The heterogeneity of obesity for risk of cardiometabolic disease. Epidemiology and clinical aspects

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The heterogeneity of obesity for risk of cardiometabolic disease – epidemiology and clinical aspects

Abstract:

The overall aim of this thesis was to explore and describe a variant of obesity with a proposed lower risk of cardiometabolic disease (CMD), and further search for possible pathways of CMD differentiating between obese individuals with variations in mortality and morbidity risk.

The epidemiological data was obtained from the Malmö Diet and Cancer Study (MDCS), MDCS-CardioVascular subcohort (MDCS-CV) and the Malmö Preventive Project – Re Examination Study (MPP-RES). All obese individuals (BMI $\geq$30 kg/m^2) were selected to be included and were further sub-divided into two different groups, depending on absence or presence of hospitalization for somatic disease prior to baseline inclusion in each cohort study, respectively. Papers I-III were conducted using data from the MDCS and the MDCS-CV cohorts. Paper IV was conducted using data from the MPP-RES cohort.

In **Paper I** we aimed to describe determinants, characterizing patterns and prognosis for cardiovascular disease (CVD) and mortality among middle-aged individuals with metabolically healthy obesity (MHO) (defined as absence or presence of hospitalization for somatic disease prior to baseline inclusion) compared to metabolically unhealthy obesity (MUO) (defined as presence of hospitalization for somatic disease prior to baseline inclusion) and non-obese participants (NOC). Compared to MUO individuals, we found that MHO individuals presented lower prospective risk of total mortality and CV morbidity during 20-years of follow-up. Notably, no significant differences could be seen in mortality and CV morbidity risks when comparing MHO subjects to NOC. Differences in characterising patterns of MHO compared to MUO included a higher level of physical activity and a more favourable lipid- and glucose profile.

In **Paper II**, we wanted to further explore the metabolic profile of previously defined MHO individuals in **Paper I** by comparing plasma levels of *metabolites* (metabolomics and lipidomics) and circulating *proteins* (proteomics) with MUO and NOC subgroups. We found that MHO individuals, compared to MUO, presented with a more favourable lipid metabolic profile, accompanied by a downregulation of potentially harmful proteomic biomarkers.

In **Paper III**, to further elucidate on possible differentiating traits between obesity phenotypes, we explored the role of anti-phosphorylcholine (anti-PC, antibodies with anti-inflammatory properties) in hospitalized (HO) versus non-hospitalized (NHO) subjects with obesity in MDCS-CV. Our findings showed that low levels of certain anti-PCs were associated with higher risk of being HO.

Finally, in **Paper IV**, we used a multiplex proteomic panel consisting of 92 proteins associated with CVD, metabolism and inflammation to find novel associations with HO compared to NHO. We found that increased Galectin-4 (Gal-4) levels were associated with a higher probability of HO. However, this association was only significant in subjects with diabetes, further implying a role for Gal-4 in diabetes and its complications.

Key words: obesity, epidemiology, cardiometabolic disease, biomarkers

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Coverphoto by Johan Korduner. *An early morning in late spring, Kävlinge.*

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MADE IN SWEDEN

To my family

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List of Papers

Paper I

Korduner J, Bachus E, Jujic A, Magnusson M, Nilsson PM. Metabolically healthy obesity (MHO) in the Malmö diet cancer study – Epidemiology and prospective risks. Obes Res Clin Pract. 2019 Nov-Dec;13(6):548-554.

Paper II

Korduner J, Nilsson PM, Melander O, Gerl MJ, Engström G, Bachus E, Magnusson M, Ottosson F. Proteomic and Metabolomic Characterization of Metabolically Healthy Obesity: A Descriptive Study from a Swedish Cohort. J Obes. 2021 Oct 6;2021:6616983.

Paper III

Jujić A, **Korduner J**, Holm H, Engström G, Bachus E, Bhattacharya P, Nilsson PM, Frostegård J, Magnusson M. Antibodies against phosphorylcholine in hospitalized versus non-hospitalized obese subjects. Sci Rep. 2021 Oct 12;11(1):20246.

Paper IV

Korduner J, Holm H, Jujic A, Melander O, Pareek M, Molvin J, Råstam L, Lindblad U, Daka B, Leosdottir M, Nilsson PM, Bachus E, Olsen MH, Magnusson M. Galectin-4 levels in hospitalized versus non-hospitalized subjects with obesity: the Malmö Preventive Project. Cardiovasc Diabetol. 2022 Jul 2;21(1):125.

Scientific contributions not included in this thesis:

Nilsson PM, **Korduner J**, Magnusson M. Metabolically Healthy Obesity (MHO)-New Research Directions for Personalised Medicine in Cardiovascular Prevention. *Curr Hypertens Rep*. 2020 Feb 17;22(2):18.

Zaghi A, Holm H, **Korduner J**, Dieden A, Molvin J, Bachus E, Jujic A, Magnusson M. Physical inactivity is associated with post-discharge mortality and re-hospitalization risk among Swedish heart failure patients – The HARVEST-Malmö Study. Front Cardiovasc Med. 2022 Feb 21;9:843029.

Nezami Z, Holm H, Ohlsson M, Molvin J, **Korduner J**, Bachus E, Zaghi A, Dieden A, Platonov PG, Jujic A, Magnusson M. The impact of myocardial fibrosis biomarkers in a heart failure population with atrial fibrillation-The HARVEST-Malmö study. Front Cardiovasc Med. 2022 Oct 19;9:982871.

Abbreviations

AHT	Antihypertensive drug treatment
Anti-PC	Anti-phosphorylcholine
BMI	Body mass index
BP	Blood pressure
BSA	Bovine serum albumin
CAD	Coronary artery disease
CI	Confidence interval
CMD	Cardiometabolic disease
CRF	Cardiorespiratory fitness
DPP-4	Dipeptidyl peptidase-4
ELISA	Enzyme-linked immunosorbent assay
EOSS	Edmonton Obesity Staging System
CV	Cardiovascular
CVD	Cardiovascular disease
DM2	Type-2 diabetes
FDR	False discovery rate
FFA	Free fatty acids
FPG	Fasting plasma glucose
FTO	Fat-mass and obesity associated
GAL	Galanin
Gal-4	Galectin 4
GIP	Glucose-dependent insulinotropic polypeptide
HDL-C	High density lipoprotein-cholesterol
HF	Heart failure
HOMA-IR	Homeostasis assessment of insulin resistance
HR	Hazard ratio
Hs-CRP	High sensitivity C-reactive protein
HF	Heart failure
HT	Hypertension
IGT	Impaired glucose tolerance
IFG	Impaired fasting glucose
IGFBP1	Insulin-like growth factor-binding protein 1
IGT	Impaired glucose tolerance
IL-6	Interleukin-6
IL-1ra	Interleukin-1 receptor antagonist
IR	Insulin resistance
LDL-C	Low density lipoprotein-cholesterol
MDCS	Malmö Diet and Cancer Study
MDCS-CV	Malmö Diet and Cancer Study – CardioVascular cohort
MetS	Metabolic syndrome

MESA	Multi-Ethnic Study of Atherosclerosis
MHO	Metabolically healthy obesity
MPP	Malmö Preventive Project
MPP-RES	Malmö Preventive Project – Re-Examination Study
MPP-RES-Echo	Malmö Preventive Project – Re-Examination Study Echocardiography cohort
MUO	Metabolically unhealthy obesity
NAFLD	Non-alcoholic fatty liver disease
NOC	Non-obese controls
OR	Odds ratio
PA	Physical activity
PC	Principal component
PCA	Principal component analysis
PEA	Proximity extension assay
PBS	Phosphate buffered saline
RAAS	Renin-angiotensin-aldosterone system
RCT	Randomized controlled trial
SBP	Systolic blood pressure
SD	Standard deviation
TG	Triglycerides
WC	Waist circumference
WHR	Waist-hip ratio
WHO	World Health Organization
WOF	World Obesity Federation

Introduction

Obesity – a global health problem

Obesity is defined, according to the World Health Organization (WHO), as excessive body fat accumulation with a body mass index (BMI) ≥30 kg/m^2 (1). Until the early 20th century, obesity was regarded as a symbol of health and wealth (2). Today, obesity is rather linked to low socioeconomic status and is one of the leading causes to our most common non-communicable diseases, such as cardiovascular disease (CVD), type 2 diabetes (DM2) and certain types of cancer (3, 4). The life expectancy of individuals with obesity is markedly reduced by 5-20 years, depending on the severity of the condition and comorbidities (5). Even more troublesome, the global prevalence of obesity has been steadily increasing since the 1970's, especially among adolescents and children, today reaching pandemic levels (3).

In a recent study from the *Non-communicable diseases* (*NCD) risk factor collaboration*, providing extensive BMI data from 128.9 million individuals, there was a global increase in the obesity prevalence between 1975 and 2016 (6). Data from the same collaboration, stated that the obesity prevalence tripled in adult men (3.2% to 10.8%) between 1975-2014 and more than doubled in adult women (6.4% to 14.9%) (7). In Sweden, the percentage of individuals aged 25-84 years with obesity follows a similar trend as globally (between 2004-2016 an increase from 11.9% to 17%) and is anticipated to increase in a linear trend until at least 2030 (8). **Figure 1** illustrates the global prevalence of obesity, including Sweden.

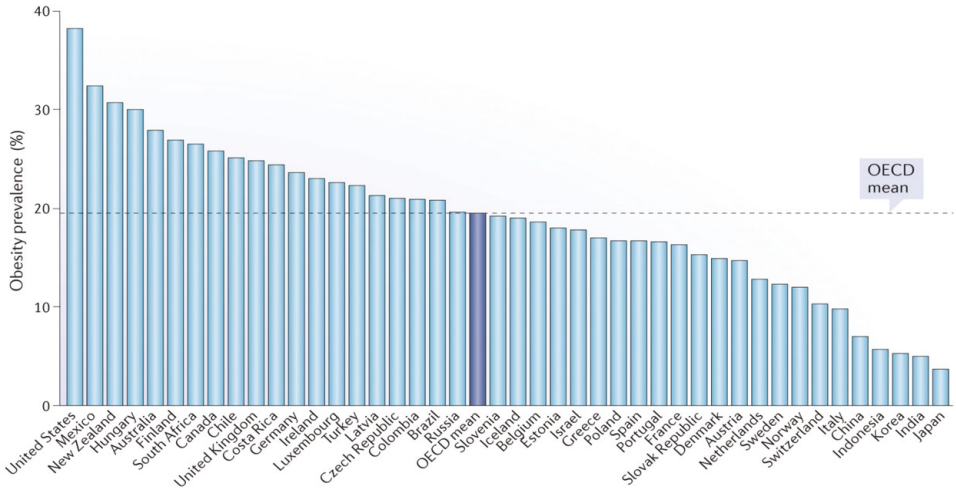

Figure 1. The prevalence (%) of obesity across different OECD (Organisation for Economic Cooperation and Development) countries, from measured data (2017). Adapted with permission from ref. (3).

Cardiometabolic morbidity and mortality

A paradigm shift in CVD prevention came with the *Framingham Heart Study* (started in 1948), where increasing BMI, apart from high blood pressure and elevated levels of blood cholesterol, was found to be an independent risk factor for CVD and premature death (9). Several well-established study cohorts confirming these findings followed, providing deeper insights into the pathophysiology and health issues of obesity (10, 11). Along these discoveries, associations between increasing BMI and the development of metabolic disturbances, most importantly DM2, could be seen and validated in randomized controlled trials (RCT), and mendelian randomisation analyses using genetic markers, all supporting causality, as well as in large-scale observational studies (12-15). This spectrum of diseases including glucose disturbances, hypertension, dyslipidaemia, non-alcoholic fatty liver disease (NAFLD) and obesity may eventually progress into CVD and DM2 – a term named *cardiometabolic disease* (CMD) (16). However, there is still no clear consensus around the definition of CMD (17, 18), which is regarded as the number-one cause of death in the world (19). Therefore, this thesis will mainly focus on different phenotypes of obesity and their link to CMD. Furthermore, the heterogeneity in risk of CMD development in individuals with obesity will also be discussed.

Pathophysiology

Throughout the human evolution, selection pressure favoured a genotype linked to behaviours of overeating, physical inactivity and low energy expenditure, leading to the ability to survive prolonged periods of undernutrition (20). Paradoxically, overnutrition has during the past decades exceeded the health consequences of undernutrition – in other words, more people are now dying from the consequences of overweight than underweight (21).

In biomedical studies with the aim to design interventions to treat obesity, a deeper understanding has been gained for the biological mechanisms promoting weight gain. Although the fundamental cause of obesity is the simple equation of too many calories consumed contra expended (22), there are several other mechanisms coexisting – i.e. how impaired satiety regulation via adipose tissue, liver and gut hormones, and consequently regulation of appetite in the hypothalamus, lead to disturbed food cravings in individuals with obesity (23, 24). Furthermore, there is a profound knowledge about how adipose tissue dysfunction promotes secondary health problems (23). Finally, through adoption and twin studies, it has been shown that obesity might be an inherited disorder of energy homeostasis (25, 26). This became even more apparent with the discovery of the fat-mass and obesity associated (*FTO*) gene, the first gene linked directly to obesity where variation in *FTO*, resulting in increased gene expression and activity, might promote feeding and obesity (27).

Atherosclerosis – an inflammatory disease

Atherosclerosis is a complex process that starts early in life and can even be detected in adolescents (28). The disease engages the intimal layer of the arterial wall promoting endothelial dysfunction. Early changes involve fatty streaks which will later develop into plaques: a thickening of the intimal layer that contains macrophages and cholesterol (so called “foam cells”), inflammatory cells, smooth muscle cells, and connective tissue. Some plaques are characterized by a large lipid core with a thin overlying cap containing high amounts of inflammatory cells (**Figure 2**). These plaques are especially vulnerable and may rupture, eventually leading to thrombosis formation – the main cause of myocardial infarction, stroke and acute limb ischemia (29).

In every step above, an inflammatory response is involved, from leukocyte adhesion molecules and chemokines promoting foam cell formation, to the secretion of pro-inflammatory cytokines from T-cells that contribute to local inflammation and growth of the plaque and eventually its rupture (30). This inflammatory activity is associated with traditional risk factors for CVD such as dyslipidaemia, hypertension, smoking, diabetes and overweight.

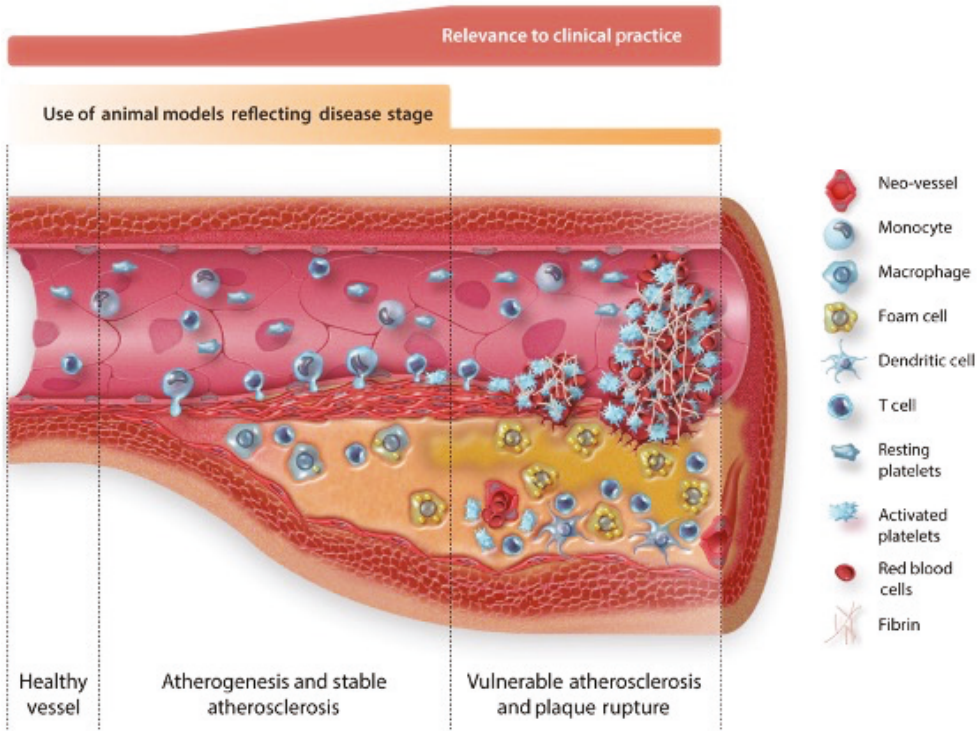

Figure 2. Illustrates the timeline of the formation of an atherosclerotic plaque in a mouse model, from the early foam cells formation to the unstable fibrous cap. With permission from ref. (31).

Adipose tissue – an endocrine organ

In the mid 1990's, along with the discovery of the leptin hormone, it became apparent that the importance of the adipose tissue was not solely as an energy storage but also as an endocrine organ, where the adipocytes and macrophages produce many different proteins – *adipokines* – with paracrine and endocrine functions (32, 33). The metabolic activity of adipose tissue depends on where it is located. Visceral or intra-abdominal fat storages are more metabolically active than peripheral or subcutaneous fat (34). With a higher lipolytic activity, this leads to elevated plasma levels of free fatty acids (FFA) and triglycerides (TG) (34). Moreover, visceral fat is drained by the portal vein circulation, exposing the liver to higher levels of FFA which in turn worsens the negative metabolic consequences (35).

The endocrine function of adipose tissue is divided into **two** major categories:

Adipose tissue-derived proteins – secreted proteins that have metabolic effects on distant cells or tissues. The most important proteins include hormones such as *leptin* (that affects the energy homeostasis through the hypothalamic pathway, where

increasing levels signalize satiety) (36), *adiponectin* (has an inverse relationship to insulin resistance and chronic inflammation, where declining levels are seen before the onset of obesity) (37), *adipsin* and *acylation stimulating protein (ASP),* which both positively correlate with adiposity and CMD (38). Furthermore, several inflammatory cytokines, promoting insulin resistance, are derived from adipocytes, such as *tumour necrosis factor α (TNF-α)* and *interleukin-6 (IL-6) (39).* Finally, several proteins from the *renin-angiotensin-aldosterone system (RAAS)* and from the haemostatic and fibrinolytic system, most importantly *plasminogen activator inhibitor (PAI)-1*, are secreted by adipocytes, further implicating their role in CVD development (40, 41).

Enzymes involved in the metabolism of steroid hormones – most of the production and secretion of steroid hormones derives from the adrenal glands and gonads. However, adipose tissue expresses impressive amounts of enzymes for activation, inactivation and interconversion of steroid hormones (42, 43). Apart from the production and secretion of sex hormones (*oestrogen* and *testosterone*), which might play a role in the fat distribution (44), increasing attention lies instead on the adipose-tissue regulation of glucocorticoid metabolism through *11β-hydroxysteroid dehydrogenase-1 (11β-HSD1).* Overexpression of *11β-HSD1* seems to promote visceral obesity and CMD development (43).

Association between obesity and cardiometabolic disease

According to a pooled analysis carried out in 2017, of individual-participant data from 120,813 adults from 16 different cohort studies in both Europe and USA, the risk to develop CMD over a 11-year time period was almost five times higher in individuals with mild obesity (BMI 30.0-34.9 kg/m^2) and 15 times higher in individuals with severe obesity (BMI ≥35 kg/m^2) as compared to individuals with a normal weight (11). There is a clear association between adiposity and the development of CMD; however, the underlying mechanisms are multifactorial and complex (45). The most established hypothesis states that visceral fat, which through its increased metabolic activity and the connection to the portal vein system, promotes atherogenic processes through both chronic inflammation (via the secretion of proinflammatory adipokines) and insulin resistance (both through adipokines and exposing the liver to FFA) (46). Moreover, higher plasma levels of FFA and TG in the portal system lead to the development of NAFLD (47). Other ectopic fat deposits have recently become a focus of interest, namely pericardial and epicardial fat, which show similar negative properties as intra-abdominal/visceral fat (48). The location of fat deposits is important to address since individuals with high waist circumference (WC) and normal BMI are at higher risk of developing CMD (49). Individuals with obesity but a predominantly peripheral fat distribution, might present with a lower CMD risk than expected based on their BMI (50).

Several major health issues are linked to obesity via CMD:

Coronary artery disease (CAD) and cerebrovascular disease – As mentioned previously, metabolically active adipocytes (with an abundance in visceral fat) induce the negative cascade of promoting atherosclerotic plaques through chronic inflammation and insulin resistance subsequently leading to the development of CAD (51) and cerebrovascular disease (52). To further clarify obesity related cerebrovascular disease, it has been shown that each unit increase of BMI was associated with a significant 6% increase of stroke risk (52).

Hypertension (HT) – Many factors linked to obesity contribute to the development of HT, mainly through the activation of RAAS due to increased sympathetic tone, adipocyte secretion of angiotensinogen, and increased insulin resistance (53).

Heart failure (HF) – Excess adipose tissue influences cardiac function directly through hemodynamic changes (larger blood volume, higher cardiac output, and reduced systemic vascular resistance) (54). Indirectly, the impact of obesity on HF development is through elevated blood pressure, myocardial changes related to metabolic disturbances (hyperglycaemia, insulin resistance, inflammation) and obesity related CAD (55) (**Figure 3**).

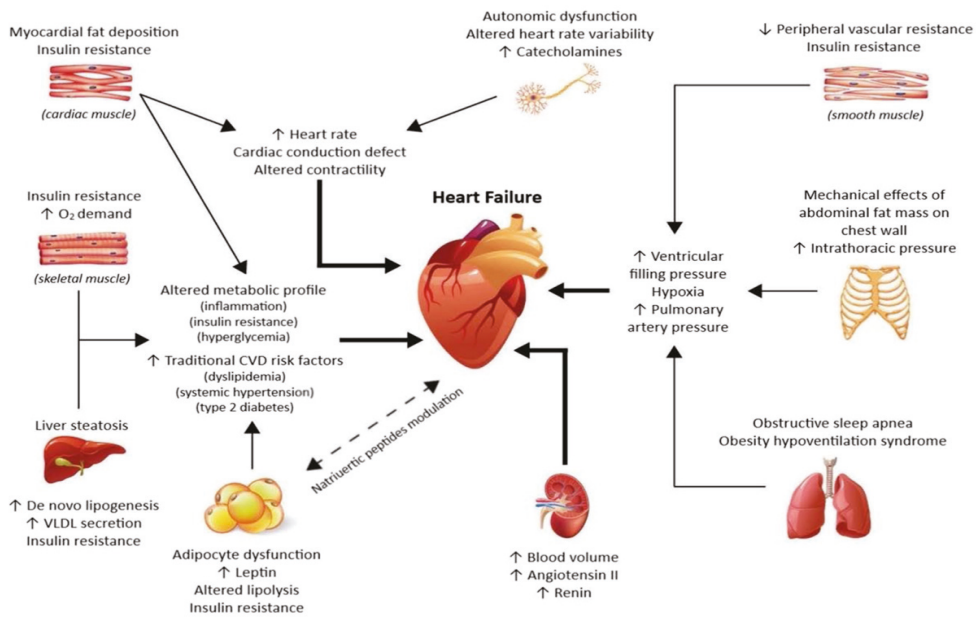

Figure 3. The pathophysiology behind HF in obesity. CVD – cardiovascular disease; VLDL – very low-density lipoprotein. Adapted from ref. (56) with permission from Taylor & Francis Ltd.

Dyslipidaemia – A range of disturbances in lipid metabolism are related to obesity. These typically include high levels of TG and FFA together with decreased levels of high-density lipoprotein cholesterol (HDL-C), named dyslipidaemia. Furthermore, there are normal or slightly increased low-density lipoprotein cholesterol (LDL-C) levels but with a higher amount of small dense LDL-C particles, which are especially atherogenic (57, 58).

Glucose disturbances – Through higher lipolytic activity and elevated levels of FFA, especially in the portal circulation of individuals with obesity, it is hypothesized that this in turn promotes insulin resistance (58). In addition, increased release of pro-inflammatory cytokines from visceral adipose tissue contributes significantly (59). Further, increasing demand for insulin secretion from the pancreatic β-cells eventually leads to a continuing decline in their function. Finally, the progression to hyperglycaemia, impaired glucose tolerance (IGT) and DM2 is inevitable in most, but not all, subjects with obesity (58, 59). It is estimated that obesity accounts for approximately 80-85% of the total risk of developing DM2 (60).

The metabolic syndrome (MetS) – A denotation for a cluster of metabolic and cardiovascular risk factors is commonly known as the MetS (also known as syndrome X or insulin resistance syndrome). An important feature of this syndrome is insulin resistance and how it shares and connects possible pathophysiological mechanisms with our most common non-communicable diseases such as obesity, DM2 and CVD (61). Since it became accepted as a syndrome by the WHO in 1998, its definition has undergone several revisions. Today the most clinically applicable definition is by the National Cholesterol Education Programme's Adult Treatment Panel-III (NCEP ATP-III), which considers five components equally important: abdominal obesity, elevated serum triglycerides, hypertension, lowered HDL-C and elevated serum glucose (62). This definition was again modified by the International Diabetes Federation (IDF) that put abdominal obesity in focus as the main component together with at least two additional CVD-risk factors mentioned above (61). However, this has been questioned by reports from the American Heart Association/National Heart, Lung, and Blood Institute where the IDF-definition seems to display a lower predictive value even though more individuals could be identified as having MetS (63). Clearly, this indicates that the syndrome is a complex disorder without a single factor of the cause. Today MetS is regarded as a controversial concept with considerable doubt about its value as a CVD risk marker (62).

Obesity – disease or decision?

In contrast to the common view that obesity results primarily from unhealthy lifestyle and dietary choices that reflect a lack of motivation and ignorance, the World Obesity Federation (WOF) has acknowledged obesity as a chronic, relapsing progressive disease (64). The arguments circle around an interaction of environmental factors (low demand of physical activity and the surplus availability of food rich in calories), genetic susceptibility, and the pathophysiology of obesity (64). The strong dilemma, in spite of targeted weight-loss strategies, of mechanisms promoting weight gain and defending a higher body weight adds further to the statement that obesity is a disease rather than an individual decision (65). In this context, the declaration of obesity as a disease would imply an enormous health benefit to both the individual and the society through counteracting stigmatization, and increased attention from healthcare professionals and politicians (66).

Weight loss strategies

We are facing a big challenge treating obesity through weight loss strategies. Traditional treatment, focusing on behavioural change (mainly improvements in diet and exercise) have unimpressive long-term effects and current pharmacological interventions are only at the range of 3-10% of weight reduction (67, 68). Recent studies have, however, shown promising results with the new "twincretin" drug tirzepatide (a combined glucagon like peptidase-1 [GLP-1] and glucose-dependent insulinotropic polypeptide [GIP] receptor agonist) where treatment resulted in substantial weight reduction of up to 15-20% as compared to a placebo group (69).

During the 20th century, pharmacological therapy of obesity has long been a story of failed interventions due to serious side effects (70-73). Through years of trying to understand the pathophysiological complexity of obesity, a paradigm shift came in the beginning of the 2010's, when it became apparent to target appetite-signalling pathways in the hypothalamus. Since then, several EMA/FDA approved drugs have been shown to be safer and more tolerable (74). In Sweden, three anti-obesity drugs are currently available: orlistat (Xenical©, that inhibits intestinal lipase and thus reduces the uptake of FFA in the gut) (75), liraglutide (Saxenda©, a GLP-1 analogue which regulate appetite and feeding centres in the brain and delaying gastric emptying actions that are dependent on GLP-1 receptor activation within the central nervous system) (76) and bupropion/naltrexone (Mysimba©, that induces appetite suppression and increased energy expenditure through stimulating hypothalamic pro-opiomelanocortin) (77). Finally, promising results have been seen in obesity treatment with the multi-faceted sodium glucose transporter-2 (SGLT-2) inhibitor empaglifozin of patients with DM2, where not only significant reduction of $HbA1_c$

was seen but also weight reduction of ≥10% was observed in 9% of patients receiving oral empaglifozin (78).

If conservative treatment fails to achieve adequate weight loss, certain individuals with obesity may be indicated to undergo surgical intervention – so called obesity surgery or bariatric surgery. Weight loss in this way is achieved through reducing the size of the stomach (gastric banding) and/or inducing malabsorption and caloric restriction through bypassing and leaving only a small portion of the stomach (Roux-en-Y gastric bypass, **Figure 4**), where the latter is the most commonly used procedure worldwide and in Sweden (79, 80).

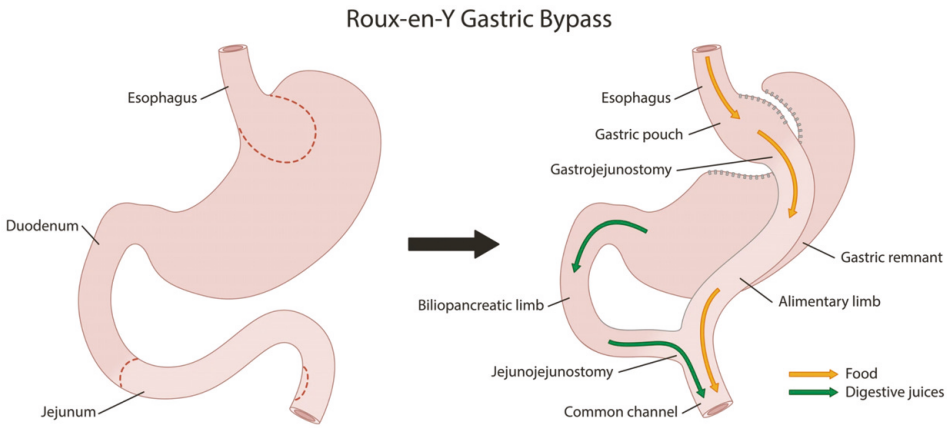

Figure 4. An illustration of the Roux-en-Y gastric bypass. A gastric pouch is created and anastomosed with part of the jejunum, thus bypassing a greater part of the ventricle. The secretion of digestive juices, such as essential gastrointestinal enzymes taking part in the digestion, is retained through anastomosing the jejunum with itself. Adapted from ref. (81), CC BY 4.0 (http://creativecommons.org/licenses/by/4.0).

Even though weight loss in individuals with obesity results in improvement of CVD risk factors (reduction in HbA_{1c}, systolic blood pressure, cholesterol and TG), there is no clear evidence from RCT's that this strategy also reduces the risk of CV-events (68, 82, 83). In the Look AHEAD clinical trial in overweight/obese volunteers with DM2, the long-term effects of an intensive lifestyle intervention program, designed to achieve and maintain weight loss by decreased caloric intake and increased physical activity, did not reduce the primary CV endpoint. There was however an observational CV mortality reduction in patients who lost more than 10% of their body weight (84). Moreover, data from the Swedish Obese Subjects Study (SOS – a prospective, non-randomized, intervention trial) revealed that bariatric surgery reduced CV mortality and the occurrence of first-time CV events (85). Finally, the ongoing SELECT-study, an RCT with subcutaneously administered semaglutide in the intervention group, is currently investigating if superiority to placebo in CV-events could be observed in patients with established CVD and overweight/obesity (86).

Weight stability

Once achieving a plausible weight loss, the short-term health effects remain positive; nevertheless, sustaining lower body weight has proven to be almost impossible for most individuals battling obesity (87). This is in part due to changes in the secretion of hormones that regulate appetite suppression (leptin, peptide YY and cholecystokinin) and hormones that promote eating and energy storage (ghrelin and GIP) (88). This in turn induces more rapid growth and hyperplasia of adipose tissue which in some instances may aggravate the body fat accumulation during weight relapse and thus worsening of cardiometabolic health compared to the initial weight gain (89). The phenomenon of weight cycling (often referred to as yo-yo dieting) should therefore be avoided and thus focus should be laid upon maintaining weight stability. The most effective way of achieving weight stability is through physical exercise, first shown in the National Weight Control Registry study where individuals who, on their own, managed to lose more than 13.6 kg in weight and maintained that weight for an average of 5 years were studied. Daily physical activity for approximately 60-90 minutes was one of the most remarkable predictors for weight stability (90). Furthermore, promising results in maintaining lost weight are seen in patients undergoing a combination of lifestyle intervention and anti-obesity medication, where a recent retrospective cohort study showed long-term weight loss (-38.8% to +12.5%) among patients compliant with 2 years of pharmacotherapy (91).

