Characterization of plasma lipoprotein subclasses in lean and obese subjects with different metabolic risk profiles

Gulla Aase Formo

Master thesis at the Department of Nutrition
Institute of Basic Medical Science
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Supervisors: Stine M. Ulven, Kirsten B. Holven and Vibeke H. Telle-Hansen

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Oslo, November 2018

Gulla Formo

Summary

Background: The prevalence of overweight and obesity has increased dramatically over the last decades. Obesity is a heterogeneous condition and obesity-related metabolic disturbances vary among obese individuals leading to differences in cardiovascular risk. One subgroup of obese individuals has been described as metabolically healthy obese (MHO). In contrast to at risk obese (ARO), the MHO phenotype is defined by a favorable lipid profile and an almost normal insulin sensitivity.

Aim: The aim of this thesis was to investigate differences in the lipid composition and distribution of lipoprotein subclasses, and branched chain amino acids (BCAAs) in MHO and ARO individuals compared with healthy, normal weight individuals.

Subjects and methods: Obese individuals (men and women; 18-70 years) with BMI ≥ 30kg/m^2 were characterized as MHO (n=8) or as ARO (n=10). In addition, normal weight individuals characterized as healthy by the same criteria as described for the MHO individuals were included (n=11). A comprehensive metabolic profiling with nuclear magnetic resonance (NMR) spectroscopy was performed on fasting plasma samples to characterize and compare distribution of lipoprotein subclasses and amino acids between the groups.

Results: ARO individuals had higher concentration of all subclasses of very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and large (L-) low-density lipoprotein (LDL) and lower concentration of extra-large (XL-), large (L-) and medium (M-) high-density lipoprotein compared with the MHO individuals. They also had higher concentration of all BCAA compared with the MHO individuals. Furthermore, MHO individuals had higher concentration of all subclasses of VLDL, M- and S-LDL and S-HDL compared with the normal weight individuals.

Conclusion: There is a difference in lipoprotein subclass profile between the MHO and the ARO phenotypes, and a difference in lipoprotein profile between MHO and the normal weight individuals. The difference in the lipoprotein subclass profile is larger between ARO and MHO individuals than between MHO and normal weight individuals. The clinical implication of these differences in relation to CVD risk and insulin sensitivity need to be further elucidated.

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Abbreviations

ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B
ARO	At-risk obese
BCAA	Branched chain amino acid
BMI	Body mass index
CE	Cholesteryl ester
CETP	Cholesteryl ester transfer protein
CVD	Cardiovascular disease
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
IR	Insulin resistance
L	Large
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
M	Medium
MetS	Metabolic Syndrome
MHO	Metabolically healthy obese
NMR	Nuclear magnetic resonance
S	Small
SAT	Subcutaneous adipose tissue
TG	Triglyceride
T2DM	Type 2 diabetes mellitus
VAT	Visceral adipose tissue
VLDL	Very low density lipoprotein
XL	extra-large
XS	extra-small
XXL	extremely large

1 Introduction

1.1 The global epidemic of obesity

Obesity is characterized by an increase in white adipose tissue mass and develops when energy intake exceeds energy expenditure. The World Health Organization (WHO) defines overweight and obesity as "abnormal or excessive fat accumulation that may impair health"(1). The body mass index (BMI) is the ratio of the weight (kg) divided by the square height (m^2). Clinically, obesity is defined as BMI of ≥ 30 kg/m² and overweight is defined as BMI of ≥ 25 kg/m² (1).

The prevalence of overweight and obesity has increased dramatically over the last decades and obesity has become a worldwide epidemic **(Figure 1)**. Worldwide obesity has nearly tripled since 1975 (1). Over the past four decades, the world has more people with obesity than with underweight in all regions except in parts of sub-Saharan Africa and Asia (2).

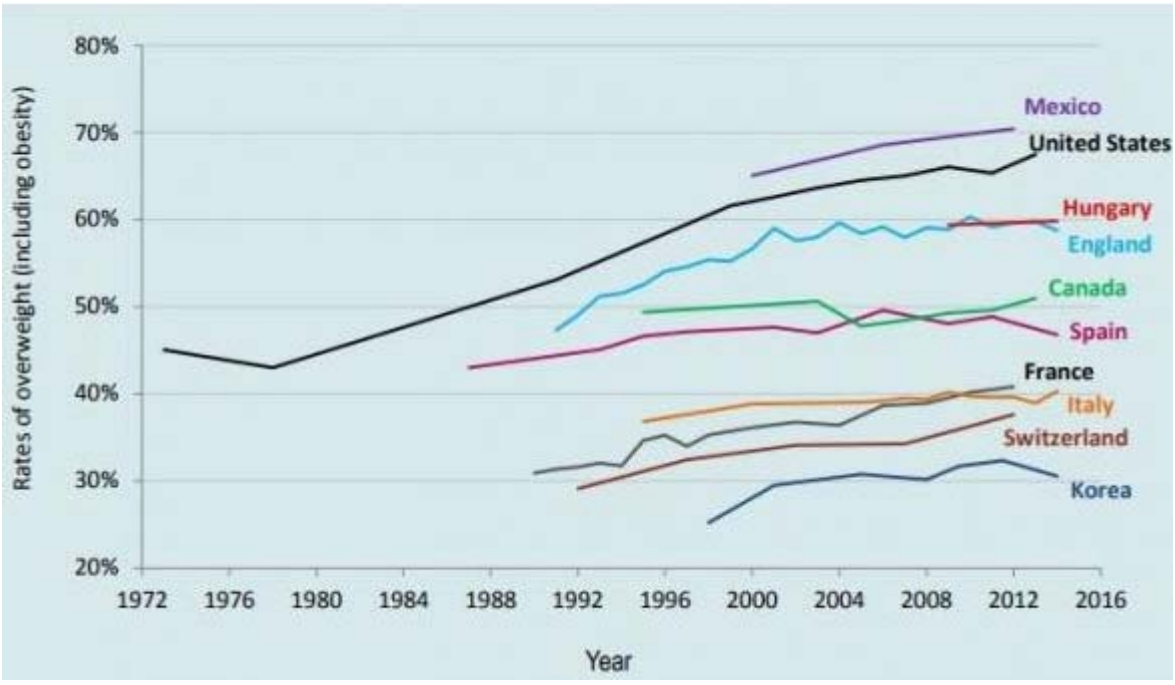

Figure 1. Rates of overweight, including obesity in adults aged 15-74 years in different countries (OECD, 2017). Reproduced with permission from OECD.

In the OECD countries, more than one in two adults and nearly one in six children are overweight or obese (2). Recent estimates indicates that about 266 million men and 375 million women are obese worldwide today, and more than one billion people or approximately 20% of the world's entire adult population are expected to be obese by 2030

(3, 4). In Norway, one in five adults has developed obesity, which represents a doubling over the last two decades (5). Excess calorie intake, altered food composition and physical inactivity are the most likely drivers of the obesity epidemic (6). However, there is a wide level of variability in susceptibility to obesity among individuals, or communities, exposed to the same environmental risk factors. This suggests that genetic differences have an appreciable role in the observed individual variation in body weight (7).

Obesity is associated with a cluster of metabolic abnormalities, such as high blood glucose, dyslipidemia and hypertension (**Figure 2**) (8). Despite the medical, economic and human cost, obesity has not been recognized a disease (9). However, it is considered an important risk factor for the development of several non-communicable diseases such as metabolic syndrome (MetS), Type 2 Diabetes Mellitus (T2DM), cardiovascular diseases (CVD), some cancers and several musculoskeletal disorders (1, 4). These diseases results in severe costs to the society, and prevention and treatment of obesity and obesity-related diseases are thus major public health challenges.

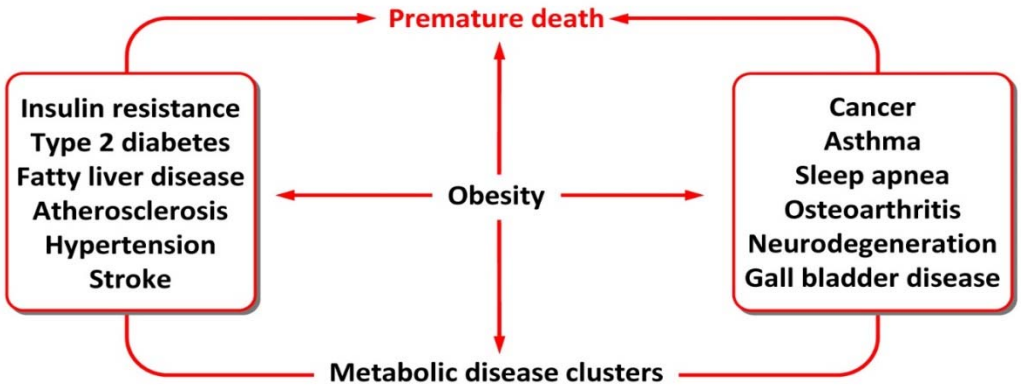

Figure 2. Obesity is considered a central feature that increases the risk for a vast array of diseases leading to premature death. Reproduced with permission from Nature Reviews (8)

Cardiovascular disease (CVD) is a general term for conditions affecting the heart and the blood vessels. CVD is one of the leading causes of mortality worldwide (1, 10). Atherosclerosis is the major cause of cardiovascular disease, and is a process of sub-endothelial lipid retention and chronic inflammation in the arterial intima developing slowly over many years (11, 12). Lipids and lipoproteins play a central role in the pathogenesis of atherosclerosis, and lipid parameters are risk factors for CVD.

1.2 Adipose tissue and its function.

Energy homeostasis in humans is achieved by a combination of processes that manage energy intake, energy storage and energy expenditure in order to maintain a stable body weight. Initially, the body will handle caloric surplus by increasing fat storage in adipose tissue.

Adipose tissue is responsible for storing excess calories as triglycerides (TGs) in cellular lipid droplets and releasing lipids in response to energy deficit. Expanding fat mass requires either increased adipocyte number (hyperplasia) or increased adipocyte size (hypertrophy). The capacity in adipose tissue to store and release lipids upon systemic metabolic demand links the cell biology of the adipocyte and adipose tissue physiology to the whole body metabolism and energy homeostasis. Adipose tissue is remarkably flexible in terms of energy storage and release (13).

Adipose tissue includes different anatomical deposits, and is divided into subcutaneous and visceral fat. Subcutaneous adipose tissue (SAT) store 80% of total body fat and high amount of SAT is referred to as “pear-shaped” obesity. Visceral adipose tissue (VAT), also referred to as central obesity, is defined as increased waist circumference. VAT represents 10–20% of total body fat in men and 5–8 % in women (14). Central obesity increases with age in both genders (15). High amount of visceral fat is often referred to as “apple-shaped” obesity. The location of adipose tissue in the body, “apple-shaped” versus “pear-shaped” obesity, has profound consequences for the metabolic impact of the excess body fat (16).

1.3 Obesity and metabolic disturbances

The accumulation of VAT correlates closely with the development of a collection of metabolic abnormalities, commonly referred to as MetS (17). VAT differs in function and production of bioactive molecules (18) and has a much greater negative metabolic effect compared with SAT (19). Due to its anatomical position, venous blood from visceral deposits is drained directly to the liver through the portal vein, while venous blood from subcutaneous fat is drained through systemic veins.

Obesity is an important risk factor for the development of metabolic diseases. Adipose tissue is not only a passive storage for excess energy, but also an active endocrine organ with a key role in the metabolism. The metabolic function is mediated through synthesis and secretion of paracrine and endocrine molecules, such as adiponectin, leptin and interleukins, collectively

referred to as adipokines (20). These signal molecules influence the metabolic activity of many tissues, including hypothalamus, pancreas, liver, skeletal muscle, kidneys and the immune system (20).

During conditions of prolonged positive energy balance, nutrients can no longer be stored in adipose tissue. Normally, new and smaller adipocytes in SAT act as a sink in the excess of energy, and these absorb free fatty acids (FFA) and triglycerides (TG). As adipocytes grow larger through hypertrophy, they become saturated with fat. When adipocyte storage capacity is exceeded, lipids "overflow" into non-adipose tissue, such as muscle, liver and pancreas, a phenomenon called ectopic lipid deposition (21) **(Figure 3)**. Ectopic fat storage causes muscle and hepatic insulin resistance and impaired insulin secretion. This results in a diabetogenic and inflammatory environment, and has been suggested to play an important role in the development of metabolic diseases (22-24).

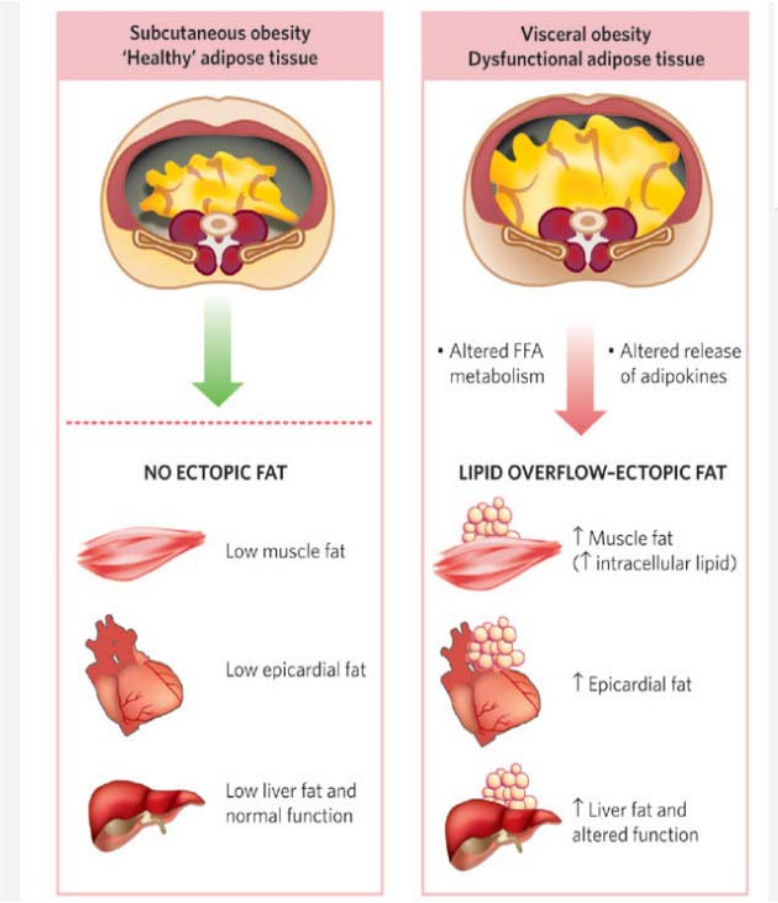

Figure 3. Ectopic fat storage. Excess visceral fat accumulation might be a marker of dysfunctional adipose tissue being unable to appropriately store the energy excess. Modified and used with permission from Nature Reviews (25).

Increased VAT with pathological growth in adipocytes, will eventually lead to adipocyte rupture and macrophage invasion. VAT responds to the metabolic stress by initiating an immune response with recruitment and infiltration of macrophages with an inflammatory phenotype and other immune cells as shown in **figure 4**. Furthermore, adipocytes and macrophages will release increased amounts of pro-inflammatory adipokines such as Tumour necrosis factor-α (TNF-α) and interleukin (IL-) 6 and suppressed secretion of anti-inflammatory cytokines, creating a low-grade inflammation (26) and an insulin-resistant milieu (16). In addition, adipokines facilitate hepatic immune response with increased production of inflammatory mediators such as C-reactive protein (CRP) (18, 27).

Accumulating evidence indicates that a state of chronic inflammation has a crucial role in the pathogenesis of obesity related metabolic dysfunction (8). Systemic concentrations of pro-inflammatory mediators are higher in obese people compared with people with normal weight (28), and these are believed to play a role in causing insulin resistance (IR) and other metabolic disturbances (29).

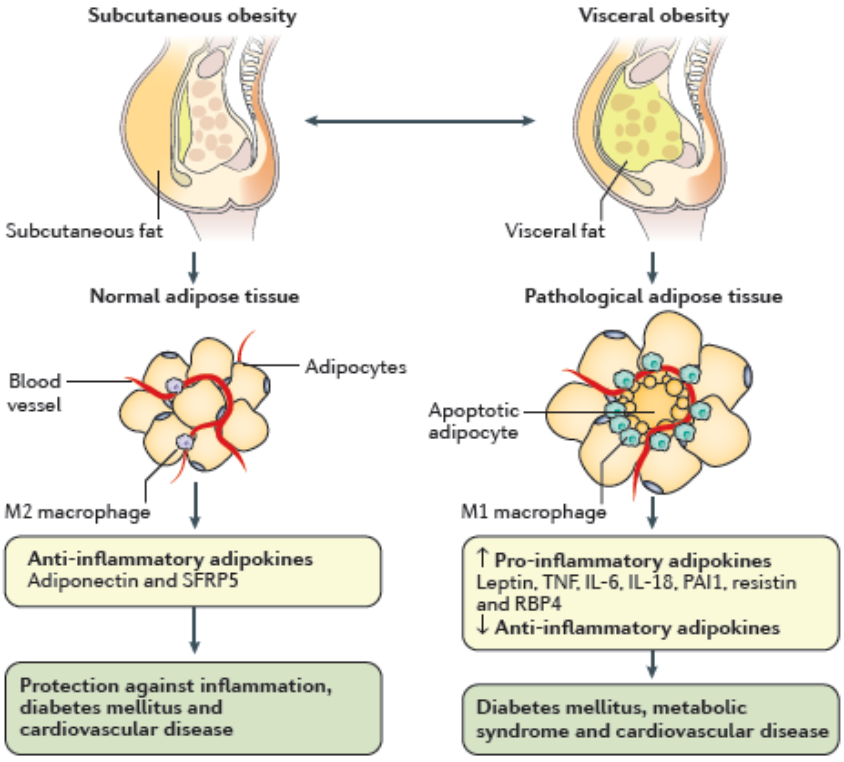

Figure 4. Pathological change in adipose tissue with increased secretion of pro-inflammatory adipokines creating a low-grade inflammation. SFRP, Secreted frizzled-related protein; TNF, Tumour necrosis factor; IL. Interleukin; PAI, plasminogen activator inhibitor; RBP, retinol-binding protein. Reproduced with permission from Nature Reviews (16)

The most common metabolic consequence of obesity is IR, which is a condition where a normal concentration of insulin is insufficient for a response in insulin target tissues (13). In the IR state, the reduced efficiency of insulin to inhibit hepatic glucose production and stimulate glucose uptake in skeletal muscle and adipose tissue leads to hyperglycemia and a subsequent compensatory hyperinsulinemia (30).

Combined with ectopic lipid accumulation in muscle and liver, the chronic inflammatory response is the main mechanism to explain IR (31, 32). However, the molecular mechanisms by which fat causes insulin resistance continues to be investigated (32).

Insulin normally blocks hormone-sensitive lipase (HSL) in adipocytes, where this enzyme stimulates hydrolysis of TGs and thereby the production of FFAs. In the IR state, insulin is no longer capable of inhibiting the action of HSL in fat stores (33), and causes an upregulation of adipose tissue lipolysis. The increased concentration of FFAs together with pro-inflammatory mediators augments IR further, and cause impaired insulin signaling. Because of the increased lipolysis, the flux of FFAs to the liver also increases profoundly (27). The presence of IR has a profound impact on lipid profiles and it has been shown that IR often precedes the onset of dyslipidemia in most obese individuals (33). IR is also a major feature of T2DM (34).

