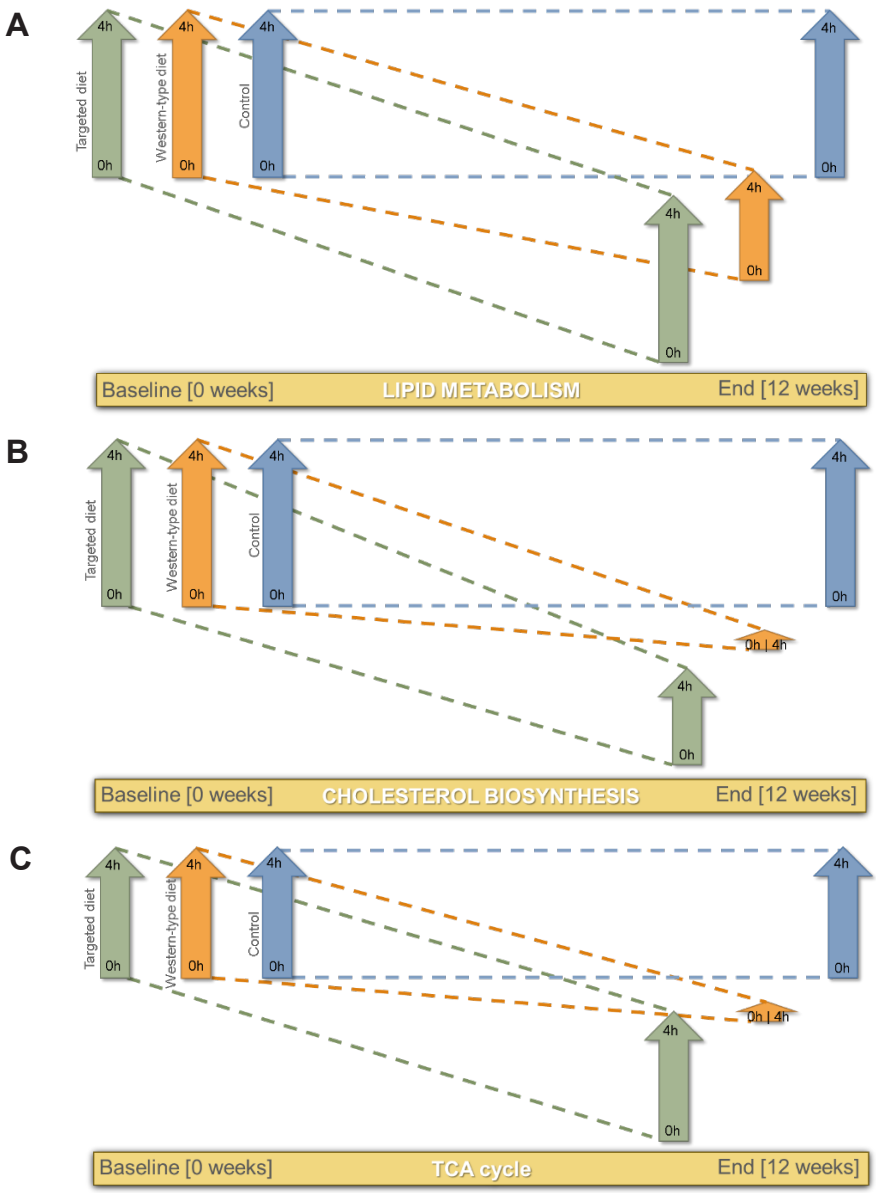


Figure 4. Effects of the dietary interventions on fasting and postprandial lipid metabolism (A), cholesterol biosynthesis (B), and TCA cycle (C)

Bottom of the arrow (0h) represents the fasting state, top of the arrow (4h) represents postprandial state, i.e. 4 hours of consumption of the mixed meal. The size of the arrow at baseline represents the relative increase in postprandial gene expression, the size of the arrow at 12 weeks showed the relative change upon intervention. In the Targeted diet, the initial increase in cholesterol biosynthesis was reduced upon intervention. In the Western-type diet, the initial increase in lipid metabolism was reduced upon intervention while the initial increase in cholesterol biosynthesis and TCA cycle was abolished upon intervention. In the control group, no changes in postprandial response were observed.

DISCUSSION

In this manuscript, we have investigated the effects of two 25% ER-diets differing in nutrient quality on fasting gene expression profiles in an important target organ for weight loss interventions; the subcutaneous adipose tissue (SAT). Furthermore, we have investigated the effects of these ER diets on the adaptive capacity of the adipose tissue to a mixed meal test. In the fasted state, we observed differential regulation in multiple pathways between the high nutrient quality ER-diet (TD), enriched with soy protein, fiber, MUFA, and *n-3* PUFA, and the low nutrient quality Western-type diet (WD). The TD induced a reduction in fasting PI3/Akt signaling which was not observed in the WD. Both ER-diets reduced fasting mitochondrial energy production as well as in lipid and cholesterol metabolism but these reductions were significantly larger in the TD than in the WD. In the postprandial phase, we also observed a differential regulation in lipid and cholesterol metabolism, due to a stronger postprandial suppression of these processes in the WD when compared to the TD.

Only the TD downregulated expression of several genes related to PI3/Akt including activators of this pathway such as EGF[22] and IGF[23] at fasting. PI3/Akt is a lipid kinase signaling pathway involved in numerous cellular functions. The pleiotropic effects of this master regulator are caused by the phosphorylation of many nuclear and cytosolic downstream targets, including mTOR and insulin receptor substrate signaling[24,25]. A reduction in mTOR activity after CR is not a novel finding. In fact, the increase in life-span observed in many animal CR-models is thought to be partly attributed to this decrease[26-28]. However, data from human studies on a reduction in mTOR signaling by weight loss is scarce. A recent study showed a downregulation in IGF-stimulated PI3/Akt/mTOR signaling in SAT from obese women upon ER, but this intervention lasted on average 4 years[29]. The accepted theory is that a decrease in mTOR signaling results in a suppression of cellular senescence. This is a state in which cells cease to divide but become metabolically overactive and insensitive to hormones and growth factors, which is a common feature of age-related metabolic disease such as type 2 diabetes[30,31]. A downregulation in mTOR and insulin receptor substrate signaling might thus indicate improved local SAT insulin sensitivity, which can be an early organ adaptation in the TD that is not reflected yet by differences in systemic parameters between the two diets, such as differences in HOMA-IR.

Both ER-diets decreased the expression of genes involved in mitochondrial energy production with significantly larger reduction in the TD when compared to the WD. A reduction in oxidative phosphorylation in SAT upon ER has been described previously and is thought to be an adaptive mechanism to the decrease in nutrients[32-34]. Both ER-diets also reduced the expression of genes involved in the synthesis of free fatty acids, triglycerides,

and cholesterol, which makes sense in the process of losing weight and has also been described in other human ER-studies[32,35]. Remarkably, fatty acid beta-oxidation was also reduced in both ER-diets with a more pronounced reduction in the TD. A shift from carbohydrate to fat metabolism is thought to be a key feature of ER that is needed to compensate for the reduction in substrate availability, mainly the lack of glucose[28,36]. This means that an upregulation in beta-oxidation after ER is expected, rather than a downregulation. Two other human ER-studies indeed showed an increase in SAT proteins involved in beta-oxidation but only after a weight maintenance phase[37,38]. In literature it has been shown that ER induces a decrease in resting energy expenditure that is larger than what can be expected from the decrease in fat mass and fat free mass[39-41], indicating a role for adaptation in adipose tissue processes such as decrease beta-oxidation. We therefore speculate that a decrease in energy expenditure in our study may be the underlying cause of the observed decrease in gene expression of fat oxidation upon ER.

The TD was significantly more effective than the WD in downregulating gene expression of metabolic processes and only the TD reduced expression of genes involved in PI3/Akt signaling. This difference is likely caused by the high quality nutrients in the TD but we cannot completely exclude effects of the extra weight loss in TD. However, the correlation analyses between weight loss and changes in gene expression yielded little substantiation for this assumption, as the change in only two genes was correlated to weight loss in the TD. The differential effects between the diets may thus be caused by the high quality nutrients for example differences in the type of dietary protein and fat between the diets. The *n-3* PUFA EPA and DHA as well as MUFA, of which the intake was high in the TD, are better agonists of PPARs than the SFA, of which the intake was high in the WD. Activation of PPARs upregulates the expression of genes involved in, for example, fatty acid oxidation[42] in the in liver. In cause uncoupled from ATP production, this might have enlarged the energy deficit in the TD which in turn could have been a stimulus for the adipose tissue to go even further into a "stand-by mode" with regard to lipid and energy metabolism. In addition, *n-3* PUFA have the ability to suppress mTOR activity, which might explain our findings on the PI3/Akt reduction in TD, although this action has so far only been demonstrated in cell line and animal cancer models[43-45]. Our study showed that consumption of SFA, which was higher in WD, induced a pro-inflammatory expression profile reflected by an increase in Toll-like and cytokine signaling in the WD but not the TD, although the difference between groups failed to reach significance. In a previous study we also showed that 8wk consumption of a SFA-rich diet led to an pro-inflammatory gene expression profile in SAT[46]. However, in the current study, no significant difference in changes in these pathways between the WD and TD was observed. In our study, fatty acid saturation might thus have a played a non-beneficial role in regulating metabolic pathways

but was not very dominant in a differential regulation of inflammatory pathways. This might have been due to the high protein content of the TD, as we previously showed that ER with a relatively high protein content abolished a reduction in inflammation related gene expression observed after a normal protein ER-diet[14].

The TD was enriched with soy protein, which contains isoflavones. These phytochemicals exert specific actions that can be described as "CR mimicking". Cell studies showed that isoflavones can reduce mTOR signaling and IGF activity[47-49] which was also observed in our study. Furthermore, van der Velpen et al showed that 8 week consumption of isoflavone supplements can induce a downregulation of energy metabolism-related genes in SAT of postmenopausal women[50]. This might also explain the stronger downregulation of energy metabolism-related genes in the TD when compared to WD. Overall, the soy protein and *n-3* PUFA in the TD might have enhanced known ER-effects such as a reduction in mTOR signaling and energy metabolism, maybe even through a synergistic effect of these nutrients. Especially as these ER-effects were absent or only minimally induced in the WD which was soy-free and low in *n-3* PUFA. With respect to the differential regulation of mTOR signaling and energy metabolism between the TD and the TD, protein source might thus have played a larger role than protein quantity. Especially since two other human intervention studies have compared to effects of high- versus normal animal protein ER-diets on SAT gene expression[13,14] and found no specific changes in mTOR or energy metabolism-related pathways.

To determine the effects of both ER diets on the adaptive capacity of the adipose tissue we also assessed changes in gene expression profiles in the postprandial phase, i.e. 4h after the MMT. The WD induced a reduction in the initially observed postprandial increase in cholesterol and lipid metabolism as well as the TCA cycle while the TD only reduced postprandial cholesterol synthesis. A postprandial increase in these metabolic pathways is a clear adaptive response to the meal, as postprandial signals stimulate anabolic pathways in SAT such as the synthesis of cholesterol and lipids. The main postprandial signal, insulin, activates the transcription factors sterol regulatory element-binding proteins (SREBPs)[51], which are indeed regulating gene expression of lipogenic pathways and cholesterol metabolism[52]. Both ER-diets improved fasting measures of glucose homeostasis and insulin sensitivity and showed a trend towards reduced postprandial insulin levels. A reduction in postprandial insulin levels could have played an important role in the reduced response of these pathways upon intervention. Remarkably, the responsiveness in cholesterol biosynthesis in the WD was completely abolished, meaning that no postprandial increase in this pathway was observed after intervention. As the WD contained high amounts of SFA (14.9En%) and dietary cholesterol, it might be that intracellular cholesterol in SAT remained high or even

increased after intervention. High cholesterol levels inhibit the cleavage of SREBP precursors into functional proteins, providing a negative feedback loop for reducing the synthesis of more cholesterol, which might have nullified the postprandial stimulatory effects of insulin.

It must be noted that the observed changes in gene expression showed great inter-individual variation. This might be because individuals respond differently to dietary interventions, for instance due to differences in (epi) genetic background. Furthermore, our study was based on dietary advice combined with supplementary key products. This might have led to more variation in the dietary intake than a completely controlled diet. Studies showing postprandial adipose tissue transcriptional data are very rare and, to the best of our knowledge, we are the first to show this data in relation to a longer-term dietary intervention. Based on other postprandial studies, we hypothesized that 4h would be sufficient to induce changes in gene expression in the postprandial phase[53,54]. Indeed we show that dietary interventions affect adaptive responses at 4h in the postprandial phase, highlighting the added value of analysis of postprandial gene expression profiles.

To summarize, a high nutrient quality 25% ER-diet rich in fiber, soy protein, MUFA, and *n-3* PUFA induced a more pronounced ER-like adipose tissue gene expression response in the fasted state when compared to a low nutrient quality Western-type ER-diet. In addition, the high nutrient quality diet decreased PI3/Akt signaling. In contrast, a Western-type ER-diet reduces the postprandial adaptive response more than a high nutrient quality ER-diet. We conclude that a high nutrient quality ER-diet enhances the benefits of weight loss in adipose tissue on the gene expression level compared to a low quality Western-type ER-diet. Therefore, we hypothesize that a high nutrient quality diet is the preferred dietary approach to obtain health benefits in the adipose tissue known to be associated with weight loss on the long run.

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SUPPLEMENTAL MATERIAL

Supplemental Table 1. Baseline characteristics and effects of dietary interventions on body composition and cardio-metabolic risk factors

	BASELINE	CHANGE After 12 weeks	Within group p-value	Difference between groups p-value
BASELINE CHARACTERISTICS				
Age, years				
Western-type diet group	64 (28)			
Targeted diet group	62 (29)			
Control group	62 (29)			
Gender, n males, %				
Western-type diet group	17, 44%			
Targeted diet group	16, 47%			
Control group	13, 48%			
BODY COMPOSITION				
Body weight, kg				
Western-type diet group	93.1 ± 13.9	-6.3 ± 3.9 [A]	**<0.001**	
Targeted diet group	94.8 ± 13.2	-8.4 ± 3.2 [B]	**<0.001**	**<0.001**
Control group	92.1 ± 12.6	0.8 ± 1.7 [C]	**0.018**	
BMI, kg/m²				
Western-type diet group	31.2 ± 3.3	-2.1 ± 1.3 [A]	**<0.001**	
Targeted diet group	31.8 ± 3.4	-2.8 ± 1.1 [B]	**<0.001**	**<0.001**
Control group	30.9 ± 3.9	0.3 ± 0.6 [C]	**0.015**	
Waist circumference, cm				
Western-type diet group	108.7 ± 9.8	-3.1 ± 5.3 [A]	**<0.001**	
Targeted diet group	109.5 ± 9.8	-4.7 ± 5.7 [A]	**<0.001**	**<0.001**
Control group	106.6 ± 8.1	4.1 ± 5.2 [B]	**<0.001**	
Abdominal SAT, cm²*				
Western-type diet group	332.5 ± 92.4	-45.3 ± 32.7 [A]	**<0.001**	
Targeted diet group	348.0 ± 106.5	-54.1 ± 55.3 [A]	**<0.001**	**<0.001**
Control group	355.6 ± 118.8	1.6 ± 36.9 [B]	0.628	
Abdominal VAT, cm²*				
Western-type diet group	149.1 ± 77.0	-44.1 ± 48.2 [A]	**<0.001**	
Targeted diet group	175.7 ± 94.2	-61.5 ± 51.3 [A]	**<0.001**	**<0.001**
Control group	115.8 ± 26.1	3.7 ± 17.9 [B]	0.551	
VAT/SAT ratio*				
Western-type diet group	50.6 ± 34.8	-9.4 ± 16.9 [A]	**0.001**	
Targeted diet group	61.2 ± 54.9	-16.0 ± 28.1 [A]	**0.001**	**0.032**
Control group	36.2 ± 16.0	0.8 ± 6.3 [B]	0.775	
Intrahepatic lipids, % of water peak*				
Western-type diet group	7.3 ± 6.5	-3.4 ± 5.1 [A]	**<0.001**	
Targeted diet group	7.1 ± 8.7	-3.9 ± 5.7 [A]	**0.001**	**<0.001**
Control group	5.9 ± 5.8	1.4 ± 1.9 [B]	**0.021**	
CARDIO-METABOLIC RISK FACTORS				
Plasma glucose, mmol/L				
Western-type diet group	5.6 ± 0.8	-0.3 ± 0.6 [A]	**0.003**	
Targeted diet group	5.7 ± 0.5	-0.3 ± 0.4 [A]	**<0.001**	**0.007**
Control group	5.6 ± 0.4	0.0 ± 0.4 [B]	0.585	
Plasma insulin, mU/L				
Western-type diet group	14.6 ± 10.3	-3.9 ± 7.5 [A]	**0.002**	
Targeted diet group	13.5 ± 7.8	-3.6 ± 5.3 [A]	**<0.001**	**0.012**
Control group	12.1 ± 5.3	0.3 ± 6.1 [B]	0.782	
HbA1c, mmol/mol				
Western-type diet group	36.3 ± 2.1 [a,b]	-0.8 ± 2.0 [A]	**0.013**	
Targeted diet group	37.3 ± 2.9 [a]	-1.5 ± 2.0 [A]	**<0.001**	**0.014**
Control group	35.1 ± 2.6 [b]	0.4 ± 1.3 [B]	0.146	