Scope of the present thesis

The heterogeneity of obesity

Despite a well-recognized pathophysiological pathway linking obesity to atherosclerosis and metabolic disturbances, there is a marked heterogeneity in CMD risk between individuals with the same degree of obesity (92). As early as in the 1950's, there were speculations on how certain obese individuals might run a lower risk than obese individuals in general for developing atherosclerotic disease and diabetes (93).

A phenomenon, known as the *obesity paradox*, suggests that patients with many types of established CVD, especially HF, may have a better prognosis if overweight or even obese compared to their leaner counterparts (94). This could in part be explained by the fact that many of these disease conditions are associated with a chronic catabolic state, where lean body mass loss carries a negative prognosis, hence the term cardiac cachexia (95).

The idea that certain individuals with obesity possess a decreased CMD risk has evolved into the concept of Metabolically Healthy Obesity (MHO, **Figure 5**). This is based on the absence of risk factors of the MetS (96, 97), defining obese but metabolically healthy individuals with a more favorable inflammatory and metabolic profile (98). Other studies that support this notion, further describe MHO individuals reporting higher degree of physical activity and showing increased cardiorespiratory fitness compared to their unhealthy counterparts with obesity (metabolically unhealthy obesity [MUO]) – thus supporting a concept known as *fat but fit* (99).

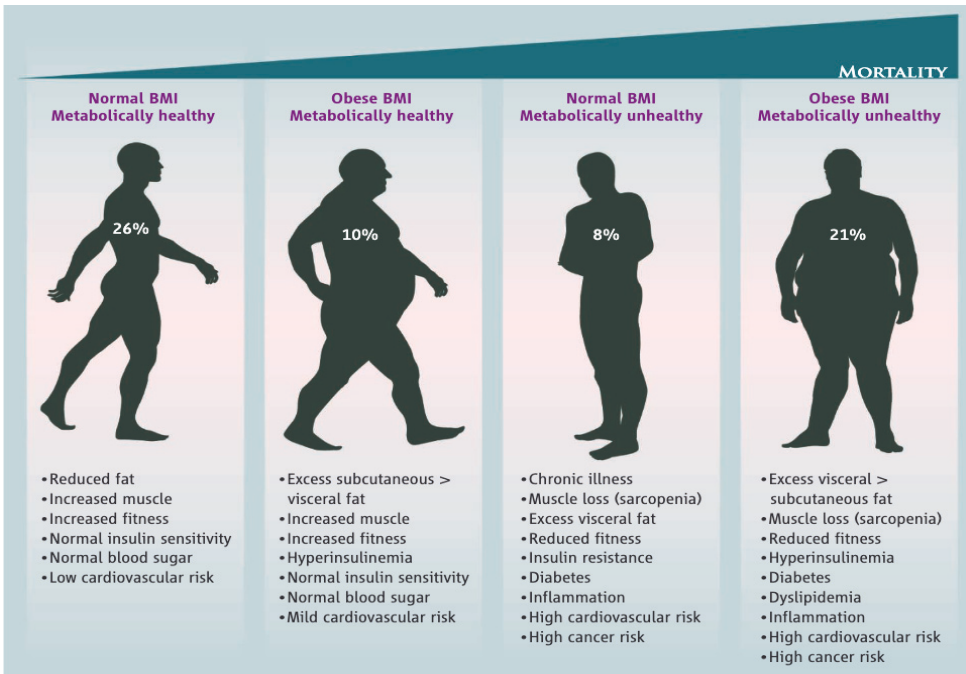

Figure 5. Depicts obesity as a heterogeneous phenomenon in regard to CMD risk. The prevalence of the different phenotypes in the United States (2013) are presented in percentages. Adapted with permission from ref. (100).

Thus, there is a need to better understand the underlying mechanisms behind obesity, as well as its heterogeneity, to be able to address the increasing prevalence and incidence of obesity in the world. By mapping individuals with obesity and lower than expected CMD risk, we can gain a deeper understanding of risk determinants for obesity as well as biological mechanisms, lifestyle and social factors, and in the extension how obesity could be treated causally and individualized (personalized medicine). Therefore, it was of interest to dig deeper and explore determinants, characterizing patterns and prognosis for CVD and

mortality among individuals with obesity and a decreased CMD risk, by introducing a new definition of metabolic health in individuals with obesity, through a status of non-hospitalization (**aim I**). Moreover, in following studies, the aims were to search for specific risk characteristics (biomarkers) differentiating between individuals with obesity possessing variation in CMD risk (**aims II-IV**). Instead of the more traditional definitions of MHO, involving the absence of risk factors of the MetS (101), we aimed to develop a more steadfast rationale for this concept. Obese individuals with no recorded history of hospitalization before the baseline examination (102) were considered MHO (**Papers I-II**) or non-hospitalized individuals with obesity (NHO, **Papers III-IV**), representing subjects with reduced CMD risk.

Aims

Overall aim

To explore and describe obesity associated with a proposed decreased risk of CMD, and to further search for possible pathways of CMD differentiating between obese individuals with variations in mortality and morbidity risk.

Specific aims

Paper I: to describe determinants, characterizing patterns and prognosis for CVD and mortality among middle-aged individuals with MHO from a population-based study.

Paper II: to better characterize the metabolic profile of previously defined MHO individuals (**Paper I**) by comparing plasma levels of *metabolites* (metabolomics and lipidomics) and circulating *proteins* (proteomics) between MHO subjects, metabolically unhealthy obesity (MUO) subjects, and non-obese controls (NOC).

Paper III: to determine if anti-phosphorylcholine (anti-PC) immunoglobulin M (IgM), G1 (IgG1) and G2 (IgG2) are associated with higher risk of being an obese subject with a history of hospitalization for somatic disease.

Paper IV: to explore possible novel associations between CVD biomarkers and a phenotype of unhealthy obesity, namely obese subjects with a history of hospitalization for somatic disease up until late mid-life, using a multiplex proteomic platform consisting of 92 proteins linked to cardiovascular disease.

Methods

Study design

Table 1. Overview of the four papers.

	PAPER I	PAPER II	PAPER III	PAPER IV
DESIGN	Observational cohort study	Observational cohort study	Observational cohort study	Observational cohort study
COHORT	MDCS and MDCS-CV	MDCS-CV	MDCS-CV	MPP-RES
SAMPLE	MHO (n=224), MUO (n=424) and NOC (n=4,459)	MHO (n=143), MUO (n=273) and NOC (n=3,027)	HO (n=96) and NHO (n=32)	HO (n=407) and NHO (n=110)
PRIMARY OUTCOME	Descriptive data All-cause mortality risk and CV incident morbidity risk	Descriptive data Biomarker profiling of 432 lipids, metabolites and proteins	Descriptive data Association between anti-PC Ig's and HO compared to NHO	Descriptive data Association between 92 proteins linked to CVD and HO compared to NHO
STATISTICAL ANALYSES	Descriptive statistics Kaplan-Meier estimates Cox-regression analysis	Descriptive statistics Principal component analysis Spearman's correlation test Multivariate logistic regression analysis	Descriptive statistics Multivariate logistic regression analysis	Descriptive statistics Benjamini-Hochberg multiple testing correction Multivariate logistic regression analysis

Study populations

Through the extensive work from research project employees in Malmö, a city situated in southern Sweden, two well acclaimed prospective cohort studies have been developed – the Malmö Diet and Cancer Study (MDCS) and the Malmö Preventive Project (MPP). Both cohorts are large, well-characterized, and population-based cohorts with follow-up times surpassing 25 years. The data collection for this thesis is derived from these two cohorts, as further described below.

The Malmö Diet and Cancer Study (MDCS)

By the start in 1991, the main purpose of this prospective cohort study was to investigate the impact of diet on cancer incidence; however, its use was extended for research also on other disease exposures and endpoints. At the end of baseline examinations in 1996, a total of 28,098 subjects had been included (41% attendance rate) with a full data set, including dietary records. The inclusion procedure involved invitations of all men born between 1923-1950) and women (born between 1923-1945) residing in the city of Malmö, Sweden, through contact by letter or local advertisements (103). At baseline, all participants underwent, at baseline, anthropometric measurements and were asked to fill in a 7-day extensive dietary assessment and a detailed self-administered questionnaire covering demographic, socio-economic and lifestyle factors (104). If any obvious abnormalities were observed in need of medical attention, such as severely uncontrolled hypertension, the participants were referred to their local general practitioner.

Every second individual of MDCS was randomly selected and re-invited to participate in a cardiovascular sub-cohort of MDCS, between 1992 and 1994, (MDCS-CV; n= 6,103; 49% attendance rate). The primary aim was to study the epidemiology of carotid artery disease, including ultrasound examination of *arteria carotis* and laboratory analyses of fasting blood samples (i.e., blood glucose, blood lipids and plasma insulin) under standardized conditions (105, 106). Blood samples were not collected at the time of the ultrasound measurement, but instead at a separate visit when a total of 5,540 individuals returned for the blood sample collection (107).

The Malmö Preventive Project (MPP)

In 1974, the Malmö Preventive Project (MPP) cohort was established at the University Hospital, Malmö, Sweden, for the purpose of investigating cardiovascular risk factors in the general population (108). Complete birth cohorts from 1921, 1926-1942, 1944, 1946, 1948, and 1949 were invited by letter to a health examination screening. This included a panel of laboratory tests, a physical

examination and a self-administered questionnaire focusing on risk conditions relevant for CVD. A total of 33,346 individuals were included at baseline 1974 to 1992 (71% attendance rate). Survivors of the original cohort were re-examined between 2002 and 2006 (n=18,240) in the MPP Re-Examination cohort (MPP-RES, attendance rate 72%) (109).

Furthermore, from this MPP-RES cohort, a sub-sample of 1,792 participants underwent echocardiography and electrocardiogram recordings, referred to as the MPP-RES-Echo. These individuals were randomly chosen from subgroups based on their glucometabolic status. Oversampling was performed within the groups with glucometabolic disturbances: impaired fasting glucose (IFG), new onset diabetes, or prevalent diabetes to ensure numerical balance (110). This resulted in approximately 1/3 normoglycemic subjects, 1/3 with IFG, and 1/3 with diabetes.

Ethical approval

The MDCS, presented in **Papers I-III**, was approved by the ethical committee at Lund University (registration number LU 51-90). Moreover, included participants gave permission to be registered and consented to long term register follow-up.

All procedures conducted in the MPP and MPP-RES (**Paper IV**), were in accordance with the ethical standards of the regional ethics committee in Lund (registration number LU 244-02) and complied with the Helsinki declaration. All participants gave informed consent prior to enrolment.

Definition of obesity with proposed decreased CMD risk

All obese individuals (BMI ≥30 kg/m^2) from the MDCS (**Paper I-III**) and MPP-RES (**Paper IV**) baseline examination, were selected to be included in the studies. These individuals were sub-divided into two different groups, depending on absence or presence of hospitalization for somatic disease, as recorded in the Swedish National Hospital Inpatient Register, up until the baseline inclusion in either cohort study. Hospitalization due to normal deliveries (**Papers I-IV**) or external injuries/intoxications were considered non-hospitalization for our aim in **Papers I-III**. Obese individuals with no recorded history of hospitalization before baseline were considered MHO, whereas obese individuals with at least one recorded history of hospitalization were considered subjects with MUO in **Papers I-II**. This definition was originally proposed by Tremmel *et al.* in MPP, although with a higher BMI cut-off (BMI ≥ 35 kg/m^2) and a different nomenclature (healthy severe obesity [HSO]) (102).

In **Papers III-IV**, as an attempt to exclude the term "healthy" in our definition, because of doubtful long-term prognosis, there was instead a re-branding of MHO and MUO into non-hospitalized subjects with obesity (NHO) and hospitalized subjects with obesity (HO). These two groups were further compared with non-obese controls (BMI <30 kg/m^2) in **Papers I** and **II**.

Paper specific methods

Paper I

In **Paper I**, an investigation was conducted in the MDCS-CV to analyse descriptive data of individuals presenting with MHO (n=224) compared to MUO (n=424) and NOC subjects (n=4,459), who had complete data available. Further, prospective risk analyses for all-cause mortality and incident CVD morbidity were carried out in the same groups (mean follow up-time 20±6 years). Specifics of the study population are presented in a flowchart together with **Paper II** (**Figure 6**).

Paper II

In this paper, there was a more detailed investigation of the same population from **Paper I**, through comparing plasma levels of 492 different *metabolites* (metabolomics and lipidomics) and circulating *proteins* (proteomics). However, the number of included individuals was further reduced (MHO, n=143; MUO, n=273; NOC, n=3,027, respectively) due to lack of complete biomarker data. Specifics of the study population are presented in a flowchart along with **Paper I** (**Figure 6**).

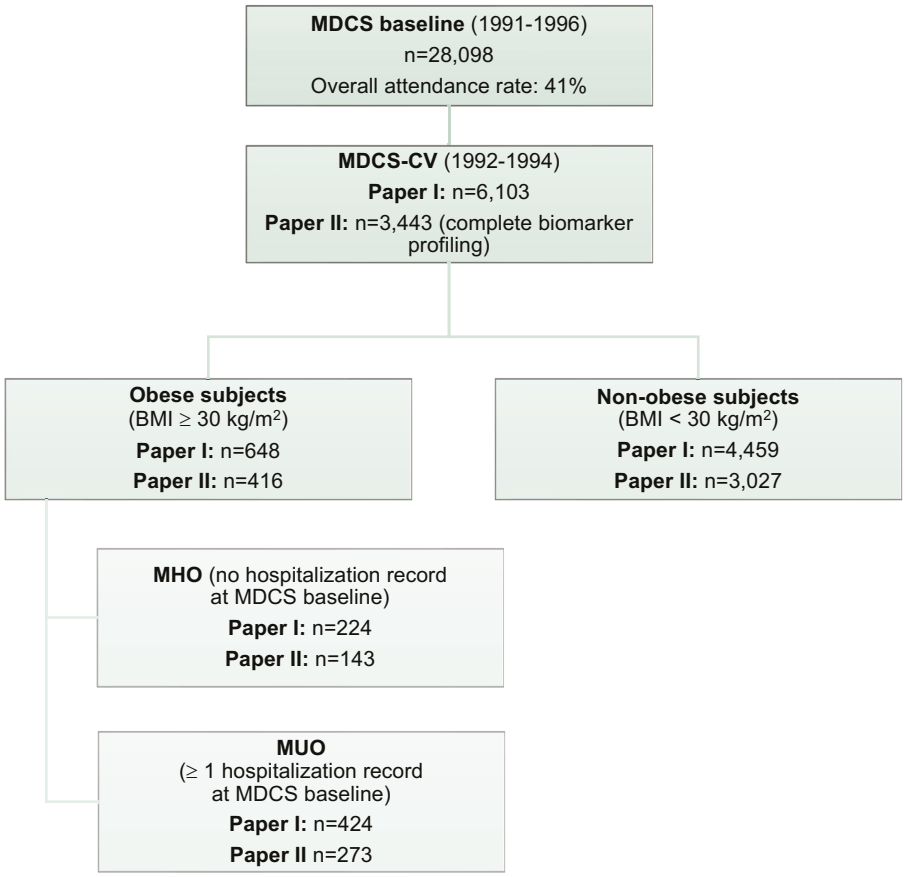

Figure 6. Flowchart of MDCS-CV in Papers I and II. BMI – body mass index; MDCS – Malmö Diet and Cancer Study; MDCS-CV – Malmö Diet and Cancer Study-CardioVascular arm; MHO – metabolically healthy obesity; MUO – metabolically unhealthy obesity.

Paper III

The purpose was to determine if anti-PC immunoglobulin M (IgM), IgG1 and IgG2 (antibodies with proposed atheroprotective properties) are associated with higher risk of being a hospitalized individual with obesity (HO). A total of 300 individuals were selected from the MDCS-CV cohort according to predefined BMI criteria, data on prior hospitalization status, and equal sex distribution. This resulted in a total of 234 included individuals, due to lack of sufficient number of individuals fulfilling applicable inclusion criteria (see **Figure 7**). In a sub-sample of 134 people with obesity (BMI ≥30 kg/m^2) subjects, anti-PC were analysed. Self-reported data on smoking was missing in six subjects, resulting in 128 subjects with complete data set. Those subjects were further sub-divided into HO (n=96) and NHO (n=32).

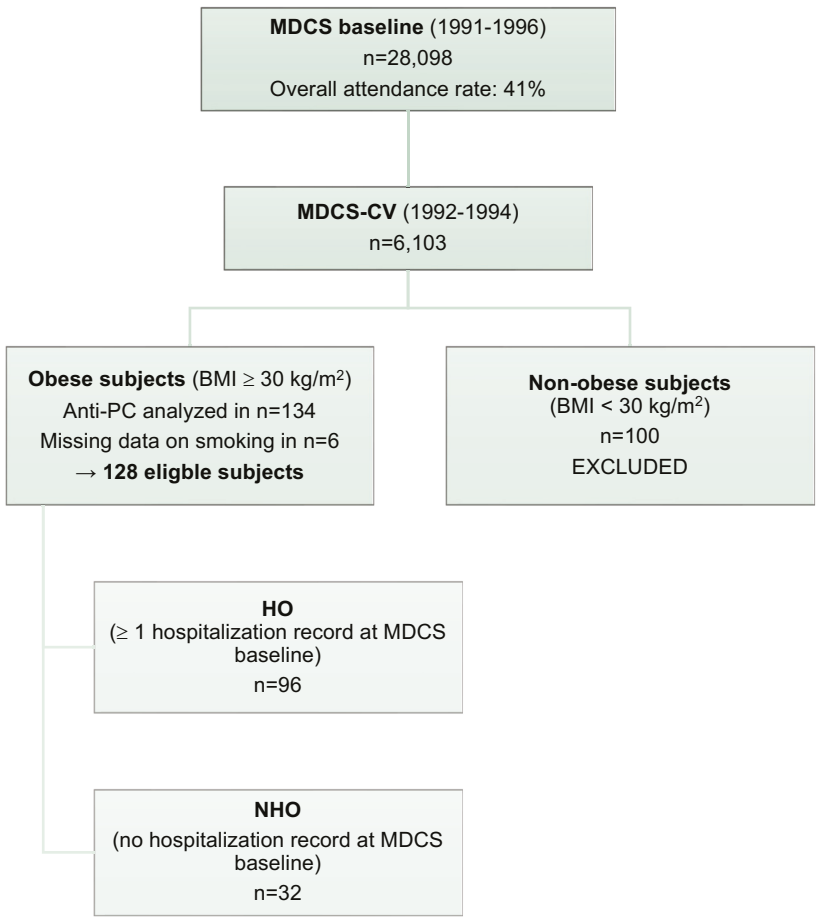

Figure 7. Flowchart of MDCS-CV in Paper III. BMI – body mass index; MDCS – Malmö Diet and Cancer Study; MDCS-CV – Malmö Diet and Cancer Study-CardioVascular arm; HO – hospitalized subjects with obesity; NHO – non-hospitalized subjects with obesity.

Paper IV

The aim of paper IV was to explore possible novel associations between CVD biomarkers and HO compared to NHO through using a multiplex proteomic platform (Proseek Multiplex CVD III 96 × 96 reagents kit, Olink Bioscience, Uppsala, Sweden) consisting of 92 proteins linked to CVD. The study was conducted in the MPP-RES-Echo sub cohort, where a total of 517 individuals with obesity and complete biomarker data were included. The participants were further subdivided into HO (n=407) and NHO (n=110). Specifics of the study population are presented in **Figure 8**.

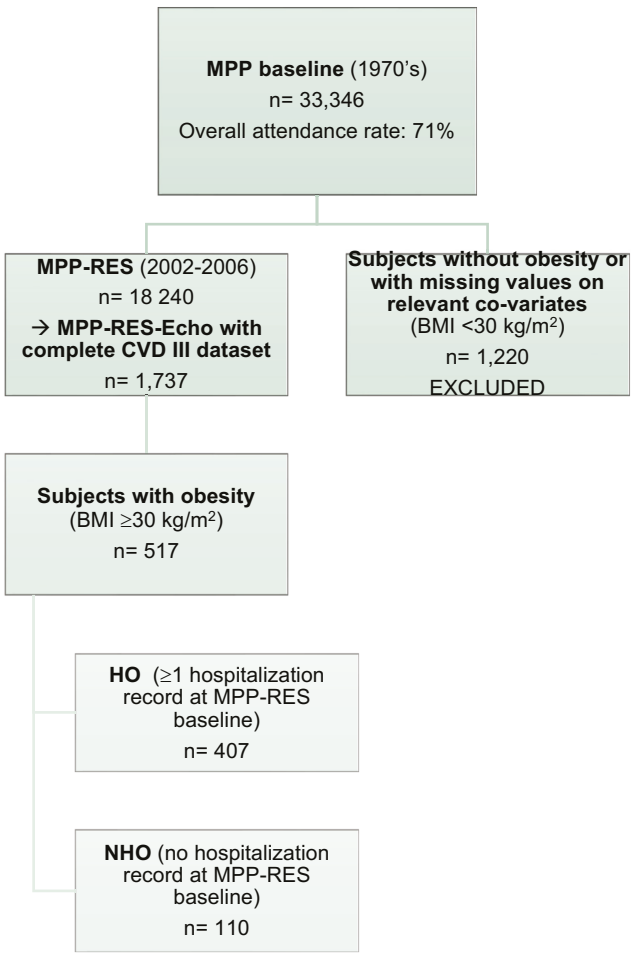

Figure 8. Flowchart of MPP-RES-Echo in Paper IV. BMI – body mass index; MPP – Malmö Preventive Project; MPP-RES – Malmö Preventive Project-Re-examination Study; MPP-RES-Echo – Malmö Preventive Project-Re-examination Study Echocardiography; HO – hospitalized subjects with obesity; NHO – non-hospitalized subjects with obesity.

Description of variables

Anthropometric measurements

At the MDCS baseline (**Paper I-III**), all participants' weight (kg) and height (cm) were measured without shoes and in light indoor clothing, and BMI was calculated (kg/m^2). Waist and hip circumference (WC; cm), including waist-o-hip ratio (WHR), was measured in the standing position without clothing. Similarly, in MPP (**Paper IV**), weight and height were measured in light indoor clothing, and BMI was subsequently calculated.

Hypertension

In **Paper III** hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg and/or diastolic ≥90 mmHg (**Paper I-II** and **IV**), SBP ≥130 mmHg and/or diastolic ≥85 mmHg (**Paper III**) and/or currently on antihypertensive drug treatment (AHT).

In MDCS (**Paper I-III**) right arm blood pressure (mmHg) was measured twice in the recumbent position after a 5-minute rest, using the Korotkoff phase V. In MPP-RES (**Paper IV**), blood pressure (mmHg) was measured twice using a validated sphygmomanometer with a mercury manometer in the supine position by trained nurses after 10-minutes of rest – the mean values were then recorded. No intra- and/or inter-observed variability calculations were performed. However, the sphygmomanometer used was validated and continuously calibrated according to clinical and research standards at the Skåne University Hospital in Malmö.

Prevalent diabetes

In **Paper III** (MDCS cohort), pooled prevalent diabetes and IFG was defined based on self-reported physician´s diagnosis per questionnaire, or current treatment with glucose-lowering drugs, or fasting whole blood glucose ≥6.1 mmol/L. In **Paper IV** (MPP-RES), prevalent diabetes was defined as either new-onset diabetes (defined by two separate measurements of FPG ≥7.0 mmol/l or one measurement ≥11.1 mmol/l), or previously known diabetes (obtained through participant self-reporting and/or reporting of current glucose-lowering medication).

Incident CVD and all-cause mortality (Paper I)

All individuals were followed from baseline until the first CV-event, death (obtained from Swedish total population register Statistics Sweden [SCB]),

migration or end of study December 31st 2016. Endpoints were retrieved through the Swedish Inpatient Registry and the Causes of Death register, administrated by the Swedish National Board of Health and Welfare. Furthermore, the definition of a stroke event was additionally supplemented by information obtained through the local STROMA-register with its Relapse-register (111). The definition of an incident CV-event (fatal or non-fatal) included *myocardial infarction* and *ischaemic heart disease* (ICD-9 codes 411-414; and ICD-10 codes I20, I24, I251-I259), *stroke* (ICD-9 430-434, 436 and ICD-10 I60-I64), *heart failure* (ICD-9 428 and ICD-10 I50, I11.0) and *atrial fibrillation/flutter* (ICD-9 427D, 4273 and ICD-10 I48).

Leisure time physical activity (Paper I)

A method used at the MDCS-baseline was adapted from the Minnesota Leisure Time Physical Activity Questionnaire. In questionnaires, participants were asked to report the amount of physical activity during their leisure time by being presented a list of eighteen different activities. They were then asked to fill in how many minutes per week they spent on average, on each activity. The result was then multiplied using an activity-specific intensity coefficient, where the product was called a physical activity score.

The variable for physical activity during leisure time provided the following answer alternatives: sedentary spare time (category 1), moderate exercise in spare time (category 2), regular exercise and training (category 3), or hard training or competition sport (category 4). This was further computed into a binary variable (sedentary=1/active=2-4), thereby creating a variable called sedentary spare time (%).

Smoking

In both MDCS and MPP, data on smoking was obtained through self-reported questionnaires.

Laboratory analyses

In MDCS-CV (**Paper I-III**) fasting venous blood samples were drawn after an overnight fast and stored in a biobank (-80°C). Laboratory blood tests for high sensitivity C-reactive protein (hsCRP) (mg/L), HbA_{1c} (%), fasting blood glucose (mmol/L), triglycerides, total cholesterol, LDL and HDL cholesterol (all mmol/L), were analysed at the Department of Clinical Chemistry, Skåne University Hospital in Malmö, that is participating in a national standardisation and quality control system.

In MPP (**Paper IV**), fasting serum total cholesterol, serum triglycerides, serum HDL-cholesterol and FPG were analyzed using Beckman Coulter LX20 (Beckman Coulter Inc., Brea, USA). Serum low-density lipoprotein concentration was calculated through Friedewald's formula (112). NT-proBNP was measured with an electrochemiluminescence immunoassay (Elecsys; Roche Diagnostics, Basel, Switzerland) at the Department of Clinical Chemistry, Akershus University Hospital, Oslo, Norway.

Retrieval of biomarkers

Lipidomic profiling (Paper II)

Lipid extraction of 1 μL of overnight fasted citrate plasma samples stored at -80°C upon collection, was followed by quantitative mass spectrometry–based lipid analysis. The analysis was performed at Lipotype GmbH using a high-throughput shotgun lipidomics technology (113). In short, lipids are obtained from organic material by automated solvent extraction and then measured using a high throughput approach. The main hallmarks of this technology is its comprehensiveness (quantifying 22 different lipid classes) and that it allows fast screening of several thousands of samples with high reproducibility (113).

Metabolomic profiling (Paper II)

Profiling of plasma metabolites was performed using a LC-QTOF-MS System (Agilent Technologies 1290 LC, 6550 MS, Santa Clara, CA, USA) and has previously been described in detail(114). Briefly, over-night fasted citrate venous plasma samples stored at -80°C were thawed and extracted by addition of 120 μl extraction solution (80:20 methanol/water) to 20 μl plasma. The samples were then incubated at 4°C for 1 hour at 1250 rpm. After 15 min centrifugation at 14 000 g, 100 μl supernatant was transferred into a glass vial for analysis. Extracted samples were separated on an Acquity UPLC BEH Amide column (1.7 μm, 2.1 $*$ 100mm; Waters Corporation, Milford, MA, USA).

Proteomic profiling (Paper II and IV)

Fasting plasma levels of 136 proteins (**Paper II**) and 92 proteins (**Paper IV**) from the Olink CVD I and II, and III panel respectively, were measured using Olink Proseek Multiplex proximity extension assay (PEA) at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala, Sweden. PEA uses two oligonucleotide-labelled antibodies per protein, which bind pairwise to each specific

protein, creating a polymerase chain reaction sequence which then can be detected and quantified (115), as illustrated in **Figure 9**. The advantage of this type of analysis is the ability to detect small-abundance proteins in a low quantity of material (i.e., blood sample) and still allow for high specificity and sensitivity results (115). One disadvantage of PEA is that you get arbitrative values and no absolute concentrations of measured proteins. However, the PEA method has been related to established immunoassays with valid correlations for biomarker levels (116).

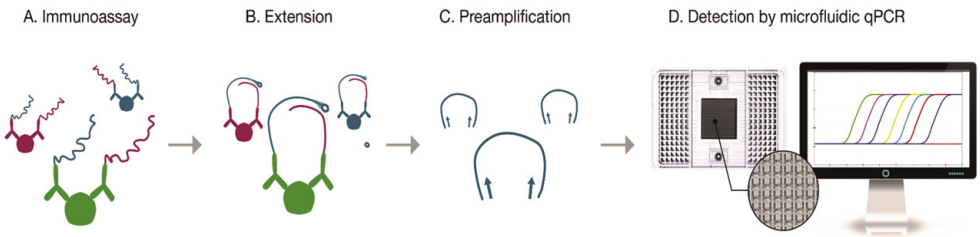

Figure 9. Overview of the PEA technique. (A) specific pairwise DNA oligonucleotide-labeled antibodies (PEA probes) bind to their target-specific antigens (e.g. protein biomarker). (B) Hybridization of the oligonucleotides occurs through pair-wise binding of matching PEA probes and addition of a DNA polymerase leads to an extension and formation of a PCR template. (C) Addition of primers results in preamplication of the new DNA sequence. (D) Each individual DNA sequence is detected an quantified by microfluidic qPCR (115). Image courtesy of ref (117).

Anti-PC antibodies retrieval (Paper III)

Antibodies such as IgM, IgG1 and IgG2 to phosphorylcholine were determined by enzyme-linked immunosorbent assay (ELISA) essentially as described previously (118-121). Briefly, pooled serum from Sigma Aldrich (St Louis, MO, USA) was used as standard in each plate. Nunc Immuno microwell plates (Thermo Labsystems, Franklin, MA, USA) were coated with PC-bovine serum albumin (BSA) antigen at a concentration of 10 µg/mL per well and incubated overnight at 4°C. After four washings with wash buffer, the plates were blocked with 2% BSA–phosphate buffered saline (PBS) for 1 h at room temperature. The same washing steps were followed throughout the assay. Serum samples were then diluted at 1:100 for IgM, IgG1 and IgG2 in 0.2% BSA–PBS and added at 100 µL/well to each plate. Plates were then incubated at room temperature for 2 h and washed as described above. Biotin-conjugated goat antihuman IgM, mouse antihuman IgG1 and mouse antihuman IgG2 (diluted 1:30,000, 1:500 and 1:5000 respectively in 1% BSA–PBS) was then added at 100 µL/well and the plates were incubated at room temperature for 2 h. After four washings, horseradish peroxidase conjugated streptavidin (diluted 1:5000, 1:3000 and 1:3000 for IgM, IgG1 and IgG2 respectively in 0.2% BSA–PBS) [Thermo Scientific, Roskilde, Denmark] were added at 100 µL/well to respective plates and they were further incubated for 20 min. The colour was developed by adding the horseradish peroxidase substrate, TMB (3,3',5,5'

tetramethylbenzidine; Sigma Aldrich, St. Louis, MO, USA), at 100 µL/well and after incubating the plates for 15 min, 20 min and 20 min for IgM, IgG1 and IgG2 respectively at room temperature in a dark place. Further reaction was stopped by adding stop solution 1N H_2SO_4 at 50 µL/well to each plate. Finally, plates were read on ELISA Multiscan Plus spectrophotometer (Spectra Max 250; Molecular Devices, CA) at both 450 nm and 540 nm. All samples were measured in duplicates within a single assay and the coefficient of variation between the duplicates was below 15% for all the antibodies.

Statistics

All statistical analyses were made in **Paper I, III** and **IV**, by using IBM SPSS Statistics version 25 (SPSS, Chicago, IL, USA). In **Paper II**, all statistical analyses were performed in R 3.6.1. In all papers, a p-value <0.05 was considered significant.