1.4 Lipoprotein metabolism

TGs serve as energy substrate in the liver and peripheral tissues, particularly in muscle. Excess energy is stored as TGs in adipose tissue (35). Cholesterol is essential for membrane integrity and structure but do also serve as a precursor of bile acids, steroid hormones and vitamin D (35). Because they are water-insoluble, cholesterol and TGs have to be transported in the circulation in special water-soluble particles, called lipoproteins.

Lipoproteins are large spherical complexes that consist of lipids and proteins. Their function is to transport lipids and cholesterol in the blood to the liver and peripheral tissues. The hydrophobic core consists of TGs and a hydrophobic form of cholesterol, cholesteryl esters. These substances are covered by a hydrophilic monolayer of free cholesterol, phospholipids and apolipoproteins (33).

In the circulation, lipoproteins form a continuum, varying in size, density, composition and function due to activity of enzymes and lipidtransporters (**Figure 5**). Each lipoprotein particle is associated with one or more apolipoproteins. Apolipoproteins are proteins located in the

outer surface monolayer consisting of phospholipids and free cholesterol (36). The apolipoproteins serve as cofactors for enzymes and as ligands for receptors. There are primarily two different classes of lipoproteins. Those containing apolipoprotein B-100 (ApoB-100) such as very low-density lipoprotein (VLDL), VLDL remnant, intermediate – density lipoprotein (IDL) and low-density lipoprotein (LDL), and those containing apolipoprotein A-1 (ApoA-1) such as high-density lipoprotein (HDL). In addition, chylomicrons contain ApoB-48 (35). Lipoproteins are traditionally classified according to their size and density, with chylomicrons, chylomicron remnants and VLDL being rich in TGs, whereas LDL and HDL are sequentially smaller and heavier, with a higher content of cholesterol (36, 37).

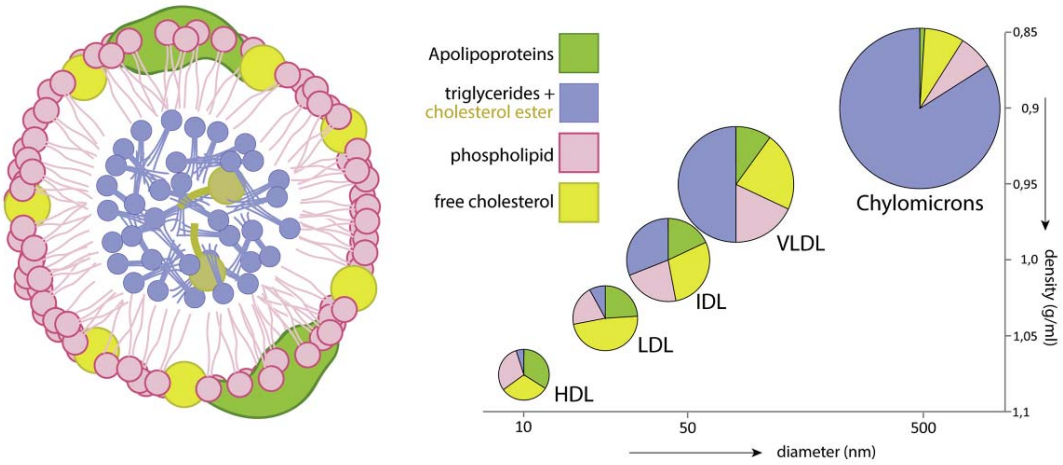

Figure 5. Composition and main physical-chemical properties of major lipoproteins classes. Left; The outer shell of lipoproteins consists of a phospholipid and cholesterol, combined with apolipoproteins, which defines that type, function and/or destination of the lipoprotein. Hydrophobic lipids (triglycerides, cholesteryl esters) are in the core of the lipoprotein. Right; Lipoproteins are classified according to their size, density and composition. HDL, high-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; VLDL, very low-density lipoprotein. Reproduced with permission from Elsevier (38)

After a meal, dietary fat is digested, and pancreatic lipase hydrolyzes TGs and phospholipids, before the intestine absorbs it. In the enterocytes, fatty acids are re-esterified to form new TG molecules **(Figure 6)** (36). TGs and cholesterol are incorporated into chylomicrons, and transported via the lymph system out in the circulation In the capillary beds of adipose tissue and muscle, chylomicrons interacts with the enzyme lipoprotein lipase (LPL) and the TG in the core of the lipoprotein is hydrolyzed, releasing glycerol and fatty acids from TGs to peripheral tissues for energy use. The depleted particle, called a remnant, is taken up by the

liver (30). In the circulation, chylomicrons also interact with other particles such as HDL and exchange surface material, such as apolipoproteins, phospholipids and cholesterol (36, 39).

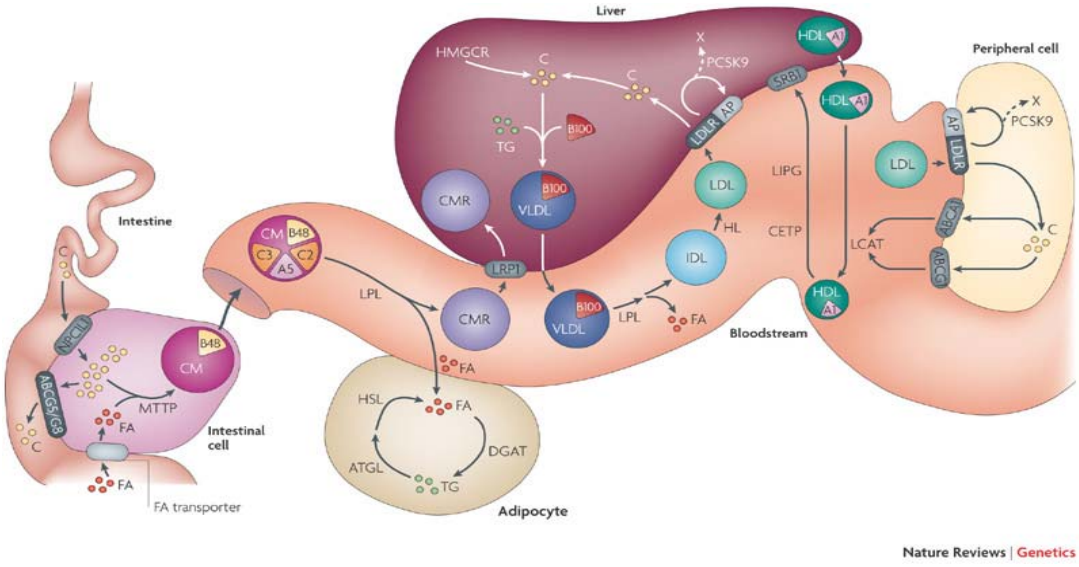

Figure 6. Overview of the lipoprotein metabolism. FA, fatty acids; TG, triglycerides; CM, chylomicrons; CMR, chylomicron remnant; LPL, lipoprotein lipase; HSL, hormone sensitive lipase; CETP, cholesteryl ester transport protein; B48, apolipoprotein B48; B100, apolipoprotein B100; A1, Apolipoprotein A1; C3, Apolipoprotein C3; C2 Apolipoprotein C2; A5, Apolipoprotein A5; VLDL, very low-density protein, IDL- intermediate-density protein, LDL, low-density protein, HDL, high-density protein; MTTP, Microsomal TG-transfer protein; DGAT, acyl CoA diacylglycerolacyltransferase; ATGL, adipose TG lipase; LRP1, LDLR-related protein-1; NPC1L1, Niemann, Pick C1-like 1 transporter, ABCG5/G8, ATP-binding cassette transporter G5/G8; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; AP, adaptor protein; PCSK9, Proprotein convertase subtilisin/kexin type 9; LIPG, endothelial lipase; SRB1, scavenger receptor class B type I. Reproduced with permission from Springer (39).

The liver produces TGs from fatty acids and glycerol, but also from excess glucose under influence of insulin. TGs from the liver are secreted into the bloodstream as VLDL. VLDL also contains free and esterified cholesterol and ApoB-100. In addition, VLDL acquires additional apolipoproteins in circulation which they obtain from HDL. The TGs in VLDL are hydrolyzed by LPL, releasing FFA to different tissues, and therefore the VLDL particle exists in different sizes depending on the lipid content the lipoprotein carries.

In the circulation, when the VLDL particle becomes depleted with lipids, they are termed a remnant or IDL. These can be removed by the liver or undergo further catabolism by hepatic lipase (HL), thereby yielding LDL. The LDL particle is the main carrier of cholesterol to peripheral tissues, and exists in a variety of sizes from small to large depending on diameter. The LDL particle leave circulation through uptake into various tissues by the LDL receptor (36, 39).

The HDL particles mediate the reverse cholesterol transport, via Apo A1, from peripheral tissues, including the arterial wall. Subsequently, the cholesterol within the HDL particle becomes esterified by lecithin-cholesterol acyltransferase (LCAT) to a long-chain fatty acid (36). Thus, the particle acquire a core of hydrophobic cholesteryl esters. A circulating protein known as cholesteryl ester transfer protein (CEPT) mediates the exchange of lipids -TGs and cholesteryl esters (CE) between different lipoprotein particles along concentration gradients in a dynamic process. The HDL particle acquires TGs from TG-rich lipoproteins, such as chylomicrons or VLDL, in exchange for CE because of the action of CEPT. Ultimately, HDL returns cholesterol to the liver through Scavenger receptor class B type 1 (SRB1) for ultimate excretion (33, 36, 40).

1.5 Alterations in lipid metabolism among obese.

The typical dyslipidemia of obesity is characterized by increased levels of plasma FFA, elevated levels of fasting and postprandial TGs, decreased levels of HDL with increased LDL and formation of small dense LDL particles (33). The concentration of ApoB is often increased, partly due to the hepatic overproduction of VLDL, an ApoB containing lipoprotein particle (40). Approximately 60-70% of obese patients have dyslipidemia, and an important component of atherogenic dyslipidemia is central obesity (41). The presence of IR has also been shown to precede the onset of dyslipidemia in most obese individuals (33).

The most significant contributing factor for obesity-related dyslipidemia is the uncontrolled FFA release from adipose tissue, especially visceral adipose tissue **(Figure 7)**. The liver will have increased delivery of FFAs. As a result, the liver will increase VLDL production, particularly large VLDL particles, to maintain TG homeostasis (33, 42, 43). Clearance of TGs is reduced because of impaired activation of LPL in the IR state, contributing to a further increase in circulating TGs levels (33). In addition, there is an increased competition for lipolysis between VLDL and chylomicrons (41).

Hypertriglyceridemia also has effect on other lipoproteins through several processes, among others the increased activity of CEPT. CEPT mediate the exchange of TGs for CEs between TG-rich lipoproteins, such as VLDL and IDL, to other lipoproteins, such as LDL and HDL, which are relatively richer in CEs. This will cause increased amounts of atherogenic cholesterol-rich VLDL remnant particles and TG-rich, cholesterol-depleted HDL particles (44). Subsequently, increased TG content in HDL and LDL is hydrolyzed by hepatic lipase or

LPL (40), leading to the formation of small, dense LDL particles that are associated with a higher risk of cardiovascular disease (45). The atherogenicity of small, dense LDL particles is attributed to their increased susceptibility to oxidation. Although, in many patients they may also be a marker for IR, or the presence of atherogenic VLDL (46). The increased secretion, assembly and decreased clearance of VLDL will also contribute to lower HDL levels through the decreased flux of apolipoproteins and phospholipids from chylomicrons and VLDL particles, which is fundamental for HDL maturation (33).

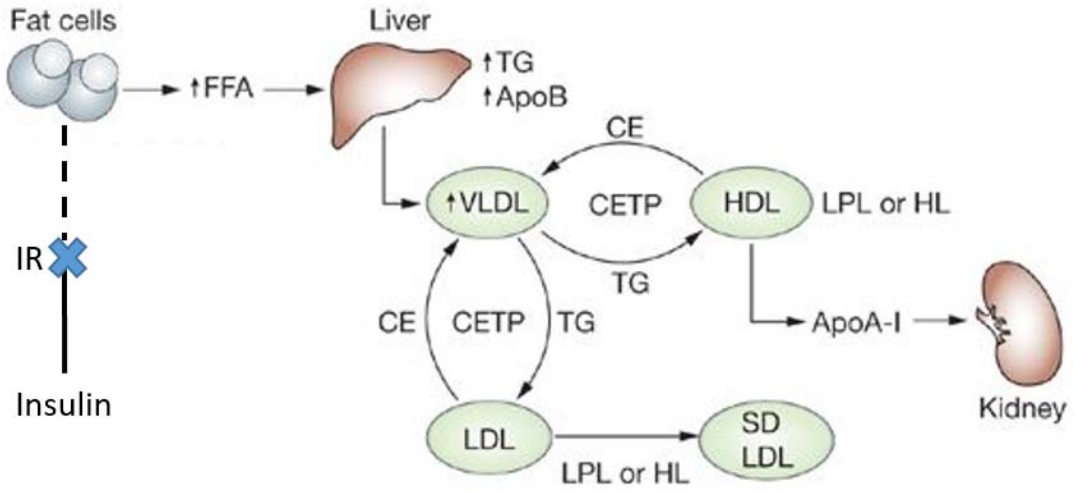

Figure 7 A simplified model relating insulin resistance to dyslipidemia and cardiovascular disease. IR, insulin resistance; FFA, free fatty acids; TG, triglycerides; CE, cholesteryl ester; Apo B, apolipoprotein B; ApoA1, apolipoprotein A1; VLDL, very low-density protein; LDL, low-density lipoprotein; SD LDL, small dense low-density lipoprotein; HDL, high-density lipoprotein; LPL, lipoprotein lipase; HL, hepatic lipase; CEPT, cholesteryl ester transfer proteins. Reproduced and modified with permission from Nature Reviews (44)

Numerous studies have demonstrated an association between lipoprotein subfractions and CVD risk (47-50). LDL cholesterol (LDL-C) have for decades served as indicator of LDL particle concentration (51), being the principal target of cholesterol treatment to reduce CVD risk. Still, there has been discovered a residual CVD risk despite the implementation of LDL-C treatment goals in the National Cholesterol Education Program (NCEP) guidelines for treatment of patients at the greatest absolute risk for coronary heart disease (52). The residual risk is most prominent in patients with metabolic syndrome and/or diabetes (53), which represents metabolic disturbances that affect both quantity and quality of lipoproteins (54).

1.6 Branched chain amino acids role in obesity and insulin resistance

High circulating levels of branched chain amino acids (BCAA) have been shown to associate with obesity and prediabetes and are considered an early marker of IR (55-59). BCAAs, including leucine, isoleucine and valine, are a subgroup of amino acids derived from the diet, which are essential for normal cell growth and function (60).

Amino acid-induced IR probably results from mechanisms that have evolved to operate in a low-calorie, high-activity environment now functioning in a high-calorie, low-activity environment. In a low-calorie environment in which high-protein meals are infrequent, BCAAs, and particularly leucine, would promote an anabolic state by inhibiting proteolysis and directly stimulating protein synthesis (61). Similarly, elevated concentrations of amino acids produce IR by disrupting insulin-mediated glucose uptake pathways, resulting in reduced glucose uptake and glycogen synthesis (55, 62). Increased circulating concentrations of BCAAs may also be explained by obesity-related decline in catabolism of BCAAs in adipose tissue (63, 64).

High concentrations of BCAAs could lead to IR, but alternatively high values could also represent a biomarker of a metabolic dysregulated status rather than an initiating event in the causal chain leading from dietary exposure to IR (65). Prospective studies have showed that BCAAs , as well as tyrosine and phenylalanine, also reflect the risk of developing T2DM (66). Soininen et al. indicated that IR plays a mediating role in the relation between BCAAs and T2DM (64). The connection between amino acid metabolism and CVD is poorly understood (60).

1.7 The metabolically healthy obese phenotype

In recent years, obesity has been recognized as a very heterogeneous condition where obesity-related metabolic disturbances vary among obese individuals (67). One subgroup of obese individuals does not display a disturbed metabolic profile or increased risk of cardiometabolic diseases despite having excessive body fat. These individuals, now known as metabolically healthy obese (MHO), display favorable metabolic profiles. A profile characterized by insulin sensitivity, normal blood pressure, as well as a favorable lipid and inflammation profile, in contrast to the at-risk obese (ARO) individuals **(Figure 8)** (68-71). Stefan et al. characterized

MHO individuals as being insulin sensitive, similarly to healthy lean individuals, with lower liver fat content and lower intima media thickness of the carotid artery than the majority of the ARO individuals (70).

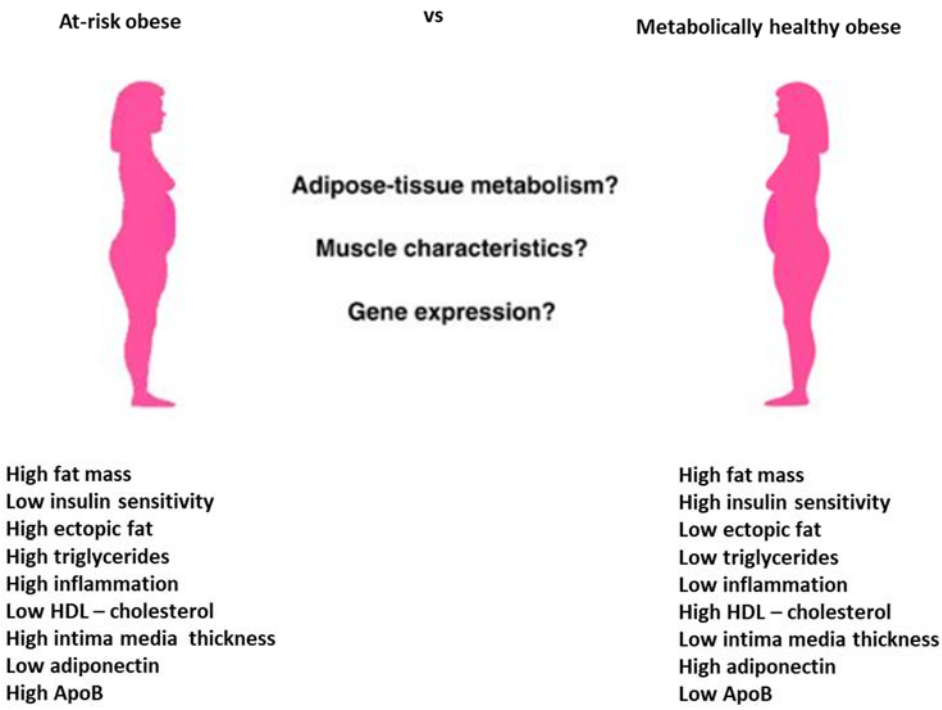

Figure 8. Factors that might distinguish metabolically healthy obese individuals from at-risk obese individuals despite similar fat mass. Modified with permission from International Journal of Obesity (72).

The ARO phenotype is characterized by a dysfunctional adipose tissue with increased immune cell infiltration and reduced capacity of subcutaneous adipose tissue to expand. This leads to increased ectopic fat deposition, IR in peripheral tissues and other metabolic abnormalities (67, 73). In MHO individuals, a preserved expandability of SAT may cause lower VAT and may explain the improved metabolic regulation in the MHO phenotype (22).

If MHO phenotype leads to a different regulation of genes involved in key metabolic pathways remains to be elucidated. However, Telle-Hansen et al. have suggested that MHO individuals have different expression levels of a number of genes linked to lipid metabolism, such as uncoupling protein 2 (UCP2), hormone sensitive lipase (HSL) and peroxisome proliferator activated receptor δ (PPAR δ), compared to ARO individuals (74).