	BASELINE	**CHANGE** After 12 weeks	Within group **p-value**	Difference between groups **p-value**
Serum total cholesterol, mmol/L				
Western-type diet group	5.7 ± 1.1	-0.2 ± 0.8 [A]	0.218	
Targeted diet group	5.5 ± 0.8	-0.5 ± 0.6 [B]	**<0.001**	**0.013**
Control group	5.4 ± 1.0	0.0 ± 0.5 [A]	0.633	
Serum HDL cholesterol, mmol/L				
Western-type diet group	1.3 ± 0.4	0.0 ± 0.2	0.096	
Targeted diet group	1.4 ± 0.4	-0.1 ± 0.2	0.084	0.878
Control group	1.4 ± 0.4	0.0 ± 0.3	0.439	
Plasma triglycerides, mmol/L				
Western-type diet group	1.8 ± 0.8	-0.2 ± 0.6 [A]	**0.018**	
Targeted diet group	1.6 ± 0.6	-0.4 ± 0.6 [B]	**0.001**	0.026
Control group	1.7 ± 0.7	-0.1 ± 0.5 [A]	0.280	
Plasma free fatty acids, mmol/L				
Western-type diet group	0.41 ± 0.21	-0.02 ± 0.20	0.522	
Targeted diet group	0.57 ± 0.37	-0.04 ± 0.49	0.471	0.389
Control group	0.42 ± 0.18	0.05 ± 0.24	0.291	

Data are presented as mean ± 1SD. For SAT and VAT: Western-type diet group *n*=28, Targeted diet group *n*=26, Control group *n*=16, for IHL: Western-type diet group *n*=34, Targeted diet group *n*=29, Control group *n*=19. For all other values: Western-type diet group *n*=39, Targeted diet group *n*=34, Control group *n*=27. Values denoted with an * are log2 transformed for statistical analyses to improve normality. Changes within group analyzed using paired t-test, changes between groups analyzed using general linear model for univariate analysis with baseline values as covariates (ANCOVA). Post hoc analyses were conducted using LSD, different letters (A, B, C) indicate significant post hoc differences ($p<0.05$) between diet group. Abbreviations: BMI: body mass index, SAT: subcutaneous adipose tissue mass (assessed by MRI), VAT: visceral adipose tissue mass (assessed by MRI), IHL: intrahepatic lipid (assessed by ^{1}H-MRS), HbA1c: glycosylated hemoglobin, HDL: high-density lipoprotein.

Supplemental Table 2. Significant correlations between weight loss, changes in HOMA-IR, and gene expression (FDR0.25)

	TARGETED DIET GROUP		WESTERN-TYPE DIET GROUP	
	Weight loss	Change in HOMA-IR	Weight loss	Change in HOMA-IR
1. METABOLIC PATHWAYS (*n*=90 genes)				
GCKR	-0.550 *p=0.003*			
SCD		0.560 *p=0.003*		
CHST2				-0.617 *p=0.001*
2. MITOCHONDRIAL ENERGY PRODUCTION (*n*=55 genes)				
GPX1		0.598 *p=0.001*		
NDUFA3			0.575 *p=0.002*	
CYCS			-0.493 *p=0.009*	
ATP6V1A			-0.493 *p=0.009*	
3. PI3/AKT SIGNALING (*n*=27 genes)				
AKT2	0.513 *p=0.006*	0.503 *p=0.009*		
PRKACA			0.514 *p=0.006*	
LEP			0.461 *p=0.016*	

Correlation between weight loss, changes in HOMA-IR (homeostatic model assessment of insulin resistance), and expression of genes from clusters of pathways differentially changed between the Targeted and Western-type diet. P-values are corrected for multiple testing using an FDR of 0.25. Genes: GCKR: glucokinase regulator, SCD: stearoyl-CoA desaturase, CHST2: carbohydrate sulfotransferase 2, GPX1: glutathione peroxidase 1, NDUFA3: NADH: ubiquinone oxidoreductase subunit A3, CYCS: cytochrome c, somatic, ATP6V1A: ATPase H+ transporting V1 subunit A, AKT2: AKT serine/threonine kinase 2, PRKACA: protein kinase cAMP-activated catalytic subunit alpha, LEP: leptin.

Supplemental Table 3. Change in pathway enrichment after Targeted diet or Western-type diet compared to control

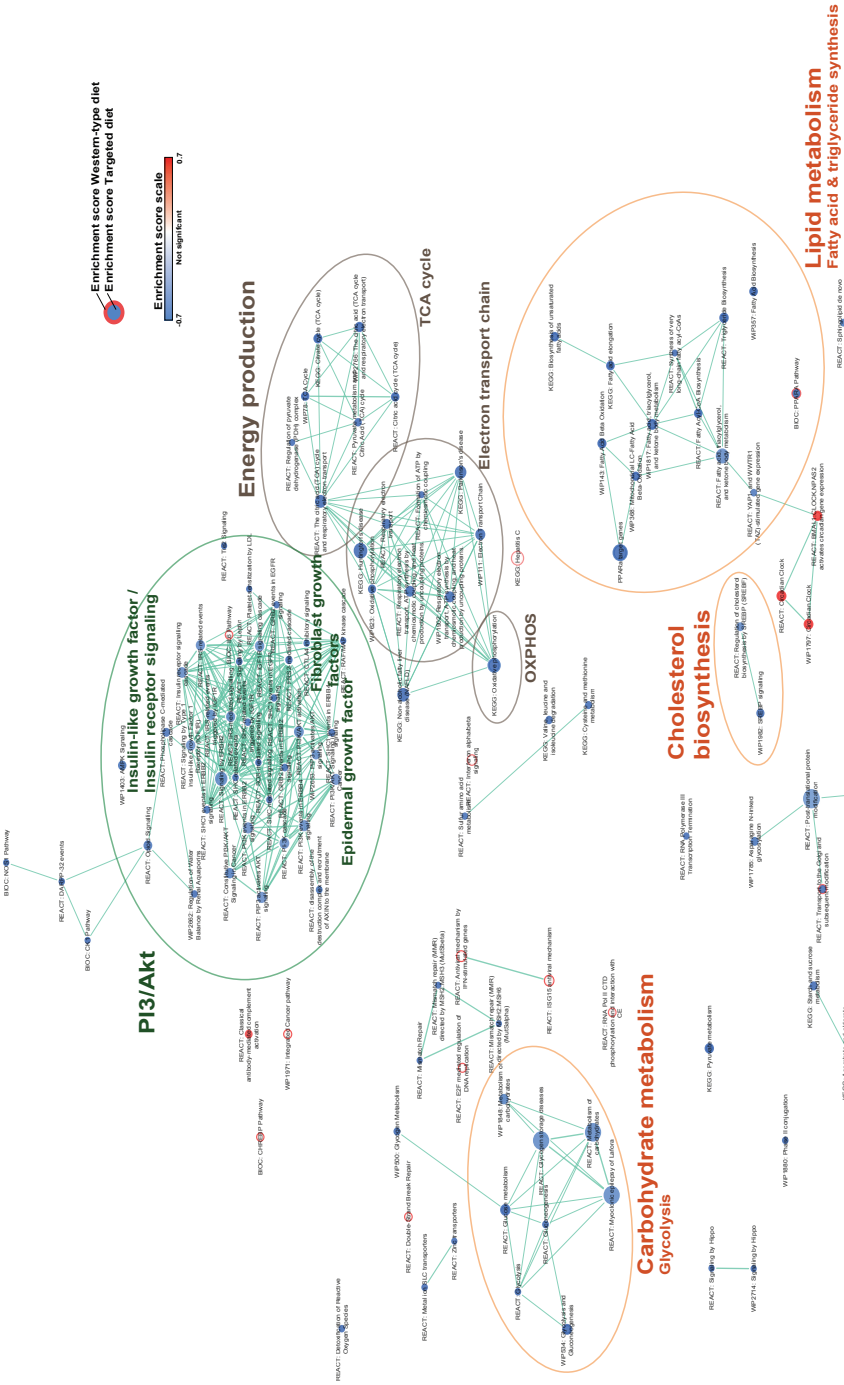
	TARGETED DIET GROUP COMPARED TO CONTROL	WESTERN-TYPE DIET GROUP COMPARED TO CONTROL
PATHWAYS increased UPON INTERVENTION		
Translation	↑	↑
Olfactory signaling	↑	-
Toll-like signaling	-	↑
Apoptosis	-	↑
Cell cycle	-	↑
Cytokine signaling	-	↑
PATHWAYS decreased UPON INTERVENTION		
FA acid synthesis, elongation, saturation	↓	↓
TG synthesis	↓	↓
Beta-oxidation	↓	-
TCA cycle	↓	-
Electron transport chain	↓	-
Oxidative phosphorylation	↓	-
Carbohydrate metabolism	↓	-
Cholesterol biosynthesis	↓	-
Phospholipid metabolism	↓	-
Collagen, extracellular matrix remodeling	↓	↓
Platelet, hemostasis	↓	↓
Vascularization (including VEGF)	↓	-
PI3/Akt signaling in relation to growth factors	↓	-
Ubiquitin proteasome pathway, degradation	↓	-
Wnt/beta-catenin signaling pathway	↓	-

Gene sets more/less enriched upon Targeted or Western-type diet compared to the control group using a False Discovery Rate Q-value <0.25. Abbreviations: FA: fatty acid, TG: triglycerides, TCA: tricarboxylic acid, VEGF: vascular endothelial growth factor.

Supplemental Table 4. Differential changes in postprandial response of pathway enrichment between Targeted diet and Western-type diet

	TARGETED DIET GROUP			WESTERN-TYPE DIET GROUP		
	4h-0h Baseline, 0wk	4h-0h End, 12wk	Significant change in response	4h-0h Baseline, 0wk	4h-0h End, 12wk	Significant change in response
1. METABOLIC PATHWAYS						
Lipid metabolism, including fatty acid elongation	↑↑	↑↑		↑↑	↑	*
Cholesterol biosynthesis	↑↑	↑	*	↑↑	-	*
2. MITOCHONDRIAL ENERGY PRODUCTION						
TCA cycle	↑	↑		↑	-	*
3. NEUROTRANSMITTERS						
Neurotransmitter release, including ghrelin	-	-		-	↓	*

Responses of gene sets more/less enriched in postprandial phase after intervention that were significantly different between the two ER-diets, shown as the effect within each diet at baseline (0 weeks) and at the end of the intervention (12 weeks). * indicates a significant change in response within the diet group (False Discovery Rate Q-value <0.25). Abbreviations: TCA: tricarboxylic acid.



Supplemental Figure 1. Clusters of gene sets (*n*=128) differentially enriched between TD and WD, colored by using the enrichment scores for wihitn TD and within WD

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REGULATION OF ANGIOPOIETIN-LIKE 4 AND LIPOPROTEIN LIPASE IN HUMAN ADIPOSE TISSUE

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ABSTRACT

Background: Elevated plasma triglycerides are increasingly viewed as a causal risk factor for coronary artery disease. One protein that raises plasma triglyceride levels and that has emerged as a modulator of coronary artery disease risk is angiopoietin-like 4 (ANGPTL4). ANGPTL4 raises plasma triglyceride levels by inhibiting lipoprotein lipase (LPL), the enzyme that catalyzes the hydrolysis of circulating triglycerides on the capillary endothelium.

Objective: The objective of the present study was to assess the association between ANGPTL4 and LPL in human adipose tissue, and to examine the influence of nutritional status on ANGPTL4 expression.

Methods: We determined ANGPTL4 and LPL mRNA and protein levels in different adipose tissue depots in a large number of severely obese patients who underwent bariatric surgery. Furthermore, in 72 abdominally obese subjects, we measured ANGPTL4 and LPL mRNA levels in subcutaneous adipose tissue in the fasted and postprandial state.

Results: ANGPTL4 mRNA levels were highest in subcutaneous adipose tissue, whereas LPL mRNA levels were highest in mesenteric adipose tissue. ANGPTL4 and LPL mRNA levels were strongly positively correlated in the omental and subcutaneous adipose tissue depots. In contrast, ANGPTL4 and LPL protein levels were negatively correlated in subcutaneous adipose tissue, suggesting a suppressive effect of ANGPTL4 on LPL protein abundance in subcutaneous adipose tissue. ANGPTL4 mRNA levels were 38% higher in the fasted compared to the postprandial state.

Conclusion: Our data provide valuable insights into the relationship between ANGPTL4 and LPL in human adipose tissue, as well as the physiological function and regulation of ANGPTL4 in humans.

ABBREVIATIONS

ANGPTL3	Angiopoietin-like 3
ANGPTL4	Angiopoietin-like 4
ANGPTL8	Angiopoietin-like 8
Endo H	Endoglycosidase H
LDLs	Low-density lipoproteins
LPL	Lipoprotein lipase
NEFA	Non-esterified fatty acids
PNGase F	Peptide:N-Glycosidase F
TG	Triglycerides
WAT	White adipose tissue

INTRODUCTION

Coronary artery disease is a major cause of morbidity and mortality worldwide[1]. Despite significant progress in the diagnosis, prevention and treatment of coronary artery disease, novel and effective treatments are needed to further reduce cardiovascular disease rates. Elevated plasma triglycerides are increasingly viewed as a causal risk factor for coronary artery disease[2]. Consequently, targeting plasma triglycerides may be a viable approach to lower coronary artery disease risk. One protein that regulates plasma triglyceride levels and that has recently emerged as a modulator of coronary artery disease risk is angiopoietin-like 4 (ANGPTL4). Specifically, studies have shown that carriers of the inactivating variant E40K in the ANGPTL4 gene are at reduced risk of developing coronary artery disease[3,4]. ANGPTL4 is a member of the angiopoietin-like protein family that also includes angiopoietin-like 3 (ANGPTL3) and angiopoietin-like 8 (ANGPTL8)[5]. ANGPTL4 likely modulates coronary artery disease risk by raising plasma triglyceride levels via inhibition of lipoprotein lipase (LPL), the enzyme that catalyzes the hydrolysis of circulating triglycerides on the capillary endothelium[6]. ANGPTL4 inhibits LPL by promoting the unfolding of LPL, which in turn leads to the dissociation of the catalytically active LPL dimer into inactive and unstable monomers[7,8]. Besides ANGPTL4, ANGPTL3 and ANGPTL8 also potently inhibit LPL activity and increase plasma triglyceride levels[5]. Notably, inactivation of ANGPTL3 via monoclonal antibodies and anti-sense oligonucleotides markedly reduces circulating levels of triglycerides and LDL cholesterol in humans[9,10]. Accordingly, ANGPTL3 and ANGPTL4 hold considerable promise as pharmacological targets for coronary artery disease.

As mentioned above, carriers of the E40K variant in the ANGPTL4 gene have significantly lower plasma triglyceride levels, as well as elevated HDL cholesterol levels. However, despite the strong genetic evidence for a role of ANGPTL4 in regulating plasma triglycerides in humans, the relation between circulating ANGPTL4 and plasma triglyceride levels remains somewhat ambiguous[11–14]. These and other observations have raised questions about the role of circulating ANGPTL4 in the regulation of LPL activity on the capillary endothelium. Indeed, several mouse studies indicate that locally expressed ANGPTL4 is important for regulating tissue LPL activity[15–17]. For example, the elevated expression of ANGPTL4 in adipose tissue during fasting likely accounts for the inhibition of adipose tissue LPL activity and the reduced uptake of TG-derived fatty acids in adipose tissue during fasting[17,18]. Moreover, we recently showed that, in mouse adipocytes, ANGTPL4 lowers the amount of LPL protein by promoting the intracellular degradation of mature glycosylated LPL[19]. Little is known about the regulation of ANGPTL4 mRNA and protein in human adipose tissue, and on its relationship with LPL. Accordingly, the objective of the current study was to assess the association between ANGPTL4 and LPL in human adipose

tissue, by using material of different adipose tissue depots that was obtained from a large number of severely obese patients undergoing bariatric surgery. In addition, we investigated the relationship between ANGPTL3, ANGPTL4 and ANGPTL8 mRNA levels in liver material obtained from a subgroup of patients. Furthermore, we investigated the correlation between the above parameters and lipid and ANGPTL4 levels in blood plasma. Finally, in a separate study, we examined the influence of nutritional status on ANGPTL4, ANGPTL8, and LPL mRNA levels.