Descriptive statistical analyses (Paper I-IV)

De-identified epidemiological data were analysed using descriptive statistical methods. Continuous variables were presented as means (±standard deviation, SD) or medians (25th-75th percentiles). Comparison analyses were made by using one-way ANOVA test for normally distributed continuous variables, Mann-Whitney U-test for continuous variables, dichotomous and category variables with non-normal distribution, and $\chi 2$ test for binary variables with normal distribution.

Paper I

In the section of descriptive statistics, the values of the variables for smoking habits (1-4) were computed into binary (no: 1/ yes: 2-4). Moreover, the variable for leisure time physical activity (1-4) variable was computed into binary (sedentary: 1/ active: 2-4).

A prospective risk analysis of mortality incidence and incident cardiovascular morbidity of MHO individuals compared to MUO and NOC subjects, from the time of start-up (baseline) until the end of follow-up, was performed using non-adjusted Kaplan-Meier curves followed by Cox-regression analyses adjusted for age, sex, smoking, blood pressure, sedentary behaviour and waist/hip ratio.

Paper II

Prior to statistical analysis, missing values for all biomarkers (maximum 20% missing allowed) were imputed using the NIPALS algorithm and were subsequently mean-centered and unit-variance scaled. Unsupervised dimension-reduction of each set of biomarker layer (metabolites, lipids and proteins) was performed using principal component analysis (PCA). PCA was first performed for each biomarker layer in all obese participants and subsequently in all non-MUO participants in the same manner. For each biomarker layer, five principal components (PC) were calculated. In the participants with obesity, logistic regression models, adjusted for age and sex, were used to find associations between PCs and MHO (compared to MUO). PCs that were significantly associated with MHO were investigated for correlations with cardiometabolic risk factors using partial Spearman's correlation tests, adjusted for age and sex. Subsequent analysis in all non-MUO participants used logistic regression models in order to find associations between PCs and MHO (compared to NOC).

Paper III

Prior to analyses, variables showing non-normal distribution were ln-transformed (anti-PC IgM, IgG1, IgG2, FBG, triglycerides and total cholesterol). Anti-PCs were further z-transformed. Unadjusted logistic regressions were carried out for anti-PC and prevalence of HO using odds ratios (OR) and 95% confidence intervals (95% CI). Multivariate logistic regressions were then carried out adjusted for age and sex (*Model 1*), and further adjusted for WC, SBP, fasting blood glucose, and smoking status (*Model 2*).

Paper IV

Prior to analysis, skewed variables were log-transformed. Unadjusted binary logistic regression models exploring associations between each of the 92 proteins and HO were carried out applying the Benjamini-Hochberg multiple testing correction (122) (false discovery rate, FDR, <0.05**)**. Significant associations were carried forward to analyses according to *Model 1* (age- and sex-adjusted), and further adjusted according to *Model 2* (total cholesterol, current smoking, hypertension, BMI, prevalent diabetes of any type, and log-transformed FPG). Finally, for associations significant in *Model 2*, a post-hoc analysis was carried out in subjects with and without diabetes using the remaining variables in *Model 2*. Lastly, to test for linearity between remaining variables with significant associations in *Model 2* and independent variables, quartile analyses were carried out.

Results

Paper I

Baseline characteristics

MHO versus MUO

Compared to MUO individuals, MHO individuals were younger and more likely to be male. Additionally, MHO individuals had a significantly lower BMI as well as lower waist and hip circumference, but no significant differences could be seen for waist/hip ratio or mean blood pressure between the two groups. Moreover, MHO individuals reported a significantly lower proportion of sedentary lifestyle than MUO. The MUO individuals were more likely to be ever smokers. Furthermore, MHO individuals had significantly lower HbA_{1c}, fasting plasma glucose and triglyceride levels, as compared to their MOU counterparts. No significant difference could be seen in cholesterol (total cholesterol, HLD-C and LDL-C) or hsCRP levels. See **Table 2** for more detailed results.

MHO versus NOC

When comparing MHO individuals to NOC subjects, differences could be seen in blood pressure levels, where NOC had a significantly lower systolic and diastolic blood pressure. Furthermore, NOC had a more favourable glycaemic profile with both significantly lower HbA_{1c} and fasting blood glucose levels. The inflammatory status, defined by measuring hsCRP, was lower in NOC. See **Table 2** for more detailed results.

Table 2. Descriptive comparison and significance testing for MHO (n=1,182) compared to MUO subjects (n=2,630); and MHO compared to NOC subjects (n=24,591), with standard deviation (SD) or percentage (%) for metric and categorical variables, respectively.

Variable	MHO	MUO	p-value	NOC	p-value
N	1,182	2,630		24,591	
Sex (men (%))	487 (41.2)	975 (37.1)	**0.016**	9,754 (39.7)	0.306
Anthropometric data (MDCS)					
Age (years (SD))	58 (7.2)	60 (7.4)	**<0.001**	58 (7.61)	0.026
BMI (kg/m2 (SD))	32.6 (2.6)	33.13 (3.06)	**<0.001**	24.64 (2.76)	**<0.001**
Waist (cm (SD))	99.7 (11.7)	101.22 (11.42)	**0.002**	81.66 (13.17)	**<0.001**
Hip (cm (SD))	111.2 (7.7)	112.47 (9.36)	**<0.001**	96.33 (6.7)	**<0.001**
Waist/hip ratio (n (25–75)) median (25–75)*	0.88 (0.82–0.98)	0.89 (0.83–0.98)	0.185	0.83 (0.77–0.92)	**<0.001**
SBP (mmHg (SD))	149 (18.6)	148 (19.13)	0.889	140 (19.9)	**<0.001**
DBP (mmHg (SD))	91 (9.4)	90 (9.59)	0.061	85 (9.84)	**<0.001**
Outcomes (MDCS)					
Mortality (n (%))	422 (36.3)	1,201 (46.6)	**<0.001**	8,178 (33.6)	0.066
Incident CV-event (n (%))	260 (22.3)	749 (29)	**<0.001**	4,957 (20.4)	0.109
IR for CV-event**	18.1	25.3	-	17.0	-
Social and lifestyle data (MDCS)					
N	800	1,612		17,837	
Smoking current or past, (n (%))	450 (56.3)	997 (61.8)	**0.008**	10,896 (61.1)	**0.006**
Regular smoking (years (SD))	21.2 (13.5)	24.5 (13.8)	**<0.001**	23.2 (14.1)	**0.006**
Alcohol intake (g/day)	11.08 (14.8)	10.25 (15.44)	0.282	11.14 (12.25)	0.988
Sedentary leisure time, (n (%))	139 (17.4)	353 (21.9)	**0.009**	1 899 (10.6)	**<0.001**
Physical Activity Score (SD)	7,933 (5660)	7,300 (5881)	0.079	8,417 (6759)	0.119
University degree, (n (%))	107 (13.4)	152 (9.4)	**0.003**	2 825 (15.8)	0.065
Married, n (%)	506 (63.2)	997 (61.8)	0.503	11,504 (64.5)	0.472
Laboratory data (MDCS-CV)					
N	224	424		4,459	
Total cholesterol (mmol/L (SD))	6.25 (1.13)	6.30 (1.13)	0.845	6.14 (1.06)	0.293
hsCRP (mg/L (SD))	0.37 (0.44)	0.45 (0.58)	0.215	0.24 (0.42)	**<0.001**
HDL-C (mmol/L (SD))	1.24 (0.30)	1.20 (0.29)	0.287	1.41 (0.37)	**<0.001**
LDL-C (mmol/L (SD))	4.96 (0.88)	4.31 (1.04)	0.989	4.15 (0.98)	0.105
Triglycerides (mmol/L (SD))	1.59 (0.72)	1.76 (0.76)	**0.011**	1.26 (0.60)	**<0.001**
Fasting glucose (mmol/L (SD))	5.49 (1.36)	5.90 (1.92)	**<0.001**	5.06 (1.19)	**<0.001**
HbA1c (% (SD))	5.09 (0.78)	5.77 (1.08)	**0.012**	4.85 (0.67)	**<0.001**
Drug treatment (MDCS-CV)					
BP lowering drugs (n(%))	53 (52)	178 (57)	0.108	676 (33)	**0.003**
Lipid-lowering drugs (n (%))	2 (2)	22 (7)	0.083	105 (5)	0.235

* Interquartile range (IQR) 25-75.
** Incident rate for any cardiovascular event per 1,000 person-years.

Prospective risk of all-cause mortality and cardiovascular events

Incident rate (IR) for developing cardiovascular disease per 1,000 person-years of follow-up was significantly lower for MHO individuals compared to MUO subjects. Additionally, when comparing the interquartile range between MHO and NOC, there were no significant differences. Cox-regression analysis adjusted for age, sex, smoking, blood pressure, sedentary lifestyle and waist/hip ratio (mean follow-up time 20±6 years) showed a significantly lower all-cause mortality risk for MHO individuals as compared to MUO, hazard ratio (HR) 0.74 (95% CI: 0.66-0.82; p=0.001), as well as lower total incident CV morbidity risk, HR 0.69 (95% CI: 0.60-0.80; p=0.001).

Interestingly, when comparing MHO individuals to NOC, there were no significant differences in neither mortality risk (p=0.90), nor incident CV morbidity risk (p=0.7), see **Table 3** for additional data. Unadjusted Kaplan Meier curves representing all-cause mortality risk and incident CV-event risk for MHO, MUO and NOC are shown in **Figure 10** and **11** respectively.

Table 3. Mortality risk in MHO (n=1,182) vs. MUO (n=2,630) and NOC (n=24,591) subjects until end of follow-up. Cox regression analysis with 95% confidence intervals.

Variables	HR	95% CI for HR		p-value
		Lower	Upper	
Total mortality risk				
MHO vs. MUO	0.80	0.70	0.92	**0.001**
Smoking status	1.20	1.05	1.37	**<0.001**
Sex (female)	0.71	0.62	0.81	**<0.001**
Age (years)	1.11	1.10	1.12	**<0.001**
SBP (mmHg)	1.01	1.00	1.01	**0.003**
Sedentary leisure time (%)	1.42	1.24	1.63	**<0.001**
Waist/hip ratio	3.00	2.03	4.34	**<0.001**
CV event risk*				
MHO vs. MUO	0.77	0.65	0.92	**0.003**
Smoking status	0.97	0.82	1.14	0.708
Sex (female)	0.58	0.48	0.69	**<0.001**
Age (years)	1.07	1.05	1.08	**<0.001**
SBP (mmHg)	1.01	1.01	1.02	**<0.001**
Sedentary leisure time (%)	1.31	1.10	1.57	**0.003**
Waist/hip ratio	2.27	1.31	3.92	**0.003**
Total mortality risk				
MHO vs. NOC	0.95	0.84	1.07	0.358
Smoking status	1.50	1.42	1.59	**<0.001**
Sex (female)	0.84	0.78	0.90	**<0.001**
Age (years)	1.12	1.12	1.13	**<0.001**
SBP (mmHg)	1.01	1.01	1.01	**<0.001**
Sedentary leisure time (%)	1.71	1.60	1.83	**<0.001**
Waist/hip ratio	4.29	3.12	5.91	**<0.001**
CV event risk*				
MHO vs. NOC	0.95	0.82	1.10	0.462
Smoking status	1.29	1.20	1.38	**<0.001**
Sex (female)	0.68	0.63	0.74	**<0.001**
Age (years)	1.08	1.07	1.08	**<0.001**
Systolic blood pressure (mmHg)	1.01	1.01	1.01	**<0.001**
Sedentary leisure time (%)	1.38	1.26	1.52	**<0.001**
Waist/hip ratio	4.38	3.23	5.94	**<0.001**

Values are presented as hazard ratios (HR) with 95% confidence interval (95% CI). SBP = systolic blood pressure.

* Incident CV-events, excluding individuals with prevalent CV-events.

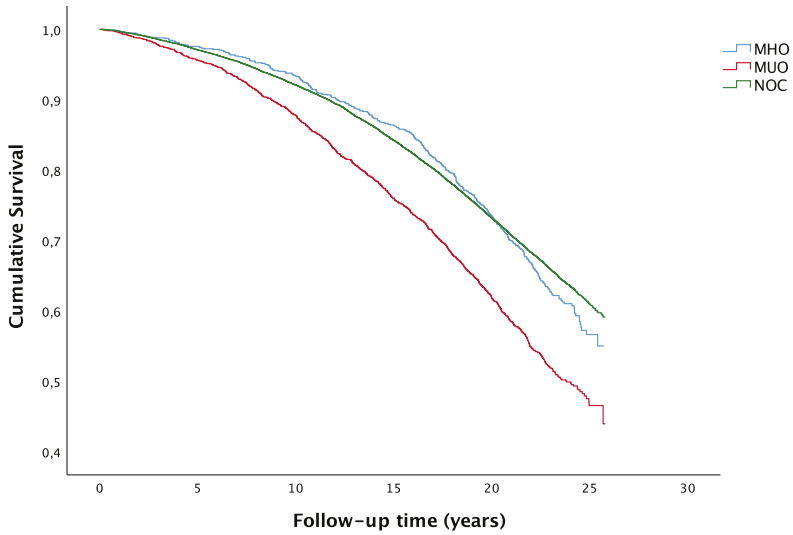

Figure 10. *Unadjusted Kaplan-Meier curves* representing all-cause mortality risk for MHO (n=1,182), MUO (n=2,630) and NOC (n=24,591) respectively.

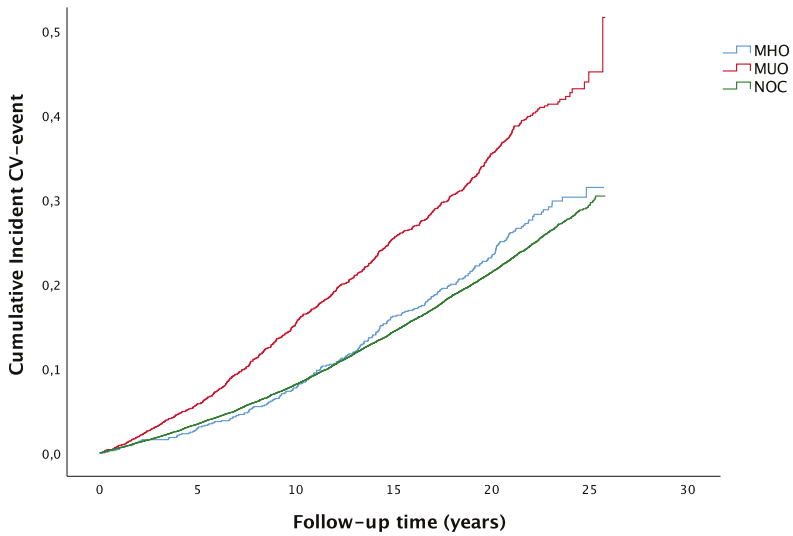

Figure 11. *Unadjusted Kaplan-Meier curves* representing incident CV-event risk for MHO (n=1,182), MUO (n=2,630) and NOC (n=24,591) respectively.

Paper II

Baseline characteristics

Similar to findings in **Paper I**, several differences in cardiometabolic risk factors between subjects with MHO compared to MUO subjects were observed (**Table 4**). MHO participants had a more favourable cardiometabolic risk factor profile compared to MUO, including lower BMI and waist circumference, proportion of prescribed antihypertensive drugs, and fasting levels of glucose and triglycerides, as well as higher levels of HDL cholesterol. Apart from lower BMI, NOC participants were characterized by lower waist circumference, systolic and diastolic blood pressure HbA_{1c}, proportion of antihypertensive drugs, fasting levels of glucose, triglycerides and LDL cholesterol, but higher levels of HDL cholesterol.

Table 4. Descriptive comparison and significance testing for MHO (n=143) compared to MUO subjects (n=273); and MHO compared to NOC subjects (n=3,027), with standard deviation (SD) or percentage (%) for metric and categorical variables, respectively.

Variable	MHO (N=143)	MUO (N=273)	p-value	NOC (N=3027)	p-value
Age (years)	57.7 (5.7)	59.1 (5.9)	**0.02**	57.4 (6.0)	0.50
Sex (% women)	65.7	65.6	0.97	59.2	0.11
BMI (kg/m^2)	32.2 (2.3)	33.4 (3.34)	**<0.001**	24.6 (2.8)	**<0.001**
Waist (cm)	97.0 (12)	99.5 (12)	0.04	80.9 (11)	<0.001
SBP (mmHg)	150 (19)	148 (19)	0.51	140 (19)	<0.001
DBP (mmHg)	90.9 (9.7)	91.0 (9.4)	0.90	86.1 (9.2)	**<0.001**
Smoker (%)	21.1	15.8	0.19	27.6	0.07
AHT drug (%)	19.6	37.7	**<0.001**	14.1	0.11
Fasting glucose (mmol/L)	5.53 (1.4)	5.90 (1.9)	**0.03**	5.09 (1.2)	**<0.001**
HbA_{1c} (%)	5.11 (0.82)	5.29 (1.0)	0.054	4.88 (0.68)	**0.001**
HOMA-IR	3.66 (7.5)	3.81 (4.0)	0.82	1.67 (1.9)	**0.002**
TG (mmol/L)	1.57 (0.72)	1.75 (0.80)	**0.02**	1.25 (0.60)	**<0.001**
HDL-C (mmol/L)	1.27 (0.32)	1.20 (0.28)	**0.03**	1.43 (0.38)	**<0.001**
LDL-C (mmol/L)	4.35 (1.1)	4.35 (1.1)	0.99	4.13 (0.97)	**0.02**
CRP (mg/L)	0.39 (0.50)	0.41 (0.44)	0.74	0.23 (0.40)	**<0.001**
MetS (%)	49.7	61.5	**0.03**	13.6	**<0.001**

Antihypertensive treatment (AHT), diastolic blood pressure (DBP), systolic blood pressure (SBP), glycated haemoglobin (HbA1c), Homeostatic model assessment for insulin resistance (HOMA-IR), C-reactive protein (CRP), Metabolic syndrome (MetS).

Biomarker profiles

Biomarker profiles (lipidomics, metabolomics and proteomics) in relation to MHO and MUO

Biomarker profiles were constructed in participants with obesity, using PCA of three different biomarker layers, including either 112 metabolites, 184 lipids or 136 proteins, respectively. The first five PC's in each biomarker layer could explain 41.8% of the metabolite variation, 63.8% of the lipid variation and 53.1% of the protein variation, respectively.

In order to investigate whether the obesity biomarker patterns were related to MHO, all 15 biomarker PCs were analyzed using logistic regression models. The second lipid PC (PL2) (odds ratio, OR 1.06, p=0.018) and the fifth protein PC (PP5) (OR 0.85, p=0.013) were associated with MHO (**Figure 12**). PL2 was dominated by positive contributions from phospholipids, such as sphingomyelins and phosphatidylcholine ethers, but negative contributions from triglycerides (**Figure 13**). The strongest positive contributions to PP5 were interleukin-1 receptor antagonist (IL1-RA) followed by leptin and fatty acid-binding protein 4, while the strongest negative contributions were from galanin (**Figure 14**). When adjusting for the combined components of the MetS, according to National Cholesterol Education Program panel III (NCEP III) criteria (123), no significant differences could be seen between the PCs.

Both MHO-associated PCs were correlated with traditional cardiometabolic risk factors (**Figure 15**). PL2 showed strong inverse correlations with plasma triglycerides (rho= -0.67, $p < 0.001$), HOMA-IR (rho= -0.36, $p < 0.001$) and glucose (rho= -0.32, $p < 0.001$) and also strong positive correlations with HDL cholesterol (rho=0.59, $p < 0.001$). PP5 was strongly correlated with CRP (rho=0.36, $p < 0.001$) and waist circumference (rho= 0.26, $p < 0.001$) but inversely correlated with HDL cholesterol (rho= -0.27, $p < 0.001$).

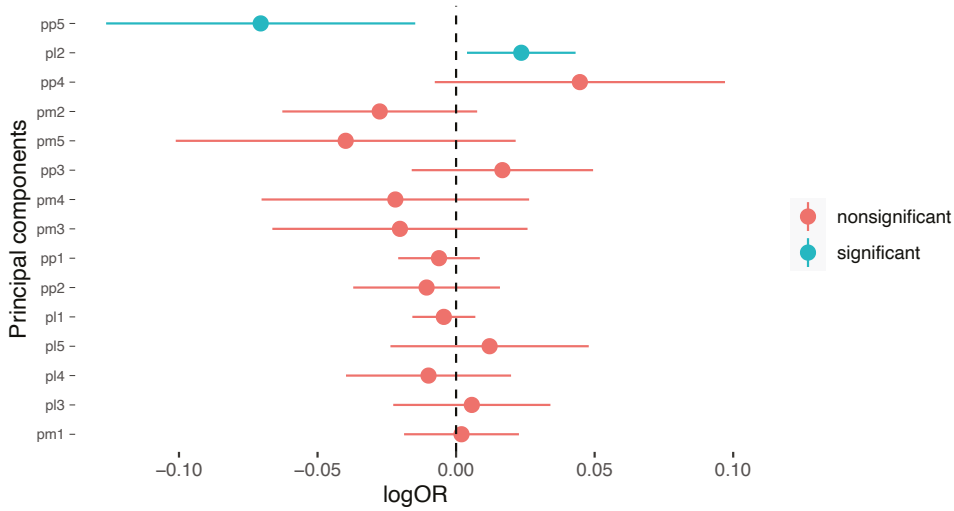

Figure 12. Logistic regression models, with significance testing, of main PC's when comparing *MHO* (right side of logOR) with *MUO* (left side of logOR) subjects.

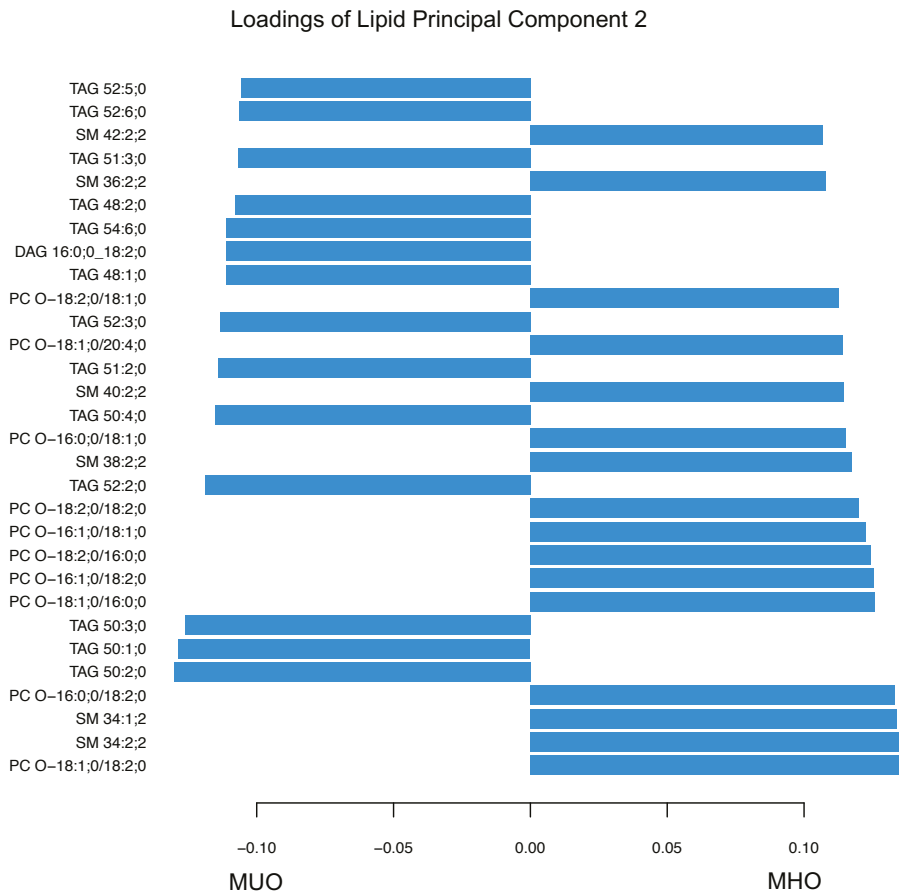

Figure 13. Main loadings for PL2, when comparing *MHO* with *MUO*.

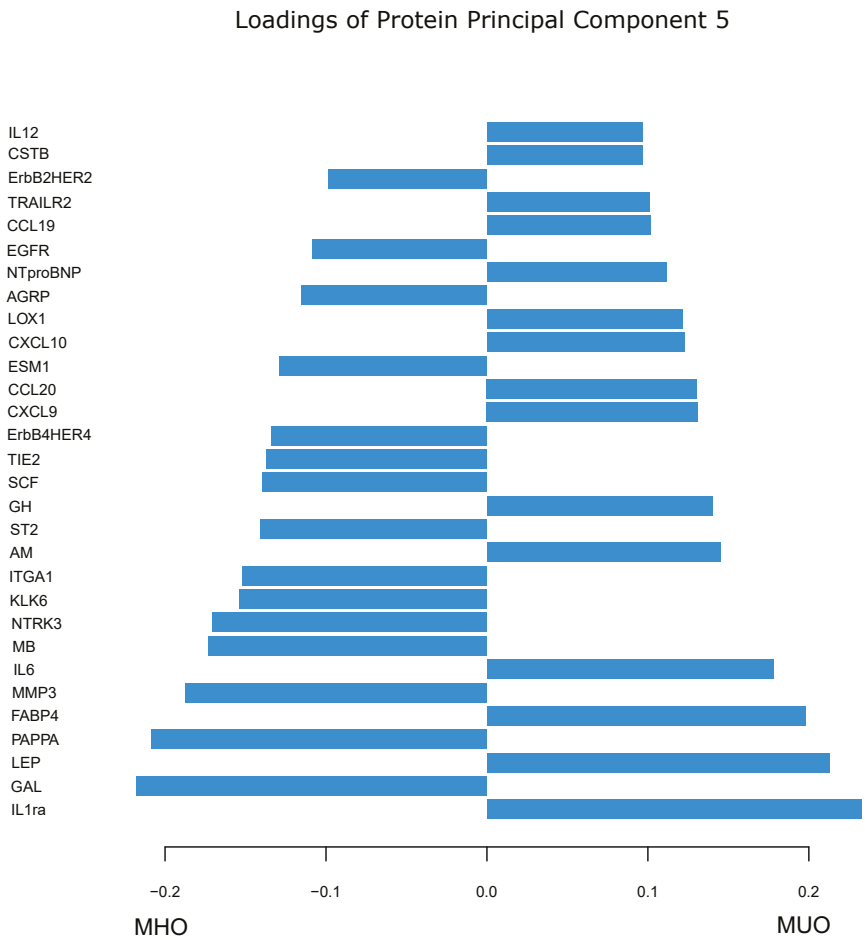

Figure 14. Main loadings for PP5, when comparing *MHO* with *MUO*.

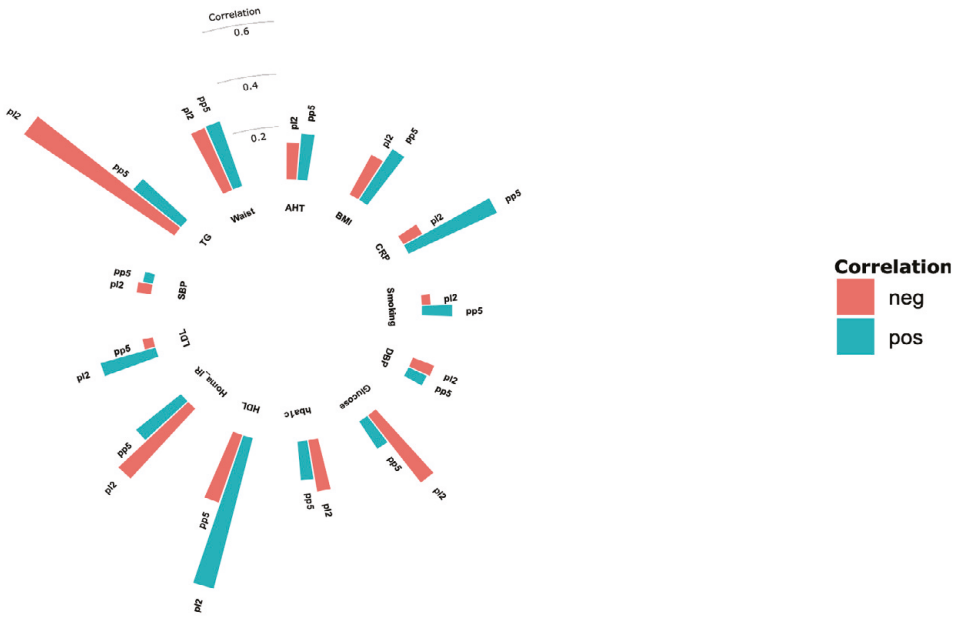

Figure 15. Correlation between biomarker principal components (PC) and cardiometabolic risk factors. Correlations between cardiometabolic risk factors and lipid principal component 2 (pl2) and protein principal component 5 (pp5) are expressed as partial Spearman's correlation coefficients, adjusted for age and sex. Anti-hypertensive treatment (AHT), C-reactive protein (CRP), diastolic blood pressure (DBP), systolic blood pressure (SBP), glycated haemoglobin (HbA1c), Homeostatic model assessment for insulin resistance (HOMA-IR).

Biomarker profiles (lipidomics, metabolomics and proteomics) in relation to MHO and NOC

PCA of three different biomarker layers was used to describe the biomarker variation of study participants without MUO. The first five PC's in each biomarker layer could explain 43.0% of the metabolite variation, 53.5% of the lipid variation, and 61.4% of the protein variation. In general, there were larger differences in the biomarker pattern between NOC and MHO subjects, than between MHO and MUO subjects. Seven PC's, three protein PC's, two lipid PC's and two metabolite PC's were associated with increased odds of MHO as compared to NOC (**Figure 16**). When investigating loadings of the PC's, data demonstrated expected findings of obesity-related parameters.

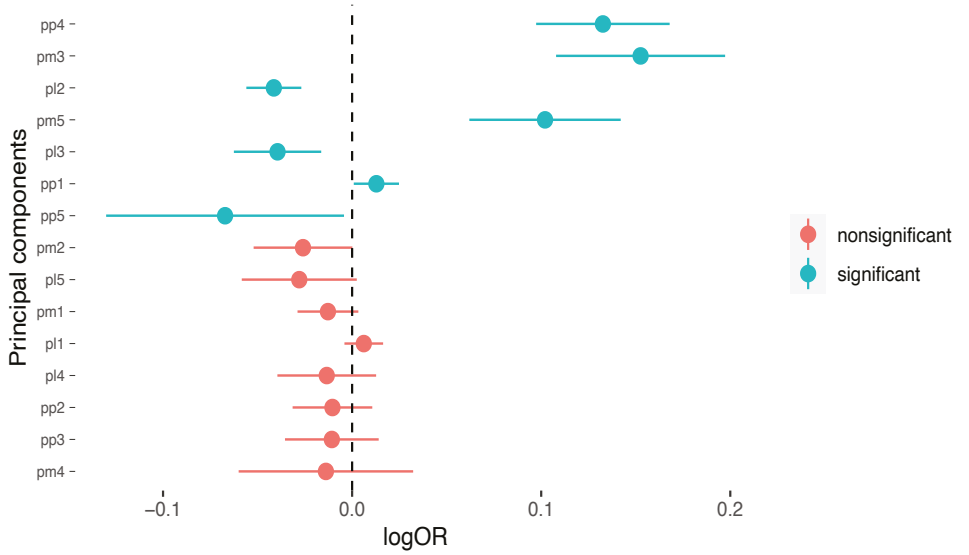

Figure 16. Logistic regression models, with significance testing, of main PC's when comparing *MHO* (right side of logOR) with *NOC* subjects (left side of logOR).

Paper III

Baseline characteristics

Characteristics of the study population are presented in **Table 5.** HO subjects were older, with higher BMI, waist circumference, SBP and DBP, but lower levels of anti-PC IgM and IgG1 than NHO subjects. Furthermore, a larger proportion of the HO subjects presented with diabetes and abdominal obesity as compared to subjects with NHO.

Table 5. Characteristics of the study population in **Paper III**.