MHO was initially regarded a static condition. It is still not clear whether MHO individuals maintain their phenotype during their entire life, or whether the MHO state represents a late onset of obesity related metabolic dysregulation. The Bogalusa Heart Study examined MHO stability over time, with 1098 subject participating, first as children (aged 5-17) and later as

young adults (aged 24-43) with an average follow-up of 24 years. This provided an opportunity to examine the MHO status for a longer period of time. Only 13% of the MHO children maintained their MHO status in adulthood. However, in adults, compared with the ARO group, the MHO group maintained a more favorable cardiometabolic profile (75). Eshtiaghi et al. demonstrated the instability of MHO status, with more than 40 % of MHO subjects developing MetS during a 10-years follow-up (76). Consistent with these findings, another long-term study performed among Japanese and Americans with MHO status, two-thirds of the population developed MetS during 10 years of follow-up (77).

Characterization of the factors that distinguish those who progress to or maintain MHO from those who transition from MHO to ARO may uncover potential intervention targets. Longitudinal follow-up (median 7.8 years) of the San Antonio Heart Study revealed that almost half (47.6%) of MHO subjects at baseline transitioned to ARO (78). Those who converted to ARO were older, had greater adiposity, and lower HDL-C levels than those with stable MHO. The authors further attempted to characterize the factors that distinguished those who progressed to MHO from those who progressed to ARO. Interestingly, BMI, waist circumference, and weight gain were not significant predictors. However, lipid profiles seemed to be the strongest determinant of future metabolic health status. Individuals with elevated TG levels had significantly higher probability to develop multiple metabolic abnormalities, while the opposite was true for individuals with elevated HDL-C (78).

There has been conflicting evidence on whether MHO individuals are at higher risk of CVD than the normal weight population. (79-83). Caleyachetty et al. used electronic health data from 3.5 million individuals to create a cohort with a mean follow up of 5.4 years (80). They concluded that MHO had a higher risk of coronary heart disease, cerebrovascular disease and heart failure than normal weight individuals did. Despite a more favorable cardiometabolic profile in MHO individuals, examination of the prevalence and severity of subclinical atherosclerosis has also produced conflicting findings (84-86). A systematic review and meta-analysis from Kramer et al. reported that obese individuals are of increased risk for adverse long-term outcomes even in the absence of metabolic abnormalities compared to normal weight individuals (84). Similarly, Khan et al. reported that MHO women have a significantly greater subclinical CVD burden than normal weight women (85). Findings from prospective studies tracking the development of CVD and mortality in MHO have been inconsistent (87-90).

2 Aim and objectives

Lipoproteins consist of a heterogeneous spectrum of particles that differ in size, density and lipid composition. Each lipoprotein class; VLDL, LDL and HDL may be divided into different subclasses. NMR spectroscopy enabled qualitative and quantitative measurement of lipoproteins, measuring the number of particles in each subclass and their size, as well as concentration of lipids in each subfraction. In addition, NMR spectroscopy measure amino acids.

The aim of this study is to expand the knowledge of cardiometabolic risk among obese individuals with different metabolic phenotypes by investigating the lipid composition and distribution of lipoprotein particles and their subclasses, and BCAAs compared with healthy, normal weight individuals using NMR spectroscopy.

In particular the objectives of the study were;

- To describe the lipoprotein subclass distribution among ARO, MHO and normal weight individuals.

- To examine if there are differences in the lipoprotein subclass particle concentration among the three groups of ARO, MHO and normal weight individuals.

- To examine if there are differences in the concentration of TGs and CEs in the lipoprotein subclasses among the three groups of ARO, MHO and normal weight individuals.

- To examine if there are differences in concentration of amino acids among the three groups of ARO, MHO and normal weight individuals.

3 Subjects and methods

3.1 Permissions

This project utilize blood samples obtained from individuals in a previous study conducted at Oslo and Akershus University College and University of Oslo (74). The study protocol in this current project has been approved by REK (#6.2008.1368)

3.2 Study population

Obese subjects (men and women; 18–70 years) with BMI ≥ 30 kg/m^2 were included in this study. They were characterized as MHO when three out of the following five criteria were fulfilled (HOMA-IR index ≤ 1.95; triacylglycerol (TAG) ≤ 1.7 mmol/L; total cholesterol ≤ 5.2 mmol/L; LDL cholesterol ≤ 2.6 mmol/L and HDL cholesterol ≥ 1.3 mmol/L) or as ARO subjects when four out of the following five criteria were fulfilled (HOMA-IR index > 1.95; TAG >1.7 mmol/L; total cholesterol >5.2 mmol/L; LDL cholesterol >2.6 mmol/L and HDL <1.3 mmol/L). The criteria used in the present study are based on the National Cholesterol Education Program's Adult Treatment Panel III report (ATP III) for lipid profiles as previously described by Karelis et al.(91). Furthermore, healthy, normal weight subjects characterized as healthy, when four out of five of the same criteria described for the MHO subjects, were included. Exclusion criteria for both obese and normal weight subjects were T2DM; kidney, liver, gall bladder, coronary, endocrine or chronic rheumatic disease; malign cancer the last 5 years; hypertension (≥ 160/100); pregnancy and lactation. Regular use of anti-inflammatory, lipid-lowering and antihypertensive medications were not permitted.

3.3 Blood sampling

The day prior to blood sampling the subjects were told to refrain from alcohol consumption and vigorous physical activity. Venous blood samples were drawn after an overnight fast (≥ 12 h). Serum was obtained from silica gel tubes [Becton–Dickinson (BD) vacutainer] and kept at room temperature for at least 30 min, until centrifugation (1,500*g*, 12 min). Serum was kept at room temperature and immediately prepared for subsequent analysis of routine laboratory analyses or aliquoted and stored at −80 °C until further analyses. Plasma was obtained from EDTA tubes (BD vacutainer), immediately placed on ice and centrifuged

within 10 min (1500*g*, 4 °C, 10 min). Plasma samples were aliquoted and stored at −80 °C until further analyses.

3.4 Routine laboratory analysis

Fasting serum high-sensitivity C-reactive protein (hsCRP), total cholesterol, LDL cholesterol, HDL cholesterol, TAG, glucose, C-peptide, insulin, HbA1c, gamma-glutamyl transpeptidase (γGT), alkaline phosphatase (ALP), alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were measured by standard methods at a routine laboratory (Fürst Medical Laboratory, Norway).

3.5 NMR spectroscopy

Fasting plasma samples were analyzed and quantified using high-throughput serum nuclear proton magnetic resonance (^{1}H-NMR) metabolomics platform. The analysis was performed at Nightingale Health Ltd (Vantaa, Finland). Sample preparation and NMR spectroscopy have been described in detail previously (64, 92). This NMR platform provides data on 14 lipoprotein subclasses which are characterized by particle size **(Figure 13)**. The fourteen lipoprotein subclasses were defined by their average diameter: extremely large VLDL with a possible contribution of chylomicrons (>75nm), five VLDL subclasses (64.0, 53.6, 36.9 and 31.3 nm), IDL (28.6 nm), three LDL subclasses (25.5, 23.0, and 18.7nm), and four HDL subclasses (14.3, 12.1, 10.9 and 8.7 nm). For each subclass, CEs and free cholesterol, TGs and phospholipids were quantified, allowing calculation of the average lipid compositions of each lipoprotein subclass as demonstrated in the figure13 (64). In addition, concentration of amino acids were quantified.

Figure 9. 14 different lipoprotein subclasses with lipid measures analyzed with nuclear magnetic resonance NMR. Particle diameters ranges from >75 nm for the largest lipoprotein particle, including chylomicrons to 8.8 nm for the smallest high-density lipoprotein (64). Used with permission from Wolters Kluwer Health Inc.

3.6 Statistical analysis.

The present study was an exploratory study designed as being

hypothesis generating. Data were checked for normality with the use of distribution plots and the Kolmogorov-Smirnov test. As the number of subjects in the study was relatively small, non-parametric ANOVA Kruskal-Wallis tests were used to test for overall difference between the three groups. When significance was observed, a post-hoc non-parametric Mann-Whitney *U*-test was used for pairwise analyses. For all analyses, a P-value of < 0.05 was considered significant. Statistical analyses were performed using SPSS software (IBM SPSS statistics 24). Data was given as median and 25th and 75th percentile.

4 Results

4.1 Characteristics of participants

In total, 29 adults were included in the present study of which 18 subjects were obese (BMI ≥ 30 kg/ m2) and 11 subjects were normal weight (BMI ≤ 25 kg/m2). Among the obese subjects, eight were characterized as MHO and 10 were characterized as ARO. The median age of the subjects were 51 y (43-64y; MHO), 52 y (43-59y; ARO) and 47 y (42-54y; normal weight) with a median BMI of 34 kg/m^2 (30-38 kg/m^2), 32 kg/m^2 (30-34 kg/m^2) and 23 kg/m^2 (21-24 kg/m^2), respectively. There were no significant difference between the obese groups in age, weight and BMI (**Table 1**). There was a skewed gender distribution in the ARO and the normal weight group. As expected, the parameters included in the inclusion criteria to define MHO and ARO were significantly different between the ARO subjects compared with the MHO subjects

Table 1. Characteristics of the study population

	ARO	MHO	Normal weight	P^*	P^1	P^2	P^3
	n=10	n=8	n=11				
Gender (*M/F)*	9/1	4/4	4/7				
Age *(years)*	52 (43-59)	51 (43-64)	47 (42-54)	0.611	-	-	-
Weight *(kg)*	102.7 (96.0-113.8)	103.5 (92.4-111.9)	65.8 (63.1-79.1)	**<0.001**	0.859	**0.001**	**<0.001**
BMI *(kg/m^2)*	32 (30-34)	34 (30-38)	23 (21-24)	**<0.001**	0.286	**<0.001**	**<0.001**
TG *(mmol/L)*	2.3 (1.7-2.7)	1.0 (0.8-1.3)	0.6 (0.4-0.9)	**<0.001**	**0.001**	**0.015**	**<0.001**
Total cholesterol *(mmol/L)*	5.8 (5.4-6.1)	5.0 (4.3-5.2)	4.7 (4.2-5.0)	**<0.001**	**0.003**	0.319	**<0.001**
HDL-C *(mmol/L)*	1.1 (0.9-1.1)	1.4 (1.2-1.5)	1.6 (1.4-2.2)	**<0.001**	**<0.001**	0.061	**<0.001**
LDL-C *(mmol/L)*	3.8 (3.4-3.9)	2.9 (2.5-3.1)	2.3 (2.1-2.6)	**<0.001**	**0.002**	**0.017**	**<0.001**
Glucose *(mmol/L)*	5.7 (5.4-6.4)	5.5 (4.9-5.7)	5.1 (4.8-5.4)	**0.037**	0.109	0.320	**0.015**
Insulin *(ρmol)*	75 (60-107)	68 (56-113)	40 (19-59)	**0.002**	0.657	**0.009**	**0.001**
$HOMA_{ir}$[4]	3.0 (2.3-4.3)	2.4 (1.8-4.0)	1.4 (0.6—2.0)	**0.001**	0.214	**0.010**	**0.001**
HbA1c *(%)*	5.5 (5.3-6.1)	5.7 (5.4-5.9)	5.1 (4.9-5.4)	**0.011**	0.754	**0.003**	**0.037**
C-peptid *(ρmol/L)*	943 (821-1171)	939 (777-1234)	482 (279-584)	**<0.001**	0.859	**<0.001**	**<0.01**
HsCRP *(mg/L)*	2.0 (1.0-3.5)	2.0 (1.0-2.8)	0.5 (0.3-1.1)	**0.009**	0.649	**0.013**	**0.008**

Data presented as median and 25th and 75th percentiles. The Kruskal-Wallis test was used to test for overall differences between the three groups.
Mann Whitney *U*-test was used to compare groups. P< 0.05 is considered statistical significant.
*Overall differences between the three study groups
[1] Between MHO and Obese at-risk
[2]Between MHO and normal weight
[3]Between obese at-risk and normal weight

4.2 Lipoprotein particle concentration and lipid-related measures

4.2.1 Concentration of lipoprotein in 14 subclasses

Lipoprotein particle concentration of the study population according to metabolic health status is presented in **Table 2**. In total, particle concentrations of six different VLDL subclasses were analyzed. The VLDL subclass analysis demonstrated that ARO individuals had significantly higher particle concentration of extremely large (XXL)-VLDL (P=0.001), very large (XL), large (L), medium (M) and small (S)-VLDL particles (P<0.001 for all), and of the extra small (XS)-VLDL subclass (P=0.003) compared with MHO individuals. Compared to the normal weight group, the MHO group also displayed significant higher particle concentration of all the VLDL particle subclasses; (P=0.013, P=0.020, P=0.013, P= 0.010, P=0.004 and P=0.006 for XXL-, XL-, L-, M-, S- and XS-VLDL, respectively. The ARO individuals also had significant higher particle concentration of all the different subclasses of VLDL compared to the normal weight group (P<0.001for all).

In total, particle concentration of one IDL subclass and three different LDL subclasses were analyzed. Compared to the MHO individuals, the ARO individuals had significantly higher particle concentrations of IDL (P=0.033) and L-LDL (P=0.041), but there was no difference in the particle concentration of the M- and S-LDL subclasses between the two obese groups. Compared to the normal weight group, there was no significant difference in the particle concentration of IDL in the MHO group. Interestingly, a trend towards significance was found in the MHO individuals for increased L-LDL particle concentration (P=0.058), as well as significantly higher particle concentration of M-LDL and S-LDL subclasses (P=0.017, P=0.021, respectively) compared to the normal weight group. The ARO individuals had significantly higher concentration of IDL, L-LDL, M-LDL and S-LDL particles than the normal group (p<0.001 for all).

Table 2. Concentration of lipoprotein particles in 14 subclasses (NMR) in at-risk obese, metabolically healthy obese and normal weight subjects.

	ARO	MHO	Normal weight	P^*	P^1	P^2	P^3
	n=10	n=8	n=11				
VLDL							
Extremely large VLDL (*nmol/L)*	0.21 (0.18, 0.26)	0.12 (0.09, 0.13)	0.07 (0.06, 0.09)	**<0.001**	**0.001**	**0.013**	**<0.001**
Very large VLDL *(nmol/L)*	0.96 (0.74, 1.2)	0.42 (0.32, 0.55)	0.07 (0.06, 0.09)	**<0.001**	**<0.001**	**0.020**	**<0.001**
Large VLDL (*nmol/L)*	5.86 (4.83, 6.95)	3.04 (2.50, 3.86)	1.89 (0.00, 2.54)	**<0.001**	**<0.001**	**0.013**	**<0.001**
Medium VLDL (*nmol/L)*	20.6 (18.3, 23.5)	13.2 (11.3,15.0)	9.3 (5.5, 11.9)	**<0.001**	**<0.001**	**0.010**	**<0.001**
Small VLDL (*nmol/L*)	32.5 (30.2, 35.7)	24.0 (21.9, 15,2)	17.3 (11.9, 20.5)	**<0.001**	**<0.001**	**0.004**	**<0.001**
Extra small VLDL (*nmol/L)*	39.2 (35.3, 41.9)	31.3 (28.8, 34.9)	26.8 (23.9, 28.4)	**<0.001**	**0.003**	**0.006**	**<0.001**
IDL *nmol/L*	98.9 (87.1, 101.8)	83.5 (77.3, 89.3)	75.7 (73.1, 82.8)	**0.001**	**0.033**	0.099	**<0.001**
LDL							
Large (*nmol/L)*	159.6 (140.7, 164.6)	136.8 (125.7,146.3)	121.2 (116.2, 132.5)	**0.001**	**0.041**	0.058	**<0.001**
Medium (*nmol/L)*	126.0 (108.7, 131.1)	107.8 (100.3, 122.6)	98.1 (92.1, 103.6)	**<0.001**	0.076	**0.017**	**<0.001**
Small	144.9 (127.8, 152.1)	127.9 (121.5, 144.2)	118.7 (111.4, 123.9)	**0.001**	0.155	**0.021**	**<0.001**
HDL							
Very large *(µmol/L)*	0.33 (0.30, 0.36)	0.42 (0.32, 0.48)	0.49 (0.41, 0.78)	**0.003**	0.051	0.099	**0.001**
Large *(µmol/L)*	0.51 (0.35, 0.62)	0.93 (0.77, 0.97)	1.10 (0.83, 1.61)	**<0.001**	**0.003**	0.099	**<0.001**
Medium *(µmol/L)*	1.51 (1.38, 1.56)	1.68 (1.61, 1.80)	1.53 (1.35, 1.74)	**0.031**	**0.010**	0.083	0.512
Small *(µmol/L)*	4.36 (4.19, 4.61)	4.43 (4.30, 4.75)	4.10 (3.65, 4.46)	**0.044**	0.374	**0.026**	0.072

Data presented as median and 25th and 75th percentiles. The Kruskal-Wallis test was used to test for overall differences between the three groups.
Mann Whitney *U*-test is used to compare groups. P< 0.05 is considered statistical significant.
*Overall differences between the three intervention groups
[1]Between MHO and Obese at-risk
[2]Between MHO and normal weight
[3]Between obese at-risk and normal weight

In total, four HDL subclasses were analyzed. The ARO individuals had borderline significantly lower particle concentrations of XL-HDL (*P*=0.051), and significant lower concentration of L-HDL (*P*=0.003) and M-HDL (*P*=0.010) subclasses compared to the MHO group, but no difference was found in S-HDL particle concentration. Compared with the normal weight group, the MHO individuals had significantly higher particle concentration of S-HDL, but there were no difference in the particle concentration of XL-, L- and M-HDL subclasses. The ARO individuals had significant lower particle concentration of XL-HDL and L-HDL (*P*=0,001, *P*<0.001 respectively), but no difference in M-HDL and S-HDL particle concentration compared to the normal weight group.

4.2.2 Lipid distribution in 14 lipoprotein subclasses

The TG concentration in the 14 different lipoprotein subclasses of the study population according to metabolic health status is presented in **table 3**.

TG is the main lipid in VLDL and in accordance with the results from VLDL subclasses, as presented in **figure 9**, the ARO individuals displayed significantly higher concentrations of TG in all VLDL subclasses; XXL-VLDL (*P*=0.001) and XL-, L-, M- and S-VLDL (*P*<0.001 for all) compared with the MHO individuals. The MHO individuals had significant higher concentration of TG in all subclasses of VLDL compared to the normal weight group (*P*=0.012, *P*=0.016, *P*=0.021, *P*=0.032, *P*=0.010, *P*=0.021 for XXL-, XL-, M- and S-VLDL, respectively). Compared to the normal weight group, ARO had significant higher concentration of TGs in all VLDL subclasses (*P*<0.001 for all).