MATERIALS & METHODS

MONDIAL study

The MONDIAL study (acronym for Markers of Organ health in Non-diabetic and Diabetics; Intestine, Adipose tissue & Liver) is a cross-sectional study in male and female patients undergoing bariatric surgery at Rijnstate hospital/ Vitalys clinics in Arnhem, the Netherlands. The study aimed to determine the health of adipose tissue depots, the liver, and the intestine, with a focus on examining potential differences in organ health between diabetics and non-diabetics, and to search for novel biomarkers for metabolic diseases. The collection of material was conducted in 15 patients in 2012 and in 61 patients in 2015. Tissue samples were obtained from residual biological material from patients who underwent either a primary laparoscopic Roux-en-Y gastric bypass or a primary laparoscopic gastric sleeve procedure, deployed to induce weight loss and to alleviate co-morbidities of obesity. Both procedures were performed laparoscopically. Patients met the criteria for surgery from the Interdisciplinary European Guidelines for Surgery for Severe (Morbid) Obesity, amongst others aged 18-60, a BMI of over 40kg/m^2 or a BMI between 35 and 40kg/m^2 with co-morbidity that is expected to improve after surgically-induced weight loss, a history of longstanding obesity (>5 years), proven failed attempts to lose weight in a conventional way, or primarily successful weight loss with eventual weight regain, and the intention to adhere to a postoperative follow-up program[20]. For the MONDIAL study, we excluded non-Caucasian patients and targeted further recruitment of patients to obtain an equal ratio of females and males, as well as diabetics and non-diabetics. Patients were informed by their surgeon and one of the researchers about the study during a pre-operative visit two months prior to the surgical procedure, after which they could give a written acknowledgement of informed consent to participate. Participation included the consent that residual material would be used for scientific research and the withdrawal of a fasting blood sample before surgery. The blood sample was drawn prior to the administration of anesthetics on the operating table. Blood was collected in a 6mL EDTA tube, after which the sample was centrifuged at 1500*g at 4°C to obtain plasma. Patients were informed

that their decision to participate was totally voluntary and that they could withdraw at any time without giving a reason. Withdrawal would not affect their regular medical treatment. The study was approved by the local ethics committee of Rijnstate hospital.

Belly Fat study

The Belly Fat study was approved by the Medical Ethics Committee of Wageningen University and registered at ClinicalTrials.gov, identifier: **NCT02194504**. In short, 110 healthy participants aged 40-70 years with abdominal obesity (BMI >27kg/m^2 or waist circumference >88cm for females, >102cm for males) were randomly assigned to one of three different energy-restricted diets. Here, we only report baseline data prior to the intervention. Subjects came to our department in the morning in the fasted state. After blood sampling and collection of a subcutaneous adipose tissue biopsy, subjects were given an in-house prepared mixed meal consisting of 76.3g carbohydrates, 17.6g protein, and 60.0g fat. Four hours later, a second subcutaneous adipose tissue biopsy was taken, after making a new incision. The subcutaneous adipose tissue samples were obtained by needle biopsy from the periumbilical area under local anesthesia. The samples were rinsed to eliminate blood and were immediately frozen in liquid nitrogen. All samples were stored in aliquots at -80 °C.

RNA isolation & qPCR

Total RNA from residual material of the liver, the subcutaneous adipose tissue, the mesenteric adipose tissue and the omental adipose tissue from patients included in the MONDIAL study was isolated using TRIzol reagent (Life Technologies Europe BV, Bleiswijk, the Netherlands) and purified using the Qiagen RNeasy Mini kit (Qiagen, Venlo, the Netherlands). Quality of the isolated RNA was verified using an Agilent 2100 bioanalyzer (Agilent Technologies, Amsterdam, NL). Next, 500 ng of RNA was reverse transcribed using the First-Strand cDNA Synthesis Kit (Thermo Scientific, Landsmeer, the Netherlands). Real-time PCR was carried out using SensiMix (Bioline, GC Biotech, Alphen aan de Rijn, the Netherlands) on a CFX 384 Bio-Rad thermal cycler (Bio-Rad, Veenendaal, the Netherlands). Primer sequences can be found in Supplemental Table 1. 36B4 and BACTIN were used as housekeeping genes for the adipose tissue depots, whereas CYPA was used as housekeeping gene for the liver.

Microarray analysis

Purified RNA (100 ng) from subcutaneous adipose tissue of subjects included in the Belly Fat study was labeled with the Ambion WT expression kit (Invitrogen) and hybridized to an Affymetrix Human Gene 1.1 ST array

plate (Affymetrix, Santa Clara, CA). Hybridization, washing, and scanning were carried out on an Affymetrix GeneTitan platform according to the instruction by the manufacturer. Analysis of microarray data was carried out as previously described[21]. The complete analysis of the microarray data from the Belly Fat study will be presented elsewhere.

Western blots

Protein lysates were made of residual material of the subcutaneous adipose tissue depot of patients included in the MONDIAL study and of whom sufficient material was available. Part of the material was lysed in RIPA lysis buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium; deoxycholate, 0.1% SDS; Thermo Scientific) supplemented with protease and phosphatase inhibitors (Roche, Woerden, The Netherlands) to make 30% protein lysates. After a 30-minute incubation on ice, the lysates were spun down at 13.000 rpm in order to get rid of non-dissolved material and fat. Following the transfer of the infranatant to a clean tube, this procedure was repeated twice to get rid of excess fat. Protein concentrations of lysates were determined with BCA reagent (Thermo Scientific) and subsequently adjusted to a concentration of 2.5 µg/µL. Next, lysates were mixed with 2x LSB loading buffer and denatured at 95 °C for 5 minutes. For each patient, 5 µg of protein was loaded per lane on 26-wells Criterion 8-16% TGX gels (Bio-Rad) and separated by SDS gel electrophoresis. Separated proteins were transferred to a PVDF membrane by means of a Transblot Turbo System (Bio-Rad). Primary antibodies [goat anti-human LPL antibody (Santa Cruz Biotechnology, #Y-20) or goat anti-LPL antibody (kind gift from Anne Beigneux, #88B8[22]); and rabbit anti-human ANGPTL4 antibody[23]) were used at a ratio of 1:750 (#Y-20) or 1:1000 (#88B8 and anti-human ANGPTL4) and incubated overnight at 4°C. Corresponding secondary antibodies (HRP-conjugated) (Sigma-Aldrich) were used at a 1:5000 dilution. Importantly, all samples were analyzed simultaneously to avoid variation. All incubations were done in Tris-buffered saline, pH 7.5, with 0.1% Tween-20 (TBS-T) and 5% dry milk, whereas all washing steps were done in TBS-T without dry milk. Blots were visualized using the ChemiDoc MP system (Bio-Rad) and Clarity ECL substrate (Bio-Rad). Quantification of bands was performed using ImageLab software (Bio-Rad). Of note, due to the absence of visual bands for ANGPTL4 (n=1) and LPL (n=2), protein data of three patients were excluded from subsequent statistical analyses.

Plasma metabolites

Plasma concentrations of triglycerides (Instruchemie, Delfzijl, the Netherlands) and free fatty acids (Wako Chemicals, Neuss, Germany; HR(2) Kit) in fasted plasma samples of patients included in the MONDIAL study were determined following the manufacturers' instructions. Plasma concentrations of glucose were determined

on the morning of the operation using Accu-Chek meters (Roche). Plasma ANGPTL4 levels were determined as described previously[24] using an anti-human ANGPTL4 polyclonal goat IgG antibody (AF3485; R&D Systems, Abingdon, United Kingdom). Briefly, 96-wells plates were coated with anti-ANGPTL4 antibody and incubated overnight at 4°C. The next day, 100 µL of 20-fold diluted human plasma was applied and incubated at room temperature for 2 hours. Next, 100 µL of biotinylated anti-human ANGPTL4 polyclonal goat IgG antibody (BAF3485; R&D Systems) was added to each well and incubated at room temperature for 2 hours, followed by the addition of streptavidin-conjugated HRP for 20 min. Tetramethylbenzidine substrate reagent was added for 6 min, where after the reaction was stopped by adding 50 µL of 10% H2SO4. The absorbance was measured at 450 nm.

Statistical analyses

Statistical analyses were performed using GraphPad software (La Jolla, California, United States). A Kruskal-Wallis test followed by a Dunn's test was used to compare ANGPTL4 and LPL expression values in different adipose tissue depots. To determine the correlation coefficients between multiple parameters, we employed non-parametric Spearman's correlations. P-values of < 0.05 were considered statistically significant. Differences in (log-transformed) subcutaneous adipose tissue gene expression between the fasted and post-prandial state were evaluated using a paired Student's t-test.

RESULTS

Patient's characteristics of the MONDIAL study

Patient characteristics of the MONDIAL study are shown in **Table 1**. Age of the participants varied between 21 and 69 years old. Participants were almost equally distributed among men (46%) and women (54%). The mean body weight and BMI were 129.0 ± 18.9 kg and 42.7 ± 5.3 kg/m^2, respectively (means ± SD). The mean fasting plasma glucose concentration was 7.8 ± 2.8 mmol/Liter.

mRNA levels of ANGPTL3, ANGPTL4 and ANGPTL8 are not correlated in human liver

ANGPTL4 is abundantly secreted into the circulation by the liver, where its expression is regulated by the transcription factor PPARA25. Interestingly, in the human liver material, no correlation was found between the mRNA levels of ANGPTL4 and PPARA (**Figure 1A**). Also, no correlation was found between mRNA levels of ANGPTL4 and ANGPTL3 or ANGPTL8 (**Figure 1B**). The correlations between the gene expression levels

of ANGPTL3, ANGPTL4, ANGPTL8 and PPARa with various plasma parameters are depicted in **Figure 1C**. Notably, no significant correlation was found between hepatic ANGPTL4 mRNA levels and the plasma ANGPTL4 concentration or between hepatic ANGPTL4 mRNA levels and the plasma triglyceride concentration (**Figure 1C, Supplemental Figure 1A & 1B**).

Table 1. Patient characteristics

PATIENT CHARACTERISTICS (*n*=76)	
Gender, *n* males (%)	35 (46%)
For females: menstrual state	
Premenopausal, *n* females (% of total females)	15 (37%)
Postmenopausal, *n* females (% of total females)	12 (29%)
Unknown, *n* females (% of total females)	14 (34%)
Diagnosed with type 2 diabetes, *n* cases (%)	30 (39%)
Medication:	
Insulin injection, *n* patients (% of total diabetics)	1 (3%)
Metformin, *n* patients (% of total diabetics)	16 (53%)
Glimepiride (sulfonylurea), *n* patients (% of total diabetics)	4 (13%)
Unknown, *n* patients (% of total diabetics)	2 (7%)
Combination therapy	
Metformin + Glimepiride (sulfonylurea), *n* patients (% of total diabetics)	3 (10%)
Metformin + Gliclazide (sulfonylurea), *n* patients (% of total diabetics)	4 (13%)
Age at surgery, range in years[1]	21 - 69
Weight, kg	129.0 ± 18.9
BMI, kg/m²	42.7 ± 5.3
Waist circumference, cm	133.6 ± 15.4
Fasting plasma glucose, mmol/L	7.8 ± 2.8
Fasting plasma triglycerides, mmol/L	2.4 ± 2.1
Fasting plasma free fatty acids, mmol/L	0.90 ± 0.34

Data are presented as mean ± SD, except [1] which is presented as range. Fasting glucose was determined by the Laboratory of Clinical Chemistry and Haematology, Rijnstate Hospital, Arnhem, The Netherlands. For plasma triglyceride and free fatty acids: *n*=59. .

Expression levels of ANGPTL4 are highest in the subcutaneous adipose tissue

Expression of ANGPTL4 in human and mouse adipose tissue is comparatively high[17,18,25]. Interestingly, in our study, ANGPTL4 mRNA levels were significantly higher in the subcutaneous adipose tissue depot than in the omental and mesenteric adipose tissue depots (**Figure 2A**). In contrast, mRNA levels of LPL were highest in the mesenteric adipose tissue depot (**Figure 2B**). Strikingly, we observed a highly significant correlation between ANGPTL4 mRNA levels in the subcutaneous adipose tissue depot and ANGPTL4 mRNA levels in the omental and mesenteric adipose tissue depots (**Figure 2C**). Similarly, the LPL mRNA levels in the subcutaneous adipose tissue were significantly correlated with LPL mRNA in the omental and mesenteric adipose tissue depots (**Figure 2D**).

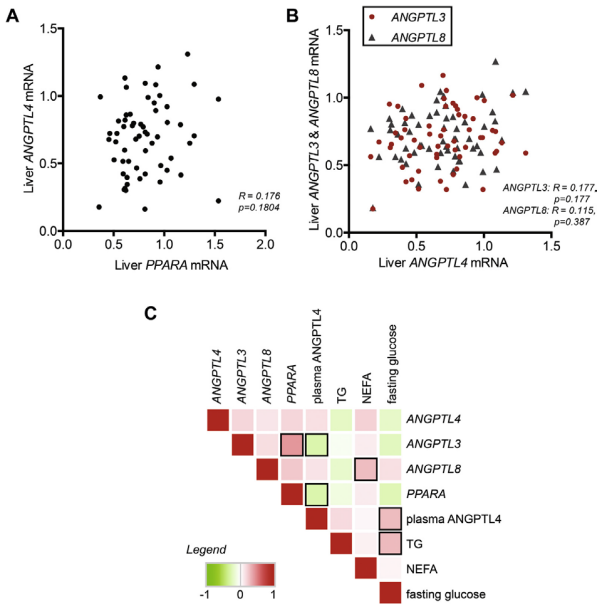

Figure 1. mRNA levels of ANGPTL3, ANGPTL4 and ANGPTL8 are not correlated in human liver

(A) Correlation between liver PPARa mRNA levels and liver ANGPTL4 mRNA levels in liver material obtained from patients undergoing bariatric surgery (*n*=59). (B) Correlation between liver ANGPTL4 mRNA levels and liver ANGPTL3 and ANGPTL8 mRNA levels in liver material obtained from patients undergoing bariatric surgery (*n*=59). (C) Correlation heat map of ANGPTL4, ANGPTL3, ANGPTL8, and PPARa mRNA levels, plasma ANGPTL4 concentrations, fasting plasma glucose levels, plasma levels of non-esterified fatty acids (NEFA) and plasma triglyceride (TG) concentration (*n*=59). Significant correlations are indicated with a black box. Correlations were analyzed by Spearman's R, p-values of <0.05 were considered statistically significant.

Expression levels of ANGPTL4 and LPL are positively correlated in the omental and subcutaneous adipose tissue.

ANGPTL4 potently inhibits LPL in the adipose tissue of mice, but data on the relationship between ANGPTL4 and LPL expression in human adipose tissue are scarce[17]. We observed a highly significant, positive correlation between ANGPTL4 mRNA levels and LPL mRNA levels in the omental and subcutaneous adipose tissue depots (**Figure 3A and Figure 3B**), but not in the mesenteric adipose tissue depot (**Figure 3C**). Given that both LPL and ANGPTL4 are under positive control of the transcription factor PPARa, we examined the association between the mRNA levels of ANGPTL4 and LPL, respectively, and PPARg mRNA levels in the different adipose tissue depots[6,25,26]. Whereas ANGPTL4 mRNA levels in the various fat depots showed only a weak or no correlation with PPARg mRNA (**Figure 3D; Supplemental Figure 2A**), LPL mRNA levels were strongly and significantly correlated with PPARg mRNA levels in all three adipose tissue depots (**Figure 3D, Supplemental Figure 2B**).

An overview of the correlations between ANGPTL4, LPL, and PPARg mRNA levels in the three different adipose tissue depots, as well as various plasma parameters, is shown in **Figure 3D**. Specifically, we found no association between the mRNA levels of ANGPTL4 in the subcutaneous, omental and mesenteric adipose tissue depots and the plasma ANGPTL4 concentration or plasma triglyceride concentration (**Figure 3D, Supplemental Figure 1C & 1D**).