	Total	HO	NHO	p-value
N	128	96	32	
Age (years)	59.8 (±5.5)	61.1 (±4.9)	55.9 (±5.4)	**<0.001**
Sex (women)	69 (53.9)	47 (49)	22 (68.8)	0.052
BMI (kg/m^2)	32,7 (±3.2)	33.2 (±3.4)	31.3 (±1.3)	**0.002**
Waist (cm)	100.5 (±12.9)	102.6 (±13.0)	94.2 (±10.5)	**0.001**
Smoking (yes/no)	22 (17.2)	15 (15.6)	7 (21.9)	0.417
Anti-PC IgM (AU)	113 (98-130)	110 (96-126)	124 (110-137)	**0.008**
Anti-PC IgG1 (AU)	126 (86-203)	110 (77-187)	174 (96-230)	**0.023**
Anti-PC IgG2 (AU)	222 (110-480)	222 (112-455)	261 (85-506)	0.741
SBP (mmHg)	148 (±17)	150 (±18)	141 (±12)	**0.017**
DBP (mmHg)	91 (±9)	92 (±9)	88 (±6)	**0.042**
Total cholesterol (mmol/L)	6.3 (5.7-7.3)	6.2 (5.6-7.4)	6.4 (5.7-7.3)	0.511
HDL-C (mmol/L)	1.2 (±0.2)	1.2 (±0.3)	1.2 (±0.3)	0.724
Triglycerides (mmol/L)	1.5 (1.1-2.9)	1.5 (1.1-2.5)	1.4 (1.0-1.9)	0.240
Fasting glucose (mmol/L)	5.2 (4.8-5.7)	5.3 (4.8-5.9)	5.0 (4.6-5.4)	**0.033**
Diabetes mellitus, n (%)	26 (20.3)	24 (25)	2 (6.3)	**0.022**
MetS, n (%)	64 (50)	51 (53.1)	13 (40.6)	0.221
Abdominal obesity, n (%)	47 (36.7)	40 (41.7)	7 (21.9)	**0.044**
High triglycerides, n (%)	53 (41.4)	43 (44.8)	10 (31.3)	0.178
Low HDL-C, n (%)	60 (46.9)	43 (44.8)	17 (53.1)	0.413
Hypertension, n (%)	121 (94.5)	92 (95.8)	29 (90.6)	0.262
Elevated glucose, n (%)	37 (28.9)	31 (32.2)	6 (18.8)	0.143

Values are means (± standard deviation), medians (25-75 interquartile range), or numbers (%). Components of the Metabolic syndrome (abdominal obesity (waist circumference ≥88 cm and ≥102 cm for women and men, respectively), elevated triglycerides (≥1.7 mmol/L), reduced high density lipoprotein cholesterol (<1.03 mmol/L in males and <1.29 mmol/L in females), hypertension (systolic blood pressure ≥130 mm Hg and/or diastolic blood pressure ≥85 mm Hg, or drug treatment), or elevated fasting glucose (≥5.6 mmol/L or glucose-lowering treatment) were defined as stated by Alberti *et al*.[13] HO – hospitalized obese; NHO – non-hospitalized obese; AU – arbitrary units; Anti-PC - Antibodies against phosphorylcholine; Ig – immunoglobulin; MetS – metabolic syndrome; SBP – systolic blood pressure; DBP – diastolic blood pressure; HDL – high density lipoprotein cholesterol

Anti-PC levels and relation to HO subjects

Each 1 SD increment in anti-PC IgM levels was associated with a lower prevalence of HO when unadjusted, OR 0.53 (CI95%: 0.31-0.90; p=0.020), and when adjusted for age and sex, OR 0.54 (CI95%: 0.30-0.99; p=0.049), but the association was attenuated upon further adjustment for waist circumference, SBP, DBP, FPG, and smoking status, OR 0.58 (CI95%: 0.30-1.15; p=0.120).

Each 1 SD increment in anti-PC IgG1 levels was associated with lower prevalence of HO in unadjusted logistic regressions (OR 0.60; CI95% 0.39-0.93; p=0.024), and further adjusted for age and sex (OR 0.58; CI95% 0.35-0.95; p=0.029). The association remained significant when waist circumference, SBP, DBP, FPG, and smoking status were entered in the model (OR 0.57; CI95% 0.33-0.99; p=0.044), **Table 6**. Further, sex-specific analyses were carried out, showing association

between high anti-PC IgG1 levels and lower prevalence of HO in men, but not in women in the fully adjusted *Model 2* (**Table 7**). There was a trend for sex-specific associations of anti-PC IgM with HO in women, but this association was attenuated after adjusting for age and sex in *Model 1* (**Table 7**).

Table 6. Associations between anti-PC and risk of being a hospitalized obese subject (HO).

	Anti-PC IgM		Anti-PC IgG1		Anti-PC IgG2	
Unadjusted	**OR (CI95%)**	**p-value**	**OR (CI95%)**	**p-value**	**OR (CI95%)**	**p-value**
Anti-PC	0.53 (0.31 - 0.90)	**0.020**	0.60 (0.39 - 0.93)	**0.024**	1.01 (0.68 - 1.51)	0.947
Model 1						
Anti-PC	0.54 (0.30 - 0.99)	**0.049**	0.58 (0.35 - 0.95)	**0.029**	-	-
Age	1.22 (1.12 - 1.34)	**<0.001**	1.23 (1.12 - 1.35)	**<0.001**	-	-
Sex	0.29 (0.11 - 0.80)	**0.017**	0.28 (0.10 - 0.79)	**0.016**	-	-
Model 2						
Anti-PC	0.58 (0.30 - 1.15)	0.120	0.57 (0.33 - 0.98)	**0.044**	-	-
Age	1.25 (1.13 - 1.39)	**<0.001**	1.27 (1.14 - 1.42)	**<0.001**	-	-
Sex	1.12 (0.24 - 5.24)	0.889	1.29 (0.26 - 6.43)	0.760	-	-
Waist circumference	1.09 (1.01 - 1.18)	**0.024**	1.10 (1.02 - 1.19)	**0.018**	-	-
Systolic blood pressure	1.03 (0.99 - 1.06)	0.158	1.03 (1.00 - 1.07)	0.083	-	-
Fasting glucose	1.34 (0.66 - 2.73)	0.416	1.12 (0.55 - 2.30)	0.753	-	-
Smoking	0.77 (0.21 - 2.77)	0.685	0.85 (0.23 - 3.11)	0.804	-	-

Values are odds ratios (OR) with 95% confidence intervals (CI95%). Anti-PC – antibodies against phosphorylcholine; IgM – Immunoglobulin M; IgG1 – Immunoglobulin G1; IgG2 – Immunoglobulin G2

Table 7. Sex-specific associations between anti-PC and risk of being a obese subject with a history of hospitalization (HO).

	Men=59		Women n=69	
Anti-PC IgM	**OR (CI95%)**	**p-value**	**OR (CI95%)**	**p-value**
Unadjusted				
Anti-PC IgM	0.60 (0.25 - 1.45)	0.257	0.51 (0.26 - 0.99)	**0.049**
Model 1				
Anti-PC IgM		-	0.46 (0.20 - 1.02)	0.057
Age		-	1.33 (1.15 - 1.53)	**<0.001**
Model 2				
Anti-PC IgM		-		-
Age		-		-
Waist circumference		-		-
Systolic blood pressure		-		-
Fasting glucose		-		-
Smoking		-		-
Anti-PC IgG1				
Unadjusted				
Anti-PC IgG1	0.28 (0.09 - 0.85)	**0.025**	0.76 (0.47 - 1.23)	0.260
Model 1				
Anti-PC IgG1	0.29 (0.09 - 0.92)	**0.036**	0.67 (0.36 - 1.27)	0.221
Age	1.12 (1.00 - 1.29)	0.052	1.32 (1.15 - 1.53)	**<0.001**
Model 2				
Anti-PC IgG1	0.26 (0.07 - 0.98)	**0.046**	0.66 (0.31 - 1.41)	0.284
Age	1.11 (0.96 - 1.30)	0.160	1.38 (1.16 - 1.63)	**<0.001**
Waist circumference	1.05 (0.92 - 1.20)	0.462	1.12 (1.00 - 1.25)	**0.045**
Systolic blood pressure	1.02 (0.96 - 1.07)	0.564	1.05 (1.00 - 1.10)	0.068
Fasting glucose	3.62 (0.68 - 19.09)	0.130	0.95 (0.31 - 2.97)	0.933
Smoking	0.10 (0.00 - 2.43)	0.159	1.60 (0.23 - 11.06)	0.635

Values are odds ratios (OR) with 95% confidence intervals (CI95%). MetS – metabolic syndrome. Anti-PC - antibodies against phosphorylcholine, IgM – Immunoglobulin M; IgG1 – Immunoglobulin G1

Paper IV

Baseline characteristics

Characteristics of the study population are presented in **Table 8**. HO individuals were older than NHO. Furthermore, lower levels of total cholesterol and LDL-C, as well as lower systolic and diastolic blood pressure levels were seen in HO when compared with NHO. However, the use of both lipid- and blood pressure lowering drugs was significantly higher ($p<0.001$) in the HO group. No difference between the two groups were seen in FPG levels, prevalent diabetes, BMI, or waist circumference.

Table 8. Characteristics of the study population in Paper IV.

	Total	HO	NHO	p-value
n	517	407	110	
Age (years)	67.2 (±5.9)	67.7 (±5.9)	65.4 (±5.9)	**<0.001**
Sex (women); n (%)	174 (33.7)	144 (35.4)	30 (27.3)	0.11
BMI (kg/m^2)	33.5 (±3.3)	33.5(±3.2)	33.4 (±3.4)	0.76
Waist (cm)	110.2 (±10.3)	110.4 (±10.1)	109.5 (±10.9)	0.46
Smoker; n (%)	67 (13.0)	54 (13.3)	13 (11.8)	0.67
SBP (mmHg)	149.6 (±20.4)	148.5 (±20.1)	153.8 (±20.9)	**0.01**
DBP (mmHg)	86.1 (±10.6)	85.6 (±10.6)	88.0 (±10.2)	**0.04**
Total cholesterol (mmol/L)	5.3 (±1.1)	5.2 (±1.1)	5.6 ±1.1)	**0.001**
LDL-C (mmol/L)	3.4 (±1.0)	3.3 (±1.0)	3.7 (±0.9)	**0.001**
HDL-C (mmol/L)	1.2 (±0.3)	1.2 (±0.3)	1.2 (±0.4)	0.98
Triglycerides (mmol/L)	1.5 (1.0)	1.5 (1.0)	1.6 (1.2)	0.33
Fasting plasma glucose (mmol/L)	7.4 (±2.2)	7.4 (±2.1)	7.2 (2.3)	0.37
Lipid-lowering drugs; n (%)	159 (30.8)	142 (34.9)	17 (15.5)	**<0.001**
Hypertension; n (%)	467 (90.3)	371 (91.2)	96 (87.3)	0.22
AHT drugs; n (%)	359 (69.4)	302 (74.2)	57 (51.8)	**<0.001**
Prevalent diabetes; n (%)	262 (50.7)	209 (51.4)	53 (48.2)	0.56

Values are means (± standard deviation), medians (IQR) or numbers (%). AHT – antihypertensive; BMI – body mass index; DBP – diastolic blood pressure; HDL-C – high density lipoprotein concentration; HO – hospitalized subjects with obesity; LDL-C – low density lipoprotein concentration; NHO – non hospitalized subjects with obesity.

Biomarker analyses

Of 92 analyzed unadjusted associations between biomarkers and HO, increased levels of two proteins were significant at an FDR <0.05: *Galectin-4* (Gal-4) and *insulin-like growth factor-binding protein 1* (IGFBP-1). When these two proteins were included in logistic regression analyses adjusted for age and sex, Gal-4 remained significant (OR 1.76; CI 95% 1.23-2.51; p=0.002) whereas IGFBP-1 did not (OR 1.24; CI95% 0.97-1.58; p=0.087). Each 1 SD increase in Gal-4 levels was associated with a higher probability of being HO in the fully adjusted logistic regression model (OR 1.72; CI95% 1.16-2.54; p=0.007) (**Table 9)**. When further excluding external trauma (n=38) as a determinant of being HO, the positive association for Gal-4 remained significant (p=0.024). An interaction analysis was performed, showing no significant interaction between Gal-4 and prevalent diabetes (p=0.16). However, given the known association between these two variables (124, 125), a post-hoc stratified analysis was carried out and revealed that the association between Gal-4 and HO was only present among patients with diabetes (**Table 10**).

Table 9. Logistic regression models displaying associations of Galectin-4 levels and odds of being HO.

	HO (n= 407) vs. NHO (n= 110)	
Unadjusted	OR (CI95%)	p-value
Galectin-4	2.03 (1.42 - 2.90)	**<0.001**
Model 1		
Galectin-4	1.85 (1.28 - 2.67)	**0.001**
Age	1.05 (1.01 - 1.09)	**0.013**
Sex	0.93 (0.56 - 1.53)	0.765
Model 2		
Galectin-4	1.72 (1.16 – 2.54)	**0.007**
Age	1.05 (1.00 - 1.09)	**0.030**
Sex	0.73 (0.42 - 1.25)	0.246
Diabetes	0.60 (0.33 - 1.10)	0.098
Total cholesterol	0.71 (0.56 - 0.86)	**<0.001**
Smoking	1.34 (0.67 - 2.65)	0.407
Hypertension	1.03 (0.50 – 2.11)	0.938
BMI	1.00 (0.93 - 1.07)	0.885
FPG	1.27 (0.92-1.75)	0.140

Values are odds ratios (OR) and 95% confidence intervals. BMI – body mass index; FPG – fasting plasma glucose; HO – hospitalized subjects with obesity

Table 10. Post-hoc analysis comparing levels of Gal-4 in obese subjects with or without prevalent diabetes.

	Subjects without diabetes		**Subjects with diabetes**	
	n= 255		n= 262	
	HO n= 198; NHO n= 57		HO n= 209; NHO n= 53	
Model 1	OR (CI95%)	p-value	OR (CI95%)	p-value
Galectin-4	1.52 (0.99-2.53)	0.111	2.45 (1.38-4.35)	**0.002**
Age	1.06 (1.01-1.12)	**0.024**	1.07 (1.01-1.13)	**0.016**
Sex	1.08 (0.56-2.09)	0.824	0.62 (0.29-1.35)	0.228
Model 2	OR (CI95%)	p-value	OR (CI95%)	p-value
Galectin-4	1.45 (0.84-2.49)	0.172	2.26 (1.25-4.07)	**0.007**
Age	1.06 (1.00-1.12)	**0.039**	1.03 (0.97-1.10)	0.279
Sex	0.93 (0.44-1.96)	0.843	0.41 (0.18-0.97)	**0.043**
Total cholesterol	0.92 (0.67-1.25)	0.574	0.60 (0.44-0.81)	0.001
Current smoker	1.97 (0.74-5.29)	0.177	1.01 (0.38-2.68)	0.991
Hypertension	1.09 (0.46-2.57)	0.849	0.84 (0.23-3.03)	0.784
BMI	1.08 (0.95-1.23)	0.250	0.94 (0.86-1.03)	0.200
FPG	1.89 (0.88-3.99)	0.063	1.15 (0.80-1.63)	0.454

Values are odds ratios (OR) and 95% confidence intervals. BMI – body mass index; FPG – fasting plasma glucose; HO – hospitalized subjects with obesity; NHO – non hospitalized subjects with obesity

General discussion

In this thesis it has been shown, through observational data, that there seem to exist a spectrum of individuals with obesity who present with differences in risk of CMD development. This finding has previously been reported in several individual observational studies based on a more traditional definition, describing a phenotype of obesity without the presence of cardiometabolic abnormalities such as insulin resistance, IFG, dyslipidaemia and hypertension (126-130). Since the early 2010's this subgroup of individuals with obesity, defined as MHO, has gained increasing attention, reaching around 160 citations (PubMed) per year. However, the concept of MHO is indeed controversial with many pitfalls, where the ultimate question is whether these individuals are truly healthy or not. In the discussion below I will try to unravel this phenomenon by analysing our own definition methodology and findings and to relate them to the scientific literature available at present.

The dilemma of a non-unanimous definition

Unfortunately, there is no unifying definition of individuals with obesity and lowered CMD risk, most commonly referred to as MHO (101, 131). Although a general consensus of a BMI $\geq$30 kg/m^2 has been reached, there are more than thirty different definitions of metabolic health, according to a systematic review (101). The most commonly used definitions rely upon the absence of one or more parameters constituting the MetS (elevated BP, WHR, TG, blood glucose and/or decreased HDL-C) together with no previous recordings of CVD. However, large variations are seen in the range of accepted cardiometabolic abnormalities and cut-off values for each parameter (101, 132, 133). This results in a vast discrepancy of the prevalence rates of MHO depending on the definition used, with a range from 9-41%, when applying different definitions on the same study population (134, 135). Moreover, most variables are measured at the baseline examination, where they could shift intra-individually during repeated measurements at different time points and thus put subjects in another category. In 2014, through the BioShare EU Healthy Obese Project, a need was addressed for a standardized definition. This could be used as a tool in clinical practice, in a pursuit to better characterize MHO. By combining data from ten different cohorts (n=163,517) in seven European countries, a strict definition was set for MHO (**Table 11**), where included

individuals presented with obesity without any criteria for the MetS or prevalent CVD (131). Even here the prevalence of MHO varied largely between the cohorts (range from 7 to 28%).

Table 11. Proposed harmonized criteria of MHO in adults, according to the BioShare EU-project (2014). MHO is considered if all of the criteria below are met.

METS COMPONENTS	CRITERIA
SYSTOLIC BLOOD PRESSURE	≤130 mmHg
DIASTOLIC BLOOD PRESSURE	≤85 mmHg
	No antihypertensive drug treatment
BLOOD GLUCOSE	≤6.1 mmol/L
	No antidiabetic drug or diagnosis of DM2
FASTING TG	≤1.7 mmol/L
NON-FASTED STATE TG	≤2.1 mmol/L
	No drug treatment for elevated TG
HDL-C	>1.03 mmol/L (men), >1.3 mmol/L (women)
	No drug treatment for reduced HDL-C
DIAGNOSIS OF CVD	No

DM2 – type 2 diabetes; HDL-C – high density lipoprotein-cholesterol; TG – triglycerides.

In an attempt to create a novel more stable definition, MHO was instead in this thesis proposed as a phenotype in subjects with no history of hospitalization (non-hospitalized) for somatic disease up until midlife in spite of obesity defined at baseline health examination. Perhaps this could be a way to enable an objectively defined phenotype which could serve as an alternative to the conventional way of defining MHO. Not to be overlooked, some individuals might be treated for chronical illnesses or risk factors in a primary care unit and thus avoiding inpatient care; however, the outpatient primary care in Sweden up until the 1990's was less developed than presently (136). Importantly, MHO should not be interpreted as a subgroup of individuals with obesity without any health impairments. Apart from the known cardiometabolic complications of obesity, there are numerous examples of obesity-related health conditions that reduce the quality of life, such as osteoarthritis, respiratory disturbances and depression, as well as certain types of cancer (4, 137). Thus, in order to avoid the word "healthy" in MHO, there was a conversion of the term in **Papers III** and **IV** into NHO and HO respectively.

The problem of BMI as predictor of cardiometabolic disease

To address obesity with a heterogeneity in cardiometabolic risk, there is a need to investigate the definition of obesity. Used as WHO standards, obesity is classified as a BMI ≥30 kg/m^2, and could further be subdivided into different classes of obesity (class I [BMI 30 to <35 kg/m^2], II [BMI 35 to <40 kg/m^2] and III [BMI ≥40 kg/m^2]) depending on the severity of increased BMI (1). One of the reasons why BMI has been chosen as the standard measurement of obesity has to do with its simplicity of only needing information about a person's anthropometric weight and height, thus making it easily applicable in both the clinic and in population-based research (138). The issue of using only weight and height data lies in its indirect nature of measurement – it does not reflect body fat directly. Examples of conflicting information include elderly people with decreasing lean muscle mass and increasing adipose tissue, where the BMI still could be within normal limits. Similarly, younger athletes with a high lean body mass, and thus a high BMI, could have an overestimation of their body fat mass. This has been elegantly shown in previous studies, comparing BMI measurements with densitometry (139), isotopically labelled water mass estimation (140), or bioelectrical impedance methods (141), directly measuring total body fat content. In **Papers I-IV**, the population included in the studies consisted of middle-aged men and women, where many individuals could have been missed or even falsely included due to the aforementioned shortcomings. Furthermore, the inaccuracy worsens if the anthropometric measurements are self-reported (142). However, in both the MDCS and MPP-RES cohorts, this was not the case.

As previously mentioned, the location of fat deposits (visceral fat in particular) is a strong predictor of CMD development. Through imaging methods, such as computed tomography (CT) scanning and dual imaging x-ray absorptiometry (DEXA) scanning, this could be assessed with precision (143, 144). However, in large population-based studies and in clinical screening programs this would be both time consuming and economically impossible. An easier, albeit more inaccurate, estimation of body fat location is through measurement of WC and WHR, which has also been shown to be a superior predictor of CVD development compared to BMI (145). This is in line with our findings in **Paper I** and **II** where MUO individuals had a higher WC (**Paper I-II**) and WHR (**Paper I**) than MHO, as well as in **Paper III** where HO had a higher WC than NHO. However, in parallel, the same pattern was seen in BMI differences between groups which could imply a bias, as increased WC and WHR correlates with increasing BMI (146).

Frequently, as in the studies included in this thesis, there is usually no consideration of the duration of obesity or whether the body weight is relatively stable or rapidly increasing (138). This is due to the fact that BMI is only calculated at the baseline

examination in most population-based studies, even though there is strong evidence suggesting that body weight and fat stores increase and that height decreases with increasing age, called biological involution (147). In addition, there is a variability in weight gain and loss of muscle mass depending on the individual (148, 149).

The heterogeneity of prospective risk for cardiovascular events and all-cause mortality in individuals with obesity

The fact that obesity is directly linked to premature death and the risk of developing CVD in general is indisputable. However, as with many other causations, such as smoking and the development of lung cancer and chronic obstructive pulmonary disease (COPD), there is a heterogeneity in risk. In **Paper I** it was shown that individuals with MHO had a significantly lower risk of all-cause mortality and incident CVD than MUO during follow-up. Moreover, when comparing MHO to NOC subjects there were no significant differences in risk. Indeed, one could postulate that MHO, as we have defined it, is a benign condition since it has been demonstrated consistently through meta-analyses that MHO is associated with a significantly lower risk of CVD and DM2 incidence (150, 151). On the contrary, there is increasing evidence challenging this view, demonstrating an increasing risk for the development of CVD, cerebrovascular disease, heart failure (152-154), DM2 (155) and all-cause mortality (96) when compared to metabolically healthy individuals with a normal BMI, based on the traditional definition.

As stated by the WOF, obesity is a chronic relapsing and progressive disease, which most likely could be applicable to MHO (64). An individual with obesity may several times, from initial weight loss to regaining weight through dieting and/or exercise, transcend from MUO to MHO and vice versa. Several large meta-analyses and observational studies have thus proposed that MHO is a transient state. From the prospective Pizarra study, approximately 30% of MHO individuals at baseline transformed into MUO after 6 years of follow-up (156). To further support this notion, in the Multi-Ethnic Study of Atherosclerosis (MESA), almost half of the participants defined as MHO at baseline had developed MetS after a 12.2-year follow-up time (157). Finally, the acclaimed Nurses' Health Study confirmed the transient state of MHO, after analyzing 90,257 participants with a median follow-up of 24 years (150). Importantly, when again looking at the MESA-study, there was interestingly no risk increase in neither all-cause mortality nor incident CVD in those individuals who kept their MHO-status constant over time as evaluated at the follow-up examinations (157), similar to what has been observed in the UK Biobank (129). This, however, included only a small number of individuals with obesity (3%

of the entire MESA cohort), but still raises concerns regarding the heterogeneity in risk (157).

In conclusion, with increasing data over the past decades concerning the MHO phenotype, it has been demonstrated that this in most cases still carries harmful long-term consequences for cardiometabolic health in most cases compared to lean subjects. Clinical events however may be postponed. Even if MHO individuals have a significantly reduced risk of CMD development compared to MUO, this should not undermine risk factor monitoring and appropriate treatment for obesity in general; however, it may serve as a useful tool in risk stratification.

Biological mechanisms of obesity with decreased cardiometabolic risk

Put aside MHO as a controversial phenotype, it may serve as a unique model of obesity without cardiometabolic abnormalities, which in turn could provide insight into the mechanisms linking factors that promote fat accumulation to obesity-related cardiometabolic complications. Possible traits differentiating MHO from MUO include the body fat distribution, grade of physical activity, adipose tissue function and systemic inflammatory activity (**Figure 17**) (92). These traits, according to Stefan *et al*, remain the same even when investigating metabolically unhealthy subjects with normal weight (158). Of note, these associations tell us nothing about which phenotypic characteristics that contribute the most to protection against CMD development (or rather postponement) in MHO subjects.

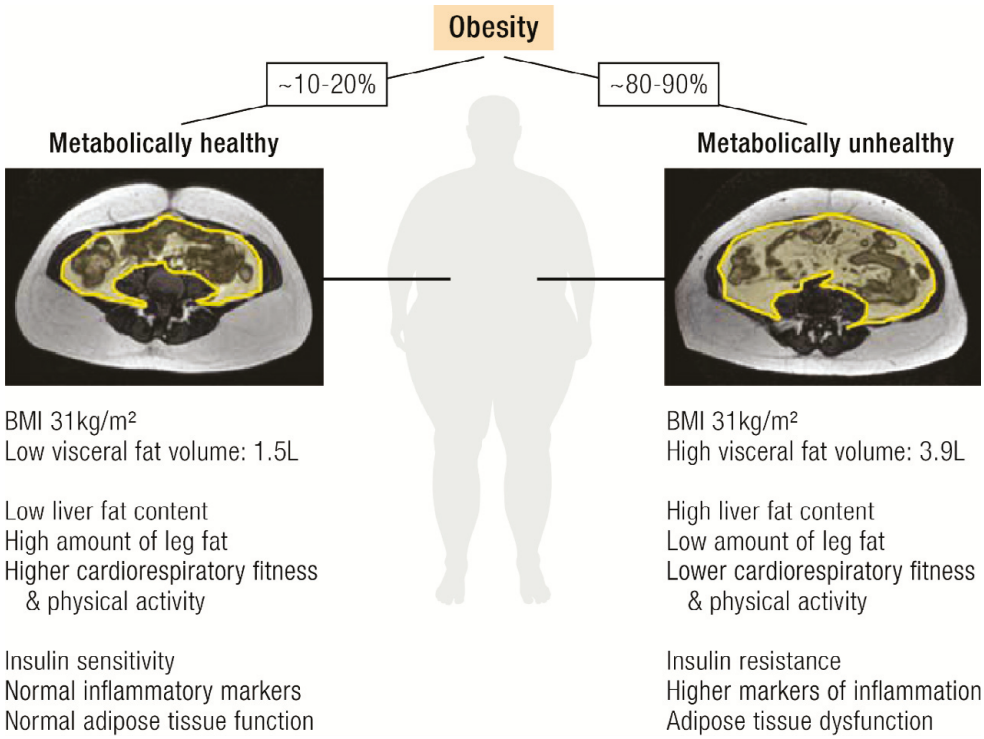

Figure 17. The physiological traits associated with MHO (prevalence 10-20%) and MUO (prevalence 80-90%). Within the illustration is shown pictures of two different women with the same BMI and age, where a transabdominal MRI demonstrates large differences in intra-abdominal fat depostis. Adapted with permission from ref. (92), CC BY 4.0 (http://creativecommons.org/licenses/by/4.0).

Body fat distribution

Adipose tissue is characterized by a variation in metabolic activity, depending on where the deposits are located (34). The most metabolically active adipocytes are found in the intra-abdominal or visceral fat, which through their higher lipolytic activity and secretion of inflammatory adipokines, promotes the development of both CVD and DM2 (34, 35). Supporting this statement, results from a recent population-based cohort study have shown that higher trunk fat predicted increased incidence of CVD in postmenopausal women, whereas higher leg fat predicted lower rates of CVD-events (159).

When comparing MHO and MUO groups for body fat percentage, there are small or no differences when matched for both BMI and sex (127, 160, 161). However, when investigating the differences in fat deposition there was a marked difference in visceral fat, with a higher abundance in MUO than MHO subjects (160-163). Furthermore, when comparing MHO to lean subjects with normal metabolic health there was a significantly higher amount of visceral fat in subjects with obesity (127,

164). In the studies of this thesis there were unfortunately no available data for the measurement of specific fat deposits. As previously stated, in **Paper I-III** there were differences in WC and WHR (which could serve as indirect measure of visceral fat mass), where MUO/HO presented with higher values than MHO/NHO. On the contrary, these differences were not adjusted for neither BMI nor sex which could imply a bias.

In conclusion, data suggest that excess body fat may not be the leading cause of metabolic alterations in MUO compared to MHO, but perhaps the difference in the adipose tissue distribution – a marker of shifting metabolic activity of fat cells.

Physical activity

Preservation of cardiorespiratory fitness (CRF) and increased physical activity (PA) are well established interventions to achieve a reduced risk of obesity-related CMD, mainly through improving insulin sensitivity and MetS abnormalities (165, 166). Higher PA and CRF has been well recognized to be associated with the MHO phenotype, in both adults and children (99, 167). In **Paper I,** it was shown that a factor that characterizes MHO, when compared to MUO (apart from a more favorable metabolic profile), was a less sedentary lifestyle (*fat but fit*). Furthermore, the same MHO individuals presented with a lower all-cause mortality and incident CVD risk than their MUO counterparts. These findings serve as important clinical implications to lower the CMD risk in individuals with obesity without the immediate need of reaching a normal BMI, a strategy of *weight stabilization*. Both CRF and PA are highly modifiable lifestyle factors, without any need of medical (drugs) or surgical interventions, and could thus be considered the low-hanging fruit for CMD prevention in individuals with obesity, which is also supported by the results from the Look AHEAD trial, as earlier mentioned (84). In addition, previous studies (including **Paper I)** have shown that perhaps only a slight increase in PA (i.e., being non-sedentary) could contribute to a great benefit of CMD reduction (99, 168, 169)

Reduced insulin resistance

Insulin resistance (IR), which is one of the leading causes of DM2 and CMD development, is strongly associated with obesity (170). In the post-prandial state, the liver and skeletal muscle are tissues responsible for most of the glucose uptake – likewise, these organs become progressively resistant to the actions of insulin in CMD (18). In obesity, a significant contributor to IR is a dysfunctional adipose tissue. Through increased lipolysis, release and further exposure of FFA to the liver, this stimulates gluconeogenesis and hyperglycaemia (18). This type of lipotoxicitiy further compromises IR in both skeletal muscle and the liver (171).

Several biomarkers have been identified to be associated with a decreased IR, through different biological mechanisms (172). Similarly, insulin sensitivity has been associated with MHO when compared to MUO, mainly through a decreased homeostasis model assessment of insulin resistance (HOMA-IR – a method for IR measurement through the implementation of FPG and insulin concentrations by use of an algorithm) and higher levels of circulating *adiponectin* (173-175). *Adiponectin* is the most abundant protein secreted by adipocytes, with a direct association with insulin sensitivity in both men and women where lower levels are associated with IR (176).

In **Paper II**, we identified *galanin* (GAL), an obesity-related neuropeptide, to be negatively related to MUO, suggesting a relationship with MHO. GAL is mainly involved in the energy homeostasis, where increased hormone levels contribute to the development of obesity, through orexigenic effects, but also obesity-associated metabolic impairments, regardless of feeding regulation (177, 178). Nevertheless, one study reports potential positive metabolic effects of this hormone, where it seems to improve glucose metabolism and uptake, thus decrease insulin resistance (179).

Furthermore, *leptin* correlated positively with MUO in **Paper II**. Encoded by the obese (ob) gene, this adipocyte-derived hormone regulates the energy balance by inhibiting sensations of hunger mediated via the hypothalamus. Hence it contributes to reduce the caloric intake and increase energy expenditure (180). In addition it has beneficial effects on the glucose-insulin metabolism by decreasing hyperglycemia and hyperinsulinemia, thereby reducing IR (181). Paradoxically, *leptin* increases with increasing BMI and adipose tissue mass, suggesting that obese individuals develop a resistance to this hormone in parallel to increasing body weight (182).