Table 3. Triglycerides in lipoprotein particles in 14 subclasses (NMR) in at-risk obese, metabolically healthy obese and normal weight subjects

	ARO	MHO	Normal weight	P^*	P^1	P^2	P^3
	n=10	n=8	n=11				
VLDL-TG							
Extremely large VLDL *(µmol/L)*	32.0 (27.0, 39.8)	18.1 (14.1, 20.3)	12.3 (9.9, 15.4)	**<0.001**	**0.001**	**0.012**	**<0.001**
Very large VLDL (*µmol/L)*	59.1 (44.6, 71.8)	26.6 (20.3, 34.3)	14.1 (0.0, 21.6)	**<0.001**	**<0.001**	**0.016**	**<0.001**
Large VLDL (*µmol/L)*	199.2 (159.4, 236.7)	101.6 (86.0, 133.2)	67.0 (0.0, 88.7)	**<0.001**	**<0.001**	**0.021**	**<0.001**
Medium VLDL *(µmol/L)*	359.1 (324.3, 414.1)	229.2 (203.6, 273.8)	171.6 (92.0, 218.7)	**<0.001**	**<0.001**	**0.032**	**<0.001**
Small VLDL *(µmol/L)*	269.7 (246.9, 302.6)	198.3 (174.8, 211,7)	150.0 (94.0, 178.1)	**<0.001**	**<0.001**	**0.010**	**<0.001**
Extra small VLDL *(µmol/L)*	110.2 (104.6, 116.8)	85.1 (83.5, 95.9)	71.1 (56.9, 85.1)	**<0.001**	**<0.001**	**0.021**	**<0.001**
IDL-TG *(µmol/L)*	107.1 (98.6, 115.1)	87.9 (79.2, 98, 0)	79.2 (67.3, 85.9)	**<0.001**	**0.003**	0.083	**<0.001**
LDL-TG							
Large *(µmol/L)*	88.7 (78.6, 92.3)	70.0 (61.2, 79.8)	67.6 (60.8, 70.4)	**0.001**	**0.008**	0.322	**<0.001**
Medium *(µmol/L)*	42.6 (38.1, 44.9)	35.1 (30.6, 39.0)	32.8 (30.6, 34.0)	**0.001**	**0.008**	0.248	**<0.001**
Small *(µmol/L)*	28.1 (26.7, 30.0)	22.4 (20.1, 24.6)	18.2 (16.9, 21.2)	**<0.001**	**0.001**	**0.008**	**<0.001**
HDL-TG							
Very large *(µmol/L)*	13.2 (10.6, 15.1)	8.8 (4.7, 11.3)	10.5 (7.7, 17.3)	**0.046**	**0.010**	0.117	0.481
Large *(µmol/L)*	12.3 (10.4, 16.4)	13.0 (9.0, 16.8)	18.2 (11.1, 27.9)	0.161	-	-	-
Medium *(µmol/L)*	47.5 (44.1 52.8)	38.7 (34.7, 41.0)	31.2 (21.3, 34.1)	**<0.001**	**0.001**	**0.003**	**<0.001**
Small *(µmol/L)*	56.4 (53.0, 59.3)	43.9 (41.2, 48.0)	39.0 (28.2, 43.8)	**<0.001**	**<0.001**	**0.026**	**<0.001**

Data presented as median and 25th and 75th percentiles. The Kruskal -Wallis test was used to test for overall differences between the three groups.
Mann Whitney *U*-test was used to compare groups. P< 0.05 is considered statistical significant.
*Overall differences between the three intervention groups
[1]Between MHO and Obese at-risk
[2]Between MHO and normal weight
[3]Between obese at-risk and normal weight

The ARO individuals had significantly higher concentration of TG in IDL compared with the MHO individuals (P=0.003). Also in all subclasses of LDL; L-LDL, M-LDL and S- LDL, the ARO individuals had higher concentration of TGs than the MHO individuals (P=0.008, P=0.008, P=0.01, respectively). Between MHO and the normal weight group there were no significant difference in the concentration of TGs in neither IDL, L- LDL nor M- LDL, but MHO had higher concentration of TG in S-LDL. The ARO individuals had significantly higher concentration of TGs in IDL, L-LDL, M-LDL and S-LDL compared to the normal weight group (P<0.001 for all).

The ARO individuals had significantly higher concentration of TG in XL-, M-HDL and S-HDL particles compared to the MHO group (P=0.010, P=0.001, P<0.001. respectively), but no difference was found in L-HDL particles. The MHO individuals had significantly higher concentration of TGs in M-HDL and S-HDL particles compared to the normal weight group (P=0.003 and P=0.026, respectively), but no differences were found in the XL-HDL and L-HDL particles. Compared to the normal weight individuals, the ARO group had significantly higher concentration of TGs in M-HDL and S-HDL particles (P<0.001 for both), but no differences were found for XL-HDL and L-HDL particles between the two groups.

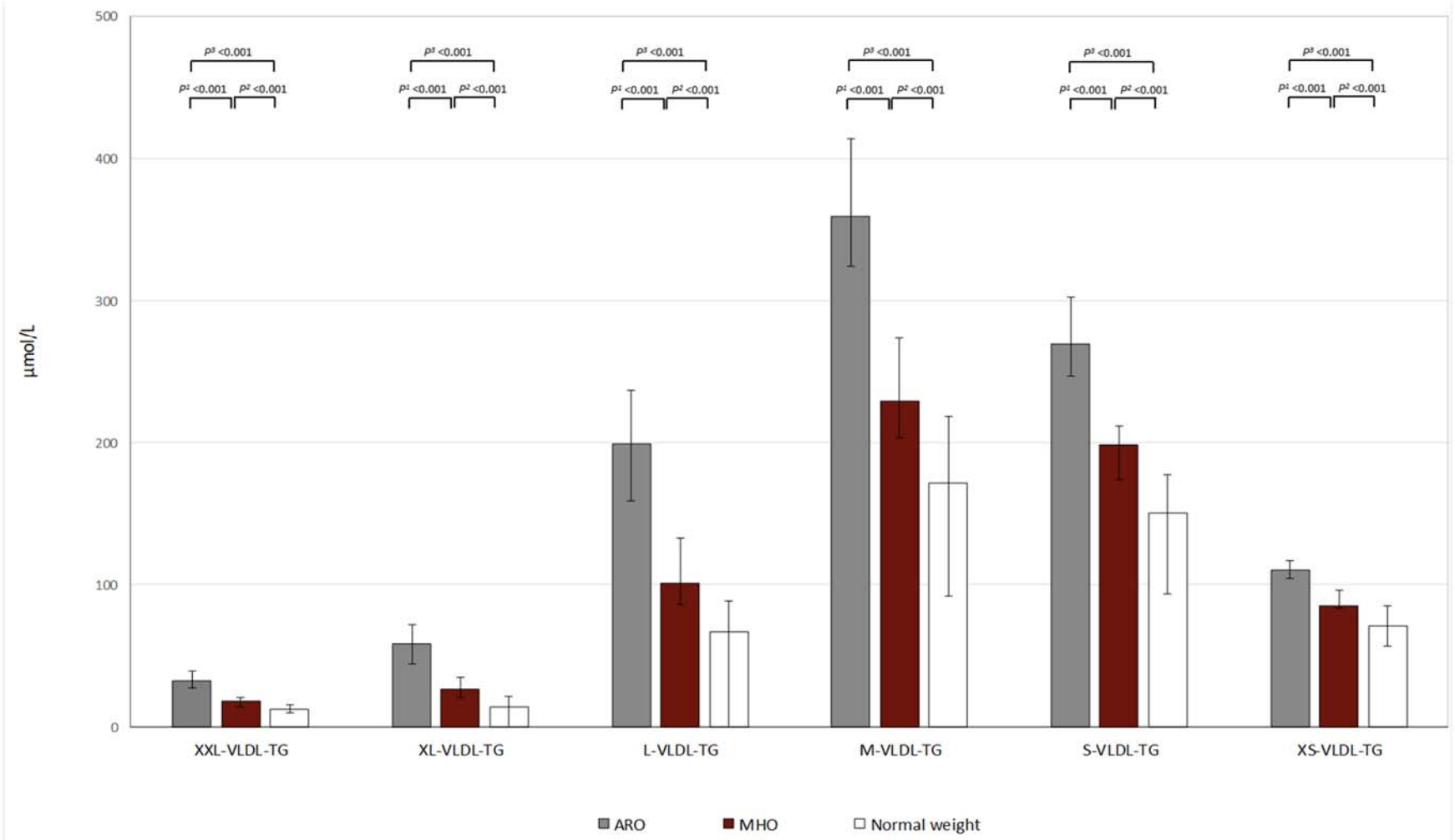

Figure 10. Concentration of triglycerides (TG) in extremely large (XXL)-VLDL, extra (XL)-VLDL, large (L)-VLDL, medium (M)-VLDL , small (S)-VLDL and extra small (XS)-VLDL according to metabolic health status. Particle concentration are expressed as mmol/L. The at-risk obese (ARO), metabolically healthy obese (MHO) and normal weight groups are depicted as grey, red and white bars, respectively. Values are median and 25th and 75th percentiles represented as vertical bars. Mann Whitney *U*-test is used to compare groups. [1] Between MHO and at-risk obese [2] Between MHO and normal weight [3] Between obese and normal weight

The concentration of CE was significantly higher in the ARO individuals in all subclasses of VLDL (*P*<0.001, *P*<0.001, *P*<0.001, *P*<0.001, *P*=0.003, *P*=0.008 for XXL-, XL-, L-, M- and S-VLDL, respectively) compared to the MHO individuals. The MHO individuals had a significantly higher concentration of CEs in all VLDL subclasses (*P*=0.010, *P*=0.016, *P*=0.006, *P*=0.003, *P*=0.001, *P*=0.017 in XXL-VLDL, XL-VLDL, L-VLDL, M-VLDL, S-VLDL and XS-VLDL, respectively). The ARO individuals had significantly higher concentration of CEs in all VLDL subclasses (*P*<0.001 for all).

The concentration of CEs was significantly higher in IDL in the ARO group compared to the MHO group (*P*=0.010), but no differences were found in the L-LDL, M-LDL and S-LDL particles between the two groups. The MHO group had significantly higher concentration of CEs in L-LDL and M-LDL compared to the normal weight group (*P*=0.026, *P*=0.026, respectively) and a tendency to significance in S-LDL (*P*=0.058). No difference was found in the concentration of CE in IDL between the two groups. The ARO group had significantly higher concentration of CEs in IDL, L-, M- and S-LDL compared to the normal weight group (*P*<0.001, *P*=<0.001, *P*=0.001, *P*=0.007, respectively) **(Figure 10).**

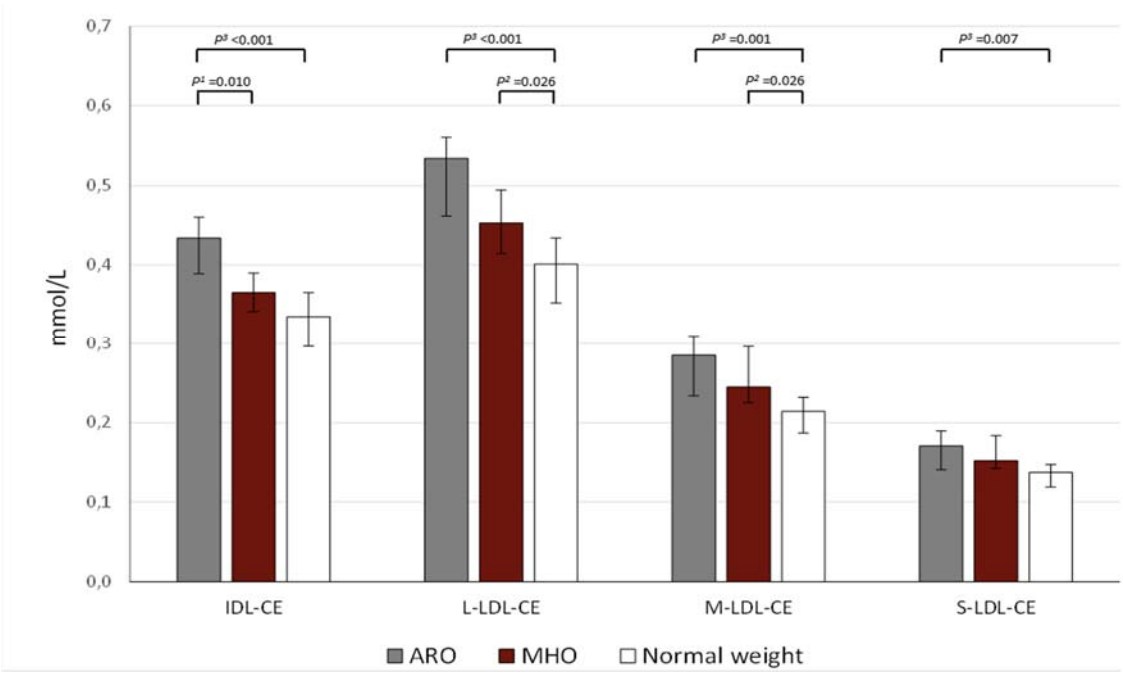

Figure 11. Concentration of cholesterol ester (CE) in IDL and LDL. Concentration of CE within intermediate-density lipoprotein (IDL) and large (L-LDL), medium (M-LDL) and small LDL(S-LDL) according to metabolic health status. Particle concentration are expressed as mmol/L. The at-risk obese (ARO), metabolically healthy obese (MHO) and normal weight groups are depicted as grey, red and white bars, respectively. Values are median and 25th and 75th percentiles represented as vertical bars. Mann Whitney *U*-test is used to compare groups. [1] Between MHO and at-risk obese [2] Between MHO and normal weight [3] Between obese and normal weight

The concentration of CE was significantly lower in L- and M-HDL particles in the ARO individuals compared to the MHO individuals (P=0.003 and P=0.003, respectively), but no difference was found in the concentration of CE in XL and S-HDL particles between the two groups. There were no differences in the concentration of CEs in any of the HDL subclasses between the MHO and the normal weight group. The ARO individuals had a significant lower concentration of CE in XL-, L- and M-HDL particles compared to the normal weight group (P=0.002, P<0.001, P=0.041, respectively), but no difference in the S-HDL particle was found **(Figure 11).**

The same pattern was observed for the concentration of cholesterol in all the different lipoprotein subclasses, as shown in supplemental table (**Appendix 1**)

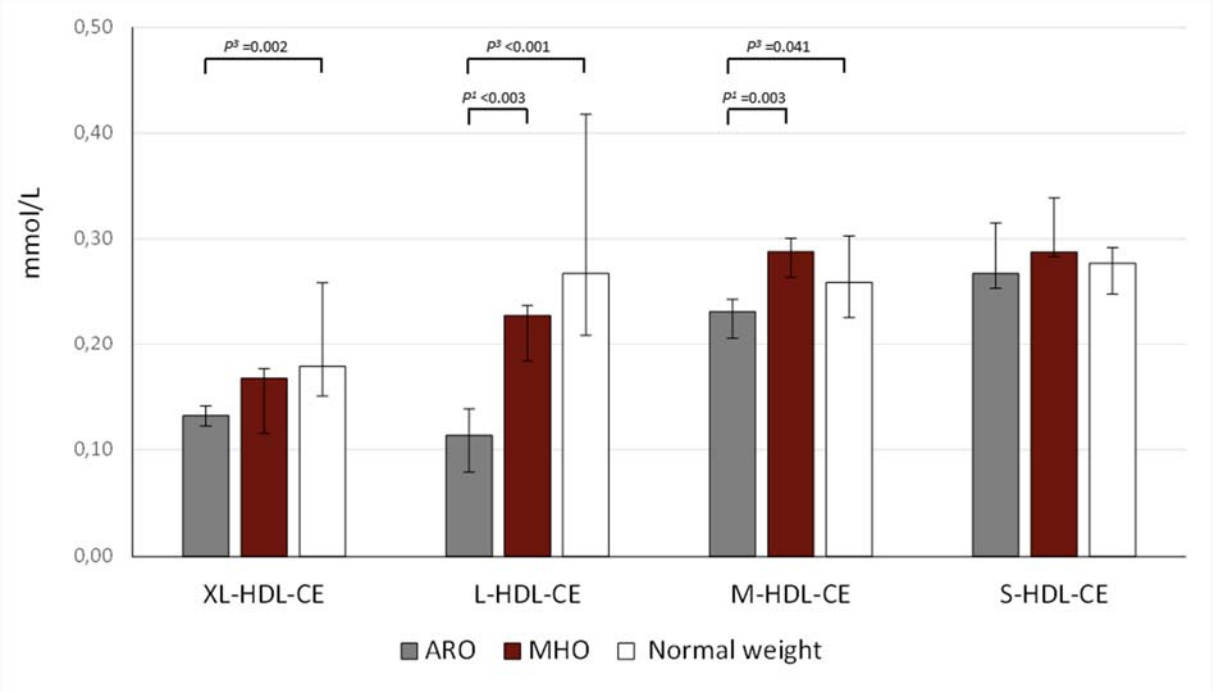

Figure 12. Concentration of cholesterol ester (CE) in HDL. Concentration of CE in large (L-HDL), medium (M-HDL), small (S-HDL) and extra small HDL (XS-HDL) according to metabolic health status. Particle concentration are expressed as mmol/L. The at-risk obese (ARO), metabolically healthy obese (MHO) and normal weight groups are depicted as grey, red and white bars, respectively. Values are median and 25th and 75th percentiles represented as vertical bars. Mann Whitney *U*-test is used to compare groups. [1] Between MHO and at-risk obese, [2] Between MHO and normal weight, [3] Between obese and normal weight

4.3 Amino acids and other metabolites

The ARO individuals had significantly higher concentration of the BCAAs; leucine, isoleucine and valine than the MHO individuals (P=0.002, P=0.001, P=0.021, respectively). There were no significant difference in concentration of leucine, isoleucine and valine between MHO and the normal weight individuals. The ARO group also had significant higher concentration compared to the normal weight group for the BCAAs (P<0.001, P<0.001, P=0.024 for leucine, isoleucine and valine, respectively)

In addition, the ARO individuals had higher concentration of tyrosine compared to the normal weight group (P=0.017) and MHO had a significantly higher concentration of phenylalanine than the normal weight individuals (P=0.017)

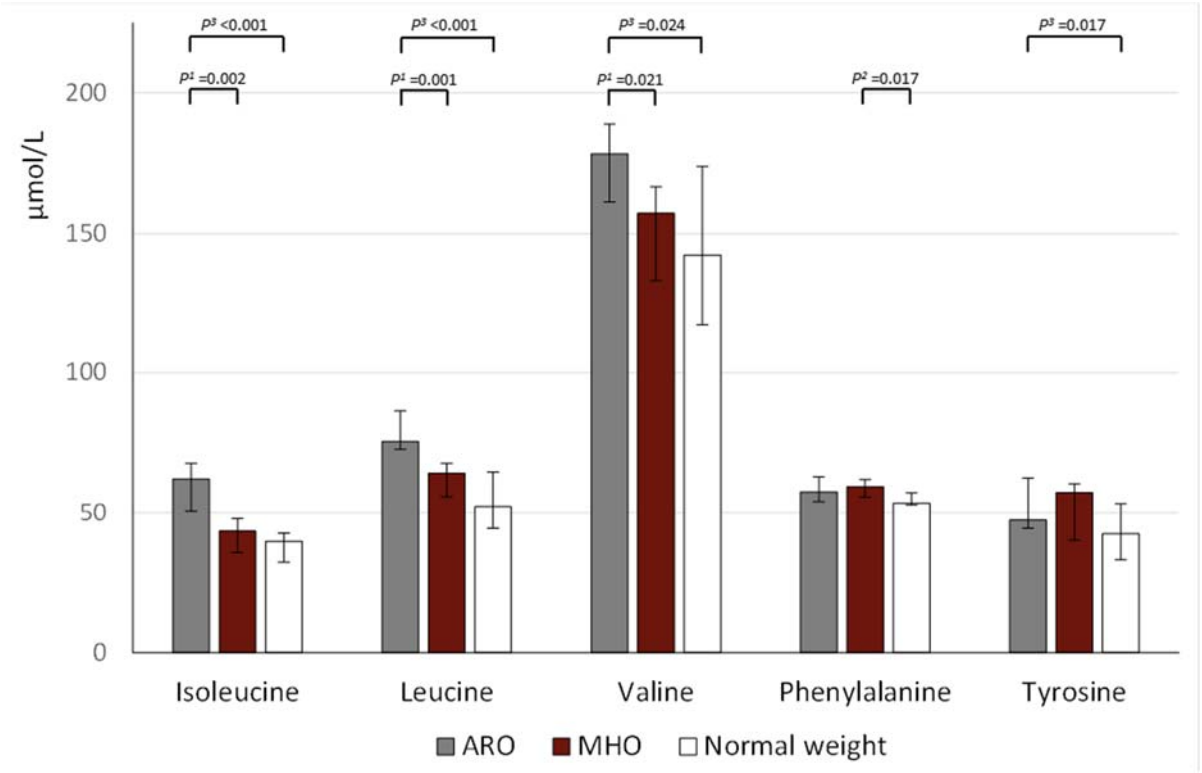

Figure 13. Concentration of amino acids. Concentration of amino acids in serum according to metabolic health status. Particle concentration are expressed as µmol/L. The at-risk obese (ARO), metabolically healthy obese (MHO) and normal weight groups are depicted as grey, red and white bars, respectively. Values are median and 25th and 75th percentiles represented as vertical bars. Mann Whitney *U*-test is used to compare groups. [1] Between MHO and at-risk obese, [2] Between MHO and normal weight, [3] Between obese and normal weight