Protein levels of LPL and ANGPTL4 are negatively correlated in subcutaneous adipose tissue

To determine the relationship between the protein levels of LPL and ANGPTL4 in human adipose tissue, we measured LPL and ANGPTL4 protein in the subcutaneous adipose tissue by Western blot using validated

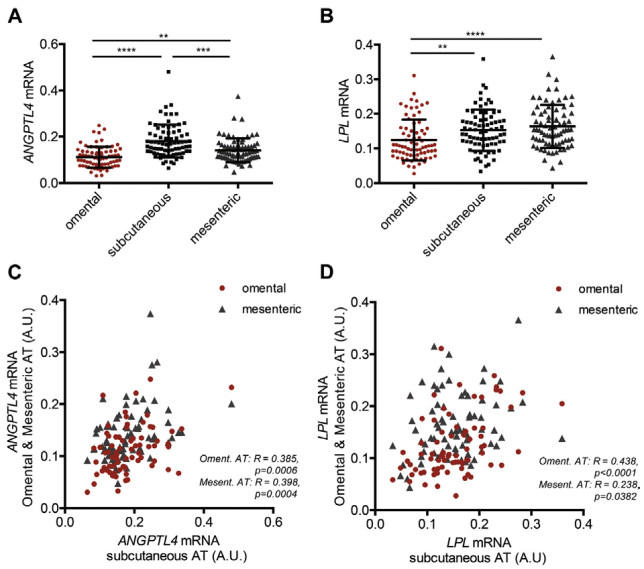

Figure 2. Expression levels of ANGPTL4 are highest in the subcutaneous adipose tissue

(A) ANGPTL4 mRNA levels in residual material of the omental, subcutaneous and mesenteric adipose tissue depots obtained from patients undergoing bariatric surgery (*n*=75). (B) LPL mRNA levels in residual material of the omental, subcutaneous and mesenteric adipose tissue depots obtained from patients undergoing bariatric surgery (*n*=75). (C) Correlation between ANGPTL4 mRNA levels in the subcutaneous adipose tissue (AT) and ANGPTL4 mRNA levels in the omental and mesenteric adipose tissue depots (n=75). A.U. signifies Arbitrary Units (D) Correlation between LPL mRNA levels in the subcutaneous adipose tissue (AT) and LPL mRNA levels in the omental and mesenteric adipose tissue depots (*n*=75). A.U. signifies Arbitrary Units. Differences in ANGPTL4 and LPL mRNA levels between different adipose tissue depots were analyzed by a Kruskal-Wallis ANOVA followed by a Dunn's test for multiple comparisons. ** p<0.01, *** p<0.001, **** p<0.0001. Correlations between ANGPTL4 and LPL mRNA levels between different adipose tissue depots were analyzed by Spearman's R, p-values of <0.05 were considered statistically significant.

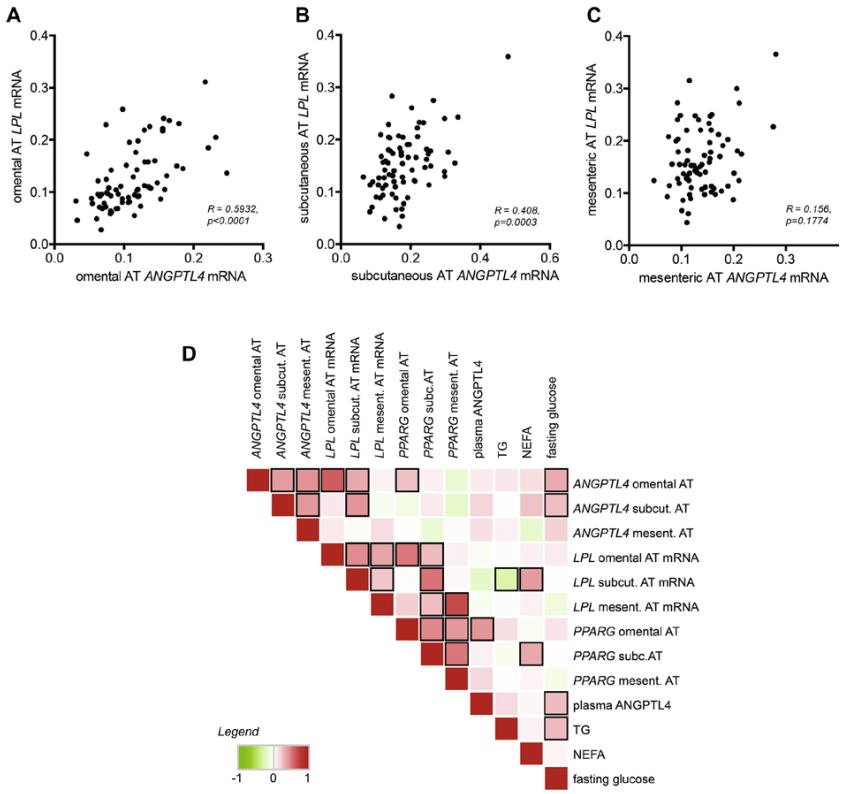

Figure 3. Expression levels of ANGPTL4 and LPL are positively correlated in the omental and subcutaneous adipose tissue

(A) Correlation between ANGPTL4 mRNA levels and LPL mRNA levels in the omental adipose tissue (AT) depots of patients undergoing bariatric surgery (*n*=75). (B) Correlation between ANGPTL4 mRNA levels and LPL mRNA levels in the subcutaneous adipose tissue (AT) depots of patients undergoing bariatric surgery (*n*=76). (C) Correlation between ANGPTL4 mRNA levels and LPL mRNA levels in the mesenteric adipose tissue depots of patients undergoing bariatric surgery (*n*=76). (D) Correlation heat map of ANGPTL4, LPL and PPARG mRNA levels in the omental, subcutaneous and mesenteric adipose tissue depots, plasma ANGPTL4 concentrations, fasting plasma glucose levels, plasma levels of non-esterified fatty acids (NEFA) and plasma triglyceride (TG) levels (*n*=76 for gene expression correlations in the omental adipose tissue, *n*=75 for gene expression analyses in the subcutaneous and mesenteric adipose tissue depots, and *n*=59 for correlations with plasma parameters). Significant correlations are indicated with a black box. Correlations were analyzed by Spearman's R, p-values of <0.05 were considered statistically significant.

antibodies against ANGPTL4 and LPL (**Supplemental Figures 3 & 4**). As previously shown, ANGPTL4 was only detectable in human adipose tissue as the full-length protein[23]. After quantification, a significant negative correlation was observed between ANGPTL4 and LPL protein level (R=-0.2314, p=0.0461) (**Figure 4, Supplemental Figure 4**). We also attempted to determine the levels of mature glycosylated LPL by means of treatment of the adipose tissue lysates with the endoglycosidase Endo H, an enzyme that removes high mannose carbohydrates from glycosylated asparagine residues and that is thus expected to act upon immature, but not

mature glycosylated LPL. Unfortunately, in contrast to mouse adipose tissue and previously published data[19], no reduction in LPL weight upon treatment with Endo H was detected, suggesting that most of the LPL found in the human subcutaneous adipose tissue is in the mature glycosylated form (**Supplemental Figure 5**)[27–30].

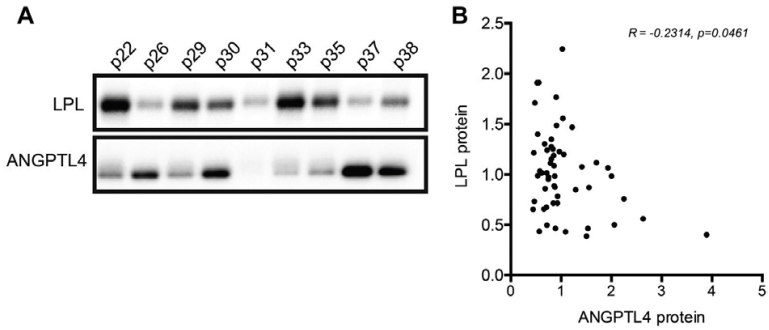

Figure 4. Protein levels of LPL and ANGPTL4 are negatively correlated in subcutaneous adipose tissue

(A) Representative Western blots for LPL and ANGPTL4 in the subcutaneous adipose tissue depot of nine patients undergoing bariatric surgery (see **Supplemental Figure 4** for the Western blots of all patients). (B) Correlation of protein levels of LPL and ANGPTL4 in the subcutaneous adipose tissue of patients undergoing bariatric surgery, as determined by the quantification of Western blots (*n*=54). Correlation was analyzed by Spearman's R, p-values of <0.05 were considered statistically significant.

ANGPTL4 mRNA levels are higher in the fasted than the post-prandial state in subcutaneous adipose tissue

ANGPTL4 was initially cloned as the Fasting-Induced Adipose Factor[25]. While the induction of ANGPTL4 mRNA and protein by fasting in adipose tissue is evident in mouse studies[17,18,25], it is unclear whether ANGPTL4 is also upregulated by fasting in human adipose tissue. To answer that question, we measured ANGPTL4 mRNA in subcutaneous adipose tissue biopsies taken from 72 human subjects after an overnight fast and 4 hours after receiving a mixed meal. ANGPTL4 mRNA levels were 38% higher in the fasted state compared to the post-prandial state (P<1*10-15) (**Figure 5**). By contrast, mRNA levels of ANGPTL8 were 64% lower in the fasted state compared to the post-prandial state (P<1*10-22) (**Figure 5**). LPL mRNA levels were not significantly different between the fasted and post-prandial state (**Figure 5**). These data indicate that ANGPTL4 expression in human adipose tissue is induced by fasting, whereas ANGPTL8 expression is reduced by fasting.

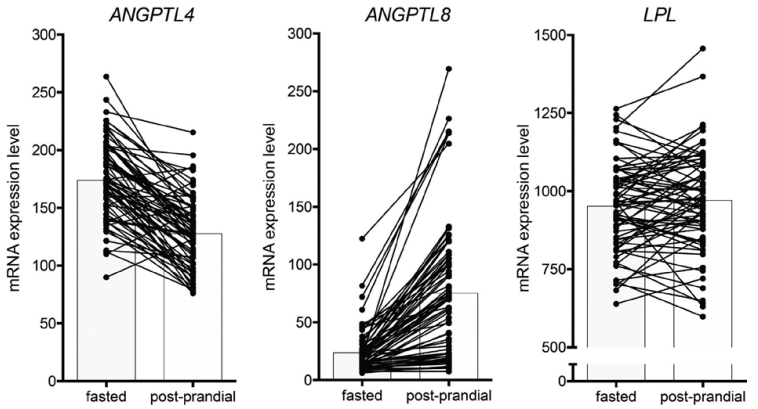

Figure 5. ANGPTL4 mRNA levels are higher in the fasted than the post-prandial state in subcutaneous adipose tissue

mRNA expression levels of ANGPTL4, ANGPTL8 and LPL in subcutaneous adipose tissue of 72 subjects for which baseline fat biopsies were available. Biopsies were taken after an overnight fast, and 4 hours after consumption of a mixed meal consisting of 76.3g carbohydrates, 17.6g protein, and 60.0g fat (post-prandial). Expression levels were determined using Affymetrix microarray analysis. A single line represents one subject. ANGPTL4 mRNA levels were 38% higher in the fasted state compared to the post-prandial state (Paired Student's t-test, P<1*10-15). ANGPTL8 mRNA levels were 64% lower in the fasted state compared to the post-prandial state (Paired Student's t-test, P<1*10-22). LPL mRNA levels were not significantly different between the fasted and post-prandial state.

DISCUSSION

This study was conducted to clarify the regulation of ANGPTL4 and LPL in human adipose tissue using residual material of omental, mesenteric and subcutaneous adipose tissue obtained from a large cohort of severely obese patients undergoing bariatric surgery. In addition, we aimed to examine the influence of nutritional status on ANGPTL4 mRNA levels in human subcutaneous adipose tissue. ANGPTL4 mRNA levels were highest in the subcutaneous adipose tissue depot, whereas LPL mRNA levels were highest in the mesenteric adipose tissue depot. ANGPTL4 and LPL mRNA levels were strongly positively associated in all three adipose tissue depots. In contrast, ANGPTL4 and LPL protein levels in subcutaneous adipose tissue were negatively correlated. Finally, consumption of a mixed meal significant reduced ANGPTL4 mRNA levels in human subcutaneous adipose tissue.

Consistent with earlier observations in mouse adipose tissue[17,18,25] our results for the first time demonstrate that fasting increases ANGPTL4 mRNA expression in human adipose tissue. Studies using mouse models have demonstrated that the upregulation of ANGPTL4 in adipose tissue during fasting leads to a reduction in local LPL activity and a concomitant decrease in the hydrolysis of circulating triglycerides[17,18,31]. As a consequence, circulating lipids are diverted away from storage in adipose tissue to other tissues. Based on the data presented

here, a similar role can be envisioned for ANGPTL4 in human adipose tissue.

Previously, we demonstrated that ANGPTL4 decreases the levels of LPL protein in mouse adipose tissue, which is likely achieved by promoting the intracellular degradation of LPL[19]. Here, we find a significant negative correlation between ANGPTL4 and LPL protein in human subcutaneous adipose tissue. It is reasonable to suggest that this negative correlation may be a reflection of the stimulatory effect of ANGPTL4 on LPL degradation, which would imply that ANGPTL4 regulates LPL protein abundance in human adipose tissue as well. To further investigate the suppressive effect of ANGPTL4 on LPL protein abundance in human fat, it would have been very worthwhile to be able to study the effect of feeding status on ANGPTL4 and LPL protein levels in human subcutaneous adipose tissue. Unfortunately, not enough adipose tissue samples were left to measure ANGPTL4 and LPL protein levels. Also, given the unhealthy metabolic phenotype of the patients included in the MONDIAL study, the negative correlation between ANGPTL4 and LPL protein levels requires confirmation in adipose tissue of lean individuals.

In our *in vitro* and mouse studies we observed that ANGPTL4 specifically reduced the level of mature glycosylated, but not immature glycosylated LPL[19]. Surprisingly, only mature glycosylated LPL could be detected in human adipose tissue. In addition, the migration of LPL was only modestly reduced following the removal of all asparagine-linked glycosylation by PNGase F, suggesting that LPL in the subcutaneous adipose tissue of our patients was only glycosylated at one of the two potential glycosylation sites[32] (**Supplemental Figure 4**). These data are in disagreement with previously published studies showing that LPL in human adipose tissue has two glycosylation chains that are primarily of the immature kind[28-30]. Further studies into the exact nature of the glycosylation side chains of human adipose tissue LPL, by using endoglycosidases with different specificities such as Endo F1, Endo F2 or PNGase A, might provide an explication for this discrepancy[28-30].

In contrast to the negative correlation between adipose tissue ANGPTL4 and LPL at the protein level, we observed a strong positive correlation between ANGPTL4 and LPL at the gene expression level. The positive correlation between ANGPTL4 and LPL mRNA may be due to a common transcription factor that drives the expression of both genes. One candidate is PPARg, as both LPL and ANGPTL4 are established PPARg target genes in adipose tissue[25,26,33]. Yet, despite a highly significant correlation between LPL and PPARg mRNA levels, we only found a modest correlation between ANGPTL4 and PPARg mRNA. An alternative candidate is the glucocorticoid receptor. Indeed, glucocorticoids have been shown to increase LPL mRNA in human adipose organ cultures[34] and to upregulate ANGPTL4 mRNA in human primary adipocytes[35].

Our study indicated that ANGPTL4 mRNA levels were highest in the subcutaneous adipose tissue depot, whereas LPL mRNA levels were highest in the mesenteric adipose tissue depot. Consequently, the ratio of LPL to ANGPTL4 mRNA was highest in the mesenteric fat. A high ratio of LPL to ANGPTL4 is expected to favor fat storage. The molecular basis for the difference in absolute ANGPTL4 mRNA levels between the fat depots is unclear, but might be related to differences in oxygen levels or inflammatory status between the depots, as hypoxia and inflammatory mediators have been shown to influence ANGPTL4 mRNA levels[36–39]. Interestingly, we observed a clear positive correlation in ANGPTL4 mRNA levels between the different adipose tissue depots, suggesting that subject-specific mechanisms driving ANGPTL4 mRNA expression are active across the three fat depots. Whether the above observations can be generalized to lean individuals or are specific to obese individuals remains unclear.