Another significant contributor to the protective mechanism of IR is the *incretin system*. Through the release of GLP-1 and GIP from the small intestine after orally consumed glucose, a greater effect on insulin response (secretion) is achieved than intravenous infusion of glucose – the *incretin effect* (183). In subjects with DM2 the incretin effect has been shown to be significantly reduced or even absent (184). Incretins are also involved in appetite control and delaying gastric emptying actions that are dependent on GLP-1 receptor activation within the central nervous system, thus having the potential to regulate body weight (185). In **Paper IV**, we found that increased levels of *Galectin-4* (Gal-4) were independently associated with a higher probability of being HO. Being part of the galectin family (consisting of 15 small peptides), Gal-4 is expressed almost exclusively in the gastrointestinal tract, where it plays a role in controlling intestinal inflammation. It reduces proinflammatory cytokine production in the intestinal mucosa, and experiments in animals with knockout of the Gal-4 peptide promotes colorectal cancerogenesis. This suggests that Gal-4 plays a significant role in the pathophysiology of the development of both inflammatory bowel disease and colorectal cancers (186). However, the physiological role of Gal-4 is multifaceted and further includes apical protein

trafficking, lipid raft stabilization, intestinal wound healing and bacterial pathogen neutralization (187). Epidemiological data also strongly support an involvement of Gal-4 in CMD, suggesting it may be considered as a predictive biomarker for the development of both CVD (109) and diabetes (124). Still, the causal pathway is poorly understood (109, 125). One possible explanation might be found at the cellular level, where Gal-4 is part of the apical protein transport from the Golgi-apparatus to the apical cell membrane of the enterocyte, including protease dipeptidyl peptidase-4 (DPP-4) (188). In mice, DPP-4 seems to be misguided and accumulates intracellularly when Gal-4 is depleted (188). DPP-4 plays a major role in promoting CMD by cleaving and thus inactivating GIP and GLP-1 (189). Modern anti-diabetes drugs such as DPP4-inhibitors and GLP-1 agonists are incretin-based and part of the standard treatment of DM2 as second-line drugs in many patients (183). Furthermore, another study of women with gestational diabetes found an overexpression of Gal-4 in the placental syncytiotrophoblast cells, compared to healthy controls (190). Thus, one proposed explanation for the main findings in **Paper IV** may be Gal-4's involvement in the development of diabetes, which also has been suggested in a previous publication with a similar approach based on proteomic exploration (191). To elucidate on this, a *post-hoc* analysis was carried out in **Paper IV**, suggesting that elevation of Gal-4 levels is associated with higher probability of being HO, but only in those with prevalent diabetes.

Dyslipidaemia

With increased abundance of fat mass together with a dysfunction of adipose tissue, the lipolytic activity of adipocytes does not only contribute to IR through the release of FFA, but also via increasing TG levels and the removal of metabolically favourable HDL-C (18). Higher levels of TG mirror a more metabolically active adipose tissue as well as atherogenic properties, and thus with the MetS (192). In **Paper II** we found, through lipidomic pattern displays, positive contributions of TGs and diacylglycerides to the MUO phenotype when compared to MHO. This confirms a more benign lipid profile (less dyslipidaemia) of MHO subjects. Furthermore, glycerphospholipids (exclusively ether phosphatidylcholine) and sphingomyelins seems to be associated in a positive manner with MHO. It is unclear whether phospholipids contribute in a positive or negative way to CVD and metabolic disorders (193, 194). However, research shows that ether phosphatidylcholine with shorter fatty acids and smaller amounts of total double bonds (more positively correlated with MHO) are increased in long lived humans (195). This suggests that MHO individuals might deal with oxygen stress better than their counterparts, a theory supported by a previously cited systematic review, revealing that plasmalogens (a subclass of cell membrane glycerophospholipids) (196) presents a negative correlation with obesity, DM2, prediabetes and CVD – all conditions associated with elevated levels of oxidative stress (193). Moreover, sphingolipids (mainly sphingomyelin) seem to contribute to adipose tissue

inflammation and the accompanying liver steatosis and insulin resistance, why their positive relationship with MHO thus appears more sophisticated than expected (193, 197, 198).

Inflammatory markers

Obesity is typically associated with a chronic low-grade inflammatory state, which in turn has been proposed to be associated with IR and CMD (199). Alterations in adipose tissue function (through the secretion of pro-inflammatory adipokines) and its immune cells are important factors to promote this obesity-associated inflammation (200). Although MHO individuals have been suggested to possess a more favourable inflammatory profile compared to MUO, there are conflicting studies on this subject. Plasma concentrations of well-known inflammatory markers, primarily CRP, PAI-1, TNF-α and IL-6 are either at higher levels (160, 161, 201, 202) or do not differ (203-205) when compared to MUO. These results may be due to alterations in the definition of metabolic health between the studies, but nonetheless they importantly question the significance of adipocyte-released cytokines in mediating the IR difference observed in MHO and MUO.

In **Paper II**, we found positive contributions of *interleukin-1 receptor antagonist* (IL-1ra) for MUO compared to MHO. It has been debated whether IL-1ra is of a benign nature or if elevated levels of this biomarker present with adverse effects (206). Indeed, it works as an inhibitor of the well-known pro-inflammatory cytokine Interleukin-1β (IL-1β), involved in the development of various chronic inflammatory disorders, as well as CVD and DM2 (207). The randomized double-blind CANTOS study contributed to the increasing evidence of positive effects of IL-1ra focused treatment, displaying that anti-inflammatory targeting with monoclonal antibodies against IL-1β significantly reduced recurrent cardiovascular events compared to placebo in post-myocardial infarction survivors (208). However, conflicting data postulates that this protein is upregulated as a protective response to increased activities of IL-1β and Interleukin-1α (IL-1α) (209). Furthermore, additional findings hypothesize that IL-1ra might have harmful cardiovascular effects of its own and additionally prevent potentially positive effects of IL-1α and IL-1β (206).

Not only the secretion of pro-inflammatory adipokines but also the immune system is involved in the development of obesity-related inflammation, where immune competent cells are known to infiltrate adipose tissue (210). In **Paper III** it was reported that levels of anti-inflammatory IgG1 and IgM anti-PC were significantly lower among HO than among NHO-individuals. Phosphorylcholine is exposed on the surface of LDL during oxidation and may play a major role in oxidized LDL-induced immune activation, which in turn is involved in atherosclerosis formation and CVD development (211). IgM anti-PC is further negatively associated with

several other chronic inflammatory conditions, including rheumatic diseases and chronic kidney disease (211). Interestingly, immunosuppressive, anti-inflammatory T regulatory cells are decreased in obesity (212). In principle, it is thus possible that low IgM anti-PC could be one factor behind low numbers of T regulatory cells in obesity. Further, inflammation can be both a cause and a consequence of obesity and ensuing metabolic changes (210, 213). An immune-deficient state with low IgM and IgG1 anti-PC could thus potentially promote obesity and related inflammation.

Methodological limitations

The results of this thesis need to be interpreted in the context of its limitations. In this section we present additional methodological limitations that have not already been dealt with in the discussion.

General limitations

All papers derive from observational cross-sectional studies, which precludes any conclusions about causality.

In the MDCS (**Papers I-III**), the modest overall attendance rate at baseline examination (41%) could imply a health selection bias. Furthermore, there is an imbalance of gender, with a majority of attending women (61%) adding to the selection bias and thus not being fully representative of the local population in general.

The studies only cover individual data collected at one research center. A multicenter study to replicate the findings would be preferable. Furthermore, most subjects were of white European descent, why the findings might not be generalizable to other populations or ethnicities.

Lastly, subjects with a non-hospitalization status prior to baseline could still suffer from cardiometabolic disturbances or a risk factor burden, since no pre-defined diagnoses of hospitalization were decided upon, and many individuals could be treated for chronic illnesses within a primary health care unit. On the other hand, these conditions could have been milder or counterbalanced by protective mechanisms in the affected subjects, leading to a status of non-hospitalization in our analyses.

Paper I

When gathering social and lifestyle information at baseline, reporting bias cannot be excluded.

Paper II

A major limitation is the small sample size, resulting in limited statistical power. Moreover, although we applied a data-reducing strategy, several derived PCs were tested with obesity phenotypes, thereby implying a risk of false-positive results. This underlines the need of replication of the reported findings.

When performing a multiple regression analysis adjusting for MetS components (123), the associations of the biomarker PCs when comparing MHO and MUO individuals were attenuated. This suggests that the difference of biomarker variation between MHO and MUO subjects in part could be explained by the presence of MetS. Thus, one might argue that in order to keep the MHO phenotypic state and avoid hospitalization, there should be an ambition of the individual for weight stability and to continue keeping a healthy lifestyle in order to avoid transformation into MUO linked to the MetS.

Paper III

This study was relatively small, and it was therefore difficult to determine associations and control for confounders due to lack of statistical power. Further, it would have been of interest to study obesity in general as compared to matched controls.

Paper IV

The population selection in this paper was based on glucometabolic disturbances, which could raise concerns of how well this cohort represents the general population. However, when compared with similar cohorts, the incidence rate of diabetes was proportional (214, 215).

The Olink CVD III panel is partially restricted to proteins associated with CVD and inflammation, why an extended analysis including biomarkers related to diabetes and/or metabolism (now lacking) would most likely add information about the pathophysiology active in HO subjects.

Future perspectives

The prevalence of obesity is progressively increasing – together with it, CMD incidence will undoubtedly increase as well, putting an enormous strain upon the healthcare systems and economy in the future (1, 6). To reduce the risk of CMD development in obesity, weight reduction/control is key of achieving this. However, individuals with obesity show a great difficulty of losing weight and maintaining a normal body weight. Furthermore, bariatric surgery (which in observational studies shows the greatest improvement in CMD risk reduction) (85) might not always be suitable, indicated, financed or even wanted by the patient. Perhaps a more pragmatic approach would be to identify individuals with obesity with a high CMD risk and convert their condition (through lifestyle interventions and pharmacotherapy) to an obesity phenotype with a lowered risk of CMD. We know that significant health improvements could be seen even by a moderate 3-10% weight loss (216). An attempt to apply this risk stratifying approach in clinical practice is through the *Edmonton Obesity Staging System* (EOSS), which not only considers the metabolic complications of obesity but also the physical and psychological aspects and thus trying to identify those individuals who will benefit the most of receiving anti-obesity treatment. This staging system has been reported to be a superior predictor of mortality compared to BMI or MetS (217, 218). The EOSS has, however, not been implemented in the Swedish guidelines for obesity treatment as of yet (219).

We should avoid the term "healthy" in MHO, since increasing evidence suggest that most of these individuals will over time convert into an unhealthier phenotype (150, 156, 157); however, there seems to be a delay in this conversion where certain protective factors may play an important role. Future research should thus take advantage of MHO as a model to understand how obesity, adipose-tissue expansion, adipocyte composition and dysfunction contribute to obesity-associated CMD, or avoidance/postponement of it.

An interesting research field, touching upon the concept of obesity with lowered CMD risk, involves genetic studies of the brown-bear in an attempt to understand its physiology during hibernation (220). During late summer/fall the brown bear changes its dietary pattern into a hyperphagic state, where it gains approximately 30% additionally to its body weight. After this, the bear goes into hibernation with almost no physical activity for 6 months. During spring it wakes up, showing no signs of aggravated parameters of CMD (**Figure 18**) (220). Bears are

physiologically closer to humans than rodents, and thus serve as a better experimental animal in medical research (221, 222). If we could understand what drives this phenomenon, then we may also be one step closer of understanding the protective mechanisms in individuals with obesity. In **Paper II** we identified several differences in lipidomic distribution and associations to certain clusters of potential harmful proteomic biomarkers when comparing MHO individuals to MUO. Many of the proposed reasons for these differences could be due to the level of physical activity or body fat distribution; however, through further research, like the one described above, we could gain knowledge from a microscopical view rather than macroscopical.

Figure 18. Displays the connection between overeating and sedentary behavior of the brown bear without any CMD complications such as atherosclerosis. With permission from ref. (220).

In **Papers III-IV** we identified an upregulation of certain anti-inflammatory anti-PC antibodies (**Paper III**) and a downregulation of Gal-4 (**Paper IV**) in NHO, where Gal-4 was further implicated as a mediator of DM2 development. Through using this type of biomarker approach, we could discover potential future treatment targets, through personalized precision medicine, thus expanding our treatment options and in parallel treatment outcomes. As an example, Gal-4 has been given a possible role within the incretin system. There are presently several incretin-based anti-obesity drugs on the market, targeting GLP-1 receptors mainly in the gut – one of them is liraglutide (a GLP-1 analogue) (76). In theory, this drug could have the

potential to influence the expression of Gal-4 and thus speed up the process of CMD risk reduction in individuals with obesity. An RCT with an endpoint of analysing Gal-4 levels in individuals with obesity receiving treatment with liraglutide or other incretin-based drugs (semaglutide, tirzepatide) compared to placebo could thus be of value. Furthermore, Gal-4 has a potential inflammatory role in the intestinal mucosa. Previous studies have linked obesity and diabetes to altered composition of the gut microbiota (223, 224). Changes in gut microbiota, i.e., through an unhealthy diet, lead to damage of the intestinal barrier, promote leakage and thus endotoxemia through higher levels of lipopolysaccharides systemically, which in turn stimulates the development of low-grade systemic inflammation associated with the negative impact of both obesity and metabolic disorders (223). Therefore, Gal-4 might, at least in theory, aggravate the pathological processes induced by the obese-diabetic microbiota. Further studies, elucidating on this hypothesis would be of interest.

Finally, there is demand for additional epidemiological studies which may identify determinants and modifiable risk factors for better prevention of the conversion from an obesity phenotype with reduced CMD risk to an increased risk of CMD. However, to be able to present comparable results, the definition of metabolic health in obesity should be harmonized. In this thesis an alternative has been proposed based on a more stable definition of overall health in subjects with obesity through a non-hospitalized status for somatic disease until upper mid-life.

Populärvetenskaplig sammanfattning (summary in Swedish)

Fetma utgör ett välkänt folkhälsoproblem av ökande betydelse i västerländska befolkningar, och är en del av en modern s.k. *syndemi* (synergism mellan fetma, malnutrition och klimatpåverkan), med stigande medel-BMI även i den svenska befolkningen. Ett flertal kroniska sjukdomar associeras med fetma, framför allt bukfetma, som till exempel typ 2 diabetes, hypertoni, kardiovaskulär sjukdom, artros, samt vissa cancerformer, förutom psykiska besvär som antingen primärt eller sekundärt kan associeras med fetma. Fetma innebär dessutom mycket stora kostnader för såväl hälso- och sjukvården som för samhället.

Trots denna kända problematik finns det även en rad paradoxer med fetma som förefaller att vara ett betydligt mer heterogent fenomen än vad som hittills klargjorts. Det har bland annat visat sig att fetma medför en bättre prognos jämfört med normalvikt eller undervikt vid redan manifest sjukdom som hjärtsvikt, KOL, demens och liknande tillstånd, där en snabb viktnedgång kan signalera dålig prognos (till exempel kardiell kakexi). Den prognostiska betydelsen av bukfetma vid hjärtsvikt är fortfarande oklar och hos hjärtsviktspatienter med fetma och samtidig typ 2 diabetes (metabolt sjuka) ses ingen bättre prognos. Samtidigt har flera studier visat att fetma ökar risken att utveckla hjärtsvikt. Fler framåtblickande studier behövs för att klarlägga dessa samband.

Man har även under senare år diskuterat konceptet om "metabolt frisk fetma" (Metabolically Healthy Obesity; MHO), och ifall detta fenomen existerar i befolkningen eller inte. Hittills har studier kring MHO baserats på avsaknad av riskfaktorer för det metabola syndromet (ett syndrom associerat med ökad risk för utveckling av typ-2 diabetes och hjärtkärlsjukdom) hos individer med övervikt/fetma, men åsikterna går isär om en reducerad risk verkligen kvarstår över tid eller inte.

Det var därför av intresse att närmare studera fetma som riskfaktor för kardiometabol sjukdom och prognos bland metabolt friska och sjuka individer i befolkningsstudier från Malmö.

I kohort-studierna inkluderat i denna avhandling (Malmö Kost Cancer, MKC, och Malmö Förebyggande Medicin, MFM) har vi använt oss av en ny definition baserat på avsaknad av sjukhusinläggningar för kroppslig sjukdom under några decennier i

medelåldern som ett kriterium på att individer med MHO inte drabbas av behandlingskrävande sjukdomar.

Liksom ovan nämnt, utnyttjades två studiepopulationer i denna avhandling. I MKC (delarbete 1-III) ingår 28 098 män och kvinnor från Malmö som undersöktes 1991–1996. Syftet med denna kohort-studie var att undersöka kostens påverkan för att utveckla cancer, men kom senare till att inkludera många fler utfallsvariabler såsom kardiovaskulär sjukdom och diabetes. Varannan individ från MKC blev återinbjuden, mellan 1992–1994, att delta i en kardiovaskulär delkohort, kallad MKC-kardiovaskulära armen (MKC-KV, n=6 103). Deltagarna genomgick mer extensiva utredningar såsom ultraljud av halskärl och ytterligare laboratorieanalyser av blodprov. I MFM ingår 33 346 män och kvinnor som under 17 år (start 1974) inkluderades i ett primärpreventivt projekt vars syfte var att studera riskfaktorer för hjärt-kärlsjukdom i en representativ population från Malmö. Vid baslinjen genomfördes bland annat kroppsundersökning, blodprovstagning och ifyllning av frågeformulär om livsstil. Efter cirka 25 år erbjöds de kvarlevande deltagarna att komma till en liknande återundersökning där 18 240 individer deltog. Här valdes 1 792 individer ut slumpmässigt och genomgick hjärtultraljud, EKG och utvidgad blodprovstagning – ifrån denna delkohort inkluderades individer till delarbete IV.

I delarbete I undersöktes, i MKC och MKC-KV, karakteristika och prognos för dödlighet och hjärtkärlhändelser hos individer klassificerade som MHO enligt vår egen definition. Här kunde vi redovisa att MHO individer kännetecknades av en mer gynnsam riskfaktorprofil (bland annat en lägre grad av stillasittande samt lägre nivåer av blodfetter och blodsocker) samt dessutom en bättre prognos för både dödlighet och hjärtkärlhändelser jämför med individer med metabolt sjuk fetma (Metabolically Unhealthy Obesity, MUO). Vidare var risken för MHO individer, för såväl dödlighet som antal hjärtkärlhändelser, jämförbar med den hos hela gruppen kontrollindivider utan obesitas.

I delarbete II byggde vi vidare på resultaten från delarbete I, genom att närmare undersöka kopplingar mellan MHO individer i MKC-KV och utvidgade analyser av totalt 432 olika biomarkörer (proteomik, metabolomik och lipidomik). Huvudfyndet var att MHO individerna hade en mer gynnsam, lipid- och proteinprofil jämfört med MUO.

För delarbete III startades under 2021 en studie av samma population i MKC-KV med analyser av ett specifikt protein, kallat IgM anti-fosfokolin antikroppar. Höga nivåer av dessa anti-inflammatoriska antikroppar har visat sig vara skyddande mot bland annat hjärtkärlsjukdom. Här kunde vi visa att sjukhusvårdade individer med fetma (hospitalized subjects with obesity, HO) hade lägre nivåer av vissa av dessa antikroppar jämfört med icke-sjukhusvårdade individer med fetma (non-hospitalized subjects with obesity, NHO).

Slutligen, i delarbete IV, analyserades 92 proteiner från plasmaprover ur en biobank valda utifrån association med kardiometabola egenskaper från Olink

kardiometabola proteomik-panel III i MFM. Här undersöktes huruvida nivåer av dessa metabola/katabola biomarkörer skiljer sig mellan HO och NHO och om de kan ha olika prognostiska betydelse. Vi fann att höga nivåer av Galectin-4 (ett protein som associeras till bland annat diabetes) ökade sannolikheten att klassificeras som HO men också att högre nivåer associerades till högre risk för utveckling av diabetes.

Slutsatsen i denna avhandling är således att individer med fetma som tidigare inte har vårdats på sjukhus förefaller ha en lägre risk än förväntat att utveckla hjärtkärlsjukdom och förtidig död jämfört med andra individer i samma viktkategori som har vårdats på sjukhus. Faktorer som kännetecknar dessa individer med reducerad risk är en mer gynnsam glukos-, lipid- och proteinprofil, en högre grad av fysisk aktivitet, en nedreglering av potentiellt skadliga biomarkörer samt högre nivåer av specifika anti-inflammatoriska antikroppar.

Om individer med fetma och som förefaller ha en lägre hjärtkärlrisk, bättre skulle kunna karakteriseras så kan detta ge vården nya möjligheter att mer inrikta sina resurser på hjärtkärlsjuka individer. Vidare behövs en ökad förståelse av detta fenomen eftersom den kliniska prognosen vid hjärtkärlsjukdom och andra liknande tillstånd verkar skilja sig åt hos patienter med olika grad och typ av fetma. Mekanismer bakom dessa observationer bör närmare studeras baserat på röntgentekniker och ultraljudsundersökningar, genetik, biomarkörer och avancerad fenotypning, d.v.s. kartläggning av kroppsfunktioner.

Errata

Paper II

In figures 3, 5 and 6 there has been a misplacement of the describing texts (legends to Figures). Correct describing text are the following:

- Figure 3 and describing text of figure 6
- Figure 5 and describing text of figure 3
- Figure 6 and describing text of figure 5

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Paper I

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Original Article

Metabolically healthy obesity (MHO) in the Malmö diet cancer study – Epidemiology and prospective risks

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ABSTRACT

Background/aims: Metabolically healthy obesity (MHO) remains controversial, since the underlying mechanisms behind this phenotype remain unclear. We aimed to investigate the characteristics of MHO, as well as prospective risks.
Method: A cross-sectional analysis was carried out in a subsample of 3812 obese subjects selected from the Malmo diet cancer study (n = 28,403). Subjects with MHO (n = 1182) were defined by having no records of hospitalization for somatic disorders prior to baseline examination. MHO subjects were further compared to subjects with metabolically unhealthy obesity, MUO (obese individuals with at least one recorded hospitalization: n = 2630), and all non-obese cohort controls (NOC; n = 24,591). Moreover, prospective risk analyses for incident cardiovascular (CV) morbidity and mortality were carried out.
Results: Compared to MUO individuals, MHO individuals reported a significantly lower proportion of sedentary life style (p = 0.009), but also significantly lower HbA_{1c} (p = 0.012), fasting glucose (p = 0.001) and triglyceride levels (p = 0.011) than MUO. Cox-regression analysis (follow-up 20 ± 6 years) showed both a significantly lower all-cause mortality risk for MHO individuals as compared to MUO (p = 0.001), as well as lower incident CV morbidity risk (p = 0.001). When comparing MHO individuals to NOC, there were no significant differences in neither mortality risk nor incident CV morbidity risk.
Conclusion: Compared to MUO individuals, MHO individuals presented with a higher level of physical activity, a more favorable lipid- and glucose profile and a lower prospective risk of total mortality and CV morbidity during 20-years follow-up. Notably, no significant differences could be seen in mortality and CV morbidity risks when comparing MHO subjects to non-obese controls.

Introduction

Obesity has become a growing global epidemic, contributing to the risk of developing numerous chronic diseases including cardiovascular disease (CVD) and diabetes type 2 (DM2) [1]. Furthermore, it has been well established that overweight and obesity are associated with a higher all-cause mortality risk compared to normal-weight subjects [2]. At least since the 1980s the prevalence of obesity and overweight individuals has been steadily increasing, today representing more than one third of the general population [3]. The definition of obesity is a Body Mass Index (BMI) ≥30 kg/m^2. Even if this measurement is not without critical remarks, it still is one of the most accurate assessment for predicting CVD mortality in overweight individuals [4].

In recent years, a controversial debate has arisen, discussing the heterogeneity of obesity and that some obese individuals might be less negatively influenced by their excess weight than others [5–7]. A phenomenon, known as the *obesity paradox*, suggests that

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; CV, cardiovascular; DM2, diabetes type 2; HF, heart failure; IR, Incidence rate; MDCS, Malmö diet cancer study; MDCS-CV, Malmö diet cancer study – cardio vascular arm; MHO, Metabolically healthy obesity; MHNW, Metabolically healthy normal weight; MUO, Metabolically unhealthy obesity; MUNW, Metabolically unhealthy normal weight; NOC, Non-obese cohort controls; SBP, Systolic blood pressure; SD, Standard deviation.

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many types of CVD, especially heart failure (HF), may have a better prognosis in the overweight or even obese patients compared to their leaner counterparts [8]. This could in part be explained by the fact that many of these disease conditions (e.g. HF) are associated with a chronic catabolic state, where lean body mass loss carries a negative prognosis, hence the term cardiac cachexia [9].

Recently the concept of 'Metabolically Healthy Obesity' (MHO) has been described, based on the absence of risk factors of the Metabolic syndrome [5,10], reporting obese but metabolically healthy individuals with a more favorable inflammatory and metabolic profile [11]. Other studies that support this notion, further describe MHO individuals having a higher degree of physical activity (PA) and cardiorespiratory fitness (CRF) compared to their unhealthy counterparts – thus supporting a concept known as *fat but fit* [12]. Additionally, obesity has been linked to a state of chronic inflammation, leading to insulin resistance and disruption of other aspects of the energy homeostasis [13] – this may be downregulated in obese individuals considered metabolically healthy, but is yet to be proven. Nevertheless, a systematic review and meta-analysis showed that MHO individuals did run an increased risk of future CV events, compared to Metabolically Healthy Normal Weight (MHNW) individuals, but not increased risk of all-cause mortality [6]. However, other studies reported that relative risks for developing CVD was not significantly higher among individuals with MHO, compared to MHNW individuals [14]. Lastly, a recent systematic review and meta-analysis did show that MHO subjects had an increased risk for all-cause mortality as well as development of CV disease compared to MHNW subjects [15].

Thus, when considering the aforementioned contradicting studies, there is a need to better understand the underlying mechanisms behind obesity, as well as its heterogeneity, to be able to address the increasing prevalence and incidence of obesity in the world. By mapping MHO, we can gain a deeper understanding of risk determinants for obesity as well as biological mechanisms, life style and social factors, and in the extension how it could be treated causally and individualized.

The *aim* of this observational study is to describe determinants, characterizing patterns and prognosis for CVD and mortality among middle-aged individuals with MHO. We will compare individuals with MHO and metabolic unhealthy obese (MUO) individuals, when groups are defined by a history of long-term non-hospitalization for somatic disease versus hospitalization, respectively, but also with non-obese controls (NOC).

Subjects

The Malmö Diet Cancer Study (MDCS) started with baseline examination between 1991 and 1996 at the University Hospital in Malmö, Sweden, with the main goal to study the contribution of dietary patterns to cancer incidence and mortality. Men born 1923–1945 and women born 1923–1950, at the time residing in Malmö, were invited to participate. In all, 28,098 subjects participated (2/3 women, 41% attendance rate) [16] (see Fig. 1). At baseline, the participants were examined with anthropometric measurements, dietary assessment, a self-administered questionnaire and blood samples. A detailed description of the baseline investigations has been previously published [16,17].

Every second individual of MDCS was re-invited to participate in a cardiovascular sub-cohort of MDCS, between 1992 and 1994, (MDC-CV; n = 6103). The primary aim was to study the epidemiology of carotid artery disease, including ultrasound examination and laboratory analyses of additional fasting blood samples [18,19].

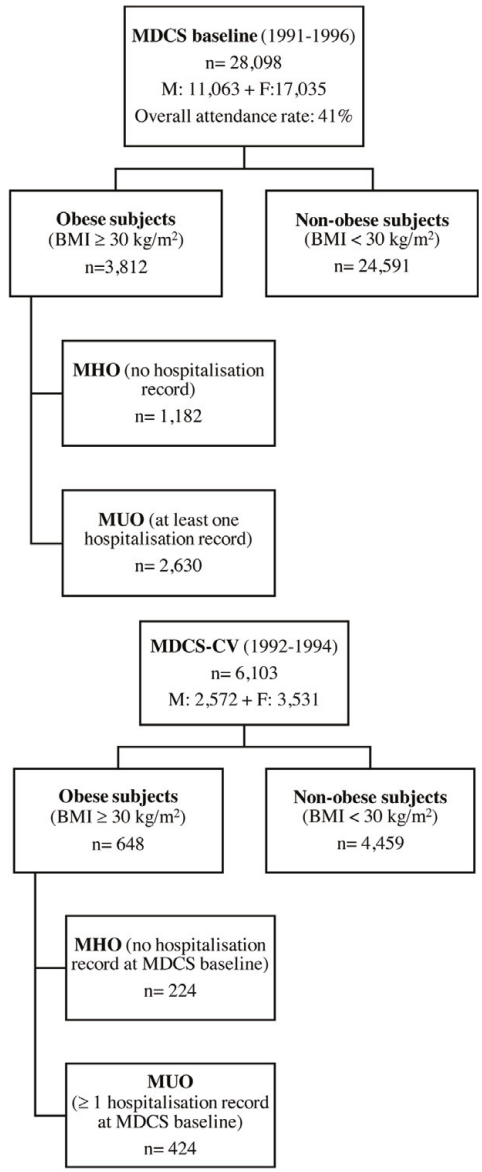

Fig. 1. Flow charts of (a) the MDCS baseline cohort and (b) the MDCS-CV sub-cohort stratified for obese and non-obese subjects respectively.

Metabolically healthy obesity (MHO)

All obese individuals (BMI ≥ 30 kg/m^2) from the MDCS baseline examination, were selected to be included in the study (n = 3812; 13.5%) (see Fig. 1). These individuals were sub-divided into two different groups, depending on absence or presence of hospitalization for somatic disease, as recorded in the Swedish National Hospital Inpatient Register, up until the baseline inclusion in MDCS. Hospitalization due to normal deliveries and external injuries/intoxications were considered non-hospitalization for our aim. Obese individuals with no recorded history of hospitalization before baseline were considered MHO (n = 1182; 4%), whereas obese individuals with at least one recorded history of hospitalization were considered MUO (n = 2630; 9.5%). These two groups were further compared with non-obese controls, NOC (n = 24,591), from the cohort. This novel approach of defining MHO individuals, has been previously applied in another local cohort from the same population [20].

Methods

Physical examination

At the MDCS baseline, all participants were examined for weight (kg) and height (cm) without shoes and in light indoor clothing and BMI was calculated (kg/m^2). Waist and hip circumference (cm), including waist-to-hip ratio, was measured in the standing position without clothing. Moreover, lean body mass and body fat was assessed by a bioelectrical impedance method. Furthermore, right arm blood pressure (mmHg) was measured twice in the recumbent position after a 5-minute rest, using the Korotkoff phase V [16,17,21].

Laboratory data

Fasting venous blood samples were drawn and stored at the biological bank −80 °C, but only in participants (n = 5540) from MDCS-CV. Laboratory blood tests for high sensitivity (hs) C-reactive protein (hsCRP) (mg/L), HbA_{1c} (%), fasting blood glucose (mmol/L), triglycerides, total cholesterol, LDL and HDL cholesterol (mmol/L), were analysed at the Department of Clinical Chemistry, Skåne University Hospital in Malmö, participating in a national standardisation and quality control system [19].

Questionnaire

The participants filled out a questionnaire including family history, demographic and socio-economic variables (including marital status and educational level), social network and support, previous and current occupation, recent stress exposure or mental problems, smoking status (yes, regularly/yes, occasionally/no, stopped smoking/no, never smoked), alcohol usage (g/day), medical history (previous and current diseases), and medications [21].

Leisure time physical activity

Method used, at the MDCS-baseline, was adapted from the Minnesota Leisure Time Physical Activity Questionnaire. The participants were asked, in the questionnaire, to report the amount of physical activity during their leisure time by being presented a list of eighteen different activities – they were then asked to fill in how many minutes per week they spent, on average, on each activity. The result was then multiplied using an activity-specific intensity coefficient, where the product was called a physical activity score. The variable for physical activity during leisure time provided the following answer alternatives: sedentary spare time (category 1), moderate exercise in spare time (category 2), regular exercise and training (category 3), and hard training or competition sport (category 4). This was further computed into binary (sedentary = 1/active = 2–4), creating a variable called sedentary spare time (%) [21].