Table 4. Concentration of amino acids (NMR) in at-risk obese, metabolically healthy obese and normal weight subjects

	ARO	**MHO**	**Normal weight**	***P****	***P*** [1]	***P*** [2]	***P*** [3]
	n=10	n=8	n=11				
Alanine (μmol/L)	344.4 (332.2, 388.0)	301.9 (293.0, 373.7)	321.2 (278.3, 343.8)	**0.050**	0.131	0.680	**0.014**
Glutamine (μmol/L)	396.6 (343.4 478.8)	429.9 (379.6, 438.8)	450.7 (432.7, 484.9)	0.208	-	-	-
Histidine (μmol/L)	62.0 (58.4, 69.5)	64.0 (56.8, 66.6)	59.8 (50.9, 64.9)	0.443	-	-	-
Isoleucine (μmol/L)	62.2 (50.6, 67.7)	43.2 (35.5, 47.6)	39.5 (32.1, 42.5)	**<0.001**	**0.002**	0.186	**<0.001**
Leucine (μmol/L)	75.5 (72.7, 86.5)	64.1 (55.7, 67.8)	52.2 (44.2, 64.6)	**<0.001**	**0.001**	0.117	**<0.001**
Valine (μmol/L)	178.5 (161.4, 189.0)	157.4 (132.9, 166.8)	142.4 (117.1, 174.1)	**0.029**	**0.021**	0.804	**0.024**
Phenylalanine (μmol/L)	57.5 (53.9, 62.9)	59.3 (55.7, 62.0)	53.4 (52.7, 57.1)	**0.037**	0.657	0.017	0.057
Tyrosine (μmol/L)	47.1 (44.1, 62.4)	57.3 (39.9, 60.4)	42.2 (33.1, 53.1)	**0.037**	1.000	0.063	**0.017**

Data presented as median and 25th and 75th percentiles. The Kruskal -Wallis test was used to test for overall differences between the three groups. Mann Whitney *U*-test was used to compare groups. P< 0.05 is considered statistical significant.

*Overall differences between the three intervention groups

[1]Between MHO and Obese at-risk

[2]Between MHO and normal weight

[3]Between obese at-risk and normal weight

5 Discussion

5.1 Discussion of study design, subjects and methods

5.1.1 Study design

In this cross-sectional study, a large number of metabolites in serum from obese and healthy normal weight subjects were measured. The purpose of the study was to investigate the composition and distribution of lipoprotein and their subclasses in two groups of obese subjects; MHO and ARO individuals at a single point in time, and to compare the results with the same measurements from healthy, normal weight subjects. Cross-sectional studies are generally used to determine prevalence. The study design does not impose causal associations and the designs ability to draw conclusion is limited. However, the study design is well suitable for descriptive research, and was therefore appropriate for this thesis.

5.1.2 Study subjects

Healthy, overweight men and women were recruited on a voluntary basis by advertisement in a local newspaper in Oslo and Akershus to a randomized controlled trial conducted at HiOA. The individuals in the ARO group (n=10) and the MHO group (n=8) were selected from this population. The normal weight group (n=11) were recruited later via newspaper advertisement in local newspapers in the Oslo and Akershus region, in order to act as a lean control group to the healthy overweight group.

Although age ranged from 36 -67 years, the youngest individuals are underrepresented, with the median age being 51 years (43-64 years) in the MHO group, 52 years (43-59 years) in the ARO group and 47 (42-54 years) in the normal weight group (**Table 1**). The findings in this thesis may not apply to individuals with different characteristics different to those described for this study sample. Both the ARO group and the normal weight group showed a skewed gender distribution, males dominated in the ARO group (90%) and women in the normal weight group (64%). The MHO group had equal representation between genders (50%). Previous studies have demonstrated stronger association between obesity and LDL-C in men compared to women, and both BMI and waist circumference had more adverse effects with a relative larger increase in TG levels and larger decrease in HDL-C with increase in BMI in

men (93). Conversely, weight loss is associated with a healthier lipoprotein profile in both men and women, but changes in HDL-C levels are more pronounced in women than in men (94). However, due to the limited number of participants in this study, there have not been conducted gender specific analyzes in the present study.

5.1.3 Statistical analysis

Our study population consisted of 29 subjects in total. Due to a small sample size, which make it impossible to verify a normal distribution of variables, non-parametric statistical methods were used. Non-parametric methods consider outliers in a small dataset using median as the middle value of the data (95). These methods are considered less powerful, but have the advantage of being less affected by extreme observations (95). Another solution would have been to log-transform the data to get the data normally distributed in order to use parametric methods. However, there is some controversy using log-transformed data in which transformed data makes the interpretation more difficult (95).

Statistical power is the probability of finding an effect if there is a true effect to be found. A power analysis can be used to estimate the minimum sample size required for a study with an adequate power to detect statistical significance (95). Calculations of statistical power have not been conducted in this study because of its explorative design. Nevertheless, the relatively small number of subjects in each group is a limitation of this study.

Since this was an explorative study, we did not adjust for multiple testing. In scientific research, the statistical significance level is frequently set to 0.05, which means there is a 5% chance that the observed difference is due to sampling or experimental errors. When running multiple statistical tests within a single study, the probability of detecting a significant finding just by chance, i.e. type I error, increases. This is called the problem of multiplicity (96). To avoid type I-errors, the Bonferroni correction is a technique frequently applied to correct p-values when making multiple comparisons. However, this correction is extremely conservative and comes to the expense of increasing the probability of a type II error. i.e. not detecting an effect even though it exists. In the present study, this might have been the case when it comes to the differences in concentration in IDL, large LDL and very large HDL particles between ARO and MHO individuals. Thus, with an increase in statistical tests, the likelihood of not detecting a real difference increases, leading to loss of statistical power. For this reason, the commonly used routine correction for multiple tests is much debated. It is

important to consider the risk of type I and type II errors before deciding to adjust p-values. In an explorative context it can be argued that it is better not to miss a possible effect, that is, to avoid a type II error, and therefore not use Bonferroni correction (96). The Bonferroni correction also becomes increasingly conservative when the outcomes are correlated with each other, such as in the post hoc test following a significant Kruskal-Wallis test. Many of the lipoprotein subclasses and their lipid constituents correlate, such as VLDL particle concentration and concentration of TGs and CEs in VLDL particles. This fact supports the decision of not conducting a Bonferroni correction.

5.1.4 High-field 1H Nuclear Magnetic Resonance-based lipoprotein profiling

Different ^{1}H-NMR based protocols for lipoproteins fractions and subfractions identification and measurement have been developed. In our study, lipoprotein subclasses were quantified by Nightingale Health ltd. This NMR platform provides data on 14 lipoprotein subclasses which are characterized by particle size **(Figure 9)**.

The lipoprotein content of a blood sample is difficult to characterize. The main reason for this is that the chemical composition, density and size of lipoproteins vary greatly, limiting the possibility of clearly establishing the relationship among these three fundamental properties. Multiple terms are used to describe lipoprotein distributions, these terms including among others – lipoprotein subclasses, particle concentration, particle number, particle diameter and particle density. These terms describe separate, but often overlapping features of the lipoprotein content in a blood sample. A variety of methods, including gel electrophoresis and ultracentrifugation, are being used to measure lipoprotein subfraction distributions, density, concentrations or diameter (97). All of the methods measures differences between lipoproteins based on different physicochemical properties. In addition, they estimate lipoprotein sizes using different assumptions and approximations. The definition of the different lipoprotein subclasses, the number and sizes of subclasses and their terminology is not uniform between the variety of analytical methodologies used, or within a single method. This makes it difficult to compare results from different tests or studies. Subsequently, some of the tests can only be performed by the company that markets the test (97). Several studies have been conducted with the aim of comparing different methods for lipoprotein subfractions determination. A systematic review by Chung et al. listed several limitations found in nine studies where different methods used for LDL subfractions determination (98). The study

confirmed that the wide variety of methodologies used, the lack of standardization among the results of NMR and the other analytical methods, and the different definitions or descriptions of LDL subfractions, limited the comparability amongst and within the analytical techniques.

Thus, there are some controversies around the usefulness of NMR spectroscopy for the characterization of lipoproteins, where one of the greatest weaknesses being lack of standardization in the characterization techniques. However, so far, the applications with coherent NMR and reference data i.e. ultracentrifugation methods, have been limited to relatively small cohorts, and the lack of appropriate standards both for the ultracentrifugation and NMR measurements have made meta-studies extremely difficult if not impossible (99). Today several commercial companies are offering NMR-based lipoprotein analysis. Two groups have taken a central position in the analysis of NMR lipoprotein profiles. The first group, led by Otvos, created a test which was commercially distributed by LipoScience Inc. This method have been widely applied in biomedical applications and has been approved by the US Food and Drug Administration (FDA) (100). In addition, Nightingale health, based on the method developed by Ala-Korpola and Würtz et al.have turned out to be useful in epidemiological cohort studies and has been widely used in research conducted in universities and medical institutions around the world (97).

However, as demonstrated in **Figure 14** each lipoprotein subclass size applied by LipoScience Inc. deviate from the measurements applied by Nightingale Ltd.

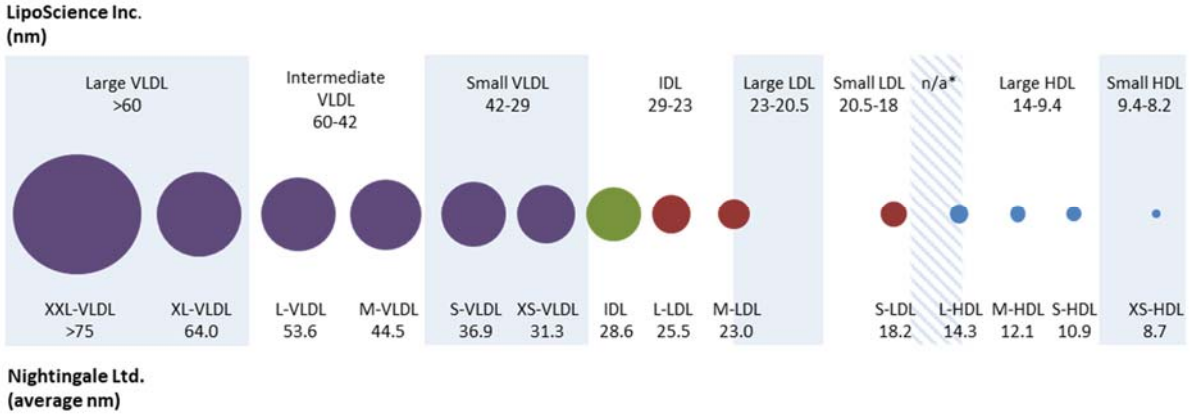

Figure 14. Size range of lipoprotein subclasses analyzed by different NMR platforms deviate and there is a need for standardization. The definition of the different lipoprotein subclasses, the number and sizes of subclasses and their terminology is not uniform within the NMR methodology. VLDL, very low-density lipoprotein; IDL, intermediate lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; XXL-, extremely large; XL-, extra-large; L-, large; M-, medium; S-, small; XS, extra-small; n/a*, non-applicable.

5.1.5 Definition of MHO

There is a wide variation in the reported prevalence of MHO (67). Comparative studies examining MHO prevalence across a range of currently used criteria reported considerable variation in MHO prevalence. In a review from 2011, Primeau et al. have reported that MHO individuals account for 20 to 44 % in different obese populations (72). A large-scale European study, including 10 large cohorts, indicated significant diversity in MHO prevalence across Europe with 7-28 % in women and 2-19 % in men (101). Calori et al. found a prevalence of MHO at only 11% in the Italian population based on the Cremona study (88).

The MHO is a well-known subset, but still there are no standard criteria for identification of MHO compared to ARO established. There is a large disparity in reported prevalence of MHO, probably caused by the lack of definition of metabolic health and differences in obesity classification (BMI vs. body fat percentage (BF%)(67). Current characterization of MHO in adults is mostly based on the absence of MetS or some of its components among individuals with excess body weight (91, 101, 102). Some definitions additionally include favorable inflammatory status determined by C-reactive protein (CRP) levels. **Table 5** demonstrates some currently used criteria to define MHO among adults.

	Aguilar–Salinas[36]	Karelis[33]	Meigs[35,a]	Meigs[35,b]	Wildman[32]	NCEP ATPIII[37]	Bioshare–EU[37]
Blood pressure, mmHg	SBP <140 and DBP <90 or no treatment	–	SBP ≥130 or DBP ≥85 or treatment	–	SBP ≥130 or DBP ≥85 or treatment	SBP >130 and/or DBP >85	SBP ≥140 and DBP ≥90 or treatment
TAG, mmol/L	–	≤1.70	≥1.70	–	≥1.70	≥1.70	≥1.70 or treatment
HDL-C, mmol/L	≥1.04	≥1.30 and no treatment	<1.04 (M) <1.30 (F)	–	<1.04 (M) <1.30 (F) or treatment	< 1.03 (M) <1.29 (F)	<1.03 (M) <1.30 (F) or treatment
LDL-C, mmol/L	–	≤2.60 and no treatment	–	–	–	–	–
Total-C, mmol/L	–	≥5.20	–	–	–	–	–
FPG, mmol/L	<7.00 and no treatment	–	≥5.60 or treatment	–	≥5.55 or treatment	≥5.6	≥7.0 or ≥7.8 nonfasting or treatment or T2DM diagnosis
HOMA	–	≤1.95	–	<75th percentile[c]	>90th percentile	–	–
Other	–	–	Waist >102 cm (M) Waist >88 cm (F)	–	CRP >90th percentile	Waist >102 cm (M) Waist > 88 cm (F)	CVD diagnosis
MH criteria	All of the above	≥4 of the above	<3 of the above	All of the above	<2 of the above	<3 of the above	None of the above

Table 5. Selection of current criteria used to define metabolic health status among adults. ATPIII, Adult Treatment Panel III; CRP, C-reactive protein; DBP, diasystolic blood pressure; F, female; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment; LDL-C, low-density lipoprotein cholesterol; M, male; MH, metabolic health; NCEP, National Cholesterol EducationProgram; SBP, systolic blood pressure; TAG, triglyceride; total-C, total cholesterol. Reproduced with permission from Annals of the New York Academy of Sciences (67).
a Using metabolic syndrome variables.
b Using homeostasis model only.

The prevalence of MHO is highly dependent on the underlying criteria used for defining the MHO phenotype. In our study, the criteria used was partly based on the National Cholesterol Education Program's Adult Treatment Panel III report (ATP III)(103) for lipid profiles with cut-off points used to define a very low risk population. Measures of insulin sensitivity were based on a study of 154 obese postmenopausal women by Karelis et al. where a cut-off point for HOMA (≤ 1.95) was suggested (91). If 4 out of 5 criteria were present, a person was diagnosed as MHO. Waist circumference was excluded as a potential marker because most obese individuals have increased waist circumferences and thus this phenotype becomes non-discriminatory in the identification of MHO individuals.

5.2 Discussion of findings

In this present study, we aimed to characterize the lipoprotein subclass profile and amino acids in individuals defined as MHO compared to ARO and normal weight individuals to further understand the differences in metabolic risk between these two groups of obese individuals.

Our main findings showed that MHO individuals displayed a lower concentration of all VLDL subclasses compared to the ARO individuals **(Figure 15).** They also had lower concentration of TG and CE in all VLDL subclasses compared to their ARO counterparts. Still the MHO individuals displayed higher concentration of all VLDL subclasses and higher concentration of TG and CE in these particles compared with the normal weight individuals.

The MHO individuals also displayed lower concentration of IDL and large LDL particles together with higher concentration of all the three largest subclasses of HDL (XL-, L-HDL and M-HDL) compared to the ARO individuals. However, MHO displayed higher concentration of M-LDL and S-LDL together with the S-HDL particle compared to the normal weight individuals. No differences were found between the other subclasses.

In addition, the MHO individuals had lower concentration of all BCAAs- valine, isoleucine and leucine, compared with the ARO individuals. Between MHO and normal weight individuals, there was no difference.

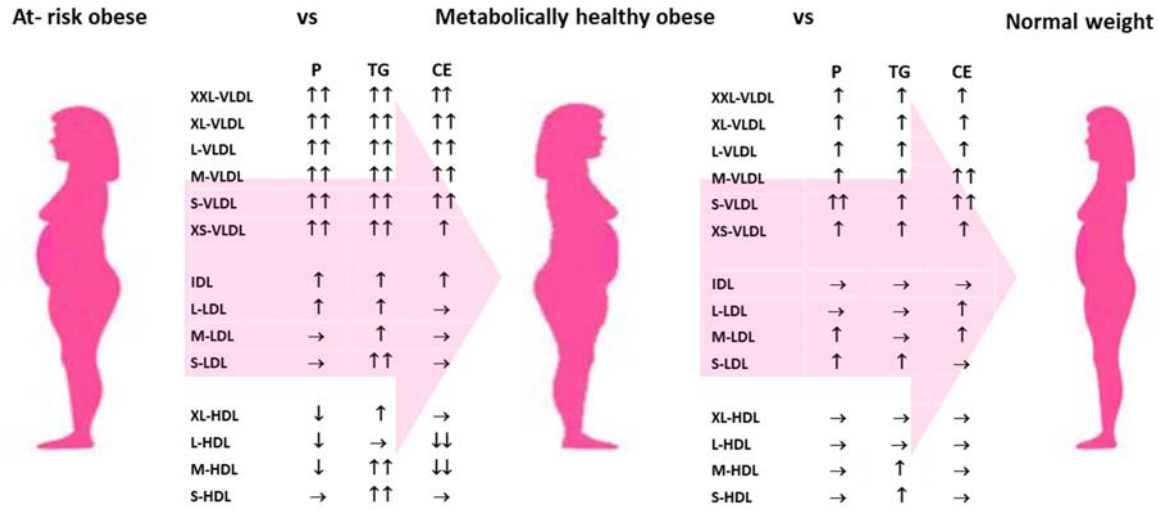

Figure 15. Summary of lipoprotein subclass findings from the present study. ARO vs MHO/ MHO vs Normal weight. ARO; at-risk obese. MHO; metabolically healthy obese. P; lipoprotein particle concentration. TG; concentration of triglyceride. CE; concentration of cholesteryl esters. ↑↑; higher concentration, $p \leq 0.005$. ↑; higher concentration, $0.005 < p < 0.05$. →; no significant difference. ↓↓; lower concentration, $p \leq 0.005$. ↓lower concentration, $0.005 < p < 0.05$. Modified and used with permission from International Journal of Obesity (72).