In our study, we failed to observe a significant positive correlation between the concentration of ANGPTL4 in plasma and ANGPTL4 mRNA levels in liver and adipose tissue. These data suggest that the plasma concentration of ANGPTL4 is not primarily driven by hepatic and/or adipose ANGPTL4 mRNA levels. Previously, we found that the liver-specific activation of ANGPTL4 transcription by the PPARa agonist fenofibrate increased plasma ANGPTL4 concentrations in human subjects[24]. These data suggest that changes in ANGPTL4 production in human liver can influence plasma ANGPTL4 concentrations. In a cross-sectional study, however, other factors may overpower the influence of tissue-specific ANGPTL4 mRNA levels on the plasma ANGPTL4 concentration. ANGPTL4 and the related ANGPTL3 and ANGPTL8 share a common structure and the capacity to regulate LPL activity and plasma triglycerides, but to what extent their physiological functions are interconnected remains unclear[5,40–42]. Our data indicate that ANGPTL3, ANGPTL4 and ANGPTL8 expression levels are differentially regulated in human liver, as we did not observe a significant correlation between liver ANGPTL4 mRNA levels and liver ANGPTL3 and ANGPTL8 mRNA levels. These data corroborate previous studies showing that ANGPTL4 mRNA, and ANGPTL8 mRNA in liver are oppositely regulated by insulin and during physiological conditions such as fasting and refeeding[43–45]. Furthermore, it has been demonstrated that ANGPTL4 is an established PPARA target gene in the liver, whereas ANGPTL3, and possibly also ANGPTL8, are LXR target genes[25,46,47]. Strikingly, we found that, opposite to ANGPTL4, ANGPTL8 mRNA in human adipose tissue is very significantly increased upon feeding. These data are consistent with previous data showing that ANGPTL8 mRNA in human adipose tissue is highly induced by insulin[48]. Taken together, the differential regulation and lack of association between ANGPTL4 mRNA and ANGPTL3/ANGPTL8 mRNA suggests that the corresponding proteins likely impact plasma LPL activity and plasma triglyceride levels during different physiological conditions[5].

In conclusion, we report a negative correlation between ANGPTL4 and LPL protein levels in human subcutaneous adipose tissue, which might reflect a suppressive effect of ANGPTL4 on LPL protein abundance. In addition, we found that adipose tissue ANGPTL4 mRNA levels were significantly lower in the fed state compared to the fasted state. These data provide valuable insights into the physiological function of ANGPTL4 in humans.

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CONFLICT OF INTEREST

None of the authors have a potential conflict of interest to report relevant to this study.

AUTHOR'S CONTRIBUTION

S.S., E.A., I.M.C.J. and L.A. conceived and executed the MONDIAL study. S.S. and L.A. conceived and executed the Belly Fat study. S.S. collected material during the MONDIAL and the Belly Fat study. W.D. and S.K conceived the idea for the study described in this paper, with input from S.S. and L.A. W.D. performed most experiments, with help of S.S. W.D. and S.K performed data analysis and wrote the initial draft of the paper. All authors approved the final draft of this article.

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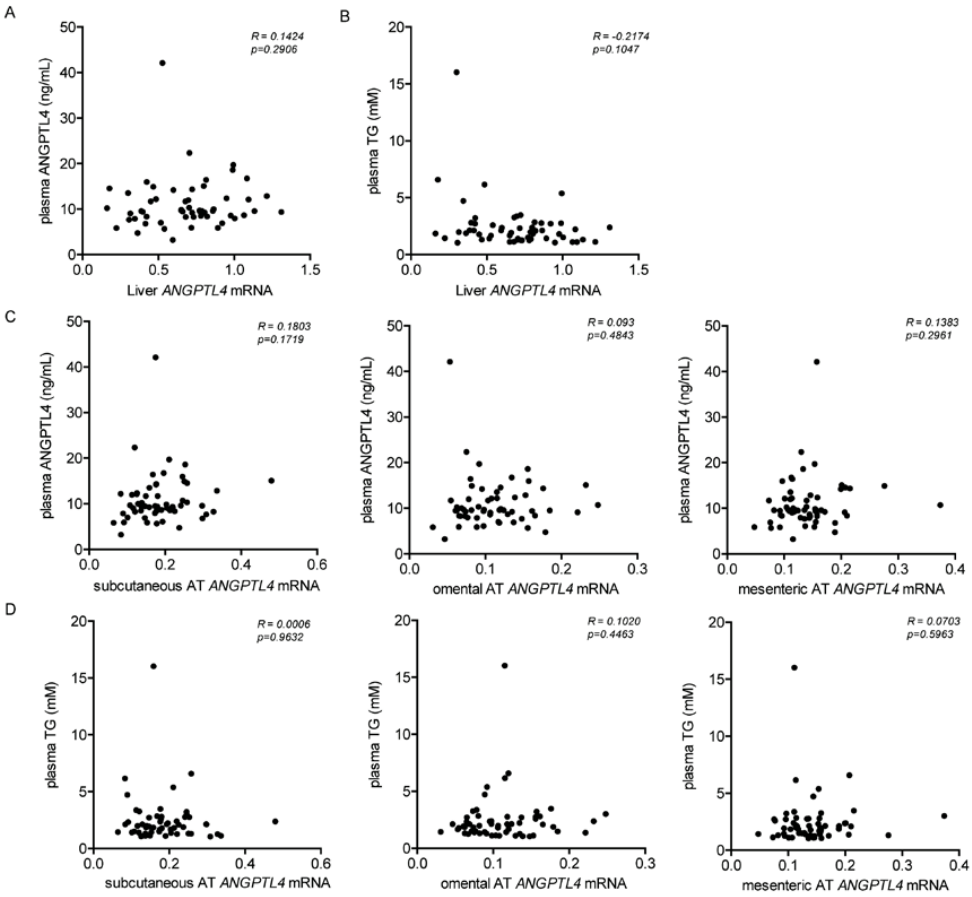
(ANGPTLs) 3 and 8 by Insulin. J Clin Endocrinol Metab. 2015;100(10):E1299-307. doi:10.1210/jc.2015-1254.

SUPPLEMENTAL MATERIAL

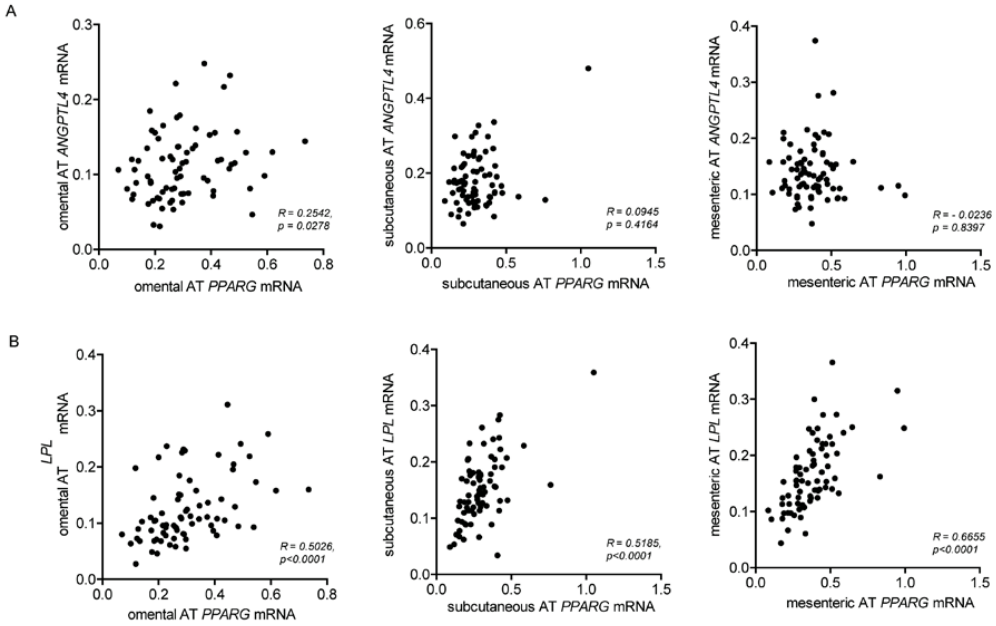
Supplemental Table 1. Primer sequences

PRIMER SEQUENCES		
Gene	**Forward primer**	**Reverse primer**
36B4	CGGGAAGGCTGTGGTGCTG	GTGAACACAAAGCCCACATTCC
BACTIN	AGAAAATCTGGCACCACACC	AGAGGCGTACAGGGATAGCA
CYPA	CCCACCGTGTTCTTCGACAT	TCTTTGGGACCTTGTCTGCAA
ANGPTL4	CACAGCCTGCAGACACAACTC	GGAGGCCAAACTGGCTTTGC
LPL	TCATTCCCGGAGTAGCAGAGT	GGCCACAAGTTTTGGCACC
PPARg	TCCATGCTGTTATGGGTGAA	TCAAAGGAGTGGGAGTGGTC
ANGPTL3	GCCAGTAATCGCAACTAGATGT	CTTCAATGAAACGTGGGAGAACT
ANGPTL8	TGGCCGCACAATAGAACTCC	TGAGTCTCCAACAGGCTTGC
PPARa	CAGAACAAGGAGGCGGAGGTC	TTCAGGTCCAAGTTTGCGAAGC



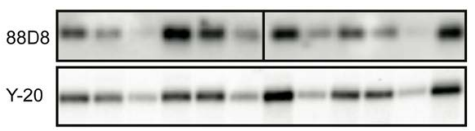
Supplemental Figure 1.

(A) Correlation between liver ANGPTL4 mRNA levels and plasma ANGPTL4 levels, measured in plasma and liver material obtained from patients undergoing bariatric surgery (*n*=59). (B) Correlation between liver ANGPTL4 mRNA levels and plasma triglyceride (TG) levels, measured in plasma and liver material obtained from patients undergoing bariatric surgery (*n*=59). (C) Correlation between ANGPTL4 mRNA levels in subcutaneous (left), omental (middle) and mesenteric (right) adipose tissue depots with plasma ANGPTL4 levels, measured in plasma and adipose tissue material obtained from patients undergoing bariatric surgery (*n*=59). (D) Correlation between ANGPTL4 mRNA levels in subcutaneous (left), omental (middle) and mesenteric (right) adipose tissue depots and plasma triglyceride (TG) levels, measured in plasma and adipose tissue material obtained from patients undergoing bariatric surgery (*n*=59). Correlations were analyzed by Spearman's R, p-values of <0.05 were considered statistically significant.



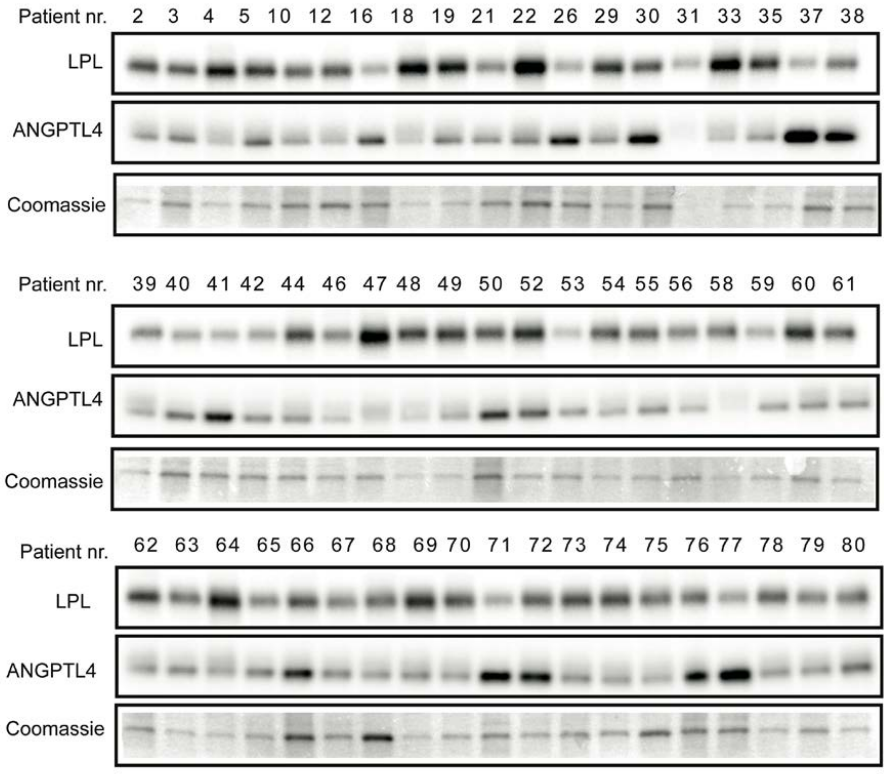
Supplemental Figure 2.

(A) Correlation between PPARg mRNA levels and ANGPTL4 mRNA levels in omental (left), subcutaneous (middle) and mesenteric (right) adipose tissue depots from patients undergoing bariatric surgery (*n*=76). (B) Correlation between PPARg mRNA levels and LPL mRNA levels in omental (left), subcutaneous (middle) and mesenteric (right) adipose tissue depots from patients undergoing bariatric surgery (*n*=76). Correlations were analyzed by Spearman's R, p-values of <0.05 were considered statistically significant.



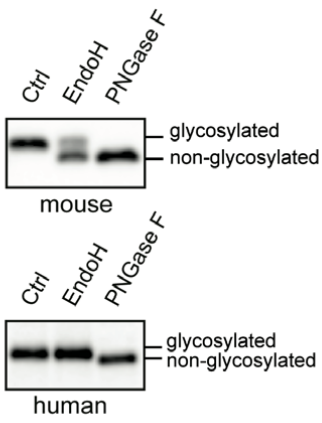
Supplemental Figure 3.

LPL protein levels in subcutaneous adipose tissue material of several patients, as detected using 2 different primary anti-LPL antibodies; 88D8 (kind gift from Anne Beigneux) and Y-20 (Santa Cruz Biotechnologies).



Supplemental Figure 4.

Western blots for LPL and ANGPTL4 in the subcutaneous adipose tissue depot of patients undergoing bariatric surgery (n=57) (anti-LPL antibody Y-20). Protein quantifications of patients 31, 53, and 58 were excluded from subsequent statistical analyses due to insufficient signal for either ANGPTL4 or LPL. Coomassie staining was performed to demonstrate approximately equal loading of protein between the different patients.



Supplemental Figure 5.

Western blots for LPL in mouse subcutaneous adipose tissue and in the subcutaneous adipose tissue depot of a patient undergoing bariatric surgery. Adipose tissue was either left untreated (Ctrl) or treated with the endoglycosidases EndoH or PNGase F to remove either high mannose N-linked oligosaccharide side chains (EndoH) or all N-linked oligosaccharide side chains (PNGase F).

7

GENERAL DISCUSSION & CONCLUSION

The aim of this thesis was to assess the effects of nutrient quality in eucaloric and energy restricted diets on metabolic health with a specific focus on the liver and adipose tissue, in overweight and obese individuals. To broadly evaluate the effects on metabolic health and to elucidate potential underlying mechanisms of nutrient quality we have applied comprehensive phenotyping, ~omics techniques, and standardized mixed meal challenge tests.

Summary of the main findings

In **Chapter 2** we showed that a 12 week intervention with refined wheat products negatively impacts liver health, mainly reflected by a massive increase in intrahepatic lipids. In contrast, a whole grain wheat intervention did not cause any change in intrahepatic lipids and reduced plasma levels of liver acute phase proteins. We concluded that refined wheat might contribute to the development of steatosis while incorporating feasible doses of whole grain wheat in the diet might prevent liver fat accumulation. The differential effects between refined and whole grain wheat consumption are summarized in **Figure 1**.

In **Chapter 3-5**, we showed that both a low quality Western-type energy restricted diet as well as a high quality energy restricted diet rich soy protein, fiber, MUFA, and *n-3* PUFA, induce clinically relevant, i.e. >5%, weight loss after 12 weeks of intervention. Weight loss was accompanied by a reduction in subcutaneous and visceral fat mass in the abdominal region as well as a reduction in intrahepatic lipids. Furthermore, both diets reduced systolic and diastolic blood pressure and improved measures of glucose homeostasis and insulin sensitivity. However, the high quality diet induced a greater body weight loss, an anti-atherogenic blood lipid profile, and more enhanced changes in adipose tissue gene expression of metabolic pathways. Thus, while both energy restricted diets alleviated several metabolic complications associated with abdominal obesity, the high quality diet might be considered as the superior whole diet approach in improving metabolic health in these subjects. The effects induced by both diets, summarized as the effects of >5% weight loss, and the additional benefits of nutrient quality are depicted in **Figure 2**.