Register end-point data

All individuals were followed from baseline until the first CV-event, death (obtained from Swedish total population register Statistics Sweden [SCB]), migration or end of study December 31st 2016. Endpoints were retrieved through the Swedish Inpatient Registry and the Causes of Death register, administrated by the Swedish National Board of Health and Welfare. Furthermore, the definition of a stroke event was additionally supplemented by information through the local STROMA-register with its Relapse-register [22]. The definition of an incident CV-event (fatal or non-fatal) included *myocardial infarction* and *ischaemic heart disease* (ICD-9 codes 411–414; and ICD-10 codes I20, I24, I251–I259), *stroke* (ICD-9 430–434, 436 and ICD-10 I60–I64), *heart failure* (ICD-9 428 and ICD-10 I50, I11.0) and *atrial fibrillation/flutter* (ICD-9 427D, 4273 and ICD-10 I48).

Statistical methods

De-identified epidemiological data was analyzed using descriptive statistical methods, comparing MHO individuals to both MUO individuals and NOC. Analysis of the difference in continuous variables was made by one-way ANOVA, whereas dichotomous and category variables were analyzed through using Mann–Whitney U-test and Chi-squared test.

The values of the variables for smoking habits (1–4) were computed into binary (No: 1/ Yes: 2–4). Moreover, the variable for physical activity in free time (1–4) variable was computed into binary (sedentary: 1/ active: 2–4). In addition, a prospective risk analysis of mortality incidence and incident cardiovascular morbidity, from the time of start-up until the end of follow-up, was performed using Cox-regression analysis. All statistical analyses were made using IBM SPSS Statistics version 25 (SPSS, Chicago, IL, USA). Statistically significance level was set at p-value less than 0.05.

Results

MHO versus MUO subjects

Compared to MUO individuals (one-way ANOVA) MHO individuals were younger (58 ± 7 years vs. 60 ± 7 years; p = 0.001) and more likely to be male (41.2% vs. 37.1%; p = 0.016). Additionally, MHO individuals had a significantly lower BMI (MHO 32.6 kg/m^2 vs. MUO 33.1 kg/m^2; p = 0.001) as well as lower waist and hip circumference (p = 0.001), but no significant differences could be seen in the waist/hip ratio. No statistically significant difference in mean blood pressure was seen between the two groups. Moreover, MHO individuals reported a significantly lower proportion of sedentary life style than MUO (17.4% vs. 21.9%; p = 0.009), and were more likely to hold a university degree (13.4% vs. 9.4%; p = 0.003). MUO individuals were more likely to be ever smokers (MUO 61.8% vs. MHO 56.3%; p = 0.008) but no significant difference was seen in alcohol consumption (p = 0.3). Furthermore, MHO individuals had significantly lower HbA_{1c} (p = 0.012), fasting plasma glucose (p = 0.001) and triglyceride levels (p = 0.011), as compared to their MOU counterparts. No significant difference could be seen in cholesterol (total cholesterol, HLD-C and LDL-C) or hsCRP levels. See Table 1 for more detailed results.

Table 1
Descriptive comparison and significance testing for MHO (n = 1182) compared to MUO subjects (n = 2630); and MHO compared to NOC subjects (n = 24,591), with standard deviation (SD) or percentage (%) for metric and categorical variables, respectively.

Variable	MHO	MUO	p-Value	NOC	p-Value
Anthropometric data (MDCS)					
N	1182	2630		24,591	
Gender (men, %)	487 (41.2)	975 (37.1)	0.016	9,754 (39.7)	0.306
Age (years, SD)	58 (7.2)	60 (7.4)	<0.001	58 (7.61)	0.026
BMI (kg/m^2, SD)	32.6 (2.6)	33.13 (3.06)	<0.001	24.64 (2.76)	<0.001
Waist (cm, SD)	99.7 (11.7)	101.22 (11.42)	0.002	81.66 (13.17)	<0.001
Hip (cm, SD)	111.2 (7.7)	112.47 (9.36)	<0.001	96.33 (6.7)	<0.001
Waist/hip ratio (n (25–75)) median (25–75)[a]	0.88 (0.82–0.98)	0.89 (0.83–0.98)	0.185	0.83 (0.77–0.92)	<0.001
SBP (mmHg, SD)	149 (18.6)	148 (19.13)	0.889	140 (19.9)	<0.001
DBP (mmHg, SD)	91 (9.4)	90 (9.59)	0.061	85 (9.84)	<0.001
Outcomes (MDCS)					
Mortality (n, %)	422 (36.3)	1201 (46.6)	<0.001	8,178 (33.6)	0.066
Incident CV-event (n, %)	260 (22.3)	749 (29)	<0.001	4,957 (20.4)	0.109
Incident rate for CV-event[b]	18.1	25.3	–	17.0	–
Prevalent diabetes (n, %)	82 (6.9)	296 (11.3)	<0.001	883 (3.6)	<0.001
Social and lifestyle data (MDCS)					
N	800	1612		17,837	
Smoking current or past, (n, %)[c]	450 (56.3)	997 (61.8)	0.008	10,896 (61.1)	0.006
Regular smoking (years, SD)	21.2 (13.5)	24.5 (13.8)	<0.001	23.2 (14.1)	0.006
Alcohol intake (g/day)	11.08 (14.8)	10.25 (15.44)	0.282	11.14 (12.25)	0.988
Sedentary leisure time, (n, %)[d]	139 (17.4)	353 (21.9)	0.009	1 899 (10.6)	<0.001
Physical Activity Score (SD)	7933 (5660)	7300 (5881)	0.079	8,417 (6759)	0.119
University degree, (n, %)[e]	107 (13.4)	152 (9.4)	0.003	2 825 (15.8)	0.065
Married, n (%)[f]	506 (63.2)	997 (61.8)	0.503	11,504 (64.5)	0.472
Laboratory data (MDCS-CV)					
N	224	424		4459	
Total cholesterol (mmol/L, SD)	6.25 (1.13)	6.30 (1.13)	0.845	6.14 (1.06)	0.293
hsCRP (mg/L, SD)	0.37 (0.44)	0.45 (0.58)	0.215	0.24 (0.42)	<0.001
HDL-C (mmol/L, SD)	1.24 (0.30)	1.20 (0.29)	0.287	1.41 (0.37)	<0.001
LDL-C (mmol/L, SD)	4.96 (0.88)	4.31 (1.04)	0.989	4.15 (0.98)	0.105
Triglycerides (mmol/L, SD)	1.59 (0.72)	1.76 (0.76)	0.011	1.26 (0.60)	<0.001
Fasting glucose (mmol/L, SD)	5.49 (1.36)	5.90 (1.92)	<0.001	5.06 (1.19)	<0.001
HbA_{1c} (%, SD)	5.09 (0.78)	5.77 (1.08)	0.012	4.85 (0.67)	<0.001
Drug treatment and diabetes (MDCS-CV)					
BP-lowering drugs (n, %)	53 (52 %)	178 (57 %)	0.108	676 (33 %)	0.003
Lipid-lowering drugs (n, %)	2 (2 %)	22 (7 %)	0.083	105 (5 %)	0.235

[a] Interquartile range (IQR) 25–75.
[b] Incident rate for any cardiovascular event per 1000 person-years.
[c] Dichotomized to 0 = no, never. 1 = yes, regularly; yes, occasionally; no, stopped smoking.
[d] Dichotomized to 0 = moderate exercise in leisure time; regular exercise and training; hard training or competition sport. 1 = sedentary leisure time.
[e] Dichotomized to 0 = no university degree. 1 = university degree.
[f] Dichotomized to 0 = unmarried. 1 = married.

MHO versus NOC subjects

When comparing MHO individuals to NOC subjects, differences could be seen in blood pressure, where NOC had a significantly lower systolic and diastolic blood pressure (140/85 ± 19.9/9.8 vs. 149/91 ± 18.6/9.4 mmHg; p = 0.001). Furthermore, NOC had a more favorable glycaemic profile with both significantly lower HbA_{1c} (NOC 4.85% ±0.67 vs. MHO 5.09% ±0.78; p = 0.001) and fasting blood glucose (p = 0.001). The inflammatory status, defined by measuring hsCRP, was lower in NOC (0.24 ± 0.42 vs. 0.37 ± 0.44 mg/L; p = 0.001). More MHO subjects had antihypertensive drugs and diabetes than NOC subjects. See Table 1 for more detailed results.

Prospective risk of all-cause mortality and cardiovascular events

Incident rate (IR) for developing cardiovascular disease during 1000 person years was significantly lower for MHO individuals (18.1) compared to MUO subjects (25.3). Additionally, when comparing the IR between MHO and NOC (17.0), there were no significant differences. Cox-regression analysis adjusted for age, gender, smoking, blood pressure, sedentary behavior and waist/hip ratio (mean follow-up time 20 ± 6 years) showed a significantly lower all-cause mortality risk for MHO individuals as compared to MUO, HR 0.74 (95% CI: 0.66-0.82; p = 0.001), as well as lower total incident CV morbidity risk, HR 0.69 (95% CI: 0.60-0.80; p = 0.001). Interestingly, when comparing MHO individuals to NOC, there were no significant differences in neither mortality risk (p = 0.9), nor incident CV morbidity risk (p = 0.7), see Table 2 for additional data. Unadjusted Kaplan Meier curves presenting all-cause mortality risk and incident CV-event risk for MHO, MUO and NOC are shown in Figs. 2 and 3 respectively.

Discussion

The concept of MHO was approached by a newly adopted definition as previously described [20]: i.e. obese individuals with the absence of hospitalization for somatic disease up until approximately 60 years of age (the average age of MHO individuals being 58 ± 7 years). The key findings from our study include MHO individuals having a more metabolically favorable profile (lower levels of fasting blood glucose and HbA_{1c}, as well as lower triglyceride levels) than their MUO counterparts. There were no significant differences in the waist/hip ratio between MHO and MUO individuals, implying that the abdominal fat distribution did not significantly differ between the two groups. Furthermore, when examining social and lifestyle data, MHO subjects were characterized by less seden-

Table 2
Mortality risk in MHO (n = 1182) vs. MUO (n = 2630) and NOC (n = 24,591) subjects until end of follow-up. Cox regression analysis with 95% confidence intervals.

Variables	HR	95% CI for HR		p-Value
		Lower	Upper	
Total mortality risk				
MHO vs. MUO	0.80	0.70	0.92	0.001
Smoking status	1.20	1.05	1.37	<0.001
Gender (female)	0.71	0.62	0.81	<0.001
Age (years)	1.11	1.10	1.12	<0.001
SBP (mmHg)	1.01	1.00	1.01	0.003
Sedentary leisure time (%)	1.42	1.24	1.63	<0.001
Waist/hip ratio	3.00	2.03	4.34	<0.001
CV event risk[a]				
MHO vs. MUO	0.77	0.65	0.92	0.003
Smoking status	0.97	0.82	1.14	0.708
Gender (female)	0.58	0.48	0.69	<0.001
Age (years)	1.07	1.05	1.08	<0.001
SBP (mmHg)	1.01	1.01	1.02	<0.001
Sedentary leisure time (%)	1.31	1.10	1.57	0.003
Waist/hip ratio	2.27	1.31	3.92	0.003
Total mortality risk				
MHO vs. NOC	0.95	0.84	1.07	0.358
Smoking status	1.50	1.42	1.59	<0.001
Gender (female)	0.84	0.78	0.90	<0.001
Age (years)	1.12	1.12	1.13	<0.001
SBP (mmHg)	1.01	1.01	1.01	<0.001
Sedentary leisure time (%)	1.71	1.60	1.83	<0.001
Waist/hip ratio	4.29	3.12	5.91	<0.001
CV event risk[a]				
MHO vs. NOC	0.95	0.82	1.10	0.462
Smoking status	1.29	1.20	1.38	<0.001
Gender (female)	0.68	0.63	0.74	<0.001
Age (years)	1.08	1.07	1.08	<0.001
Systolic blood pressure (mmHg)	1.01	1.01	1.01	<0.001
Sedentary leisure time (%)	1.38	1.26	1.52	<0.001
Waist/hip ratio	4.38	3.23	5.94	<0.001

Values are presented as hazard ratios (HR) with 95% confidence interval (95% CI). SBP = systolic blood pressure.
[a] Incident CV-events, excluding individuals with prevalent CV-events.

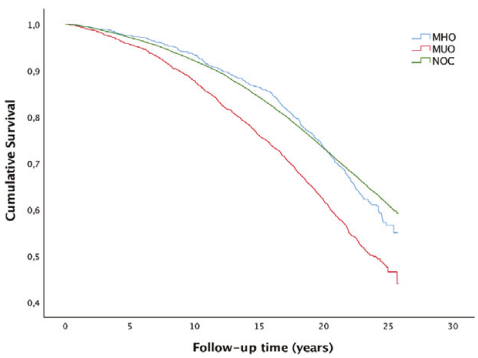

Fig. 2. All-cause mortality risk for MHO (n = 1182), MUO (n = 2630) and NOC (n = 24,591) respectively.

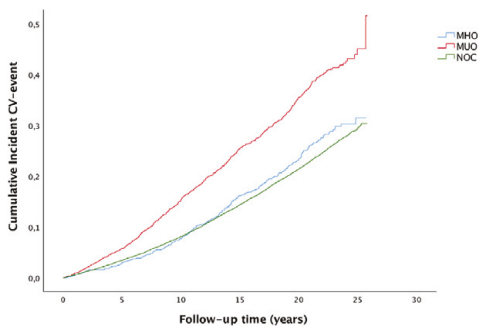

Fig. 3. Incident CV-event risk for MHO (n = 1182), MUO (n = 2630) and NOC (n = 24,591) respectively.

tary behavior, a lower proportion of smokers and additionally a higher educational level than MUO subjects. These traits could play an important role when analyzing the prospective risks, which revealed a significantly lower mortality risk for MHO individuals along with a lower risk of incident, non-fatal CV events, compared to MUO.

On the other hand, when comparing MHO with NOC individuals the latter displayed a more benign metabolic status, with lower levels of glucose, inflammatory protein and lipids in their blood and additionally lower blood pressure, apart from having a lower BMI. Despite these differences, prospective risk analyses (mean follow up-time 20 ± 6 years) for both all-cause mortality and incident CV events could not detect any significant differences between these two groups.

The concept of MHO has been eagerly debated during recent years, casting doubt on its mere existence. Even the media has tried to illustrate this phenomenon, citing findings from the *Nurses' Health Study*, supporting the notion that there is still a significantly higher risk of developing CV disease in obese individuals regardless

of metabolic health status [23]. Furthermore, accumulating evidence is clarifying the MHO phenotype, based on the absence of risk factors, to be a transient state [23–25], where MHO with time will transform into MUO. We hypothesize, however, that in some individuals this phenotype (MHO) is perhaps more stable than in others based on a more strict definition of MHO. Thus, an evident pitfall is how MHO is defined. In general, the definition of MHO focuses on the presence or absence of the metabolic syndrome (MetS) or whether the individual has developed a certain number of risk factors for MetS or not [26]. Furthermore, another common way of defining MHO is based upon insulin resistance levels by using the insulin sensitivity Index (HOMA-IR), with a certain cut-off point [27,28]. The dilemma of these definitions is that many of the risk factors involved and used to define MetS (i.e. triglyceride- and HDL-C levels, fasting glucose), shift intra-individually during repeated measurements at different time points. Moreover, accumulating evidence points out that the absence of MetS in obese subjects is not an entirely harmless condition [5].

By using this definition of MHO as being non-hospitalized for somatic disease up until midlife in spite of obesity, i.e. the MDCS baseline examination, we were able get an objectively defined phenotype which could serve as an alternative to the conventional way of defining MHO. Not to be overlooked, some individuals might be treated for chronical illnesses in a primary care unit and thus avoiding inpatient care; however, the outpatient care in Sweden up until the 1990's was limited [29] and not as developed as presently.

There are several limitations of this study. First, the poor overall attendance rate at the MDCS baseline examination (41%) could imply a health selection bias. Furthermore, there is an imbalance of gender, with a majority of women (61%) adding to the selection bias and thus not being fully representative of the local population. When gathering social and lifestyle information at baseline, reporting biases cannot be excluded. We also acknowledge that BMI was only measured at the baseline examination and that this variable indeed could shift intra-individually over time, but mostly as an increase in mid-life. Additionally, BMI does neither measure body composition nor fat distribution [30]. Lastly, another limitation of the study was that subjects with non-hospitalisation prior to baseline could still have prevalent hypertension or diabetes, two risk factors for clinical events and not really compatible with the concept of MHO. On the other hand, these conditions could have been milder or counterbalanced by protective mechanisms in the affected subjects leading to a status of "non-hospitalisation" in our analyses. Furthermore, we do not include data on risk factors during follow-up as the analyses were focused on clinical events only.

On the other hand, the MDCS is a large (n = 28,098), well-characterized prospective cohort, and not to forget, population-based, with a follow-up time of 20 years. Moreover, the MDCS-baseline and MDCS-CV sub-cohort have been linked to excellent national and well validated register data on hospitalization, why it was possible to apply our new approach to define MHO. Hospitalization as a marker of poor health could serve as a better indicator to describe an individual's health status than changing risk factor levels.

In this observational study we have shown obesity to be a heterogenous phenomenon, where certain obese individuals that escape hospitalization for somatic disease up until mid-life have a more benign prognosis than other obese subjects. At the same time, individuals characterized as MHO do not seem to have an increased risk of developing CV-disease, during a follow up time period of approximately 20 years, compared to non-obese individuals. This suggests an alternative interpretation as compared to several other studies [6,12,23,25,31], where subjects with MHO had an increased risk of developing CV-disease, when compared to MHNW individuals. What characterizes MHO, when compared to MUO (apart from a more favorable metabolic profile) is a less sedentary lifestyle. This supports the notion of MHO individuals being *fat but fit*, which is in line with a recent systematic review [12].

It would indeed be interesting to define MHO with a higher cut-off BMI value (i.e. BMI $\geq 35\ kg/m^2$), like in our previous study in another cohort [20], but too few individuals could be included when studying the MDCS-CV population. Likewise, it would be compelling to analyze MHO individuals with more precise body measurements, such as CT- and MRI-scanning for fat and muscle distribution. Furthermore, a meta-analysis describing MHO individuals' prospective risks would likewise be interesting; however, this requires additional studies adopting the same concept of a non-hospitalization status. Another interesting research aim would be to examine differences regarding socioeconomic factors, biomarkers and genetic variants between MHO and MUO subjects.

Conclusion

By applying a novel approach to define MHO as non-hospitalization for somatic disease individuals up until approximately 60 years of age, we observed a more favorable metabolic profile, a less sedentary lifestyle and a higher educational level compared to their MUO counterparts. Prospective risk analyses for all-cause mortality and incident CV morbidity confirmed this phenotype as more benign, as MHO individuals had, for both outcomes, a decreased risk compared to MUO. Interestingly, when comparing MHO to non-obese individuals, there were no significant differences in neither total mortality nor incident CV risk. Our results are in line with previous research in this field, based on other definitions, but differ regarding a more favorable incident CV risk for MHO individuals.

Conflicts of interest

The following authors report no conflict of interest: JK, AJ, MM, and PMN. One author is an employee of AstraZeneca: EB.

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Ethical statement

We have read and have abided by the statement of ethical standards for manuscripts submitted to the Obesity Research & Clinical Practice.

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Paper II

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Research Article

Proteomic and Metabolomic Characterization of Metabolically Healthy Obesity: A Descriptive Study from a Swedish Cohort

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Background/Aims. Obesity is a well-established risk factor for the development of numerous chronic diseases. However, there is a small proportion of obese individuals that seem to escape these aforementioned conditions—Metabolically Healthy Obesity (MHO). Our aim was to do a metabolic and biomarker profiling of MHO individuals. *Method*. Associations between different biomarkers (proteomics, lipidomics, and metabolomics) coupled to either MHO or metabolically unhealthy obese (MUO) individuals were analyzed through principal component analysis (PCA). Subjects were identified from a subsample of 416 obese individuals, selected from the Malmö Diet and Cancer study—Cardiovascular arm (MDCS-CV, $n = 3{,}443$). They were further divided into MHO ($n = 143$) and MUO ($n = 273$) defined by a history of hospitalization, or not, at baseline inclusion, and nonobese subjects (NOC, $n = 3{,}027$). Two distinctive principle components (PL2, PP5) were discovered with a significant difference and thus further investigated through their main loadings. *Results*. MHO individuals had a more metabolically favorable lipid and glucose profile than MUO subjects, that is, lower levels of traditional blood glucose and triglycerides, as well as a trend of lower metabolically unfavorable lipid biomarkers. PL2 (lipidomics, $p = 0.02$) showed stronger associations of triacylglycerides with MUO, whereas phospholipids correlated with MHO. PP5 (proteomics, $p = 0.01$) included interleukin-1 receptor antagonist (IL-1ra) and leptin with positive relations to MUO and galanin that correlated positively to MHO. The group differences in metabolite profiles were to a large extent explained by factors included in the metabolic syndrome. *Conclusion*. Compared to MUO individuals, corresponding MHO individuals present with a more favorable lipid metabolic profile, accompanied by a down-regulation of potentially harmful proteomic biomarkers. This unique and extensive biomarker profiling presents novel data on potentially differentiating traits between these two obese phenotypes.

1. Introduction

Although obesity is a well-established risk factor for the development of endemic modern Western public health problems, including cardiovascular disease (CVD) and type 2 diabetes (DM2) [1], accumulating evidence is suggesting that there is a small proportion of individuals with excess weight (body mass index (BMI) $\geq 30\,kg/m^2$) that seem to escape these aforementioned conditions—a concept known as Metabolically Healthy Obesity (MHO) [2, 3]. Along with this phenomenon, there has been a debate concerning the heterogeneity of obesity, and the negative consequences of excess fat seem to be more complex and individually patterned than previously thought [2, 4]. However, there is no doubt that obesity in the majority of cases represents a state of increased risk. Even in obese individuals defined as MHO that seem to experience less negative effect of their excess weight, increasing evidence is suggesting that this could be a

transient state and will eventually in due time transform into its unhealthier counterparts—Metabolically Unhealthy Obesity (MUO) [5–7].

There exists no agreed definition of MHO, but most studies on this topic suggest that it should involve a lack of risk factors for the metabolic syndrome (MetS) [8]. In a recent paper [9], we defined MHO as obese individuals (BMI $\geq 30\,kg/m^2$) who had never been hospitalized for a somatic disease before study baseline (at mean age of 56 years) and described the prognosis regarding incident CVD and mortality risk of MHO subjects compared to MUO and nonobese controls (NOC) in a Swedish cohort from the 1990s—the Malmö Diet and Cancer Study (MDCS; $n = 28{,}098$). Our findings suggested that MHO individuals had a significantly lower risk of total mortality and developing CVD during a 20-year follow-up period, compared to MUO individuals. Interestingly, no differences in prospective risks could be seen when comparing MHO to NOC individuals. Descriptive data from the study showed that MHO individuals presented with a less sedentary lifestyle, held a higher educational level, and displayed a more favorable glucose and lipid blood profile [9]. These descriptive findings were in line with earlier publications [10, 11], although our definition of MHO was novel and differed from previous ones [9].

There is still no clear explanation as to which factors contribute to the development of MHO contra MUO, but many theories exist. One common assumption is that a chronic inflammatory state, commonly associated with obesity, is downregulated in MHO individuals [12]. This in turn could be interpreted as influenced by less pronounced nonalcoholic fatty liver disease (NAFLD), determined by genetic factors and/or a diversity of the gut microbiota [4]. Other benign attributes that attract one's attention is the distribution patterns (peripheral vs. central obesity) [13] and the expandability of adipose tissue [14], as well as the glucose and triglyceride index [15, 16], but also specific biomarkers associated with obesity such as adiponectin [17] and neurotensin [18].

With this in mind, there is an urge to better understand the true benign nature of obesity presented in some selected individuals and the factors associated with it, to improve and individualize the treatment and care of individuals with excess body weight. From our recent paper [9], we concluded that a sedentary lifestyle and higher levels of blood glucose and lipids, combined with adverse lower socioeconomic conditions, contribute negatively to unhealthy obesity. Nonetheless, we would like to elucidate further on the descriptive profile of MHO individuals and thus analyze selected biomarkers associated with this specific phenotype.

Consequently, this observational study aimed to better characterize the metabolic profile of previously defined MHO individuals [9] by comparing plasma levels of metabolites (metabolomics and lipidomics) and circulating proteins (proteomics) between MHO, MUO, and NOC subgroups.

2. Materials and Methods

2.1. Subjects. A total of 28,098 individuals were selected (41% attendance rate) to participate in the baseline examination of the MDCS between 1991 and 1996, which included risk factor assessment through laboratory testing, physical examination, and a questionnaire. A detailed description of the inclusion criteria [9] and methodological aspects has been previously published [19,20]. In short, obese individuals (BMI $\geq 30\,kg/m^2$) were selected from the subcohort MDCS-Cardio Vascular arm (MDCS-CV). This subcohort was derived from the original MDCS, when every other individual included in the baseline examination was reinvited during 1992–1994 to participate in MDCS-CV ($n = 6{,}103$). The primary aim was to study the epidemiology of carotid artery disease, when also laboratory analyses of additional fasting blood samples were carried out [21, 22].

The number of included individuals was further reduced due to the lack of complete biomarker profiling data ($n = 3{,}443$). The obese individuals were then subdivided into two groups consisting of MHO and MUO, based upon the absence of hospitalization for somatic disease up until the inclusion at MDCS-baseline examinations (MHO) [9]. Hospitalization status was obtained through the Swedish National Hospital Inpatient Register, where external injuries/intoxications and normal deliveries were considered nonhospitalization and excluded. Furthermore, hospitalization status included only records for somatic disease. Obese individuals with no recorded history of hospitalization were considered MHO ($n = 143$), whereas MUO individuals were characterized as individuals with at least one record of hospitalization for somatic disease prior to inclusion at MDCS-baseline ($n = 273$). Moreover, selected MHO individuals were further compared with NOC subjects from the same subcohort ($n = 3{,}027$); see Figure 1 for a detailed flowchart. This novel approach of defining MHO individuals has been previously applied in the same cohort from an urban population [9].

2.2. Metabolite Profiling. Profiling of plasma metabolites was performed using a LC-QTOF-MS System (Agilent Technologies 1290 LC, 6550 MS, Santa Clara, CA, USA) and has previously been described in detail [23]. Briefly, overnight fasted citrate venous plasma samples stored at −80°C were thawed and extracted by addition of 120 μl extraction solution (80 : 20 methanol/water) to 20 μl plasma. The samples were then incubated at 4°C for 1 hour at 1250 rpm. After 15 min centrifugation at 14 000 g, 100 μl supernatant was transferred into a glass vial for analysis. Extracted samples were separated on an Acquity UPLC BEH Amide column (1.7 μm, 2.1 $*$ 100 mm; Waters Corporation, Milford, MA, USA). Metabolite identification, quality control, and normalization were performed as described previously [24].

2.3. Lipid Profiling. Lipid extraction of 1 μL of overnight fasted citrate plasma samples was stored at −80°C upon collection, followed by quantitative mass spectrometry-based lipid analysis. The analysis was performed at Lipotype GmbH using a high-throughput shotgun lipidomics technology [25]. Lipid identifiers of the SwissLipids database [26] (https://www.swisslipids.org) are provided in Table S1.

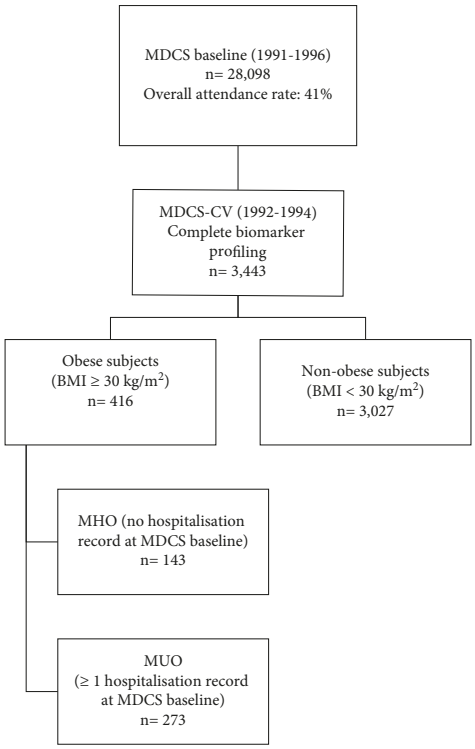

Figure 1: Flowchart of the MDCS-CV subcohort stratified for obese and nonobese subjects, respectively.

2.4. Protein Profiling. Fasting plasma levels of 136 proteins were measured using Olink Proseek Multiplex proximity extension assay (PEA) at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala, Sweden. PEA uses two oligonucleotide-labelled antibodies per protein, which form a PCR reporter sequence when both antibodies are bound to the target protein. The reported sequence is quantified by real-time quantitative polymerase chain reaction [27].

2.5. Statistical Analysis. Prior to statistical analysis, missing values for all biomarkers (maximum 20% missing allowed) were imputed using the NIPALS algorithm and were subsequently mean-centered and unit-variance scaled. Unsupervised dimension-reduction of each set of biomarker layers (metabolites, lipids, and proteins) was performed using principal component analysis (PCA). PCA was first performed for each biomarker layer in all obese participants and subsequently in all non-MUO participants in the same manner. For each biomarker layer, five principal components (PC) were calculated. In the participants with obesity, logistic regression models, adjusted for age and sex, were used to find associations between PCs and MHO (compared to MUO). PCs that were significantly associated with MHO were investigated for correlations with cardiometabolic risk factors using partial Spearman's correlation tests, adjusted for age and sex. Subsequent analysis in all non-MUO participants used logistic regression models to find associations between PCs and MHO (compared to NOC). All statistical analyses were performed in R 3.6.1. PCA and imputation were performed in the mixOmics [28] package and the partial Spearman's correlation tests in the ppcor [29] package. A p value <0.05 was considered significant.

3. Results

We observed differences in several cardiometabolic risk factors between subjects with MHO subjects compared to MUO (Table 1). MHO participants had a more favorable cardiometabolic risk factor profile compared to MUO, including lower BMI and waist circumference, proportion of prescribed antihypertensive drugs, and fasting levels of glucose and triglycerides, as well as higher levels of HDL cholesterol. Apart from lower BMI, NOC participants were characterized by lower waist circumference, systolic and diastolic blood pressure HbA_{1c}, proportion of antihypertensive drugs, and fasting levels of glucose, triglycerides, and LDL cholesterol, but higher levels of HDL cholesterol.

Biomarker profiles were constructed in participants with obesity (BMI > 30 kg/m^2), using PCA of three different biomarker layers, including either 112 metabolites, 184 lipids, or 136 proteins. The first five principal components (PC) in each biomarker layer could explain 41.8% of the metabolite variation, 63.8% of the lipid variation, and 53.1% of the protein variation, respectively (Table S2). To investigate whether the obesity biomarker patterns were related to MHO, all 15 biomarker PCs were analyzed using logistic regression models. The second lipid PC (PL2) (odds ratio, OR 1.06, $p = 0.018$) and the fifth protein PC (PP5) (OR 0.85, $p = 0.013$) were associated with MHO (Figure 2). PL2 was dominated by positive contributions from phospholipids, such as sphingomyelins and phosphatidylcholine ethers, and negative contributions from triacylglycerides (Figure 3). The strongest positive contribution to PP5 was interleukin-1 receptor antagonist (IL1-RA) followed by leptin and fatty acid-binding protein 4, while the strongest negative contributions were from galanin (Figure 4). Loadings for all PCs are presented in Tables S3–S5.