5.2.1 Discussion of lipoprotein subclass findings

Despite the inclusion of lipid profiles in most definitions of MHO individuals, limited data exists on the lipoprotein particle distribution of the MHO phenotype compared to ARO phenotype. Very few studies have been performed using NMR spectroscopy to analyze fasting plasma samples of a MHO population (104, 105), and consequently limited data regarding lipoprotein particle profiles in MHO exist (104, 106). However, there are a number of studies demonstrating the association between lipoprotein subclasses and CVD risk in population cohorts and nested case-control studies using NMR technology (50, 107-112).

In an analysis conducted of Würtz et al. of multi-metabolic profiles obtained by NMR spectroscopy in healthy young adults, three different phenotypes were associated with high carotid intima media thickness, which is a surrogate marker of CVD (112). The phenotype, with the highest CVD risk, was characterized by high concentrations of all subclasses of VLDL, IDL and LDL, as well as low concentrations of large HDL particles. This is in line with our findings when characterizing the lipoprotein profile of the ARO group compared with the MHO group. In the study from Würtz et al., they demonstrated that the phenotype with the highest CVD risk was associated with the highest prevalence of MetS, which is the most common used indicator to define the ARO individuals (112).

5.2.2 VLDL

Our study demonstrate that MHO individuals display a more favorable lipid profile than the ARO individuals, including lower concentration of all VLDL subclasses. ARO individuals displayed a 130% and 93% higher concentration of XL- and L-VLDL, respectively (See **Table 6** for percentage (%) differences in VLDL subclasses). Still, the MHO individuals had a higher concentration of all VLDL subclasses compared to the normal weight individuals, however the differences was smaller.

Difference between:	XXL-VLDL	XL-VLDL	L-VLDL	M-VLDL	S-VLDL	XS-VLDL
[1]ARO vs. MHO	79%	130%	93%	56%	36%	25%
[2]MHO vs. normal weight	61%	103%	60%	42%	39%	17%

Table 6. Difference in median particle concentration of VLDL subclasses between [1] ARO vs. MHO, [2] MHO vs. normal weight. VLDL, very low-density lipoprotein, XXL, Extremely large; XL-, very large; L-, large; M-, medium; S-, small; XS-, extra small.

These findings are consistent with the findings from Phillips et al. (104). In a cross-sectional study of 1834 middle-aged MHO and ARO individuals were included. They demonstrated that the MHO individuals had reduced numbers of L-, M- and S-VLDL particles compared to ARO counterparts. The study used approximately equal definition of the MHO phenotype, but another NMR platform (LipoScience Inc.) **(Figure 14)** as in our study. This findings is also in line with the results from Sheng et al., who examined phenotypic characteristics of MHO individuals in a sample of obese, non-diabetic patients with schizophrenia and found significantly lower levels of L-VLDL in the MHO group compared to the ARO group (105). Contradictory to our findings, Sheng et al. found significantly higher levels of intermediate VLDL, in MHO compared to the ARO individuals, using another NMR platform (LipoScience Inc.) **(Fig 14)**, where intermediate VLDL subclass corresponds to the L- and M-VLDL subclasses in the present study. Our data showed higher concentration of all VLDL subclasses in the ARO individuals.

High concentration of L-VLDL have been associated with atherosclerosis and premature CVD risk and may be more important for atherogenic risk than M- and S-VLDL particles, as they are associated with the small dense LDL phenotype (113). Strong associations with increased CVD risk were also found in individuals with higher concentration of M- and S-VLDL particles in an observational study conducted in a cohort of 7256 individuals from the population-based National Finnish FINRISK Study (111).This may indicate that the ARO

individuals in the present study may have a higher CVD risk compared to the MHO individuals since they have a higher concentration of all VLDL subclasses compared to MHO. Since there was also a pronounced difference between the MHO and the normal weight individuals, this may also indicate an increased CVD risk also for the MHO individuals.

VLDL overproduction is a hallmark of dyslipidemia in obesity and IR. At the same time, the catabolism of TG-rich lipoproteins, including VLDL, is reduced (114). Chylomicrons and VLDL are also removed by LPL from the circulation by common pathways and therefore compete for clearance, which contribute to higher concentration of the large VLDL subclasses. Evidence suggest that an overproduction of large subclasses of VLDL particles is the initiator of lipoprotein changes in obesity-related dyslipidemia, resulting in a decrease in levels and particle size of HDL and smaller-denser LDL particles (115). The synthesis and secretion of VLDL particles is dependent on TG availability, which is derived from multiple processes including TG synthesis from FFA re-esterification, *de novo* lipogenesis and uptake of remnant particles (116). The contribution of visceral lipolysis to the FFA pool increases as a function of increased visceral fat volume (117).

In a large study including 12 664 adolescents and young adults from 4 population based cohorts in Finland, associations between BMI and several metabolites were investigated (59). Mendelian randomization was used to estimate causal effects of BMI on 82 different metabolites. The study suggested that BMI had causal effects on multiple metabolic pathways, including different atherogenic lipoprotein subclasses. The strongest associations were observed for the VLDL subclasses, with strong association demonstrated in all VLDL subclasses (59). This demonstrates the causal effect of elevated BMI on VLDL as a cardiovascular risk marker. All VLDL subclasses were significantly higher in the ARO group, compared to the MHO and the normal weight group in the present study. This might demonstrate that MHO individuals are prevented against some of the metabolic abnormalities associated with obesity.

Very few studies have been performed using NMR spectroscopy to analyze lipid content in lipoprotein subclasses in the context of metabolic health. In the present study, we demonstrated that the ARO individuals had significantly higher TG content in all subclasses of VLDL compared to the MHO individuals. Subsequently, lipid enriched VLDL particles are more efficiently hydrolyzed by LPL, thereby generating smaller particles with greater

capacity to penetrate the endothelial wall and thereby enhancing intimal accumulation of TGs and CEs (118).

The ARO individuals displayed significantly higher concentration of TGs in all subclasses of VLDL compared to the MHO individuals. Still, the MHO individuals displayed higher concentration of TGs than the normal weight individuals did. However, the between groups differences in concentration of TGs were larger between the ARO and MHO individuals especially for the largest VLDL subclasses, than between the MHO and the normal weight individuals **(Table 7).**

Difference between:	**XXL-VLDL-TG**	**XL-VLDL-**	**L-VLDL-TG**	**M-VLDL-TG**	**S-VLDL-TG**	**XS-VLDL-TG**
[1]ARO vs. MHO	77%	123%	96%	57%	36%	30%
[2]MHO vs. normal weight	48%	88%	52%	34%	32%	20%

Table 7. Difference in median TG concentration of VLDL subclasses between [1] ARO vs. MHO, [2] MHO vs. normal weight. TG, triglycerides; VLDL, very low-density lipoprotein; XXL, Extremely large; XL-, very large; L-, large; M-, medium; S-, small; XS-, extra small.

Amor et al. conducted a study in a group of 96 newly diagnosed T2DM individuals with normal HDL-C and TG concentrations measured by standard lipid panel, compared with a matched control group. It was discovered additional atherogenic abnormalities i.e. particle number, size and lipid composition of VLDL and HDL (119). The newly diagnosed T2DM individuals displayed a higher concentration of all VLDL subclasses, as well as higher concentration of TG and cholesterol in VLDL particles, which is consistent with our data characterizing the ARO individuals, compared to the MHO and the normal weight individuals.

To our knowledge, this is the first study to compare concentration of CE and cholesterol in VLDL particle subclasses between MHO, ARO and normal weight individuals. The CE content in VLDL subclasses mirrored the picture from VLDL concentration in all three study groups. The ARO individuals displayed a significant higher content of CEs in all VLDL subclasses compared to the MHO individuals. Similarly, the MHO individuals displayed a significant higher concentration of CE compared to the normal weight individuals. The clinical implication of this is unclear.

In summary, the ARO individuals display a higher concentration of all subclasses of VLDL compared to both the MHO and the normal weight individuals. They also had higher concentration of TG and CE in all subclasses of VLDL. This demonstrates the difference in the ARO and the MHO phenotypes and the increased CVD risk related to the ARO phenotype.

5.2.3 IDL and LDL

In our study population, we found a trend of lower concentration of IDL and all of the LDL subclasses among the MHO individuals compared to the ARO individuals **(Table 8)**.

Difference between:	IDL	L-LDL	M-LDL	S-LDL
[1]ARO vs. MHO	18.4%	16.6%	n.s.	n.s.
[2]MHO vs. normal weight	n.s.	n.s.	9.9%	7.7%

Table 8. Difference in median particle concentration of IDL and LDL subclasses between [1] ARO vs. MHO, [2] MHO vs. normal weight. IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; l-, large; M-, medium; S-, small; n.s., non significant.

Surprisingly, there were only significant differences in the concentration of larger particles - IDL and L-LDL. This is in contradiction to previous findings. Phillips et al. found a significant higher concentration of L-LDL particles in the MHO group and lower concentration of small LDL compared to the ARO group, which are in line with current knowledge (104). Consistent with Phillips et al. findings, Kim et al. demonstrated higher concentration of L-LDL particles in a group of MHO in a from a Korean study population of 472, all patients in an obesity clinic in Korea. Here, only LDL subclasses were examined by electrophoresis (106). Würtz et al. described the causal association between BMI and LDL particle concentration as weak, with the strongest association for the S-LDL subclass (59). Our data demonstrated significant higher concentration of S-LDL in ARO and MHO individuals compared with the normal weight group, but showed no significant difference in the concentration of S-LDL between the ARO and the MHO individuals.

IDL is the remnant from VLDL and act as an unstable, transitional particle that are taken up by the liver through the LDL receptor. Alternatively, IDL act as a substrate for HL where the remaining TG is removed from the core of the lipoprotein particle and emerges from the liver as intact LDL (120). As shown in the comparison of characterization techniques **(Figure 14)**,

there is a discrepancy in the size definition of the IDL particle, which makes comparisons to other studies difficult.

The concentration of TG in IDL and all LDL subclasses were lower in the MHO individuals compared to their ARO counterparts **(Table 9).**

Difference between:	IDL-TG	L-LDL-TG	M-LDL-TG	S-LDL-TG
[1]ARO vs. MHO	22 %	27 %	21%.	26%
[2]MHO vs. normal weight	n.s.	n.s.	n.s.	23 %

Table 9. Difference in median particle concentration of IDL and LDL subclasses between 1 ARO vs. MHO, 2 MHO vs. normal weight. IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; l-, large; M-, medium; S-, small; n.s., non significant.

This might indicate a higher activity of CEPT in the ARO group. ARO displayed TG enriched LDL particles in all subclasses, which is might be the precursor for small dense LDL, depending on hepatic lipase activity (121) .There was no significant difference in concentration of TG between the MHO and the normal weight individuals except for small LDL, where the concentration of TG was higher in the MHO individuals. Regarding the CE content, a different pattern was observed. There was no difference between the ARO and the MHO group in any of the LDL subclasses. Surprisingly the MHO individuals displayed a higher concentration of CE in all LDL subclasses, except S-LDL compared to the normal weight group.

The main reason for the variability in LDL composition is related to the plasma TG levels (51). The core lipid composition in the LDL particle is driven by a reaction that modulates the relative amounts of CE and TG contained in the core of the particle. The variability in particle size also affects how LDL is composed. Smaller LDL particles contain less cholesterol than larger ones. When plasma TG levels are elevated, even modestly, a reaction catalyzed by CEPT becomes important, in which TG-rich lipoproteins, mainly VLDL, exchange one TG molecule with one CE molecule in the core of LDL. When large LDL thus becomes depleted in cholesterol and enriched in TG, the particle becomes a substrate for HL and become transformed into a smaller and denser LDL particle. A small difference in diameter in the LDL particle, typically up to about 3nm, causes a different lipid composition with about 40% less core cholesterol (51).

Various mechanisms have been suggested to explain the enhanced atherogenic activity of small dense LDL particles. These mechanisms include lower affinity for the LDL receptor, facilitated entry into the arterial wall, major arterial retention, major susceptibility to oxidation and longer half-time (122). Increased levels of small dense LDL particles represent increased numbers of artherogenic particles, which may not be reflected by the traditional LDL measurement as the small dense LDL particle contains less cholesterol. It have been discovered that people with the same LDL-C concentration can have discrepant LDL particle size and cholesterol content (123). S-LDL and L-LDL particles may play distinct roles in driving vascular disease (124). Our present data showed no significant difference in M- and S-LDL between MHO and ARO individuals. However, both ARO and MHO individuals displayed a higher concentration of S- and M-LDL particles compared to the normal weight individuals, which indicate a more atherogenic LDL profile in the obese groups compared to the normal weight group.

In the Multi- Ethnic Study of Atherosclerosis (MESA) study participants with discordant measurements of LDL particle concentration were compared to LDL-C. It was demonstrated that elevated LDL particle concentration was more strongly associated with carotid IMT and CVD events than elevated LDL-C. Previous studies have mostly demonstrated a relationship between small LDL particles and CVD risk (123).The largest study on the relationship between lipoprotein subfractions and CVD risk was the Women's Health Study. This study utilized NMR analyses in 27673 initially healthy women, then following up for incident CVD after a 11-year period (125). Hazard ratios were significant related to particle concentrations of total LDL and S-LDL as well as IDL. However, when S-LDL and L-LDL were examined in a model that included all NMR-measured lipoprotein particle concentrations, both were significantly associated with CVD to a similar degree, suggesting that total LDL particle number, rather than levels of individual subfractions, is the primary LDL determinant of CVD risk. Würtz et al. found no evidence of higher atherogenicity for S-LDL subclasses than for other LDL subclasses or cholesterol content in LDL (110). However, significant associations for IDL and all subclasses of LDL were demonstrated (110). Discrepancies in the findings among these studies may be attributed to differences in subject characteristics or analytical platform used in lipoprotein subclass measurements. Therefore, the clinical implications of our findings concerning LDL, where ARO displayed higher concentration of L-LDL compared to MHO and no significant difference in the other subclasses (M- and S-LDL), is difficult to interpret.

In summary, the results from the present study draw a complex picture concerning the LDL particle and its role in obesity and the risk of CVD. An interpretation of these results are difficult, because of the complexity in the interface of different metabolic pathways, discrepancies in findings and the use of different analytical methods. Furthermore, the role of TGs as a cardiovascular risk factor is a matter of debate (126). However, newer genetic data indicate a potential causal role of TG-rich lipoprotein in the development of CVD (127, 128). To our knowledge, little to no research have been conducted on TGs and CEs content in the various lipoprotein classes in relation to metabolic phenotypes and CVD risk.

5.2.4 HDL

In the ARO individuals, we observed a lower concentration of the XL-, L- and M-HDL subclasses, compared to the MHO individuals, with an especially large difference in the concentration of XL- and L-HDL between the two groups (-22% and -45%, respectively) **(Table 10)**. Between the MHO and the normal weight individuals there were no significant difference in the concentrations of XL-, L- and M-HDL particles. However, differences in the medians between the groups according to metabolic health demonstrated a clear tendency of a cardiometabolic favorable lipoprotein profile in the MHO individuals.

Difference between:	XL-HDL	L-HDL	M-HDL	S-HDL
[1]ARO vs. MHO	-22 %	-45 %	-10 %	n.s.
[2]MHO vs Normal weight	n.s.	n.s.	n.s.	8%

Table 10. Difference (%) in median particle concentration in HDL subclasses between [1] ARO vs. MHO, [2] MHO vs. normal weight. HDL, high-density lipoprotein; XL-, extra-large; L-, large; M-, medium; XS-, extra small; n.s., non significant.

Consistent with our findings, Phillips et al. reported greater number of large HDL particles among the MHO individuals compared to the ARO individuals, which is comparable with the largest HDL subclasses (XL-, L- and M-HDL) in our data. However, lower concentration of small HDL was also reported, which is contradictory to our findings. Our data showed no significant difference between the ARO and the MHO individuals in S-HDL particle concentration. The difference in findings is possibly related to a different NMR spectroscopy platform used. Similarly, Amor et al. reported higher concentrations of large HDL in healthy normal weight controls compared to newly diagnosed T2DM individuals. In concentration of

S- HDL and M-HDL subclasses, there were no difference when adjusted for age, sex, BMI and lipid-lowering medications.

Results from the Women`s Health Study, showed that total concentration of HDL particles was not significantly associated with CVD (125). Large HDL particles were significantly and inversely associated with CVD, while medium and small HDL particles had no significant associations (125). Moreover, Würtz et al. also demonstrated the strongest inverse association with cardiovascular risk within the large HDL subclass and for HDL-C (111). This, support the hypothesis of MHO having a favorable cardiometabolic lipoprotein profile than the ARO phenotype.

Increased levels of large VLDL, as demonstrated in the ARO individuals, is associated with increased catabolism of HDL particles. Subsequently, increased concentration of large VLDL particles alter the composition of HDL through the enhanced activity in CEPT and HL in IR, leading to the formation of small dense HDL (129). The largest HDL subclasses have been suggested to be the most important in reverse cholesterol transport since they are involved in cholesterol efflux and transfer of cholesterol to the liver. The size of HDL is important, because larger particles can transport more cholesterol to the liver whereas small HDL particles are less functional, and do not capture as much cholesterol as the larger ones due to their size (130). However, in a systematic review by Taskinen et al. (2015) they showed that a high concentration of TG-rich lipoproteins or remnant cholesterol are causal factors for CVD, and low HDL is probably an bystander, and thus low HDL might merely be a long time marker of raised TG and remnant cholesterol (131)

The ARO individuals in our study had higher concentration of the largest subclasses of VLDL compared to the MHO individuals, which may in part explain the pattern of HDL subclasses between the ARO (low concentration of the largest subclasses of HDL) and the MHO individuals (higher concentration of the largest subclasses of HDL) in the present study.

The ARO individuals had significantly higher concentration of TG in all HDL subclasses except in L-HDL subclass compared to the MHO individuals **(Table 11).** The MHO individuals had significantly higher concentration of TG in small HDL subclasses (M- and S-HDL).

Difference between:	XL-HDL-TG	L-HDL-TG	M-HDL-TG	S-HDL-TG
[1]ARO vs. MHO	51 %	n.s.	23 %	29%
[2]MHO vs Normal weight	n.s.	n.s.	24%	12%

Table 11. Difference in median particle concentration in HDL subclasses between 1 ARO vs. MHO, 2 MHO vs. normal weight. HDL, high-density lipoprotein; TG, triglycerides; XL-, extra-large; L-, large; M-, medium; XS-, extra small; n.s., non significant.

Furthermore, CE concentrations were significantly lower in L-HDL and M-HDL in the ARO individuals compared to the MHO individuals. These data may suggest a higher CEPT activity in the ARO group, which could potentially shuffle TG to HDL particles, and cholesteryl esters in the opposite direction.