In **Chapter 6**, we examined the regulation of ANGPTL4 and LPL in the liver and adipose tissue. We demonstrated a strong positive correlation between gene expression of ANGPTL4 and LPL in subcutaneous and omental adipose tissue of obese males and females. In contrast, protein levels of ANGPTL4 and LPL were negatively associated in the subcutaneous depot, which might reflect the inhibitory effects of ANGPTL4 on LPL by intracellular degradation. This effect had been previously demonstrated in mouse studies but not in human

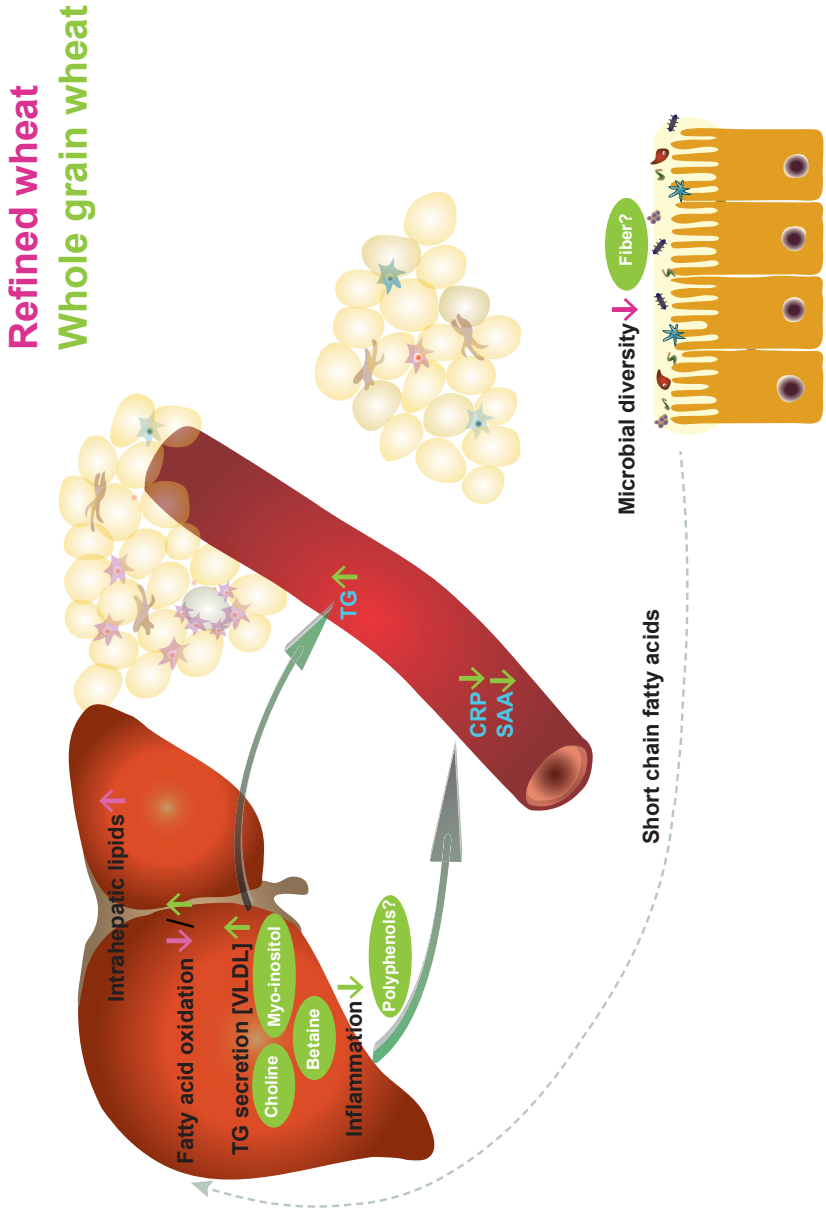


Figure 1. Differential effects of refined and whole grain wheat consumption on metabolic health

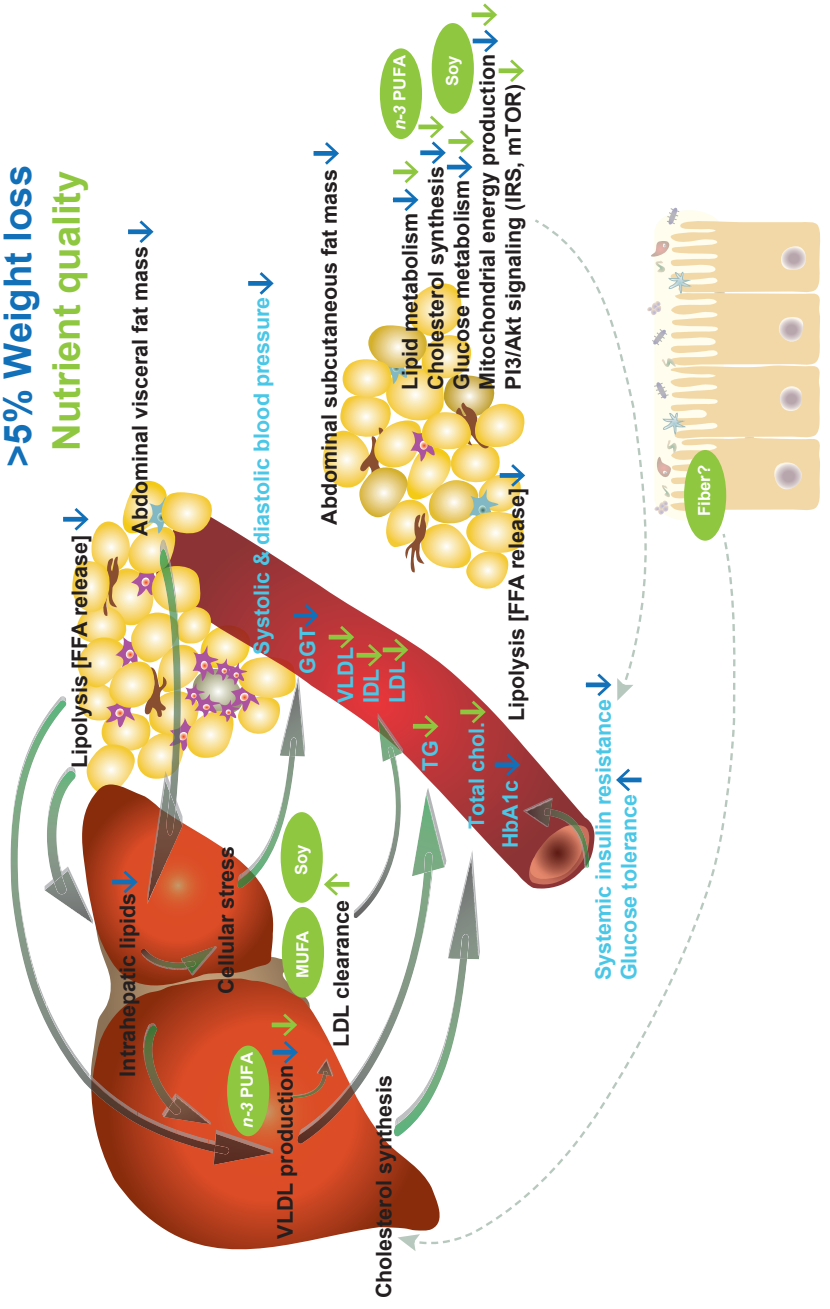


Figure 2. Additional effects of nutrient quality within weight loss diets on metabolic health

tissue. Furthermore, we showed for the first time that a mixed meal test induces a decrease in gene expression of ANGPTL4 in subcutaneous adipose tissue of overweight and obese males and females. This might abolish the suppressive effect of ANGPTL4 on LPL and allows the adipose tissue to take up triglyceride-rich lipoproteins in the postprandial phase.

The observed effects of nutrient quality on metabolic health with the liver and adipose tissue as important protagonists can be divided into three main areas. These will be discussed below and include insulin sensitivity and glucose homeostasis, intrahepatic lipids and liver health, and lipid metabolism.

Role of nutrient quality in insulin sensitivity and glucose homeostasis

The effects of both dietary interventions on parameters related to insulin sensitivity and glucose homeostasis are summarized in **Table 1**. In line with other studies[1], weight loss directly resulted in the improvements in parameters that reflect glucose homeostasis and insulin sensitivity. Beneficial effects were not enhanced by nutrient quality in the weight loss diets, nor observed in the high nutrient quality whole grain intervention. For both the high quality ER-diet, which contained more dietary fiber than the Western-type ER-diet, as well as for the whole grain diet we hypothesized to expected a beneficial effect on insulin sensitivity because of a lower glycemic load. A reduced glycemic load prevents exaggerated glucose responses and might improve overall glucose homeostasis on the long-term[2]. Furthermore, this lower glycemic load can reduce *de novo* lipogenesis in the liver[3], which leads to lower levels of intrahepatic lipids. As high levels of intrahepatic lipids are described as in independent risk factor whole body insulin resistance[4], it might be that the liver plays a pivotal role in mediating potential positive effects of whole grains or negative effects of refined products with a high glycemic load on insulin sensitivity. Although we observed a large increase in intrahepatic lipids after refined wheat consumption, there were no indications for a decrease in insulin sensitivity. This could indicate that the increase in liver fat is an early adaptation on organ level which can precede changes in systemic parameters. Likewise, there were no differences in improvements in insulin sensitivity between the two ER-diets but we did reveal an overall downregulation of the insulin-related PI3/Akt signaling pathway[5,6] in adipose tissue specifically in the high nutrient quality diet. This reduction might also reflect an early organ adaptation preceding changes in blood levels of glucose or insulin. It would be interesting to see if the high quality diet could further enhance insulin sensitivity after an extended intervention, beyond improvements induced by the Western-type diet.

Table 1. Insulin sensitivity & glucose homeostasis

	EUCALORIC DIETS Chapter 2		ENERGY RESTRICTED DIETS Chapter 3-5 ER		No ER
	Refined wheat	Whole grain wheat	Western-type diet	High quality diet	Control
Glucose ⊙ Fasting ⊙ Postprandial	↔	↔	↓ ↓	↓ ↓	↔ ↔
Insulin ⊙ Fasting ⊙ Postprandial	↔	↔	↓ ↔	↓ ↔	↔ ↔
Free fatty acids ⊙ Fasting ⊙ Postprandial	↔ ↔	↔ ↔	↔ ↓	↔ ↓	↔ ↔
Surrogate markers ⊙ HOMA-IR ⊙ QUICKI	↔	↔	↓ ↓	↓ ↓	↔ ↔
HbA1c			↓	↓	↔
Adipose tissue gene expression ⊙ Pathways related to insulin signaling			↔	↓ PI3/Akt	↔

Effects of nutrient quality on parameters related to insulin sensitivity and glucose homeostasis. ↑: significantly increased, ↓: significantly decreased, ↔: no change. Abbreviations: ER: energy restriction, HOMA-IR: homeostatic model assessment of insulin resistance, QUICKI: quantitative insulin-sensitivity check index.

Role of nutrient quality in intrahepatic lipids and liver health

We observed a large increase in intrahepatic lipids after refined wheat consumption of 49.1%, while energy restriction reduced intrahepatic lipids by 46% in the Western-type diet and 55% in the high quality ER-diet (**Table 2**). Studies indeed show that moderate weight loss of 5-10% is already associated with large, up to 80%, reductions in liver fat through quick mobilization of these triglycerides to fuel other tissues[7,8]. The differential effects of refined and whole grain wheat on liver health indicate that whole grain wheat consumption might protect against an increase in liver fat. If both energy restriction and nutrient quality can play a role in the reduction of intrahepatic lipids, adding whole grain wheat to a weight loss diet can be a strategy to enhance beneficial effects on liver fat. The high quality ER-diet was enriched with fiber from multiple whole grain sources including wheat, but this did not result in an additional reduction in liver fat in this diet group. This is remarkable, as more nutrients in the high quality ER-diet were selected based on their ability to decrease liver fat, such as MUFA and PUFA, simultaneously increasing hepatic beta-oxidation through activation of PPARa and decreasing de novo lipogenesis by inhibiting SREBP[9]. There could be a true absence of a difference, for instance due to the dominant improvements by energy restriction. Another explanation for the lack of a difference can be analytical issues including the slightly smaller subset of participants in which ^{1}H-MRS measurements were performed, a

large inter-individual variation in liver fat specifically in the high quality diet group, and several confounding factors in ^{1}H-MRS measurements such as noise bias[10].

Table 2. Intrahepatic lipids & liver health

	EUCALORIC DIETS Chapter 2		ENERGY RESTRICTED DIETS Chapter 3-5 ER		No ER
	Refined wheat	**Whole grain wheat**	**Western-type diet**	**High quality diet**	**Control**
Intrahepatic lipids	↑	↔	↓	↓	↑
Liver enzymes					
⊙ ALT	↔	↔	↔	↔	↔
⊙ AST	↔	↔	↔	↔	↔
⊙ GGT	↔	↔	↓	↓	↔
Liver acute phase proteins					
⊙ SAA	↔	↓			
⊙ CRP	↔	↓			
Hepatic beta-oxidation					
⊙ Beta-hydroxybutyrate*	↓/↔	↑/↔			
Potential organ cross-talk					
Adipose tissue					
⊙ SAT mass	↔	↔	↓	↓	↔
⊙ SAT adipocyte size	↔	↔			
⊙ VAT	↔	↔	↓	↓	↔
⊙ VAT/SAT ratio	↔	↔	↓	↓	↔
⊙ SAT gene expression; Pathways related to storage capacity / tissue remodeling	↔ TGFB1, MMP9, LOX	↔ TGFB1, MMP9, LOX	↓ Collagen formation, ECM remodeling	↓ Collagen formation, ECM remodeling	↔
Gut					
⊙ Gut microbiota diversity	↓	↔			

Effects of nutrient quality on parameters related to liver health. ↑: significantly increased, ↓: significantly decreased, ↔: no change. * Changes in beta-hydroxybutyrate were borderline significantly different between refined and whole grain wheat (p=0.063) Abbreviations: ER: energy restriction, ALT: alanine transaminase, AST: aspartate transaminase, GGT: gamma-glutamyltransferase, SAA: serum amyloid A, CRP: C-reactive protein, SAT: subcutaneous adipose tissue, VAT: visceral adipose tissue, TGFB1: transforming growth factor beta 1, MMP9: matrix metallopeptidase, LOX: lysyl oxidase, ECM: extracellular matrix

As the liver is central in metabolism, the health of other organs might impact hepatic processes. Important potential organ cross-talk exists between the liver, the adipose tissue, and the gut. Improvements in subcutaneous and visceral adipose tissue health can prevent ectopic fat storage in the liver by proper storage in the subcutaneous depots and decreased free fatty acid supply from visceral depots to the liver. The changes in liver fat in the wheat study did not coincide with changes in abdominal fat mass while those changes were observed in the ER-diets. It might be that whole grain wheat specifically targets the liver, but it is also likely that nutrient quality within an eucaloric diet is not potent enough to drive changes in fat mass. In line with this finding, whole grain or refined wheat did not affect expression of genes in the adipose tissue related to extracellular matrix (ECM) remodeling. Impaired ECM dynamics can lead to fibrosis and limited the storage

capacity of the tissue[11]. In contrast, the reduction in subcutaneous fat mass in both ER-diets was accompanied by a downregulation of the expression of adipose tissue pathways related to collagen formation and extracellular matrix remodeling. However, it must be noted that these findings were observed after an unbiased analysis using whole genome adipose tissue transcriptomics, compared to a targeted qPCR of only 3 genes in the wheat study.

Gut health, mainly in relation to microbiota composition, can also affect hepatic metabolism. We demonstrated that refined wheat decreased overall microbial diversity but did not specifically decrease the relative abundance of several known short chain fatty acid producers, while these fermentation products have been demonstrated in animal studies to increase hepatic beta-oxidation[12-14]. Most research in this area has focused on the impact of dietary fiber on gut microbiota but some studies demonstrate that dietary fat such as MUFA and *n-3* PUFA also beneficially affect microbiota composition, although underlying mechanisms are poorly understood[15-17]. To gain better insight in the effects of nutrient quality on gut microbiota it would be interesting to determine the effects of our two ER-diets on microbiota composition.

Role of nutrient quality in lipid metabolism

A reduction in intrahepatic lipids might decrease the secretion of triglyceride-rich lipoproteins, i.e. VLDL, to the circulation. Interestingly, pronounced effects on fasting triglycerides and lipoproteins, mainly larger VLDL particles, were observed for the high quality ER-diet while no such affects were observed in the Western-type diet (**Table 3**). Specific high quality nutrients such as *n-3* PUFA might have affected secretion of VLDL, apparently without affecting total lipid content in the liver. The *n-3* PUFA EPA and DHA have repeatedly demonstrated to be able to lower plasma triglycerides, which is thought to be caused by a decrease in free fatty acid availability in the liver as well as a stimulation of the degradation of ApoB, together lowering hepatic triglyceride-VLDL output[18,19]. The reductions in the high quality diet coincided with a more pronounce decrease in fasting gene expression of lipid-related pathways in adipose tissue, mainly fatty acid and triglyceride synthesis. In the wheat study, we observed no effect on fasting plasma triglycerides or differences in expression of genes related to lipid metabolism upon either refined or whole grain wheat consumption.