Both MHO-associated PCs were correlated with traditional cardiometabolic risk factors (Figure 5, Table S6). PL2 showed strong inverse correlations with plasma triglycerides (rho = −0.67, $p < 0.001$), HOMA-IR (rho = −0.36, $p < 0.001$), and glucose (rho = −0.32, $p < 0.001$) and strong positive correlations with HDL cholesterol (rho = 0.59, $p < 0.001$). PP5 was strongly correlated with CRP (rho = 0.36, $p < 0.001$) and waist circumference (rho = 0.26, $p < 0.001$) but inversely correlated with HDL cholesterol (rho = −0.27, $p < 0.001$). All correlations between MHO-related PC and cardiometabolic risk factors are depicted in Figure 5.

When adjusting for combined components of the MetS, according to National Cholesterol Education Program panel III (NCEP III) criteria [30] (systolic blood pressure, plasma glucose, HDL cholesterol, triglycerides, and waist

Table 1: Descriptive comparison and significance testing for MHO ($n = 143$) compared to MUO subjects ($n = 273$) and MHO compared to NOC subjects ($n = 3{,}027$), with standard deviation (SD) or percentage (%) for metric and categorical variables, respectively.

Variable	MHO ($N = 143$)	MUO ($N = 273$)	p	NC ($N = 3027$)	p
Age (years)	57.7 (5.7)	59.1 (5.9)	**0.02**	57.4 (6.0)	0.50
Sex (% women)	65.7	65.6	0.97	59.2	0.11
BMI (kg/m^2)	32.2 (2.3)	33.4 (3.34)	**<0.001**	24.6 (2.8)	**<0.001**
Waist (cm)	97.0 (12)	99.5 (12)	**0.04**	80.9 (11)	**<0.001**
SBP (mmHg)	150 (19)	148 (19)	0.51	140 (19)	**<0.001**
DBP (mmHg)	90.9 (9.7)	91.0 (9.4)	0.90	86.1 (9.2)	**<0.001**
Smoker (%)	21.1	15.8	0.19	27.6	0.07
AHT drug (%)	19.6	37.7	**<0.001**	14.1	0.11
Glucose (mmol/L)	5.53 (1.4)	5.90 (1.9)	**0.03**	5.09 (1.2)	**<0.001**
HbA_{1c} (%)	5.11 (0.82)	5.29 (1.0)	0.054	4.88 (0.68)	**0.001**
HOMA-IR	3.66 (7.5)	3.81 (4.0)	0.82	1.67 (1.9)	**0.002**
TG (mmol/L)	1.57 (0.72)	1.75 (0.80)	**0.02**	1.25 (0.60)	**<0.001**
HDL-C (mmol/L)	1.27 (0.32)	1.20 (0.28)	**0.03**	1.43 (0.38)	**<0.001**
LDL-C (mmol/L)	4.35 (1.1)	4.35 (1.1)	0.99	4.13 (0.97)	**0.02**
CRP (mg/L)	0.39 (0.50)	0.41 (0.44)	0.74	0.23 (0.40)	**<0.001**
MetS (%)	49.7	61.5	**0.03**	13.6	**<0.001**

AHT: antihypertensive treatment; DBP: diastolic blood pressure; SBP: systolic blood pressure: HbA1c: glycated haemoglobin; HOMA-IR: homeostatic model assessment for insulin resistance; CRP: C-reactive protein; MetS: metabolic syndrome.

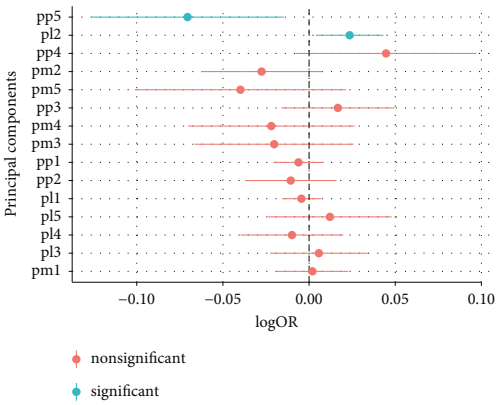

Figure 2: Logistic regression models, with significance testing, of the main PCs when comparing MHO (a) with MUO (b) subjects.

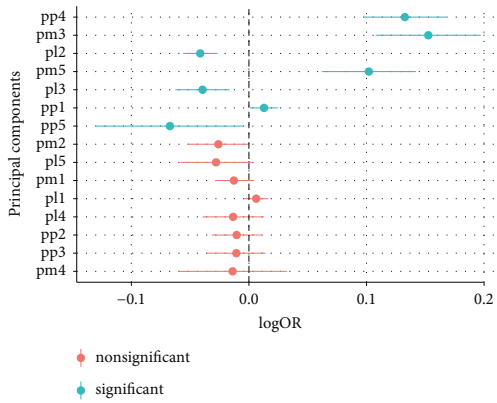

Figure 3: Main loadings for PL2, when comparing MHO with MUO.

circumference), no significant differences could be seen between the PCs. Strong contributors to differences in PL2 were HDL cholesterol and triacylglycerides (Table 2).

PCA of three different biomarker layers was used to describe the biomarker variation of study participants without MUO. The first five principal components (PC) in each biomarker layer could explain 43.0% of the metabolite variation, 53.5% of the lipid variation, and 61.4% of the protein variation (Table S2). In general, there were larger differences in the biomarker pattern between NOC and MHO subjects, than between MHO and MUO subjects. Seven PCs, three protein PCs, two lipid PCs, and two metabolite PCs were associated with increased odds of MHO as compared to NOC (Figure 6). Similar to PP4 in the obese individuals, PP5, which was the PC that was most strongly associated with increased odds of MHO over NOC, had strong positive contributions from IL1-RA and IL6, but negative contributions from galanin and pappalysin-1. The lipid PC, showing the largest differences between MHO and NOC, PL2, was dominated by negative contributions from triacylglycerides and positive contributions from phosphatidylcholine ethers, similar to PL2 in the obese population (Tables S7–S9).

4. Discussion

This observational study from an urban population ran an extensive biomarker profiling of 432 lipids, metabolites, and proteins across two distinct obese subgroups, MHO and MUO—an unexplored field as of yet. Key biomarker pattern findings include additional evidence of MHO individuals holding a more metabolically favorable lipid and glucose profile, that is, lower levels of traditional blood glucose and

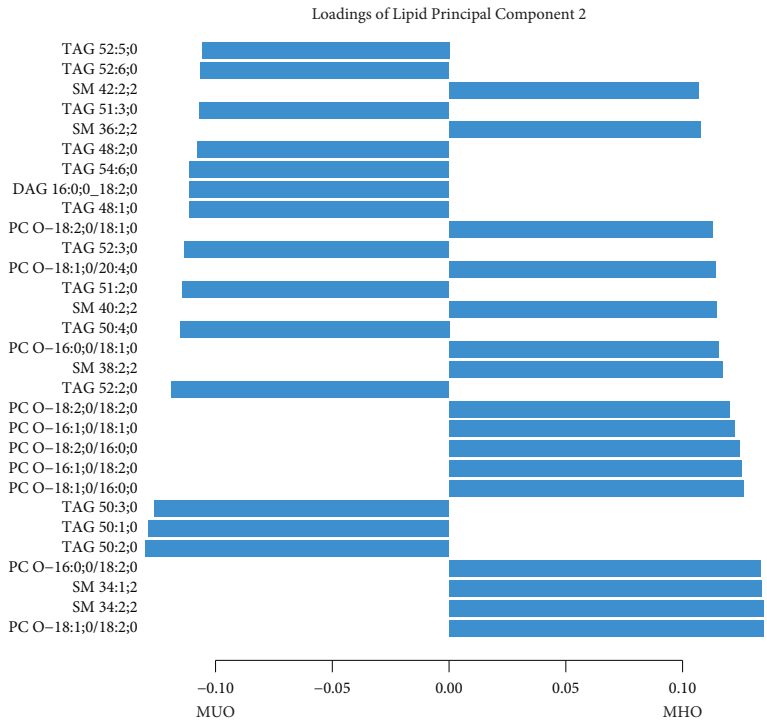

Figure 4: Main loadings for PP5, when comparing MHO with MUO.

triglycerides, as well as a trend of lower levels of metabolically unfavorable lipid biomarkers. Even if significance levels were modest, PCA discovered nominally significant proteomic and lipidomic biomarkers that differed between the MHO and MUO subgroups. These differences were to a large extent explained by factors related to the MetS. When comparing MHO individuals to NOC, PCA of selected biomarkers and descriptive data demonstrated expected findings of obesity-related parameters.

4.1. Lipidomics. Lipidomic patterns display MHO-related principal component (PL2) with negative contributions of triacylglycerides and diacylglycerides when compared to MUO. This supports the notion of a more benign lipid profile of MHO subjects, since higher levels of triglycerides mirror a more metabolically active adipose tissue as well as atherogenic properties, thus with the MetS [31]. Furthermore, glycerphospholipids (exclusively ether phosphatidylcholine) and sphingomyelins seem to be associated positively with the MHO-related PL2. It is unclear whether phospholipids contribute positively or negatively to cardiovascular disease and metabolic disorders [32, 33]. However, research shows that ether phosphatidylcholine with shorter fatty acids and smaller amounts of total double bonds (more positively correlated with MHO) is increased in long-lived humans [34]—suggesting that perhaps MHO individuals deal better with oxygen stress than their counterparts, a theory supported by a previously cited systematic review, revealing that plasmalogens present a negative correlation with obesity, DM2, prediabetes, and CVD—all conditions associated with elevated levels of oxidative stress [32]. Moreover, sphingolipids (mainly sphingomyelin) seem to contribute to adipose tissue inflammation and the accompanying liver steatosis and insulin resistance; that is why their positive relationship with MHO appears more sophisticated than expected [32, 35]. The finding should be interpreted with caution given the relatively modest level of significance and number of PCs analyzed.

4.2. Proteomics

4.2.1. Interleukin-1 Receptor Antagonist (IL-1ra). Our study presented positive contributions of IL-1ra with the MUO-related PP5, compared to MHO. It has been debated whether IL-1ra is benign or if elevated levels of this biomarker present with adverse effects [36]. Indeed, it works as an inhibitor of the well-known proinflammatory cytokine interleukin-1β (IL-1β), involved in the development of various chronic inflammatory disorders, as well as CVD and DM2 [37]. The randomized double-blind CANTOS study

Figure 5: Correlation between biomarker principal components and cardiometabolic risk factors. Correlations between cardiometabolic risk factors and lipid principal component 2 (pl2) and protein principal component 5 (pp5) are expressed as partial Spearman's correlation coefficients, adjusted for age and sex. AHT: antihypertensive treatment; CRP: C-reactive protein; DBP: diastolic blood pressure; SBP: systolic blood pressure; HbA1c: glycated haemoglobin; HOMA-IR: homeostatic model assessment for insulin resistance.

contributed to the increasing evidence of positive effects of IL-1ra focused treatment, displaying that anti-inflammatory targeting with monoclonal antibodies against IL-1β significantly reduced recurrent cardiovascular events compared to placebo in postmyocardial infarction survivors [38]. However, conflicting data postulates that this protein is upregulated as a protective response to increased activities of IL-1β and interleukin-1α (IL-1α) [39]. Furthermore, additional findings hypothesize that IL-1RA might have harmful cardiovascular effects of its own and additionally prevent potentially positive effects of IL-1α and IL-1β [36].

4.2.2. Galanin. An obesity-related neuropeptide was found negatively related to the MUO-correlated PP5, suggesting a relationship with MHO. Galanin is mainly involved in energy homeostasis, where increased hormone levels contribute to the development of obesity, through orexigenic effects, and also obesity-associated metabolic impairments, regardless of feeding regulation [40, 41]. Nevertheless, one study reports potential positive effects of this hormone, where it seems to improve glucose metabolism and uptake, thus decreasing insulin resistance [42].

4.2.3. Leptin. Being a well-known biomarker for obesity, leptin correlated positively with MUO-related PP5. This adipocyte-derived hormone, increases with BMI and adipose tissue mass, suggesting that obese individuals develop an insensitivity to this hormone with increasing weight [43]. The hormone regulates the energy balance by inhibiting hunger mediated through the hypothalamus; hence, it works to reduce caloric intake and increase energy expenditure [44], suggesting that such obesity-promoting mechanisms might be more pronounced in MUO than in MHO.

4.3. Study Limitations. This is the first study of its kind, including 432 metabolites and proteins aiming to describe their relationship with metabolically healthy versus unhealthy obesity. Still, several limitations should be considered in this study. MDCS, although being a well-characterized, population-based prospective cohort with a large number of included individuals, had a relatively poor overall attendance rate (41%) which could imply a health selection bias. Furthermore, both at baseline examination and reflected in our study sample, there exists a gender imbalance with a predominance of women.

Table 2: Multiple regression model (linear logistic regression) displaying odds ratios which indicate associations between biomarker principal components and MHO, compared to MUO.

Model	PP5		PL2	
	p	OR	p	OR
Model 1 (age + sex)	0.01*	0.85	0.02*	1.06
Model 1 + systolic blood pressure	0.01*	0.85	0.02*	1.06
Model 1 + plasma glucose	0.02*	0.86	0.06	1.05
Model 1 + HDL cholesterol	0.06	0.88	0.35	1.03
Model 1 + triglycerides	0.04	0.87	0.24	1.04
Model 1 + waist circumference	0.07	0.91	0.08	1.04
Model 1 + metabolic syndrome	0.17	0.91	0.91	1.00

Odds ratios (OR) indicate associations between biomarker principal components and MHO (=1), compared to MUO (=0). Model 1 was adjusted for age and sex. PL2: lipidomic principal component 2. PP5: proteomic principal component 5. The model adjusted for metabolic syndrome was adjusted for all factors of the metabolic syndrome according to National Cholesterol Education Program panel III (NCEP III) criteria [30] (systolic blood pressure, fasting plasma glucose, HDL cholesterol, triglycerides, and waist circumference). * Significant at $p < 0.05$.

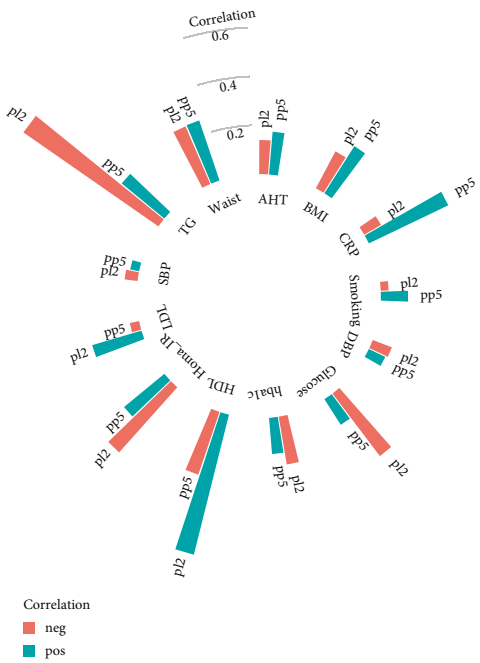

Figure 6: Logistic regression models, with significance testing, of the main PCs when comparing MHO (a) with NOC subjects (b).

A major limitation is the small sample size, resulting in limited power. Moreover, although we applied a data-reducing strategy, several derived PCs were tested with obesity phenotypes with the risk of false-positive results. This underlines the need for replication of the reported findings.

When performing a multiple regression analysis, adjusting for the components of the MetS [30], the associations of the biomarker PCs when comparing MHO and MUO individuals were attenuated (Table 2). This suggests that the difference of biomarker variation between MHO and MUO subjects in part could be explained by the MetS. Thus, one might argue that to keep the MHO phenotypic state and avoid hospitalization, there should be an ambition of the individual for weight stability and keeping a healthy lifestyle to avoid transformation into MUO linked to the MetS.

5. Conclusion

We have performed a plasma metabolic and protein profiling of MHO and MUO individuals, defined by absence (MHO) or presence (MUO) of a history of hospitalization for a somatic disease until midlife. Despite relatively weak associations, this novel approach confirms that MHO individuals present with a positive association with phosphatidylcholine ethers and sphingomyelins, as well as negative associations with triacyl- and diacylglycerides compared to MUO subjects. Furthermore, MHO individuals are characterized by the downregulation of potentially harmful proteomic biomarkers, compared to their MUO counterparts. A large part of the difference could be explained by the influence of MetS. Our research is in line with previous findings, although a unique and extensive biomarker profiling presents novel data on potential differentiating traits between these two obese phenotypes.

Abbreviations

CVD: Cardiovascular disease
CRP: C-reactive protein
DM2: Type 2 diabetes
FABP4: Fatty acid-binding protein 4
HOMA-IR: Homeostatic model assessment for insulin resistance
IL-1RA: Interleukin-1 receptor antagonist
MDCS: Malmö diet cancer study
MDCS-CV: Malmö diet cancer study—cardiovascular arm
MHO: Metabolically Healthy Obesity
MUO: Metabolically unhealthy obesity
NAFLD: Nonalcoholic fatty liver disease
NOC: Nonobese controls
PAPPA: Pappalysin-1
PC: Principal component
PCA: Principal component analysis.

Data Availability

All requests for data access should be addressed to the corresponding author. Proposals requesting data access will have to specify how they plan to use the data.

Conflicts of Interest

JK, PMN, OM, GE, MM, and FO report no conflicts of interest. MJG is an employee of Lipotype GmbH. EB is an employee of AstraZeneca.

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Supplementary Materials

Supplementary tables (S1–S9): index and contain additional data as follows: S1: table of SwissLipids IDs. S2: proportion of explained variance for the first 5 PCs in each biomarker layer (metabolite, lipid, and protein). The proportion of explained variance is presented for the principal component analysis performed for the obese participants ($n = 416$) (MHO + MUO) and the non-MUO (MUO + NOC) ($n = 3{,}027$). S3: loadings of metabolite PCs 1–5 (MHO vs. MUO). S4: loadings of lipid PCs 1–5 (MHO vs. MUO). S5: loadings of protein PCs 1–5 (MHO vs. MUO). S6: correlation between biomarker PCs (PL2, PP5) and cardiometabolic risk factors. S7: loadings of metabolite PCs 1–5 (MHO vs. NOC). S8: loadings of lipid PCs 1–5 (MHO vs. NOC). S9: loadings of protein PCs 1–5 (MHO vs. NOC). (*Supplementary Materials*)

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Paper III

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Antibodies against phosphorylcholine in hospitalized versus non-hospitalized obese subjects

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Obesity associates with reduced life expectancy, type 2 diabetes, hypertension and cardiovascular disease, and is characterized by chronic inflammation. Phosphorylcholine (PC) is an epitope on oxidized low-density lipoprotein, dead cells and some microorganisms. Antibodies against PC (anti-PC) have anti-inflammatory properties. Here, we explored the role of anti-PC in hospitalized versus non-hospitalized obese. One-hundred-and-twenty-eight obese (BMI ≥ 30 kg/m^2) individuals (59.8 (± 5.5) years, 53.9% women) from the Malmö Diet and Cancer Cardiovascular Cohort were examined and IgM, IgG1 and IgG2 anti-PC were analyzed by ELISA. Individuals with at least one recorded history of hospitalization prior to study baseline were considered hospitalized obese (HO). Associations between IgM, IgG1 and IgG2 anti-PC and HO (n = 32)/non-hospitalized obese (NHO) (n = 96), but also with metabolic syndrome and diabetes were analysed using logistic regressions. Both IgM and IgG1 anti-PC were inversely associated with HO, also after controlling for age and sex. When further adjusted for waist circumference, systolic blood pressure, glucose levels and smoking status, only IgG1 anti-PC remained significantly associated with HO. In multivariate models, each 1 standard deviation of increment in anti-PC IgG1 levels was inversely associated with prevalence of HO (odds ratio 0.57; CI 95% 0.33–0.98; p = 0.044). IgG2 anti-PC did not show any associations with HO. Low levels of IgM and IgG1 anti-PC are associated with higher risk of being a HO individual independent of sex and age, IgG1 anti-PC also independently of diabetes and metabolic syndrome. The anti-inflammatory properties of these antibodies may be related to inflammation in obesity and its complications.

Abbreviations

Anti-PC	Antibodies against phosphorylcholine
CVD	Cardiovascular disease
HO	Hospitalized obese
MDCS-CV	Malmö Diet Cancer Study-Cardiovascular cohort
NHO	Non-hospitalized obese
MetS	Metabolic syndrome
OxLDL	Oxidization of low-density lipoproteins

Obesity is rapidly becoming one of the most alarming public health hazards worldwide, accounting for an increasing negative impact on health due to its deleterious effects of excess body fat accumulation[1]. It is one of the leading risk factors for developing several debilitating comorbidities, such as various atherosclerotic processes (including cardiovascular disease, CVD) and type 2 diabetes[2]. However, although obesity is commonly associated with deleterious metabolic profiles there are individual differences, displaying a heterogeneous phenomenon of obesity. These individuals typically present with a more favorable lipid- and glucometabolic profile along with the absence of other components usually associated with the metabolic syndrome (MetS)[3,4].

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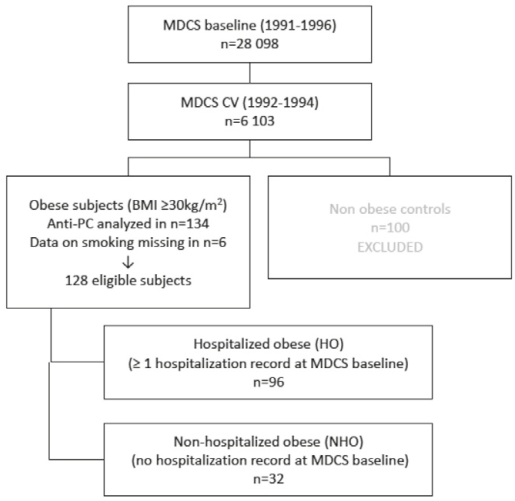

Figure 1. Flow chart of the MDCS-CV sub-cohort stratified for obese and non-obese subjects, respectively.

Although insulin resistance is an immensely important risk factor for the development of CVD through the promotion of atherosclerotic processes[5], other harmful elements may include immunological mechanisms which through inflammatory responses interact with the atherosclerotic plaque, subsequently leading to its rupture and the development of CVD caused by tissue ischemia[6]. Atherosclerotic plaques are characterized by accumulation of oxidized low-density lipoprotein (OxLDL), dead cells and a low-grade inflammation where immune competent cells as T cells, macrophages and dendritic cells represent major contributors. OxLDL is taken up by macrophages which develop into inert foam cells[6]. OxLDL is also pro-inflammatory, and phosphorylcholine (PC), exposed on LDL surface during oxidation, may play a major role, also in OxLDL-induced immune activation. PC is also exposed on dead cells and on some microorganisms, including both bacteria, parasites and nematodes, and is both a danger- and pathogen-associated molecular pattern (DAMP and PAMP)[6]. Antibodies against PC (anti-PC) are present in healthy adults; as much as 5–10% of circulating immunoglobulin M (IgM) consists of IgM anti-PC[6,7]. IgM anti-PC is negatively associated with several chronic inflammatory conditions, including atherosclerosis, CVD, rheumatic diseases and chronic kidney disease (CKD). Potential underlying mechanisms have been described, including anti-inflammatory[6]. As atherosclerosis and is subsequent pro-inflammatory induction is one of the main pathophysiological mechanisms linked to obesity-related mortality and morbidity[8], one interesting aspect would be to investigate if the levels of anti-PC play a protective role in obesity. Thus, the aim of this observational, cross-sectional study was to determine if anti-PC immunoglobulin M (IgM), G1 (IgG1) and G2 (IgG2) are associated with higher risk of being a hospitalized obese subject.

Subjects and methods

The Malmö Diet and Cancer Study (MDCS) is a population-based study that enrolled 28 449 individuals between 1991 and 1996 in the city of Malmö, Sweden. A random sample (every second individual between 1992 and 1994) of the study subjects were invited to participate in a sub-study on the epidemiology of carotid artery disease. This sub-sample comprised the MDCS-Cardiovascular Cohort (MDCS-CV; n = 6103). For this study, the purpose was to randomly select a total of 300 individuals from the MDCS-CV cohort with predefined BMI criteria, data on prior hospitalization status, and equal sex distribution. This resulted in a total of 234 included individuals, due to lack of sufficient number of individuals fulfilling applicable inclusion criteria (see Fig. 1). In a sub-sample of 134 people with obesity (BMI ≥ 30 kg/m^2) subjects, anti-PC were analyzed. Self-reported data on smoking was missing in six subjects, resulting in 128 subjects with complete data. Those subjects were further sub-divided into two different categories: absence or presence of hospitalization for somatic disease prior to study entrance, as recorded in the Swedish National Hospital Inpatient Register. Hospitalizations due to intoxications/external injuries or normal deliveries were considered non-hospitalizations. People with obesity with no recorded history of hospitalization prior to study entrance (n = 32; 25%) where defined as non-hospitalized obese (NHO). Corresponding individuals with at least one recorded history of hospitalization prior to study entrance (n = 96; 75%) were defined as hospitalized obese (HO)[9].

The study was approved by the University of Lund research ethics committee (LU 51/90) and is in accordance with the Declaration of Helsinki. All subjects gave informed consent before entering the study. All experiments were performed in accordance with relevant guidelines and regulations.

Anthropometric measurements of weight (kg) and height (cm) were carried out without shoes and in light indoor clothing. Waist circumference (cm) was measured in the standing position without clothing. Right-arm blood pressure (mmHg) was measured twice in the recumbent position after a 5-min rest (Korotkoff phase V). Diabetes was defined as self-reported physician´s diagnosis per questionnaire, or current treatment with anti-diabetic drugs or fasting whole blood glucose ≥ 6.1 mmol/L. Data on medication and smoking status (current smoker yes/no) was retrieved through questionnaires.

Definitions. General obesity was defined as BMI ≥ 30 kg/m^2. Abdominal obesity was defined as waist circumference ≥ 88 cm and ≥ 102 cm for women and men, respectively. Metabolic syndrome (MetS) was defined as presence of any three of the following five criteria: abdominal obesity, elevated triglycerides (≥ 1.7 mmol/L), reduced high density lipoprotein (HDL) cholesterol (< 1.03 mmol/L in males and < 1.29 mmol/L in females), increased blood pressure (BP) (systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg, or drug treatment), or elevated fasting glucose (≥ 5.6 mmol/L or glucose-lowering treatment)[10].

Laboratory assays. Venous blood samples were drawn and stored at − 80 °C until later analysis (2020). Fasting blood glucose (FBG), triglycerides, total cholesterol, LDL and HDL cholesterol were all analyzed at the Department of Clinical Chemistry, Skåne University Hospital, Malmö, participating in a national standardization and quality control system.

Antibody determinations. Antibodies such as IgM, IgG1 and IgG2 to PC were determined by ELISA essentially as described previously[6,11–13]. Briefly, pooled serum from Sigma Aldrich (St Louis, MO, USA) was used as standard in each plate. Nunc Immuno microwell plates (Thermo Labsystems, Franklin, MA, USA) were coated with PC-bovine serum albumin (BSA) antigen at a concentration of 10 µg/mL per well and incubated overnight at 4 °C. After four washings with wash buffer, the plates were blocked with 2% BSA–phosphate buffered saline (PBS) for 1 h at room temperature. The same washing steps were followed throughout the assay. Serum samples were then diluted at 1:100 for IgM, IgG1 and IgG2 in 0.2% BSA–PBS and added at 100 µL/well to each plate. Plates were then incubated at room temperature for 2 h and washed as described above. Biotin-conjugated goat antihuman IgM, mouse antihuman IgG1 and mouse antihuman IgG2 (diluted 1:30,000, 1:500 and 1:5000 respectively in 1% BSA–PBS) was then added at 100 µL/well and the plates were incubated at room temperature for 2 h. After four washings, horseradish peroxidase conjugated streptavidin (diluted 1:5000, 1:3000 and 1:3000 for IgM, IgG1 and IgG2 respectively in 0.2% BSA–PBS) [Thermo Scientific, Roskilde, Denmark] were added at 100 µL/well to respective plates and they were further incubated for 20 min. The colour was developed by adding the horseradish peroxidase substrate, TMB (3,3′,5,5′ tetramethylbenzidine; Sigma Aldrich, St. Louis, MO, USA), at 100 µL/well and after incubating the plates for 15 min, 20 min and 20 min for IgM, IgG1 and IgG2 respectively at room temperature in a dark place. Further reaction was stopped by adding stop solution 1N H_2SO_4 at 50 µL/well to each plate. Finally, plates were read on ELISA Multiscan Plus spectrophotometer (Spectra Max 250; Molecular Devices, CA) at both 450 nm and 540 nm. All samples were measured in duplicates within a single assay and the coefficient of variation between the duplicates was below 15% for all the antibodies.

Statistics. Variables are presented as means (± standard deviation, SD) or median (25–75 interquartile range, IQR). A stratified random sample was created for identification of eligible subjects for the study. NHO and HO subjects were compared using one-way ANOVA tests for normally distributed continuous variables, Mann–Whitney U test for continuous variables with non-normal distribution, or χ^2 tests for binary variables. Prior to analyses, variables with non-normal distribution were ln-transformed (anti-PC IgM, IgG1, IgG2, FBG, triglycerides and total cholesterol). Anti-PCs were further z-transformed. Unadjusted logistic regressions were carried out for anti-PC and prevalence of HO using odds ratios (OR) and 95% confidence intervals (95% CI). Multivariate logistic regressions were then carried out adjusted for age and sex (*Model 1*), and further adjusted for waist circumference, systolic blood pressure (SBP), FBG, and smoking status (*Model 2*). All analyses were carried out using SPSS 25.0. A p-value of less than 0.05 was considered significant.