In summary, the MHO individuals displayed a higher concentration of the largest subclasses of HDL (XL-, L- and M-HDL) compared to the ARO individuals, no difference was observed between the MHO and the normal weight group. This relates the MHO individuals to a favorable cardiometabolic profile and lower CVD-risk despite their excess body fat.

5.2.5 Amino Acids

Our data indicate an association of increased levels of the BCAAs with obesity and metabolic dysregulation. We found that MHO individuals had lower concentration of all the BCAAs - valine, isoleucine and leucine, compared with ARO individuals. Between the MHO and the normal weight individuals there were no difference.

Many studies have focused on the concentration of plasma amino acids in the context of obesity (59, 65, 132-135). Würtz et al. demonstrated that increased BMI was positively associated with elevated concentration of BCAAs and aromatic amino acids in plasma (59). This was recently confirmed by a Finnish twin study in healthy young adults where the association between 56 different metabolites and abdominal obesity, low-grade inflammation and IR where studied (135). They showed that abdominal obesity and IR were strongly associated with the concentration of BCAAs, phenylalanine and tyrosine. Only a few studies have investigated the differences in metabolomics profiles, including amino acids, of the

MHO and the ARO subsets. Chen et al. investigated the metabolites, with the use of liquid chromatography, to distinguish the MHO from ARO metabolic state. The study was performed in a group of 68 obese patients from a weight management clinic in Taiwan in a matched study design (57). The study participants were matched by sex, age and BMI, and divided into two groups, according to their metabolic health status. It was demonstrated significantly elevated levels of the branched BCAAs valine and isoleucine in the ARO group, which are in line with our findings. Leucine, which also showed significant higher concentration in the ARO group in our data, was not included in the metabolite panel examined in the study from Chen et al.

Elevated BCAA levels has also shown to be a predictor of diabetes incidence (66). In addition, dysfunction of amino acid metabolism in adipose tissues has previously been reported to correlate with IR in obesity (136).

Thus, it has been proposed that decreased BCAA metabolism in fat contributes to increased plasma levels of BCAAs in obese individuals with IR or untreated T2DM. Moreover, an increase in BCAA metabolites is a result of mitochondrial dysfunction (65). Furthermore, decreased numbers of mitochondria and mitochondrial dysfunction have been observed in obese or diabetic patients (137). This may indicate the importance of mitochondrial function, and these metabolites potential role in regulating metabolic status in obesity. However, in this present study the ARO individuals displayed a higher concentration of all BCAAs compared to the MHO individuals, despite the same BMI. There were no significant difference between the MHO and the normal weight, which demonstrate that the MHO individuals do not experience the same metabolic dysfunction despite the same fat mass as the ARO individuals. The issue of whether other organs than adipose tissue have altered BCAA metabolism in obesity and IR state is starting to be addressed (65).

Phenylalanine was also identified by Chen et al. as an important metabolite for distinguishing ARO from MHO subjects. Furthermore, the downstream product of the phenylalanine catabolism, tyrosine, also exhibited significant difference between the ARO and the MHO individuals in Chen et al. study. High levels of phenylalanine have been observed in obese subjects of previous metabolomics studies (58, 138). Contradictory to these findings, we did not observe any difference in concentration of phenylalanine between ARO and MHO individuals. However, we observed a significant higher concentration of phenylalanine in MHO individuals compared to the normal weight group.

CVD risk assessment has traditionally been based on serum lipids. However, NMR spectroscopy has enabled detection of many low-molecular-weight metabolites, such as amino acids. In a multimetabolic assessment, Würtz et al. discovered distinct metabolic phenotypes associated with increased risk of CVD. In these analyses, isoleucine was elevated for all the risk phenotypes, and these phenotypes appeared to be linked to MetS and obesity (112) . In a recent case-control study with 970 participants from the Predimed trial, Ruiz-Canela et al. found that baseline circulating BCAA concentrations were positively associated with CVD (139). This corresponds with the findings from other studies, which have demonstrated an association between BCAA and aromatic amino acids and the risk for CVD and T2DM (66, 140).

6 Conclusion

In conclusion,

- ARO individuals had significantly higher concentration of all VLDL subclasses, IDL and the large LDL subclass compared to the MHO individuals. Furthermore, the ARO individuals had lower concentration of extra-large, large and medium HDL compared to the MHO individuals. MHO had significantly higher concentration of all VLDL subclasses compared to the normal weight individuals. MHO individuals also had higher concentration of medium and small LDL compared to the normal weight individuals.

- ARO individuals had significantly higher concentration of TGs and CEs in all VLDL subclasses compared to MHO. They also had higher concentration of TG in all subclasses of LDL and HDL, except in large HDL where no difference was observed between the ARO and the MHO individuals. Compared to the normal weight individuals, the MHO individuals displayed significantly higher concentration of TG in all subclasses of VLDL.

- ARO individuals had significantly higher concentrations of the branched chain amino acids isoleucine, leucine and valine compared to the MHO individuals. No differences were observed between the MHO and the normal weight individuals.

The clinical relevance of our findings is unclear, and further research must elucidate if NMR-based lipoprotein subclass measurements can improve risk classification in the obese population. Prevention and treatment of obesity and obesity related diseases are major public health challenges. Several strategies to reduce body weight and obesity-associated risk factors have not been successful (141-143). It would be advantageous with early identification of those patients who will benefit the most from diet and exercise, pharmacological or bariatric surgery, to reduce the medical and socioeconomic burden associated with obesity and obesity-treatment (144). Further research is highly needed to demonstrate if diet or exercise may modulate the lipoprotein subclasses profile in ARO individuals towards the more cardiometabolic favorable MHO phenotype.

References

1. WHO. Obesity and overweight fact sheets [updated 16.02 2018. Available from: http://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight.
2. OECD. Obesity-Update-2017. 2017.
3. Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. International journal of obesity (2005). 2008;32(9):1431-7.
4. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. The Lancet.387(10026):1377-96.
5. Helsedirektoratet. Nasjonal faglig retningslinje for forebygging, utredning og behandling av overvekt og fedme hos voksne. 2011(IS-1735).
6. El-Sayed Moustafa JS, Froguel P. From obesity genetics to the future of personalized obesity therapy. Nature reviews Endocrinology. 2013;9(7):402-13.
7. Friedman JM. A war on obesity, not the obese. Science (New York, NY). 2003;299(5608):856-8.
8. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444(7121):860-7.
9. John-Sowah J. Implications of viewing obesity as a disease. The virtual mentor : VM. 2005;7(11).
10. WHO. Global burden of disease estimates 2016 2016 [Available from: http://www.who.int/gho/mortality_burden_disease/causes_death/top_10/en/.
11. Libby P, Bornfeldt KE, Tall AR. Atherosclerosis: Successes, Surprises, and Future Challenges. Circulation research. 2016;118(4):531-4.
12. Hansson GK, Hermansson A. The immune system in atherosclerosis. Nature immunology. 2011;12(3):204-12.
13. Rutkowski JM, Stern JH, Scherer PE. The cell biology of fat expansion. The Journal of cell biology. 2015;208(5):501-12.
14. Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2010;11(1):11-8.
15. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocrine reviews. 2000;21(6):697-738.
16. González-Muniesa P, Mártinez-González M-A, Hu FB, Després J-P, Matsuzawa Y, Loos RJF, et al. Obesity. Nature Reviews Disease Primers. 2017;3:17034.
17. Kotani K, Tokunaga K, Fujioka S, Kobatake T, Keno Y, Yoshida S, et al. Sexual dimorphism of age-related changes in whole-body fat distribution in the obese. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 1994;18(4):207-2.
18. Bergman RN, Kim SP, Catalano KJ, Hsu IR, Chiu JD, Kabir M, et al. Why visceral fat is bad: mechanisms of the metabolic syndrome. Obesity (Silver Spring, Md). 2006;14 Suppl 1:16s-9s.
19. Bays HE, Gonzalez-Campoy JM, Bray GA, Kitabchi AE, Bergman DA, Schorr AB, et al. Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. Expert review of cardiovascular therapy. 2008;6(3):343-68.
20. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nature reviews Immunology. 2011;11(2):85-97.

21. Bays H, Mandarino L, DeFronzo RA. Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. The Journal of clinical endocrinology and metabolism. 2004;89(2):463-78.
22. Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. The Journal of clinical investigation. 2007;117(9):2621-37.
23. Wang Y, Rimm EB, Stampfer MJ, Willett WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. Am J Clin Nutr. 2005;81(3):555-63.
24. Gonzalez-Muniesa P, Martinez-Gonzalez MA, Hu FB, Despres JP, Matsuzawa Y, Loos RJF, et al. Obesity. Nature reviews Disease primers. 2017;3:17034.
25. Després J-P, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006;444:881.
26. Hotamisligil GS. Inflammation, metaflammation and immunometabolic disorders. Nature. 2017;542(7640):177-85.
27. Roos CJ, Quax PH, Jukema JW. Cardiovascular metabolic syndrome: mediators involved in the pathophysiology from obesity to coronary heart disease. Biomarkers in medicine. 2012;6(1):35-52.
28. Herder C, Peltonen M, Koenig W, Kraft I, Muller-Scholze S, Martin S, et al. Systemic immune mediators and lifestyle changes in the prevention of type 2 diabetes: results from the Finnish Diabetes Prevention Study. Diabetes. 2006;55(8):2340-6.
29. Hajer GR, van Haeften TW, Visseren FL. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. European heart journal. 2008;29(24):2959-71.
30. Ginsberg HN. Insulin resistance and cardiovascular disease. The Journal of clinical investigation. 2000;106(4):453-8.
31. Methi T, Thorsby PM. Insulin resistance - the body's defence against caloric intoxication? Tidsskrift for den Norske laegeforening : tidsskrift for praktisk medicin, ny raekke. 2016;136(11):1015-7.
32. Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. The New England journal of medicine. 2014;371(23):2237-8.
33. Franssen R, Monajemi H, Stroes ES, Kastelein JJ. Obesity and dyslipidemia. The Medical clinics of North America. 2011;95(5):893-902.
34. Pandey A, Chawla S, Guchhait P. Type-2 diabetes: Current understanding and future perspectives. IUBMB life. 2015;67(7):506-13.
35. Ginsberg HN. LIPOPROTEIN PHYSIOLOGY. Endocrinology and Metabolism Clinics.27(3):503-19.
36. Frayn KN. Metabolic regulation: a human perspective. Third edition ed2010.
37. Ridker PM. LDL cholesterol: controversies and future therapeutic directions. Lancet (London, England). 2014;384(9943):607-17.
38. van Leeuwen EM, Emri E, Merle BMJ, Colijn JM, Kersten E, Cougnard-Gregoire A, et al. A new perspective on lipid research in age-related macular degeneration. Progress in retinal and eye research. 2018.
39. Hegele RA. Plasma lipoproteins: genetic influences and clinical implications. Nature Reviews Genetics. 2009;10:109.
40. Klop B, Elte JW, Cabezas MC. Dyslipidemia in obesity: mechanisms and potential targets. Nutrients. 2013;5(4):1218-40.
41. Feingold KR, Grunfeld C. Obesity and Dyslipidemia. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, et al., editors. Endotext. South Dartmouth (MA): MDText.com, Inc.; 2000.

42. Jung UJ, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. International journal of molecular sciences. 2014;15(4):6184-223.
43. Adiels M, Olofsson SO, Taskinen MR, Boren J. Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. Arteriosclerosis, thrombosis, and vascular biology. 2008;28(7):1225-36.
44. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. Nature clinical practice Endocrinology & metabolism. 2009;5(3):150-9.
45. Guendouzi K, Jaspard B, Barbaras R, Motta C, Vieu C, Marcel Y, et al. Biochemical and physical properties of remnant-HDL2 and of pre beta 1-HDL produced by hepatic lipase. Biochemistry. 1999;38(9):2762-8.
46. Kreisberg RA. Diabetic dyslipidemia. The American journal of cardiology. 1998;82(12a):67U-73U; discussion 85U-6U.
47. Krauss RM. Lipoprotein subfractions and cardiovascular disease risk. Current opinion in lipidology. 2010;21(4):305-11.
48. Freedman DS, Otvos JD, Jeyarajah EJ, Barboriak JJ, Anderson AJ, Walker JA. Relation of lipoprotein subclasses as measured by proton nuclear magnetic resonance spectroscopy to coronary artery disease. Arteriosclerosis, thrombosis, and vascular biology. 1998;18(7):1046-53.
49. Ip S, Lichtenstein AH, Chung M, Lau J, Balk EM. Systematic review: association of low-density lipoprotein subfractions with cardiovascular outcomes. Annals of internal medicine. 2009;150(7):474-84.
50. El Harchaoui K, Arsenault BJ, Franssen R, Despres JP, Hovingh GK, Stroes ES, et al. High-density lipoprotein particle size and concentration and coronary risk. Annals of internal medicine. 2009;150(2):84-93.
51. Otvos JD, Jeyarajah EJ, Cromwell WC. Measurement issues related to lipoprotein heterogeneity. The American journal of cardiology. 2002;90(8a):22i-9i.
52. Detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III).pdf. 2001. Report No.: Third report of the National Cholesterol Education Program (NCEP).
53. Davidson MH, Ballantyne CM, Jacobson TA, Bittner VA, Braun LT, Brown AS, et al. Clinical utility of inflammatory markers and advanced lipoprotein testing: advice from an expert panel of lipid specialists. Journal of clinical lipidology. 2011;5(5):338-67.
54. Laakso M. Lipids and lipoproteins as risk factors for coronary heart disease in non-insulin-dependent diabetes mellitus. Annals of medicine. 1996;28(4):341-5.
55. Felig P, Marliss E, Cahill GF, Jr. Plasma amino acid levels and insulin secretion in obesity. The New England journal of medicine. 1969;281(15):811-6.
56. Boulet MM, Chevrier G, Grenier-Larouche T, Pelletier M, Nadeau M, Scarpa J, et al. Alterations of plasma metabolite profiles related to adipose tissue distribution and cardiometabolic risk. American journal of physiology Endocrinology and metabolism. 2015;309(8):E736-46.
57. Chen HH, Tseng YJ, Wang SY, Tsai YS, Chang CS, Kuo TC, et al. The metabolome profiling and pathway analysis in metabolic healthy and abnormal obesity. International journal of obesity (2005). 2015;39(8):1241-8.
58. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell metabolism. 2009;9(4):311-26.

59. Wurtz P, Wang Q, Kangas AJ, Richmond RC, Skarp J, Tiainen M, et al. Metabolic signatures of adiposity in young adults: Mendelian randomization analysis and effects of weight change. PLoS medicine. 2014;11(12):e1001765.
60. Huang Y, Zhou M, Sun H, Wang Y. Branched-chain amino acid metabolism in heart disease: an epiphenomenon or a real culprit? Cardiovascular Research. 2011;90(2):220-3.
61. Patti ME, Brambilla E, Luzi L, Landaker EJ, Kahn CR. Bidirectional modulation of insulin action by amino acids. The Journal of clinical investigation. 1998;101(7):1519-29.
62. Huffman KM, Shah SH, Stevens RD, Bain JR, Muehlbauer M, Slentz CA, et al. Relationships Between Circulating Metabolic Intermediates and Insulin Action in Overweight to Obese, Inactive Men and Women. Diabetes care. 2009;32(9):1678-83.
63. Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. Cell metabolism. 2012;15(5):606-14.
64. Soininen P, Kangas AJ, Wurtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circulation Cardiovascular genetics. 2015;8(1):192-206.
65. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. Nature reviews Endocrinology. 2014;10(12):723-36.
66. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, et al. Metabolite Profiles and the Risk of Developing Diabetes. Nature medicine. 2011;17(4):448-53.
67. Phillips CM. Metabolically healthy obesity across the life course: epidemiology, determinants, and implications. Annals of the New York Academy of Sciences. 2017;1391(1):85-100.
68. Brochu M, Tchernof A, Dionne IJ, Sites CK, Eltabbakh GH, Sims EA, et al. What are the physical characteristics associated with a normal metabolic profile despite a high level of obesity in postmenopausal women? The Journal of clinical endocrinology and metabolism. 2001;86(3):1020-5.
69. Karelis AD, Faraj M, Bastard JP, St-Pierre DH, Brochu M, Prud'homme D, et al. The metabolically healthy but obese individual presents a favorable inflammation profile. The Journal of clinical endocrinology and metabolism. 2005;90(7):4145-50.
70. Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, et al. Identification and characterization of metabolically benign obesity in humans. Archives of internal medicine. 2008;168(15):1609-16.
71. Lynch LA, O'Connell JM, Kwasnik AK, Cawood TJ, O'Farrelly C, O'Shea DB. Are natural killer cells protecting the metabolically healthy obese patient? Obesity (Silver Spring, Md). 2009;17(3):601-5.
72. Primeau V, Coderre L, Karelis AD, Brochu M, Lavoie ME, Messier V, et al. Characterizing the profile of obese patients who are metabolically healthy. International journal of obesity (2005). 2011;35(7):971-81.
73. McMorrow AM, Connaughton RM, Lithander FE, Roche HM. Adipose tissue dysregulation and metabolic consequences in childhood and adolescent obesity: potential impact of dietary fat quality. The Proceedings of the Nutrition Society. 2015;74(1):67-82.
74. Telle-Hansen VH, Halvorsen B, Dalen KT, Narverud I, Wesseltoft-Rao N, Granlund L, et al. Altered expression of genes involved in lipid metabolism in obese subjects with unfavourable phenotype. Genes & nutrition. 2013;8(4):425-34.
75. Li S, Chen W, Srinivasan SR, Xu J, Berenson GS. Relation of childhood obesity/cardiometabolic phenotypes to adult cardiometabolic profile: the Bogalusa Heart Study. Am J Epidemiol. 2012;176 Suppl 7:S142-9.
76. Eshtiaghi R, Keihani S, Hosseinpanah F, Barzin M, Azizi F. Natural course of metabolically healthy abdominal obese adults after 10 years of follow-up: the Tehran Lipid and Glucose Study. International journal of obesity (2005). 2015;39(3):514-9.