Based on the findings on liver fat, we hypothesized that consumption of refined grain wheat might increase fasting plasma triglycerides. Interestingly, we observed an increase in 4h postprandial plasma triglycerides after the whole grain wheat intervention. VLDL particles were not measured in this study but studies show that the contribution of VLDL to plasma triglycerides peaks at 4h in the postprandial phase[20]. This might thus mean that

Table 3. Lipid metabolism

	EUCALORIC DIETS Chapter 2		ENERGY RESTRICTED DIETS Chapter 3-5 ER		No ER
	Refined wheat	Whole grain wheat	Western-type diet	High quality diet	Control
Triglycerides					
⦿ Fasting	↔	↔	↔	↓	↔
⦿ Postprandial	↔	↑	↔	↔	↔
Total cholesterol					
⦿ Fasting	↔	↔	↔	↓	↔
HDL cholesterol					
⦿ Fasting	↔	↔	↔	↔	↔
VLDL-related particles					
⦿ Fasting			↔	↓	↔
⦿ Postprandial			↓	↓↓	↔
IDL-related particles					
⦿ Fasting			↔	↓	↔
⦿ Postprandial			↓	↓↓	↔
LDL-related particles					
⦿ Fasting			↔	↔	↔
⦿ Postprandial			↓	↓↓	↔
HDL-related particles					
⦿ Fasting			↔	↓	↔
⦿ Postprandial			↔	↔	↔
Adipose tissue gene expression					
Pathways related to cholesterol synthesis					
⦿ Fasting			↓	↓↓	↔
⦿ Postprandial			↓↓	↓	↔
Pathways related to lipid metabolism					
⦿ Fasting	↔ ANGTPL4, LPL, CD36	↔ ANGTPL4, LPL, CD36	↓ synthesis	↓↓ synthesis	↔
⦿ Postprandial			↓ synthesis ↔ uptake	↔ synthesis ↔ uptake	↔ ↔

Effects of nutrient quality on parameters related to liver health. ↑: significantly increased, ↓: significantly decreased, ↔: no change. Abbreviations: ER: energy restriction, HDL: high density lipoprotein, VLDL: very low density lipoprotein, IDL: intermediate density lipoprotein, LDL: low density lipoprotein, ANGPTL4: angiopoietin-like 4, LPL: lipoprotein lipase, CD36: fatty acid translocase (cluster of differentiation 36)

the increased triglycerides levels might reflect increased hepatic VLDL-output or a decreased VLDL clearance. Elevated plasma triglycerides are considered a risk factor for developing coronary artery disease[21] but an increase in VLDL secretion could also be favorable, as it might lower intrahepatic lipids and diverts triglycerides from the liver to the desired storage in the adipose tissue. However, there were no changes in fasting expression of genes related to lipid uptake upon either refined or whole grain wheat consumption, but postprandial regulation might be more relevant. It remains to be elucidated whether an increase in postprandial triglycerides is beneficial or not. In contrast to the effects of whole grain wheat, both ER-diets reduced postprandial VLDL levels with

larger reductions observed in the high quality ER-diet. The decrease in postprandial VLDL-related particles was mainly observed at 4h, hence we speculated that a decreased hepatic secretion may be an important underlying mechanism. Another mechanism that contributes to a reduction in postprandial VLDL is an increased uptake of triglyceride-rich lipoproteins by peripheral tissues such as the adipose tissue. We did not specifically analyze changes in postprandial adipose tissue gene expression induced by ER in general but only compared the two ER-diets in **Chapter 5**. Therefore, we conducted a sub-analysis but found no changes in the pathway related to lipoprotein uptake upon ER, but it might be that these pathways were underrepresented in the used databases. However, the decrease in postprandial expression of ANGPTL4, as showed in **Chapter 6**, was not altered upon ER and neither was the postprandial expression of LPL or CD36. So far, indications for an increased clearance of triglyceride-rich lipoproteins are limited but it might be that regulation on protein level is more relevant, as we showed in **Chapter 6**.

In addition to effects on plasma VLDL and triglycerides, we observed a clear effect of nutrient quality on fasting cholesterol, as the high quality ER-diet but not the Western-type diet reduced fasting total cholesterol. Furthermore, pathways related to cholesterol synthesis in the adipose tissue were significantly more downregulated in the fasted state by the high quality diet when compared to the Western-type diet. Both MUFA and soy protein have shown to lower levels of cholesterol[22-24]. This effect is mainly observed for LDL cholesterol, while the largest reductions induced by the high quality diet were on fasting cholesterol-containing medium/large VLDL particles. However, a human study using stable-isotope tracers showed that a MUFA-rich diet also strongly affects both the secretion and clearance of triglyceride-rich lipoproteins such as VLDL[25]. In addition to MUFA and soy protein, soluble fiber also has showed to have a small, but significant decreasing effect on total and LDL cholesterol levels[26]. This is likely caused by an increased excretion of bile acids, leading to a decrease in hepatic cholesterol and an upregulation of the LDL receptor[27]. This upregulation results in increased removal of LDL cholesterol from the circulation while the decrease in hepatic cholesterol causes a reduction in secretion of VLDL cholesterol into the circulation, thereby lowering plasma total and LDL cholesterol levels[26]. An increased fiber intake might thus have contributed to the cholesterol-lowering effects of the high quality diet. Remarkably, whole grain wheat consumption did not affect cholesterol levels. We specifically included individuals with slightly elevated cholesterol levels in this study to enable improvements in this parameter, as studies show that whole grain diets indeed tend to lower total and LDL cholesterol[28]. Furthermore, the differences in fiber intake from the intervention products were quite large, with 2.5g/day for refined and 10.3g/day for whole grain wheat. According to the Dutch national Food Consumption Survey 2007-2010, bread and cereals contribute up to 43%

of the daily fiber intake[29]. In comparison, total fiber intake in the high quality and Western-type diet was 27.9, respectively 18.4g/day. The difference in fiber intake between the diets in both studies can be seen as relatively comparable. It might be that dietary fat plays a larger role in modulating blood lipid profile when compared to fiber or that ER is a prerequisite for the effects.

In the postprandial phase, both ER-diets decreased postprandial responses of cholesterol-containing IDL and LDL-particles. The effects were again more pronounced in the high quality ER-diet. Remarkably, gene expression of pathways related to cholesterol synthesis strongly decreased in the postprandial phase after the Western-type diet intervention. The question remains whether an increase or decrease in adipose tissue cholesterol synthesis has an effect on plasma cholesterol levels, as the adipose tissue is not the primary site for cholesterol synthesis and the tissue might utilize cholesterol for intracellular processes only.

The application of phenotypic flexibility in our studies

The concept of applying a stressor to challenge homeostasis has become increasingly popular over the last decade (**Figure 3**). The ability to deal with this stressor, i.e. phenotypic flexibility, might be a better indicator of health status than fasting measurements, as fasting parameters are usually under tight homeostatic control. Therefore, we were interested in how our dietary interventions would affect phenotypic flexibility, assessed by determining the response to a high fat/sugar/protein mixed meal. The postprandial measurements did yield a wealth of information but we also revealed several issues that need to be recognized when using this approach in human intervention studies.

First, it is important to define the healthy response to a challenge test. Diminished phenotypic flexibility can be described as a decreased ability to fine-tune postprandial processes to the requirements, which will lead to perturbations and exaggerated responses that exceed the range of what we still consider healthy. However, while the fact that postprandial glucose levels are best kept as low as possible is logical, responses of other parameters might not be as intuitive. An example from our studies is the decrease in the initial postprandial increase in cholesterol synthesis in the adipose tissue after both ER-diet. The Western-type diet induced a more pronounced decrease when compared to the high quality diet. Lower cholesterol synthesis can be beneficial for adipose tissue health, as high intracellular cholesterol levels trigger lipogenesis and local insulin resistance[30-32]. On the other hand, this decrease can reflect a diminished capacity to respond to the meal challenge, pointing toward a reduced flexibility of the adipose tissue. In this case, a lower postprandial response might not be the

preferred outcome for health.

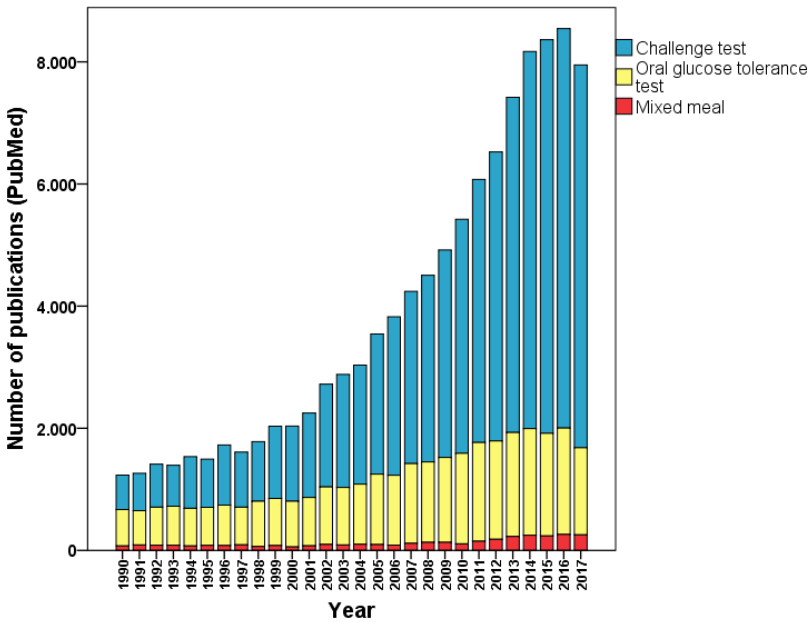


Figure 3. Number of publications on challenge tests, oral glucose tolerance tests, and mixed meal tests 1990-2017

Number of publications in the MEDLINE database

Second, responses to nutritional challenges show high inter-individual variation and might be relatively resistant to change. In 2015, Zeevi et al published a much-discussed paper in which they continuously monitored glucose levels of 800 individuals and determined the postprandial responses of glucose to >45,000 meals[33]. Surprisingly, they observed huge differences in glucose response between individuals to identical meals. In contrast, postprandial responses within persons were very well correlated, thus reproducible. In line with these findings, we observed in our weight loss study that some features of the postprandial glucose response were relatively robust, i.e. not easily altered upon the dietary intervention. For instance, some participants returned to fasting glucose values 2h after consumption of the mixed meal while others retained high postprandial glucose levels at 2h. This classification of 'returners' and 'non-returners' remained valid in ~75% of the cases after intervention, despite the fact that both ER-diets significantly lowered postprandial glucose levels. Although postprandial responses can thus be considered as a relatively rigid individual trait, we can use it to our advantage by tailoring dietary interventions to response-phenotype. With this 'personalized nutrition' approach we might be able to

maximize health benefits from dietary interventions[34].

A third issue we would like to discuss is the assumption that postprandial responses might form a more sensitive measure than fasting measurements. Results from our weight loss study show that the majority of classical parameters that we assessed already showed improvements in the fasted state. Weight loss itself was already a potent driver of improvements in metabolic health. An important exception was the analysis of postprandial plasma metabolites. The postprandial metabolomics analyses magnified the effects of the dietary interventions, especially for the Western-type diet in which the effects in the fasted state were minimal. In addition, postprandial measurements can yield more insight in adaptive responses that might involve different processes or even different organs than those implicated in the fasted state. Ever since Zilversmit in 1979 stated that atherosclerosis is essentially a postprandial phenomenon[35], it has become widely accepted that assessing postprandial pro-atherogenic particles such as chylomicron remnants and LDL particles is superior over measurements in the fasted state in determining cardiovascular disease risk[36-38]. Likewise, fasting glucose levels provide information about glucose homeostasis, mainly regulated through hepatic glycogenolysis and gluconeogenesis, while postprandial glucose levels provide information about insulin sensitivity, in which other glucoregulatory mechanisms play a role including pancreatic beta-cell functioning, gut incretin secretion, and insulin responsiveness of peripheral tissues[39].

Fourth, the composition of the nutritional challenge test is important. The composition of the mixed meal used in our studies was based on a systematic review aimed to develop the optimal nutritional stress test[40]. Mixed meals have slowly gained ground as challenge test over the past decade (**Figure 3**) as they challenge multiple metabolic organs and their interplay, requiring adaptation at several levels. A mixed meal calls for a more complex and well-orchestrated response when compared to simple challenges such as the OGTT. Furthermore, a mixed meal is more representative for actual meals than single nutrient challenges. In addition to the fat/sugar/protein ratio, it is important which fatty acids are emphasized in the mixed meal, as fatty acid saturation impacts the nature of the postprandial response. For example, studies show that MUFA challenges elicit higher postprandial responses of plasma triglycerides when compared to SFA challenges[41,42]. MUFA was most dominant in our challenge, covering 45% of the fat fraction. Indeed, we observed a high and prolonged postprandial triglyceride response in both studies with increasing levels up to 6h in the postprandial phase.

Finally, although the fact that a mixed meal test can stimulate an array of processes can be considered an asset, it also complicates the analysis of its read-outs. We have spent considerable time and effort to optimize the statistical analyses but it remains challenging to proper quantify postprandial responses. Responses can be measured using a type of repeated measure analysis or by determining specific aspects of the response curve, such as the area or incremental area under the curve (AUC), the peak, or the slope. We argued that a repeated measure analysis would suit our data best as we did not measure continuously, which leads to an estimation the peak and slope, while determining the AUC means discarding important data on the shape of the curve. The AUC can best be used for response curves with a relatively predictable/straight-forward nature, such glucose responses after an OGTT. However, we observed that a mixed meal can evoke erratic glucose responses (**Figure 4**), with some individuals even displaying a decrease in glucose levels 1h after consumption of the meal. The high caloric density of the meal as well as the combination of macronutrients affect the rate of gastric emptying and secretory processes such as GLP1 and C-peptide responses in a different way than glucose ingestion alone[43,44]. It is reasonable to assume that these processes are also subject to inter-individual variation, leading to high variety in responses.

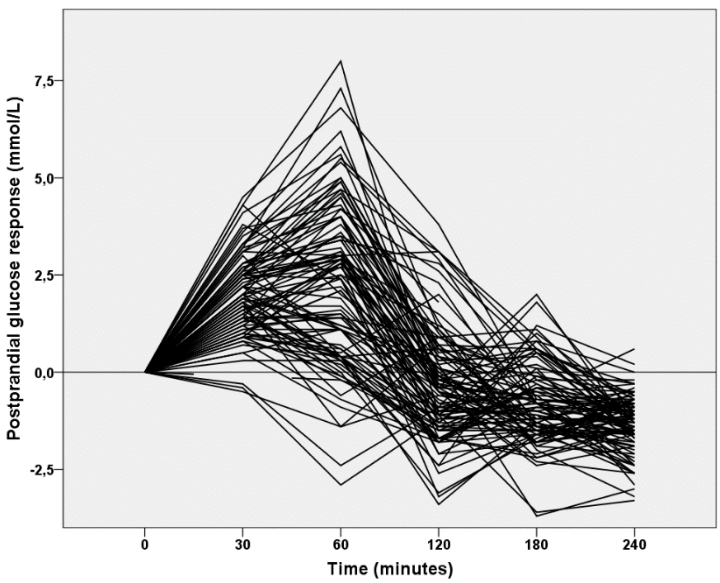


Figure 4. Individual postprandial glucose responses to the mixed meal test at baseline

Derived from data from Chapter 3

Strengths and limitations

Within the studies described in this thesis we have made full use of the toolbox of nutrition research by combining classical markers with sensitive approaches such as phenotypic flexibility, comprehensive techniques such as transcriptomics and metabolomics, and state of the art imaging techniques such as MRI and ^{1}H-MRS to completely phenotype the effects of our dietary interventions on metabolic health. In addition, studies showing postprandial adipose tissue transcriptional data are rare and, to the best of our knowledge, we are the first to show this data in relation to a longer-term dietary intervention. The results from both our dietary interventions studies can be generalized to a broad population as we included participants with a common metabolic risk profile, at least in the Netherlands. Population screening shows that in 2016 almost half (49.2%) of the Dutch population was overweight while 14.2% suffered from obesity[45].

Studying a whole diet approach has both pros and cons. Whole diets reflect habitual intake better than single nutrients and the effects of nutrition cannot always be reduced to the sum of the effects of nutrients. This indicates the importance of food matrices and the fact that foods/nutrients interact with each other and might even exert synergetic effects on metabolic health. On the other hand, whole diets and mechanistic research might form a paradoxical combination, as mechanistic research usually takes a reductionistic approach and decomposes complex pathways and foods to find an explanatory mechanism. We speculated on potential underlying but a next step would be studying ER diets enriched with only high quality nutrient, to further strengthen the evidence of a role of this nutrient in the observed health improvements and to see whether a synergistic effect of nutrients exists.

Future perspectives

We have compared the effects our high quality nutrient diet to a low quality Western-type diet, which also induced several health improvements but some benefits of weight loss could have been counteracted by poor quality nutrients such as SFA and fructose. Although this approach have led to interesting insights in which metabolic parameters are sensitive to weight loss and/or which to nutrient quality, the addition of a more 'neutral' weight loss diet, for instance in line with the Dutch dietary guidelines[46], could place the results of our high quality diet in a broader perspective. The same holds true for an eucaloric high quality diet containing soy protein, fiber, MUFA, and *n-3* PUFA.