77. Hwang YC, Hayashi T, Fujimoto WY, Kahn SE, Leonetti DL, McNeely MJ, et al. Visceral abdominal fat accumulation predicts the conversion of metabolically healthy obese subjects to an unhealthy phenotype. International journal of obesity (2005). 2015;39(9):1365-70.
78. Achilike I, Hazuda HP, Fowler SP, Aung K, Lorenzo C. Predicting the development of the metabolically healthy obese phenotype. International journal of obesity (2005). 2015;39(2):228-34.
79. Aung K, Lorenzo C, Hinojosa MA, Haffner SM. Risk of developing diabetes and cardiovascular disease in metabolically unhealthy normal-weight and metabolically healthy obese individuals. The Journal of clinical endocrinology and metabolism. 2014;99(2):462-8.
80. Caleyachetty R, Thomas GN, Toulis KA, Mohammed N, Gokhale KM, Balachandran K, et al. Metabolically Healthy Obese and Incident Cardiovascular Disease Events Among 3.5 Million Men and Women. Journal of the American College of Cardiology. 2017;70(12):1429-37.
81. Ogorodnikova AD, Kim M, McGinn AP, Muntner P, Khan U, Wildman RP. Incident cardiovascular disease events in metabolically benign obese individuals. Obesity (Silver Spring, Md). 2012;20(3):651-9.
82. Eckel N, Meidtner K, Kalle-Uhlmann T, Stefan N, Schulze MB. Metabolically healthy obesity and cardiovascular events: A systematic review and meta-analysis. European journal of preventive cardiology. 2016;23(9):956-66.
83. Sung KC, Ryu S, Cheong ES, Kim BS, Kim BJ, Kim YB, et al. All-Cause and Cardiovascular Mortality Among Koreans: Effects of Obesity and Metabolic Health. American journal of preventive medicine. 2015;49(1):62-71.
84. Kramer CK, Zinman B, Retnakaran R. Are metabolically healthy overweight and obesity benign conditions?: A systematic review and meta-analysis. Annals of internal medicine. 2013;159(11):758-69.
85. Khan UI, Wang D, Thurston RC, Sowers M, Sutton-Tyrrell K, Matthews KA, et al. Burden of subclinical cardiovascular disease in "metabolically benign" and "at-risk" overweight and obese women: the Study of Women's Health Across the Nation (SWAN). Atherosclerosis. 2011;217(1):179-86.
86. Kwon BJ, Kim DW, Her SH, Kim DB, Jang SW, Cho EJ, et al. Metabolically obese status with normal weight is associated with both the prevalence and severity of angiographic coronary artery disease. Metabolism. 2013;62(7):952-60.
87. Hamer M, Stamatakis E. Metabolically healthy obesity and risk of all-cause and cardiovascular disease mortality. The Journal of clinical endocrinology and metabolism. 2012;97(7):2482-8.
88. Calori G, Lattuada G, Piemonti L, Garancini MP, Ragogna F, Villa M, et al. Prevalence, metabolic features, and prognosis of metabolically healthy obese Italian individuals: the Cremona Study. Diabetes care. 2011;34(1):210-5.
89. Kuk JL, Ardern CI. Are metabolically normal but obese individuals at lower risk for all-cause mortality? Diabetes care. 2009;32(12):2297-9.
90. Durward CM, Hartman TJ, Nickols-Richardson SM. All-cause mortality risk of metabolically healthy obese individuals in NHANES III. Journal of obesity. 2012;2012:460321.
91. Karelis AD, Brochu M, Rabasa-Lhoret R. Can we identify metabolically healthy but obese individuals (MHO)? Diabetes & metabolism. 2004;30(6):569-72.
92. Soininen P, Kangas AJ, Wurtz P, Tukiainen T, Tynkkynen T, Laatikainen R, et al. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. The Analyst. 2009;134(9):1781-5.

93. Hu D, Hannah J, Gray RS, Jablonski KA, Henderson JA, Robbins DC, et al. Effects of obesity and body fat distribution on lipids and lipoproteins in nondiabetic American Indians: The Strong Heart Study. Obesity research. 2000;8(6):411-21.
94. Krauss RM, Winston M, Fletcher RN, Grundy SM. Obesity: impact of cardiovascular disease. Circulation. 1998;98(14):1472-6.
95. JAC KBaS. Relaxing model assumptions. Essential Medical Statistics: Blackwell Science Ltd; 2003. p. 343-54.
96. Streiner DL. Best (but oft-forgotten) practices: the multiple problems of multiplicity-whether and how to correct for many statistical tests. Am J Clin Nutr. 2015;102(4):721-8.
97. Mallol R, Rodriguez MA, Brezmes J, Masana L, Correig X. Human serum/plasma lipoprotein analysis by NMR: application to the study of diabetic dyslipidemia. Progress in nuclear magnetic resonance spectroscopy. 2013;70:1-24.
98. Chung M, Lichtenstein AH, Ip S, Lau J, Balk EM. Comparability of methods for LDL subfraction determination: A systematic review. Atherosclerosis. 2009;205(2):342-8.
99. Aru V, Lam C, Khakimov B, Hoefsloot HCJ, Zwanenburg G, Lind MV, et al. Quantification of lipoprotein profiles by nuclear magnetic resonance spectroscopy and multivariate data analysis. TrAC Trends in Analytical Chemistry. 2017;94:210-9.
100. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. Clinics in laboratory medicine. 2006;26(4):847-70.
101. van Vliet-Ostaptchouk JV, Nuotio ML, Slagter SN, Doiron D, Fischer K, Foco L, et al. The prevalence of metabolic syndrome and metabolically healthy obesity in Europe: a collaborative analysis of ten large cohort studies. BMC endocrine disorders. 2014;14:9.
102. Meigs JB, Wilson PW, Fox CS, Vasan RS, Nathan DM, Sullivan LM, et al. Body mass index, metabolic syndrome, and risk of type 2 diabetes or cardiovascular disease. The Journal of clinical endocrinology and metabolism. 2006;91(8):2906-12.
103. NCEP Expert panel. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106(25):3143-421.
104. Phillips CM, Perry IJ. Lipoprotein particle subclass profiles among metabolically healthy and unhealthy obese and non-obese adults: does size matter? Atherosclerosis. 2015;242(2):399-406.
105. Sheng J, Li C, Wang J, Chiang C, Harrington A, Yi Z, et al. Phenotypic characteristics in metabolically healthy but obese patients with schizophrenia. Psychiatry research. 2014;220(1-2):71-5.
106. Kim S, Lee H, Lee DC, Lee HS, Lee JW. Predominance of small dense LDL differentiates metabolically unhealthy from metabolically healthy overweight adults in Korea. Metabolism. 2014;63(3):415-21.
107. Mora S, Buring JE, Ridker PM. Discordance of low-density lipoprotein (LDL) cholesterol with alternative LDL-related measures and future coronary events. Circulation. 2014;129(5):553-61.
108. Kujala UM, Makinen VP, Heinonen I, Soininen P, Kangas AJ, Leskinen TH, et al. Long-term leisure-time physical activity and serum metabolome. Circulation. 2013;127(3):340-8.
109. Mantyselka P, Niskanen L, Kautiainen H, Saltevo J, Wurtz P, Soininen P, et al. Cross-sectional and longitudinal associations of circulating omega-3 and omega-6 fatty acids with lipoprotein particle concentrations and sizes: population-based cohort study with 6-year follow-up. Lipids in health and disease. 2014;13:28.
110. Wurtz P, Raiko JR, Magnussen CG, Soininen P, Kangas AJ, Tynkkynen T, et al. High-throughput quantification of circulating metabolites improves prediction of subclinical atherosclerosis. European heart journal. 2012;33(18):2307-16.

111. Wurtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, et al. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. Circulation. 2015;131(9):774-85.
112. Wurtz P, Soininen P, Kangas AJ, Makinen VP, Groop PH, Savolainen MJ, et al. Characterization of systemic metabolic phenotypes associated with subclinical atherosclerosis. Molecular bioSystems. 2011;7(2):385-93.
113. Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, et al. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. Diabetes. 2003;52(2):453-62.
114. Satoh A, Adachi H, Tsuruta M, Hirai Y, Hiratsuka A, Enomoto M, et al. High Plasma Level of Remnant-Like Particle Cholesterol in the Metabolic Syndrome. 2005;28(10):2514-8.
115. Wang H, Peng DQ. New insights into the mechanism of low high-density lipoprotein cholesterol in obesity. Lipids in health and disease. 2011;10:176.
116. Otero YF, Stafford JM, McGuinness OP. Pathway-selective insulin resistance and metabolic disease: the importance of nutrient flux. The Journal of biological chemistry. 2014;289(30):20462-9.
117. Havel RJ, Kane JP, Balasse EO, Segel N, Basso LV. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. The Journal of clinical investigation. 1970;49(11):2017-35.
118. Schreier L, Berg G, Zago V, Gonzalez AI, Wikinski R. Kinetics of in vitro lipolysis of human very low-density lipoprotein by lipoprotein lipase. Nutrition, metabolism, and cardiovascular diseases : NMCD. 2002;12(1):13-8.
119. Amor AJ, Catalan M, Perez A, Herreras Z, Pinyol M, Sala-Vila A, et al. Nuclear magnetic resonance lipoprotein abnormalities in newly-diagnosed type 2 diabetes and their association with preclinical carotid atherosclerosis. Atherosclerosis. 2016;247:161-9.
120. German JB, Smilowitz JT, Zivkovic AM. Lipoproteins: When size really matters. Current opinion in colloid & interface science. 2006;11(2-3):171-83.
121. Zambon A, Austin MA, Brown BG, Hokanson JE, Brunzell JD. Effect of hepatic lipase on LDL in normal men and those with coronary artery disease. Arteriosclerosis and thrombosis : a journal of vascular biology. 1993;13(2):147-53.
122. Hoogeveen RC, Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, et al. Small dense low-density lipoprotein-cholesterol concentrations predict risk for coronary heart disease: the Atherosclerosis Risk In Communities (ARIC) study. Arteriosclerosis, thrombosis, and vascular biology. 2014;34(5):1069-77.
123. Otvos JD, Mora S, Shalaurova I, Greenland P, Mackey RH, Goff DC, Jr. Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. Journal of clinical lipidology. 2011;5(2):105-13.
124. Mora S. Advanced lipoprotein testing and subfractionation are not (yet) ready for routine clinical use. Circulation. 2009;119(17):2396-404.
125. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. Circulation. 2009;119(7):931-9.
126. Emerging Risk Factors C, Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, et al. Major lipids, apolipoproteins, and risk of vascular disease. Jama. 2009;302(18):1993-2000.
127. Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjaerg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. The New England journal of medicine. 2014;371(1):32-41.

128. Tg, Hdl Working Group of the Exome Sequencing Project NHL, Blood I, Crosby J, Peloso GM, Auer PL, et al. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. The New England journal of medicine. 2014;371(1):22-31.
129. Chan DC, Barrett PH, Ooi EM, Ji J, Chan DT, Watts GF. Very low density lipoprotein metabolism and plasma adiponectin as predictors of high-density lipoprotein apolipoprotein A-I kinetics in obese and nonobese men. The Journal of clinical endocrinology and metabolism. 2009;94(3):989-97.
130. Rodriguez-Garcia E, Ruiz-Nava J, Santamaria-Fernandez S, Fernandez-Garcia JC, Vargas-Candela A, Yahyaoui R, et al. Characterization of lipid profile by nuclear magnetic resonance spectroscopy (1H NMR) of metabolically healthy obese women after weight loss with Mediterranean diet and physical exercise. Medicine. 2017;96(27):e7040.
131. Taskinen MR, Boren J. New insights into the pathophysiology of dyslipidemia in type 2 diabetes. Atherosclerosis. 2015;239(2):483-95.
132. Sampey BP, Freemerman AJ, Zhang J, Kuan PF, Galanko JA, O'Connell TM, et al. Metabolomic profiling reveals mitochondrial-derived lipid biomarkers that drive obesity-associated inflammation. PloS one. 2012;7(6):e38812.
133. Szymanska E, Bouwman J, Strassburg K, Vervoort J, Kangas AJ, Soininen P, et al. Gender-dependent associations of metabolite profiles and body fat distribution in a healthy population with central obesity: towards metabolomics diagnostics. Omics : a journal of integrative biology. 2012;16(12):652-67.
134. Wahl S, Yu Z, Kleber M, Singmann P, Holzapfel C, He Y, et al. Childhood obesity is associated with changes in the serum metabolite profile. Obesity facts. 2012;5(5):660-70.
135. Bogl LH, Kaye SM, Ramo JT, Kangas AJ, Soininen P, Hakkarainen A, et al. Abdominal obesity and circulating metabolites: A twin study approach. Metabolism. 2016;65(3):111-21.
136. Hanzu FA, Vinaixa M, Papageorgiou A, Parrizas M, Correig X, Delgado S, et al. Obesity rather than regional fat depots marks the metabolomic pattern of adipose tissue: an untargeted metabolomic approach. Obesity (Silver Spring, Md). 2014;22(3):698-704.
137. Ritov VB, Menshikova EV, He J, Ferrell RE, Goodpaster BH, Kelley DE. Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes. 2005;54(1):8-14.
138. Swierczynski J, Sledzinski T, Slominska E, Smolenski R, Sledzinski Z. Serum phenylalanine concentration as a marker of liver function in obese patients before and after bariatric surgery. Obesity surgery. 2009;19(7):883-9.
139. Ruiz-Canela M, Toledo E, Clish CB, Hruby A, Liang L, Salas-Salvadó J, et al. Plasma branched-chain amino acids and incident cardiovascular disease in the PREDIMED trial. Clinical chemistry. 2016;62(4):582-92.
140. Magnusson M, Lewis GD, Ericson U, Orho-Melander M, Hedblad B, Engstrom G, et al. A diabetes-predictive amino acid score and future cardiovascular disease. European heart journal. 2013;34(26):1982-9.
141. Wing RR, Bolin P, Brancati FL, Bray GA, Clark JM, Coday M, et al. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. The New England journal of medicine. 2013;369(2):145-54.
142. Wadden TA. Treatment of obesity by moderate and severe caloric restriction. Results of clinical research trials. Annals of internal medicine. 1993;119(7 Pt 2):688-93.
143. Sharma AM, Kushner RF. A proposed clinical staging system for obesity. International journal of obesity (2005). 2009;33(3):289-95.
144. Bluher M. Are there still healthy obese patients? Current opinion in endocrinology, diabetes, and obesity. 2012;19(5):341-6.

Appendices

Appendix 1 Cholesterol in lipoprotein particles in 14 subclasses according to metabolic health

Appendix 2 Approval from the Regional Committee of Medical Ethics #6.2008.1368

Appendix 1. Cholesterol in lipoprotein particles in 14 subclasses (NMR) in at-risk obese, metabolically healthy obese and normal weight subjects

	ARO	MHO	Normal weight	P^*	P^1	P^2	P^3
VLDL-C	n=10	n=8	n=11				
Extremely large VLDL *(µmol/L)*	7.3 (5.9, 9.2)	3.3 (2.7, 4.0)	1.7 (1.1, 2.5)	**<0.001**	**<0.001**	**0.006**	**<0.001**
Very large VLDL (*µmol/L)*	20.2 (16.4, 26.3)	8.8 (7.2, 11.4)	3.5 (0.0, 7.5)	**<0.001**	**<0.001**	**0.013**	**<0.001**
Large VLDL (*µmol/L)*	79.5 (70.7, 100.3)	43.3 (33.0, 50.4)	22.5 (0.0, 32.2)	**<0.001**	**<0.001**	**0.006**	**<0.001**
Medium VLDL *(µmol/L)*	179.5 (163.7, 210.5)	119.5 (98.3, 125.2)	71.2 (53.3, 94.5)	**<0.001**	**<0.001**	**0.005**	**<0.001**
Small VLDL *(µmol/L)*	210.2 (185.7, 234.1)	155.3 (137.9, 178.0)	98.8 (74.9, 124.3)	**<0.001**	**0.002**	**0.001**	**<0.001**
Extra small VLDL *(µmol/L)*	239.8 (205.9, 254.4)	183.8 (174.8, 222.4)	167.1 (156.6, 175.5)	**<0.001**	**0.006**	**0.006**	**<0.001**
IDL-C *(mmol/L)*	0.60 (0.53, 0.64)	0.52 (0.48, 0.56)	0.48 (0.43, 0.51)	**0.004**	**0.041**	0.215	**0.002**
LDL-C							
Large (*mmol/L)*	0.74 (0.65, 0.78)	0.65 (0.59, 0.70)	0.58 (0.52, 0.63)	**0.002**	0.076	**0.048**	**0.001**
Medium (*mmol/L)*	0.41 (0.34, 0.44)	0.36 (0.33, 0.42)	0.32 (0.29, 0.34)	**0.004**	0.374	**0.026**	**0.002**
Small	0.24 (0.21, 0.26)	0.22 (0.21, 0.26)	0.21 (0.18, 0.21)	**0.021**	0.424	**0.058**	**0.009**
HDL-C							
Very large (*mmol/L)*	0.18 (0.16, 0.19)	0.22 (0.16, 0.24)	0.24 (0.20, 0.36)	**0.006**	0.131	0.099	**0.002**
Large (*mmol/L)*	0.14 (0.09, 0.17)	0.29 (0.23, 0.30)	0.34 (0.27, 0.54)	**<0.001**	**0.003**	0.063	**<0.001**
Medium (*mmol/L)*	0.28 (0.24, 0.29)	0.35 (0.32, 0.36)	0.31 (0.27, 0.37)	**0.008**	**0.003**	0.137	**0.049**
Small (*mmol/L)*	0.37 (0.34, 0.41)	0.39 (0.38, 0.44)	0.37 (0.33, 0.39)	0.165	-	-	-

Data presented as median and 25th and 75th percentiles. The Kruskal -Wallis test was used to test for overall differences between the three groups. Mann Whitney *U*-test is used to compare groups. P< 0.05 is considered statistical significant.

*Overall differences between the three intervention groups

[1]Between MHO and Obese at-risk

[2]Between MHO and normal weight

[3]Between obese at-risk and normal weight

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Gjøril Bergva	22845529	09.06.2017	2009/2387/REK sør-øst D
			Deres dato:	Deres referanse:
			10.05.2017	

Vår referanse må oppgis ved alle henvendelser

Stine Marie Ulven
Universitetet i Oslo

2009/2387 S-08377 Overvekt og fettkvalitet 6.2008.1368

Forskningsansvarlig: Høgskolen i Akershus og Mills DA
Prosjektleder: Stine Marie Ulven

Vi viser til søknad om prosjektendring datert 10.05.2017 for ovennevnte forskningsprosjekt. Søknaden er behandlet av leder for REK sør-øst på fullmakt, med hjemmel i helseforskningsloven § 11.

Endringene omfatter:
-Ny medarbeider: Gulla Aase
-Forlengelse av prosjektets varighet til 31.12.2019.
-Det søkes om å gjøre mer utvidede lipid- og metabolitt analyser slik at man i tillegg til rutineanalyser av total kolesterol, LDL-kolesterol, HDL-kolesterol og triglyecerider kan få målt blant annet 14 lipoprotein subklasser (kylomikroner, 5 VLDL, 3 LDL, 4 HDL, IDL subklasser) med konsentrasjonen av forskjellige lipider (fosfolipider, kolesterol, kolesterol ester, fritt kolesterol og triglyserider) i disse, samt størrelsen på lipoproteinene ved bruk av NMR teknologi.

Vurdering
Komiteen godkjenner forlengelse av prosjektet til 31.12.2019. Komiteen vil likevel bemerke at det påligger prosjektleder og forskningsansvarlig institusjon å påse at prosjektet gjennomføres i tråd med godkjenning som er gitt, blant annet at forlengelse søkes i rett tid.

Komiteen har ingen innvendinger til de nye analysene som skal gjøres. Deltagerne er informert om at prøvene skal sendes til Finland, og komiteen vurderer at analysene faller inn under det opprinnelige formålet med studien.

Komiteen har ingen innvendinger til at ny medarbeider går inn i prosjektet.

Vedtak

REK godkjenner prosjektet slik det nå foreligger, jfr. helseforskningsloven § 11, annet ledd.

Tillatelsen er gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknaden, endringssøknad, oppdatert protokoll og de bestemmelser som følger av helseforskningsloven med forskrifter.

REKs vedtak kan påklages, jf. forvaltningslovens § 28 flg. Klagen sendes til REK sør-øst. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK sør-øst, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Vi ber om at alle henvendelser sendes inn med korrekt skjema via vår saksportal: http://helseforskning.etikkom.no. Dersom det ikke finnes passende skjema kan henvendelsen rettes på e-post til: post@helseforskning.etikkom.no.

Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen

Finn Wisløff
Professor em. dr. med.
Leder

Gjøril Bergva
Rådgiver

Kopi til: *postmottak@hioa.no*