Although we made the first steps towards combining the collected data into a framework of how nutrient

quality affects metabolic health (**Figure 1 & 2**), underlying mechanisms are complex and how organ interplay is affected by diet is poorly understood and remains speculative. An ultimate strategy to further enlarge our understanding is to integrate ~omics data from multiple sources, for instance transcriptomics, metabolomics, epigenetic data, proteomics, or microbiome data[47,48] using software packages for specific multivariate analyses such as the R package mixOmics[49]. This software can model correlations between data points of different data sets. This potentially reveals interesting relationships between the measurements, which might lead to a better understanding of organ crosstalk and how processes in different metabolic organs affect things at a systemic level, i.e. in the blood. Furthermore, with this approach we might be able to identify a specific molecular signature/fingerprint that forms a distinct read-out of our dietary interventions.

We observed pronounced lipid-lowering effects of our high quality ER-diet and speculated on the role of the liver and adipose tissue in these improvements. By studying fatty acids fluxes we might be better able to further elucidate the role of these organs in modulating plasma lipids[50]. Therefore, in future studies it would be interesting to make use of labeled fatty acid in the mixed meal test, to be trace their fate and gain better understanding of postprandial lipid metabolism.

By using a comprehensive phenotyping approach we now have a wealth of information and a fairly good indication of the effects of our dietary interventions on metabolic health on a group level. However, we could also analyze this data on the individual level to assess whether baseline phenotypic traits or even genotype affect the way individuals respond to dietary interventions. These traits can range from baseline body composition or liver fat to gut microbiota composition or, as mentioned earlier in this chapter, postprandial responses to the mixed meal. It would be interesting to see whether we can identify groups of individuals having the same phenotypic traits and the same response to a dietary intervention. If we are, to some extent, able to predict the response a certain group of individuals on the intervention we might be able to optimize this intervention and maximize its health benefits. With personalized nutrition we can thus use inter-individual variation to our advantage. We have performed such an analysis in **Chapter 2**, in which we tried to predict changes in intrahepatic lipids upon intervention based on baseline microbiota composition. There was no association but this could be due to the small sample size. Both our dietary intervention studies have yielded substantial and interesting data that can be used to elucidate potential phenotype-diet response interactions thereby potentially supporting other, more personalized dietary interventions in the future.

CONCLUSION

In this thesis I have phenotyped the effects of nutrient quality in both eucaloric and energy restricted diets on metabolic health in overweight and obese individuals. The liver was identified as an important target organ in eucaloric refined/whole grain wheat modulations as it was shown that whole grain wheat protects against the massive increase in intrahepatic lipids that was observed after refined wheat consumption. Furthermore, my results show that a high nutrient quality energy restricted diet enriched with soy protein, fiber, MUFA, and *n-3* PUFA provides additional health benefits over a Western-type diet, mainly by inducing a greater body weight loss and promoting an anti-atherogenic blood lipid profile, characterized by lower plasma triglycerides, total cholesterol, and ApoB-containing lipoproteins. Sensitive high through-put techniques and postprandial analyses enabled us to partly elucidate potential underlying mechanisms of the lipid-lowering effects of the high quality diet, which included a strong reduction in VLDL production and secretion as well as a pronounced downregulation of pathways related to lipid metabolism in the adipose tissue. As the lipid-lowering effects were not demonstrated for eucaloric high quality whole grain wheat diet, dietary fat, i.e. MUFA and *n-3* PUFA, might have played a large role in the observed effects.

To conclude, consumption of whole grain wheat might prevent intrahepatic lipid accumulation and thus lower non-alcoholic fatty liver disease risk, while a weight loss diet rich in soy protein, fiber, MUFA, and *n-3* PUFA holds great potential in lowering the risk of cardiovascular diseases, such as atherosclerosis. This thesis shows that it does matter which nutrients are emphasized within diets. Diets rich in high quality nutrients provide clear health benefits when compared to Western-style diets characterized by high intake of refined wheat, fructose, and SFA. My findings have increased the understanding of the effects of nutrient quality on metabolic health, mediated by the liver and adipose tissue, and can aid in designing optimal dietary strategies to combat pathologies associated with obesity. Moreover, this thesis yields interesting data that can be further analyzed in the context of personal responses, with the optimization of these dietary strategies through personalized nutrition as ultimate goal.

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ENGLISH SUMMARY

The growing burden of obesity-related pathologies calls for effective dietary strategies that improve metabolic health of overweight and obese individuals. Chronic diet-related diseases are the result of perturbations in different metabolic organs and/or their elegant organ interplay, important metabolic organs in this respect are the liver and the adipose tissue. An evidently effective dietary strategy to correct metabolic parameters associated with chronic diseases is limiting the intake of daily calories, i.e. energy restriction (ER). In addition to lowering the energy quantity of the diet, increasing the quality of the diet by consuming more high quality nutrients can also be an appealing dietary approach. Poor quality Western-type diets rich in refined wheat, sugars including fructose, and SFA are an important risk factor of ill health. Combining multiple high quality nutrients within an ER-diet might be an even more powerful *whole diet approach*.

However, measuring the effects of dietary strategies is challenging as effects of nutrition on health are usually relatively subtle may take a long time to manifest. In addition, classical blood markers assessed in nutritional intervention studies are typically late disease markers. To be able to more precisely assess the effect of dietary interventions and to elucidate underlying mechanisms, we need to adopt a comprehensive phenotyping approach in which we combine classical markers with sensitive measures, such as the ability of an individual to deal with a dietary stressor, i.e. phenotypic flexibility, and extensive high-throughput techniques. The aim of this thesis was to assess the effects of nutrient quality on metabolic health with a specific focus on the liver and adipose tissue, in overweight and obese individuals. Therefore, we applied comprehensive phenotyping, ~omics techniques, and standardized mixed meal challenge tests in two human intervention studies, one in which we studied nutrient quality within eucaloric diets and one in which nutrient quality within ER-diets was examined In addition, we executed an observational study to elucidate the physiological function and regulation of two important metabolic regulators in the liver and adipose tissue, ANGPTL4 and LPL.

The effects of nutrient quality within eucaloric diets were studied in **Chapter 2**, in which we examined the effects of a 12 week intervention with either refined wheat or whole grain wheat bread and cereals in overweight and obese individuals. Consumption of the low nutrient quality refined wheat resulted in a pronounced increase in intrahepatic lipids. Furthermore, refined wheat consumption was associated with a decrease in gut microbiota diversity. Whole grain wheat consumption induced a trend towards lower fasting levels of liver acute phase proteins and an increase in postprandial triglycerides after a mixed meal, which may indicate greater hepatic triglyceride output. Incorporating feasible doses of whole grain wheat in the diet at the expense of refined wheat thus favorably affects liver health.

The effects of nutrients quality within ER-diets were studied in **Chapter 3-5**, in which we examined the effects of a high quality ER-diet rich in soy protein, fiber, MUFA, and *n-3* PUFA and a low nutrient quality Western-type ER-diet in subjects with abdominal obesity. Both diets induced clinically relevant weight loss after 12 weeks, accompanied by reductions in subcutaneous and visceral fat mass and intrahepatic lipids. Despite an equal ER of 25%, participants in the high quality diet group lost a third more (2.1kg) when compared to participants in the Western-type diet. Both ER-diets alleviated metabolic complications associated with abdominal obesity, such as reductions in systolic and diastolic blood pressure and improvements in systemic markers related to glucose homeostasis and insulin sensitivity. Only the high quality diet induced a downregulation in gene expression of the insulin signaling pathway PI3/Akt in the adipose tissue, which might be an early organ adaptation that could induce further insulin sensitizing effects on whole-body level on the longer term. In addition, the high quality diet was more potent in reducing other metabolic pathways in the adipose tissue, such as lipid and energy metabolism when compared to the Western-type diet. Only the high quality diet beneficially affected fasted blood lipid profile by reducing plasma triglycerides and total serum cholesterol, which was mainly caused by a reduction in medium/large VLDL particles. Both ER-diets reduced postprandial responses of VLDL, IDL, and LDL after a mixed meal but these effects were more pronounced in the high quality diet.

In **Chapter 6** we revealed a strong positive correlation between gene expression of ANGPTL4 and LPL in subcutaneous and omental adipose tissue of obese individuals. In contrast, protein levels of ANGPTL4 and LPL were negatively associated in the subcutaneous depot, which might reflect the inhibitory effects of ANGPTL4 on LPL by intracellular degradation. Furthermore, we showed that a mixed meal induces a decrease in the expression of ANGPTL4 in subcutaneous adipose tissue of overweight and obese individuals. This might abolish the suppressive effect of ANGPTL4 on LPL and allows the adipose tissue to take up triglyceride-rich lipoproteins in the postprandial phase.

To conclude, consumption of whole grain wheat might prevent intrahepatic lipid accumulation and thus lower non-alcoholic fatty liver disease risk, while a weight loss diet rich in soy protein, fiber, MUFA, and *n-3* PUFA holds great potential in lowering the risk of cardiovascular diseases, such as atherosclerosis. This thesis shows that it does matter which nutrients are emphasized within diets. The findings have increased our understanding of the effects of nutrient quality on metabolic health, mediated by the liver and adipose tissue, and can aid in designing optimal dietary strategies to combat pathologies associated with obesity.

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Sophie

ABOUT THE AUTHOR

CURRICULUM VITAE

Sophie Schutte was born on November 7, 1988 in Apeldoorn, the Netherlands. In 2007, she completed pre-university secondary education with a curriculum based on biology and natural sciences at the Christelijk Lyceum in Apeldoorn. Thereafter she started the bachelor program Nutrition & Health at Wageningen University. During this program she followed a minor course program in Immunological aspects of Nutrition and wrote a BSc thesis based on a literature study on the induction of Toll-like receptor-mediated inflammation by saturated fatty acids. In 2010, she received her degree and was appointed by the governing board of Wageningen University & Research to take place in the organization of the Annual Introduction Days (AID) 2011 for all new students at the university. This full-time board position was preceded by several part-time board positions and being a member in several committees in academic student life.

At the end of 2011 she continued her academic career by joining the master program Nutrition & Health at Wageningen University, in which she specialized in Molecular Nutrition & Toxicology and followed a minor course program in Innovation & Entrepreneurship. She conducted research for her MSc thesis at the Nutrition, Metabolism & Genomics group within the Division of Human Nutrition & Health at Wageningen University. This research focused on the use of circulating microRNAs as metabolic markers. Early 2013 she was appointed as a PhD candidate for the Belly Fat project at the same research group, under supervision of Prof. Dr. Sander Kersten and Dr. Lydia Afman. Therefore, she completed her master program by a second MSc thesis in which she performed several small pilot projects for the Belly Fat study, awaiting the start of her contract in September 2013. Her PhD project included the execution of two human intervention studies and one observational study, all designed to examine the role of nutrient quality on liver and adipose tissue health. During her PhD project, she performed several educational tasks including the supervision of several MSc and BSc (Hbo) students during their thesis research and she attended several national and international conferences and post-graduate courses. She was also a member of a PhD committee of the Division of Human Nutrition & Health. In 2017, she was selected to participate in the 23rd Essentials seminar of the European Nutrition Leadership Platform (ENLP) and was awarded the Foppe ten Hoor young investigator award following a presentation at the Dutch Nutritional Science Days.

LIST OF PUBLICATIONS

This thesis

Schutte S, Esser D, Siebelink E, Boshuizen HC, Mensink M, Afman LA. *The effects of two energy restricted diets differing in nutrient quality on metabolic health in obese men and women; a randomized controlled trial.* In preparation.

Schutte S, Esser D, Michielsen CJR, Mensink M, Afman LA. *Plasma metabolite and lipoprotein profiling reveals additional cardio-metabolic benefits of nutrient quality within weight loss diets; a randomized controlled trial in obese subjects.* In preparation.

Schutte S, Esser D, Matualatupauw JC, Mensink M, Afman LA. *A high quality nutrient ER-diet induces a distinct adipose tissue gene expression pattern when compared to a low quality Western-type ER-diet.* In preparation.

Schutte S*, Esser D*, Hoevenaars FPM, Hooiveld GJEJ, Priebe MG, Vonk RJ, Wopereis S, Afman LA. *A 12 week whole grain wheat intervention protects against hepatic fat; the Graandioos study, a randomized trial in overweight subjects.* The American Journal of Clinical Nutrition. In press.

Dijk W*, **Schutte S***, Aarts EO, Janssen IMC, Afman LA*, Kersten S*. *Regulation of angiopoietin-like 4 and lipoprotein lipase in human adipose tissue.* Journal of Clinical Lipidology 2018; 12(3): 773-783

* authors contributed equally

Other

Hoevenaars FPM, Esser D, **Schutte S**, Priebe MG, Vonk RJ, van der Kamp JW, Stroeve JHM, Afman LA, Wopereis S. *Improving metabolic resilience with whole grain wheat products; application of the PhenFlex challenge method in a randomized controlled trial.* Submitted.

Esser D, **Schutte S**, Priebe MG, Vonk RJ, Wopereis S, Afman LA. *Effect of whole grain wheat consumption on endothelial health; outcomes of a 12 week randomized trial in overweight middle-aged subjects with mildly elevated cholesterol levels.* In preparation.

Mensink M, **Schutte S**, Esser D, Siebelink E, Afman LA, Grasman J. *Application of a simple differential equation model of blood glucose dynamics to evaluate changes in insulin action and secretion upon a mixed meal challenge.* In preparation.

Wang Y, Esser D, Balvers MGJ, **Schutte S**, Mensink M, Vincken JP, Gruppen H, Afman LA, Witkamp RF, Meijerink J. *Changes in endocannabinoids and related compounds in peripheral plasma and adipose tissue of humans with abdominal obesity; a randomized controlled trial comparing different energy restricted diets.* In preparation.

Wang Y, Esser D, Balvers MGJ, Chen Y, **Schutte S**, Mensink M, Vincken JP, Gruppen H, Afman LA, Witkamp RF, Meijerink J. *Postprandial effects of a high calorie mixed meal test on plasma profiles of endocannabinoids and related compounds in abdominal obese humans before and after a 12 week intervention with two different energy restricted diets.* In preparation.

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

Discipline specific activities

Courses

- Advanced visualisation, integration and biological interpretation of ~omics data

VLAG, Wageningen (NL) 2014

- Nutrigenomics studies in humans: from epidemiology to intervention

NuGO/Universitat de Barcelona, Barcelona (ES) 2015

- Personalised Nutrition: from scientific discovery to interventions

NuGO/New Castle University, Newcastle (UK) 2016

- Use of large scale data in dietary intervention studies

NuGO/Københavns Universitet, Copenhagen (DK) 2016

Conferences

- (Dutch) Nutritional Science Days

Independent/rotating organization, Deurne (NL) 2013, Heeze (NL) 2015, 2016, 2017

- Netherlands Association for the Study of Obesity (NASO) meeting

NASO, Oosterbeek (NL) 2014, Den Bosch (NL) 2015, Utrecht (NL) 2016, 2017

- NuGO week *Nutrigenomics & more*

NuGO/Technische Universität München, Freising (DE) 2013

- NuGO week *Mechanisms of a long-life health*

NuGO/Universitat de Barcelona, Barcelona (ES) 2015

- NuGO week *Phenotypes & Prevention: The interplay of genes, lifestyle factors and gut environment*

NuGO/Københavns Universitet, Copenhagen (DK) 2016

- Alpro 20 years symposium *The moment for plant-based eating is now*

Alpro foundation, Brussels (BE) 2017

General courses & activities

- VLAG PhD week

VLAG, Baarlo (NL) 2013

- PhD competence assessment

WGS, Wageningen (NL) 2014

- PhD peer consultation - a powerful tool to tackle PhD challenges

WGS, Wageningen (NL) 2015

- Scientific writing

WGS, Wageningen (NL) 2016

- Multivariate analysis for food data/sciences

VLAG/BIOMETRIS, Wageningen (NL) 2016

- Pitch training

Wageningen into Languages, Wageningen (NL) 2017

- Master class Mixed Models

VLAG/Division of Human Nutrition & Health, Wageningen (NL) 2017

- European Nutrition Leadership Platform 23rd Essentials seminar

ENLP, Luxembourg city (L) 2017

Optional activities

- Preparation of research proposal

2013

- Weekly group meetings + orals

Division of Human Nutrition & Health

Nutrition, Metabolism & Genomics group and Nutrition & Pharmacology group 2013-2018

- Staff seminars

Division of Human Nutrition & Health 2013-2018

- PhD study tour to East coast USA

2015

COLOPHON

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