Results

Characteristics of the study population are presented in Table 1. HO subjects were older, with higher BMI, waist circumference, SBP and DBP, but lower levels of anti-PC IgM and IgG1 than NHO subjects. Further, a larger proportion of the HO subjects presented with DM and abdominal obesity as compared to subjects with NHO. Anti-PC IgM and IgG1 levels along with waist circumference and BMI in NHO/HO subjects are illustrated in Fig. 2. The 96 HO subjects were hospitalized prior to study entrance for following reasons based on ICD8: Morbi infectiosi ex origine intestinali (n = 1), Tuberculosis (n = 1), Morbi bacterici alii (n = 1), Gonococcal infection (acute) of lower genitourinary tract (n = 1), Neoplasma malignum baseos oris (n = 1), Neoplasma malignum intestini crassi, recto except (n = 1), Neoplasma malignum mammae (n = 1), Neoplasma malignum cervicis uteri (n = 1), Neoplasma benignum systematis respirationis (n = 2), Myoma uteri (n = 3), Neoplasma benignum ovarii (n = 2), Struma nodosa atoxica (n = 1), Morbi glandularum aliarum systematis endocrine (n = 1), Morbi parathyreoideae (n = 1), Morbi glandulae suprarenalis (n = 1), Functio laesa metabolismi proteini plasmatis (n = 1), Persona pathologica asthenica (n = 1), Alcoholismus (n = 1), Perturbationes fortuitae psychogenes accidentals (n = 1), Morbi nervorum et gangliorum periphericorum (n = 1), Alii morbi nervorum cranialium (n = 1), Alii morbi inflammatorii auris (n = 1), Hypertonia benigna essentialis (n = 2), Hypertonia non indicata (n = 1), Angina pectoris (n = 1), Morbus cordis ischaemicus asymptomaticus (n = 1), Ischaemia cerebralis transitoria (n = 1), Varices venarum extremitatum inferiorum (n = 3), Bronchopneumonia (n = 2), Bronchitis chronica (n = 2), Asthma bronchiale (n = 1), Alii morbi tractus respiratorii superioris (n = 1), Laryngitis chronica (n = 1), Rhinitis

	Total	HO	NHO	p
N	128	96	32	
Age (years)	59.8 (± 5.5)	61.1 (± 4.9)	55.9 (± 5.4)	**1.0×10^{-6}**
Sex (women)	69 (53.9)	47 (49)	22 (68.8)	0.052
BMI (kg/m^2)	32.7 (± 3.2)	33.2 (± 3.4)	31.3 (± 1.3)	**0.002**
Waist (cm)	100.5 (± 12.9)	102.6 (± 13.0)	94.2 (± 10.5)	**0.001**
Smoking (yes/no)	22 (17.2)	15 (15.6)	7 (21.9)	0.417
Anti-PC IgM (AU)	113 (98–130)	110 (96–126)	124 (110–137)	**0.008**
Anti-PC IgG1 (AU)	126 (86–203)	110 (77–187)	174 (96–230)	**0.023**
Anti-PC IgG2 (AU)	222 (110–480)	222 (112–455)	261 (85–506)	0.741
SBP (mmHg)	148 (± 17)	150 (± 18)	141 (± 12)	**0.017**
DBP (mmHg)	91 (± 9)	92 (± 9)	88 (± 6)	**0.042**
Total cholesterol (mmol/L)	6.3 (5.7–7.3)	6.2 (5.6–7.4)	6.4 (5.7–7.3)	0.511
HDL-C (mmol/L)	1.2 (± 0.2)	1.2 (± 0.3)	1.2 (± 0.3)	0.724
Triglycerides (mmol/L)	1.5 (1.1–2.9)	1.5 (1.1–2.5)	1.4 (1.0–1.9)	0.240
Fasting blood glucose (mmol/L)	5.2 (4.8–5.7)	5.3 (4.8–5.9)	5.0 (4.6–5.4)	**0.033**
Diabetes mellitus, n (%)	26 (20.3)	24 (25)	2 (6.3)	**0.022**
MetS, n (%)	64 (50)	51 (53.1)	13 (40.6)	0.221
Abdominal obesity, n (%)	47 (36.7)	40 (41.7)	7 (21.9)	**0.044**
High triglycerides, n (%)	53 (41.4)	43 (44.8)	10 (31.3)	0.178
Low HDL-C, n (%)	60 (46.9)	43 (44.8)	17 (53.1)	0.413
Hypertension, n (%)	121 (94.5)	92 (95.8)	29 (90.6)	0.262
Elevated glucose, n (%)	37 (28.9)	31 (32.2)	6 (18.8)	0.143

Table 1. Characteristics of the study population. Bold indicates significance (p≤0.05) Values are means (± standard deviation), medians (25–75 interquartile range), or numbers (%). Components of the Metabolic syndrome (Abdominal obesity (waist circumference ≥ 88 cm and ≥ 102 cm for women and men, respectively), elevated triglycerides (≥ 1.7 mmol/L), reduced high density lipoprotein cholesterol (< 1.03 mmol/L in males and < 1.29 mmol/L in females), hypertension (systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg, or drug treatment), or elevated fasting glucose (≥ 5.6 mmol/L or glucose-lowering treatment) were defined as stated by Alberti et al.[13]. *HO* hospitalized obese, *NHO* non-hospitalized obese, *AU* arbitrary units, *Anti-PC* antibodies against phosphorylcholine, *Ig* immunoglobulin, *MetS* metabolic syndrome, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HDL* high density lipoprotein cholesterol. Bold indicates significance (p ≤ 0.05)

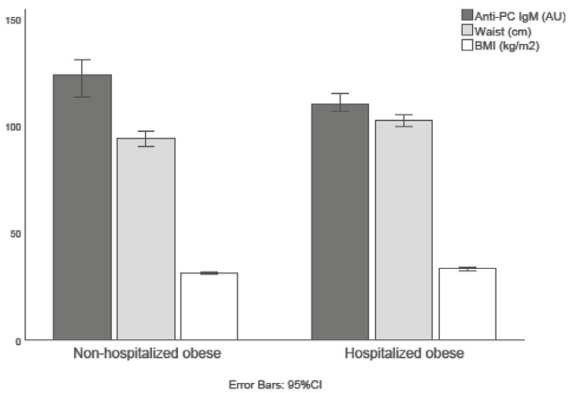

Figure 2. Anti-PC levels, waist circumference and BMI in non-hospitalized obese subjects vs hospitalized obese subjects. Values are median (anti-PC IgM) or mean (waist circumference and BMI). Error bars represent the 95% confidence interval.

	Anti-PC IgM		Anti-PC IgG1		Anti-PC IgG2	
	OR (CI 95%)	p	OR (CI 95%)	P	OR (CI 95%)	p
Unadjusted						
Anti-PC	0.53 (0.31–0.90)	0.020	0.60 (0.39–0.93)	0.024	1.01 (0.68–1.51)	0.947
Model 1						
Anti-PC	0.54 (0.30–0.99)	0.049	0.58 (0.35–0.95)	0.029	–	–
Age	1.22 (1.12–1.34)	1.8×10^{-5}	1.23 (1.12–1.35)	1.4×10^{-5}	–	–
Sex	0.29 (0.11–0.80)	0.017	0.28 (0.10–0.79)	0.016	–	–
Model 2						
Anti-PC	0.58 (0.30–1.15)	0.120	0.57 (0.33–0.98)	0.044	–	–
Age	1.25 (1.13–1.39)	1.9×10^{-5}	1.27 (1.14–1.42)	1.8×10^{-5}	–	–
Sex	1.12 (0.24–5.24)	0.889	1.29 (0.26–6.43)	0.760	–	–
Waist circumference	1.09 (1.01–1.18)	0.024	1.10 (1.02–1.19)	0.018	–	–
Systolic blood pressure	1.03 (0.99–1.06)	0.158	1.03 (1.00–1.07)	0.083	–	–
Fasting blood glucose	1.34 (0.66–2.73)	0.416	1.12 (0.55–2.30)	0.753	–	–
Smoking	0.77 (0.21–2.77)	0.685	0.85 (0.23–3.11)	0.804	–	–

Table 2. Associations between anti-PC and risk of being a hospitalized obese subject (HO). Values are odds ratios (OR) with 95% confidence intervals (CI 95%). *Anti-PC* antibodies against phosphorylcholine, *IgM* immunoglobulin M, *IgG1* immunoglobulin G1, *IgG2* immunoglobulin G2.

allergica (n = 1), Ulcus duodeni (n = 1), Appendicitis acuta (n = 3), Hernia abdominalis (n = 4), Gastro-enteritis et colitis non ulcerosa (n = 1), Alii morbi intestinorum et peritonei (n = 1), Cholelithiasis (n = 2), Alii morbi systematis urinarii (n = 2), Morbi organorum genitalium viri (n = 1), Orchitis et epididymitis (n = 1), Morbi mammae, ovarii, tubae, parametrii (n = 1), Alii morbi mammae (n = 1), Morbi ovarii et tubae alii (n = 1), Morbi cervicis uteri alii (n = 1), Aliae complicationes gravidarum (n = 1), Morbi cutis alii (n = 1), Arthritis rheumatoides et morbi similes (n = 1), Osteo-arthritis (arthrosis) et morbi similes (n = 1), Arthritis (n = 1), Rheumatismus alius non articularis (n = 1), Morbi meniscorum et alii morbi cartilagines articuli (n = 5), Alii morbi articulorum (n = 1), Alia symptomata systematis nervosi et organorum sensum (n = 1), Syncope (lipothymia) vasovagalis (n = 1), Symptomata tractus digestionis inferioris (n = 3), Febris incertae causae (n = 1), Nervosimus (n = 1), Cephalalgia (n = 1), Casus mentales pro abortu provocato sive sterilisatione (n = 1), Laceratio et vulnus extremitatis superioris (n = 2), Contusio loci alterius, multiplex sive NUD (n = 1), Contusio sive compressio, cute intacta (n = 2), Investigation of circulatory system (n = 1) and Investigation of genito-urinary system (n = 1). The remaining three hospitalizations had no ICD code recorded.

Anti-PC levels and metabolically unhealthy obesity. Each 1 SD increment in anti-PC IgM levels was associated with a lower prevalence of HO when unadjusted, OR 0.53 (CI 95% 0.31–0.90; p = 0.020), and when adjusted for age and sex, OR 0.54 (CI 95% 0.30–0.99; p = 0.049), but the association was attenuated upon further adjustment for waist circumference, SBP, DBP, FBG, and smoking status, OR 0.58 (CI 95% 0.30–1.15; p = 0.120).

Each 1 SD increment in anti-PC IgG1 levels was associated with lower prevalence of HO in unadjusted logistic regressions (OR 0.60; CI 95% 0.39–0.93; p = 0.024), and further adjusted for age and sex (OR 0.58; CI 95% 0.35–0.95; p = 0.029). The association remained significant when waist circumference, SBP, DBP, FBG, and smoking status were entered in the model (OR 0.57; CI 95% 0.33–0.99; p = 0.044), Table 2. Further, sex-specific analyses were carried out, showing association between high anti-PC IgG1 levels and lower prevalence of HO in men, but not in women in the fully adjusted *Model 2* (Table 3). There was a trend for sex-specific associations of anti-PC IgM with HO in women, but this association was attenuated after adjusting for age and sex in *Model 1* (Table 3).

Anti-PC IgG2 was not associated with HO in the unadjusted analyses (p = 0.9) and was therefore not further analyzed.

Anti-PC IgG1, the metabolic syndrome and diabetes mellitus. Anti-PC levels were neither associated with prevalence of diabetes mellitus (27 cases; OR 0.78; CI 95% 0.42–1.43; p = 0.414), nor with MetS (OR 0.86; CI 95% 0.52–1.41; p = 0.555). No associations were seen in analyses of associations between anti-PC and each component of MetS, except for elevated glucose levels being associated with anti-PC IgG1 levels (including adjustment for glucose lowering treatment) (Table 4).

Discussion

We here report that levels of anti-inflammatory IgG1 and IgM anti-PC are significantly lower among HO than among NHO-individuals. When we controlled for non-modifiable risk factors (age and sex) these associations remained significant. However, when also other factors independently associated with HO (waist circumference, systolic blood pressure, fasting blood glucose and smoking), were included in the model, only IgG1 anti-PC remained significantly associated with protection against HO. In contrast, IgG2 anti-PC was not associated with HO, before or after adjustment for potential confounders. We have not been able to determine IgG3 and IgG4

Anti-PC IgM	Men = 59		Women n = 69	
	OR (CI 95%)	p	OR (CI 95%)	p
Unadjusted				
Anti-PC IgM	0.60 (0.25–1.45)	0.257	0.51 (0.26–0.99)	0.049
Model 1				
Anti-PC IgM		–	0.46 (0.20–1.02)	0.057
Age		–	1.33 (1.15–1.53)	1.2×10^{-4}
Model 2				
Anti-PC IgM		–		–
Age		–		–
Waist circumference		–		–
Systolic blood pressure		–		–
Fasting blood glucose		–		–
Smoking		–		–
Anti-PC IgG1				
Unadjusted				
Anti-PC IgG1	0.28 (0.09–0.85)	0.025	0.76 (0.47–1.23)	0.260
Model 1				
Anti-PC IgG1	0.29 (0.09–0.92)	0.036	0.67 (0.36–1.27)	0.221
Age	1.12 (1.00–1.29)	0.052	1.32 (1.15–1.53)	1.2×10^{-4}
Model 2				
Anti-PC IgG1	0.26 (0.07–0.98)	0.046	0.66 (0.31–1.41)	0.284
Age	1.11 (0.96–1.30)	0.160	1.38 (1.16–1.63)	2.5×10^{-4}
Waist circumference	1.05 (0.92–1.20)	0.462	1.12 (1.00–1.25)	0.045
Systolic blood pressure	1.02 (0.96–1.07)	0.564	1.05 (1.00–1.10)	0.068
Fasting blood glucose	3.62 (0.68–19.09)	0.130	0.95 (0.31–2.97)	0.933
Smoking	0.10 (0.00–2.43)	0.159	1.60 (0.23–11.06)	0.635

Table 3. Sex-specific associations between anti-PC and risk of being a hospitalized obese subject (HO). Values are odds ratios (OR) with 95% confidence intervals (CI 95%). *MetS* metabolic syndrome, *anti-PC* antibodies against phosphorylcholine, *IgM* immunoglobulin M, *IgG1* immunoglobulin G1.

	Anti-PC IgM		Anti-PC IgG1	
	OR (CI 95%)	p	OR (CI 95%)	p
MetS	0.78 (0.54–1.12)	0.173	0.86 (0.52–1.42)	0.555
Abdominal obesity	0.83 (0.58–1.19)	0.316	1.03 (0.61–1.72)	0.921
High triglycerides	0.73 (0.51–1.04)	0.084	0.66 (0.39–1.11)	0.116
Low HDL-C	0.98 (0.69–1.39)	0.913	1.34 (0.81–2.23)	0.254
Hypertension	1.29 (0.67–2.53)	0.443	1.54 (0.52–4.52)	0.433
Elevated glucose	0.91 (0.62–1.32)	0.608	0.49 (0.27–0.88)	0.017

Table 4. Associations between anti-PC and MetS, including each component of MetS. Values are odds ratios (OR) with 95% confidence intervals (CI95%). MetS – metabolic syndrome. Components of the Metabolic syndrome (Abdominal obesity (waist circumference ≥ 88 cm and ≥ 102 cm for women and men, respectively), elevated triglycerides (≥ 1.7 mmol/L), reduced high density lipoprotein cholesterol (< 1.03 mmol/L in males and < 1.29 mmol/L in females), hypertension (systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg, or drug treatment), or elevated fasting glucose (≥ 5.6 mmol/L or glucose-lowering treatment) were defined as stated by Alberti et al.[13]. *Anti-PC* antibodies against phosphorylcholine, *IgM* immunoglobulin M, *IgG1* immunoglobulin G1, *HDL* high density lipoprotein cholesterol, *MetS* metabolic syndrome.

anti-PC at any significant levels previously and these were therefore not included in the present study[13]. Total IgG anti-PC was not included (since both IgG1 and IgG2 were).

We have previously described metabolically healthy obesity in an observational study, based on a definition of obesity (BMI ≥ 30 kg/m^2) with no history of hospitalization for somatic disease until mid-life (mean age 56 years) at MDCS baseline[9]. In that study, we observed that metabolically healthy obese individuals had a significantly lower risk of total mortality and incident CVD than metabolically unhealthy individuals. Notably, metabolically healthy obese subjects did not have an increased risk of these end-points when compared to non-obesity controls.

Descriptive data suggested that metabolically healthy individuals presented with lower levels of lipids and glucose in blood plasma, alongside with a less sedentary behavior than their HO counterparts[9].

In a recent study, we investigated subjects from the MDCS-CV further by comparing biomarker associations (lipidomics, metabolomics and proteomics) between metabolically healthy obese and metabolically unhealthy obese where we observed similar descriptive results (unpublished data).

Our findings in relation to anti-PC are in line with previous publications on the role of these antibodies in chronic inflammatory conditions. Most studies have involved IgM anti-PC. We previously reported that IgM anti-PC is associated with protection in atherosclerosis progress among hypertensives[12], CVD (including both stroke and MI)[14–16], rheumatic diseases, especially SLE, and other systemic rheumatic diseases, but also RA[6,17,18] and mortality in chronic kidney disease[19]. In general, these findings have been confirmed by other researchers[20–25], and extended to other chronic diseases as osteoarthritis[24]. Less is known about other subclasses and isotypes of anti-PC than IgM, even though we determined that IgG1, but not IgG2, shows comparable associations with protection as IgM, in atherosclerosis[13], SLE[26], and for mortality in CKD[11].

Experimental studies support that anti-PC may protect against atherosclerosis, its complications and other types of chronic inflammatory clinical conditions. Anti-PC inhibits pro-inflammatory effects of oxidized and modified lipids exposing PC on endothelial cells (studied on IgG anti-PC)[18]. Another example is immunomodulatory properties with anti-inflammatory effects by IgM anti-PC, promoting polarization of anti-inflammatory T regulatory cells, from healthy donors, atherosclerotic plaques, and also SLE-patients[27]. Mechanisms related to atherosclerosis include IgM anti-PC induced inhibition of uptake of oxLDL by macrophages, which could be an important factor in plaque build-up and development[14]. Since accumulation of dead cells is a major feature of atherosclerosis, IgM anti-PC-induced inhibition of cell death caused by an important inflammatory phospholipid, lysophosphatidylcholine[13] and increased clearance of dead cells by both IgM[7] and IgG1 anti-PC[26] could also play a causative and protective role, inhibiting plaque development.

Animal studies also support an atheroprotective role of anti-PC in atherosclerosis development both using active[28] and passive[29] immunization, and also in both SLE[30], and RA[31]. In line with this is a study where pneumococcal vaccination in a mouse model of atherosclerosis caused increases in different antibodies including anti-PC and a modest but significant decrease of atherosclerosis[32]. We recently demonstrated that brown bears (*Ursus arctos*) which hibernate for 5–6 months during winter, gain weight considerably before hibernation, but despite kidney insufficiency, dyslipidemia and inactivity do not develop atherosclerosis or cardiovascular disease (CVD), have strikingly high levels of IgM and IgG1 anti-PC, thus a potential natural immunization against atherosclerosis[33].

Obesity is a chronic inflammatory condition, affecting different organs including the adipose tissue. Also the immune system is involved, and immune competent cells are known to infiltrate adipose tissue[34]. Adipose tissue is known to be an endocrine organ where different cell types, including immune competent cells, secrete an array of hormones and cytokines, where the net effect is pro-inflammatory[35]. Interestingly, immunosuppressive, anti-inflammatory T regulatory cells are decreased in obesity. In principle, it is thus possible that low IgM anti-PC could be one factor behind low T regulatory cells in obesity. Further, inflammation can be both a cause and effect of obesity and ensuing metabolic changes[34,36]. An immune-deficient state with low IgM and IgG1 anti-PC could thus potentially promote obesity and related inflammation. Even though IgM anti-PC only remained significant after adjustment for non-modifiable risk factors (age and sex), but not when adjusted for waist circumference, systolic blood pressure, fasting blood glucose and smoking, we consider this finding to be relevant for the difference between NHO and HO. The cross-sectional nature of the study precludes us from drawing any conclusion about causation but still the underlying properties of IgM and IgG1 anti-PC makes causation possible, even plausible, although larger, prospective and experimental studies are needed to prove this.

Human anti-PC's are often referred to as natural antibodies, based on data from laboratory mice, which we determined as germ-line encoded, with a dominant clone, TI5. In humans, however, we could not detect such a dominant clone, but instead human anti-PC are characterized somatic mutations with Ig-switch and also T cell dependency[7,37]. Humans are born with very low levels of anti-PC, which are not close to their mothers' levels even after 2 years. This suggests that environmental factors, especially the gut microbiome, could play an important role in development of anti-PC, but that genetic programs also may contribute[38]. Recently, associations between four gut microbiota genera and BMI-predictive plasma metabolites were determined and were found to be possible mediators between gut microbiota and obesity[39]. The possibility that the microbiome is a regulating factor behind low levels of anti-PC in hospitalized obese subjects HO therefore deserves further study.

Other properties of IgM and IgG1 anti-PC as clearance of dead cells, inhibition of cell death caused by inflammatory phospholipids and increased uptake of OxLDL could have a direct effect on complications of obesity, where atherosclerosis and CVD are of major importance.

Individuals from Kitava, New Guinea, were studied in the early 1990s, and were found to have a very favorable metabolic profile, where obesity, metabolic disorders, hypertension and type 2 diabetes were virtually absent. Undoubtedly, one explanation could be differences in lifestyle, diet and exercise. We also reported that levels of IgG and IgM anti-PC are significantly lower among Swedish sex- and age-matched controls than Kitavans and based on these findings we proposed a development of the Hygiene/Old Friends hypothesis. This states that a lack of exposure to PC-bearing microorganisms such as nematodes, parasites, and also some bacteria (including *Treponema*) results in low levels of anti-PC and ensuing increased risk of atherosclerosis, CVD, and other chronic inflammation. Here, we could add obesity and metabolic alterations, based on the present data[6,40–42].

Another finding is that IgG1 and IgG2 anti-PC differ completely in relation to HO and NHO: while IgG1 was a significant marker of protection even after controlling for several other potential confounders, IgG2 was not. This finding is in line with our previous studies on these antibodies, where IgG1, but not IgG2 anti-PC, was associated with protection in atherosclerosis progress[13], SLE[11] and mortality in CKD[6].

PC can also be presented as p-nitrophenyl phosphorylcholine (NPPC)[13] and anti-PC may be divided into group I (IgM and IgG1) and group II (IgG2)[13]. Group I anti-PC recognizes both forms of PC but group II antibodies only recognize NPCC, where the phenyl-ring attached to PC is involved in the antigenicity. IgG2 anti-PC is directed against capsulated bacteria, recognizes carbohydrate antigens, and has bactericidal properties[13,43,44]. It is thus likely that the most protective immune response to PC is not derived from PC on carbohydrate structures of capsulated bacteria.

Further, the present finding that IgG1 anti-PC was significantly protective against hospitalization among obese men but not women, also after controlling for potential confounders, is in line with our previous findings, where associations among men are more prominent[6]. IgM anti-PC was a significant protective marker among women (not controlled for confounders), why it is difficult to draw conclusions about sex differences in this context.

There are limitations to this study. One is that it is relatively small, and it is therefore difficult to determine associations and also control for confounders due to lack of power. Further, the cross-sectional nature of the study precludes any conclusions about causation. It would have been of interest to study obesity in general as compared to matched controls, which is not included herein.

In conclusion, we here demonstrate that anti-inflammatory IgM and IgG1, but not IgG2 anti-PC, are inversely associated with higher risk of being HO, also after controlling for sex and age. However, only IgG1 anti-PC remained significant when also other potential confounders were controlled for. We still think that also IgM is of interest due to its other properties, especially anti-inflammatory, which could be causally related to these factors. In general, IgM and especially IgG1 could be protective for obesity complications, a mechanism that could have implications for prediction of risk, but also for prevention through immunization with PC.

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Author contributions

A.J., J.K. and J.F. wrote the manuscript, A.J., J.K. and P.B. prepared figures and tables, P.B. developed methods and performed experiments, P.M.N. and J.F. conceived the study, P.M.N. and M.M. were responsible for cohorts, all authors reviewed the manuscript.

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Competing interests

JF is named as inventor on patents related to anti-PC. EB is employed by AstraZeneca. None for other authors.

Additional information

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Cardiovascular Diabetology

RESEARCH

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Galectin-4 levels in hospitalized versus non-hospitalized subjects with obesity: the Malmö Preventive Project

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Abstract

Background: Obesity is strongly associated with the development of cardiovascular disease (CVD). However, the heterogenous nature of obesity in CVD-risk is still poorly understood. We aimed to explore novel CVD biomarkers and their possible association with presumed unhealthy obesity, defined as hospitalized subjects with obesity (HO).

Methods: Ninety-two proteins associated with CVD were analyzed in 517 (mean age 67 ± 6 years; 33.7% women) individuals with obesity (BMI ≥30 kg/m^2) from the Malmö Preventive Project cohort, using a proximity extension array technique from the Olink CVD III panel. Individuals with at least one recorded hospitalization for somatic disease prior to study baseline were defined as HO phenotypes. Associations between proteins and HO (n = 407) versus non-hospitalized subjects with obesity (NHO, n = 110), were analyzed using multivariable binary logistic regression, adjusted for traditional risk factors.

Results: Of 92 analyzed unadjusted associations between biomarkers and HO, increased levels of two proteins were significant at a false discovery rate < 0.05: Galectin-4 (Gal-4) and insulin-like growth factor-binding protein 1 (IGFBP-1). When these two proteins were included in logistic regression analyses adjusted for age and sex, Gal-4 remained significant. Gal-4 was independently associated with the HO phenotype in multivariable logistic regression analysis (OR 1.72; CI95% 1.16–2.54). Post-hoc analysis revealed that this association was only present in the subpopulation with diabetes (OR 2.26; CI95% 1.25–4.07). However, an interaction analysis was performed, showing no significant interaction between Gal-4 and prevalent diabetes (p = 0.16).

Conclusions: In middle-aged and older individuals with obesity, increased Gal-4 levels were associated with a higher probability of HO. This association was only significant in subjects with diabetes only, further implying a role for Gal-4 in diabetes and its complications.

Keywords: Obesity, Cardiovascular disease, Biomarkers, Diabetes, Galectin-4

Introduction

Obesity (body mass index, BMI ≥30 kg/m^2) contributes to health complications and reduces life expectancy with up to approximately 20 years [1]. This is mainly due to the significantly increased risk of developing numerous non-communicable diseases, such as type 2 diabetes (DM2), cardiovascular disease (CVD) and certain types of cancer

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[2, 3]. Even more troublesome, the global prevalence of obesity has been steadily increasing since the 1970s, especially among adolescents and children, today reaching pandemic levels [4]. Even though the link between obesity and increased CVD risk is not a matter of debate per se, there have long been speculations regarding how certain individuals with obesity possess a lower risk of developing CVD and diabetes type 2 (DM2), thus showing a heterogeneity of obesity as a risk factor [5].

Furthermore, although the cardiometabolic complications of obesity are well established from an epidemiological perspective, the underlying pathophysiological mechanisms are not fully understood, particularly when taking into consideration the heterogeneity of obesity [6]. Recently, there have been considerable technological advances in the incorporation of multiomics into exploring alterations in specific cell types and identifying modifications in signaling events that promote disease development [7]. To better understand the mechanisms behind disease progression in obesity, we applied proximity extension assay (PEA) technology to measure 92 proteins (biomarkers) associated with inflammation and CVD [8]. This represents an appealing approach to explore associations between multiple proteins and biological systems, which could in turn present possible diagnostic, prognostic, and therapeutic implications.

The *aim* of this cross-sectional, population-based study was to explore possible novel associations between CVD biomarkers and a phenotype of unhealthy obesity, namely obese subjects with a history of hospitalization for somatic disease up until late mid-life, [9–11] using a multiplex proteomic platform consisting of 92 proteins linked to cardiovascular disease.

Methods

Study population

In the 1970s, the Malmö Preventive Project (MPP) cohort was established at the University Hospital, Malmö, Sweden, for the purpose of investigating cardiovascular risk factors in the general population [12]. A total of 33,346 individuals were included at baseline (71% attendance rate, 2/3 men), and survivors of the original cohort were re-examined between 2002 and 2006 (n = 18,240) in the MPP Re-Examination cohort (MPP-RES, attendance rate 72%) [13]. Furthermore, from this MPP-RES cohort, a sub-sample of 1,792 participants was selected to undergo echocardiography and electrocardiogram (ECG) recordings. These individuals were randomly chosen from groups based on their glucometabolic status. Oversampling was performed within the groups with glucometabolic disturbances (impaired fasting glucose, IFG (≥ 6.1 mmol/L or a single measurement of 7.0–11.0 mmol/l of fasting plasma glucose (FPG); new onset diabetes; and prevalent diabetes) to ensure numerical balance, as described previously, [14] resulting in approximately 1/3 normoglycemic subjects, 1/3 with IFG, and 1/3 with diabetes). Prevalent diabetes was defined as either new-onset diabetes (defined by two separate measurements of FPG ≥ 7.0 mmol/l or one measurement ≥ 11.1 mmol/l) or previously known diabetes (obtained through participant self-reporting and/or reporting of current anti-diabetic medication) [14].

From the MPP-RES echocardiography sub-cohort, a total of 517 individuals with obesity and complete biomarker data were included in the present study. This subsample was further sub-divided into two different categories based on hospitalization history. Individuals with obesity with at least one recorded history of hospitalization prior to study baseline (n = 407) were defined as hospitalized subjects with obesity (HO). Correspondingly, individuals who had no history of hospitalization for somatic disease up until inclusion at MPP-RES baseline (n = 110) in late mid-age were defined as non-hospitalized subjects with obesity (NHO), (Fig. 1). Data on prior hospitalization was obtained through the Swedish National Hospital Inpatient Register. Normal deliveries were considered non-hospitalization; otherwise, all diagnoses were included. A detailed list of included/excluded diagnoses can be found in Additional file 1: Table S1.

As described in previous publications, [13, 14] data on medical history and lifestyle (including physical activity, alcohol consumption, dietary habits, and smoking status) were acquired through a self-administered questionnaire. Weight (kg) and height (m) were measured in light indoor clothing, and BMI (kg/m^2) was subsequently calculated. Blood pressure (mmHg) was measured twice using a validated sphygmomanometer with a mercury manometer in the supine position by trained nurses after 10-minutes of rest—the mean values were then recorded. No intra- and/or inter-observed variability calculations were performed; however, the sphygmomanometer used was validated and continuously calibrated according to research standards at Malmö University hospital. Blood samples were acquired after an overnight fast and stored at − 80 °C [15].

Proteomic profiling

Plasma samples were analyzed by the Proximity Extension Assay (PEA) technique, using the Proseek Multiplex CVD III 96 × 96 reagents kit (Olink Bioscience, Uppsala, Sweden). The technique uses two antibodies that bind pairwise to each specific protein, creating a polymerase chain reaction sequence which then can be detected and quantified. The CVD III panel consists of 92 markers with established or proposed involvement in metabolism, inflammation, or cardiovascular disease (Additional

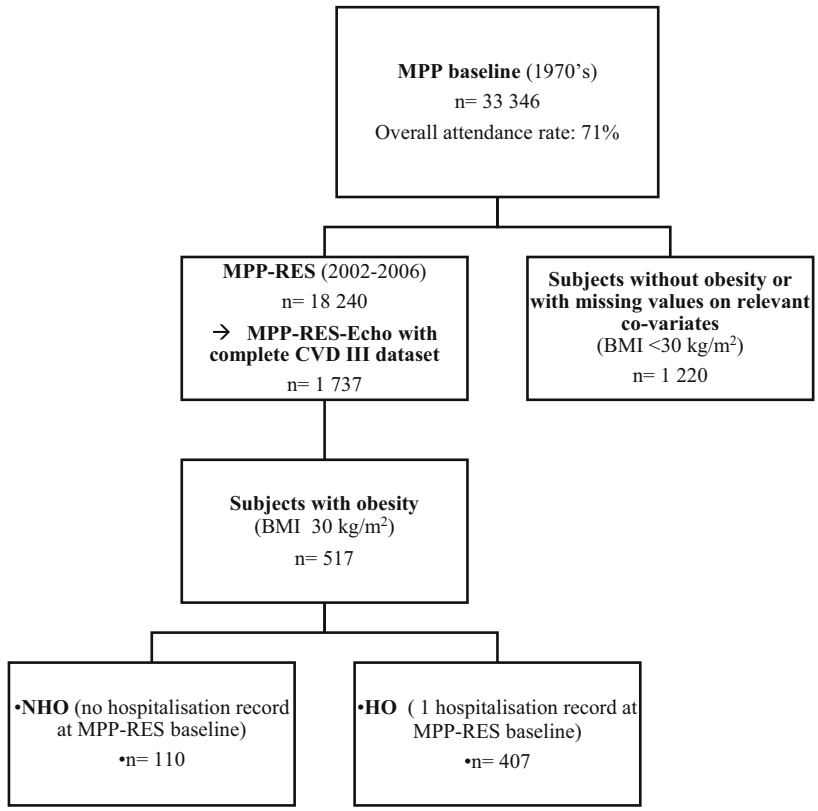

Fig. 1 Flow-chart of the MPP-RES cohort stratified for individuals with and without obesity, as well as history of hospitalization for somatic disorders in subjects with obesity, respectively

file 2: Table S2). One protein was below the limit of detection in >15% samples (N-terminal pro-B-type natriuretic peptide, NT-proBNP) and thus excluded; instead, NT-proBNP measurement with an electrochemiluminescence immunoassay was used. The mean intra- and inter-assay variations were 8.1% and 11.4%, respectively. Further information on the assays is available on the Olink homepage (www.olink.com).

Laboratory analyses

Fasting serum total cholesterol, serum triglycerides, serum high-density lipoprotein and FPG were analyzed using Beckman Coulter LX20 (Beckman Coulter Inc., Brea, USA). Serum low-density lipoprotein concentration (LDL-C) was calculated through Friedewald's formula [16]. NT-proBNP was measured with an electrochemiluminescence immunoassay (Elecsys; Roche Diagnostics, Basel, Switzerland) at the Department of Clinical Chemistry, Akershus University Hospital, Lorenskog, Norway.

Statistical analysis

Continuous variables are presented as means (±standard deviation, SD) or medians (25th-75th percentiles). A stratified random sample was created for identification of eligible study subjects. HO and NHO subjects were compared using one-way ANOVA test for normally distributed continuous variables, Mann-Whitney U-test for continuous variables with non-normal distribution, and $\chi 2$ test for binary variables. Prior to analysis, skewed variables (FPG) were log-transformed. Unadjusted binary logistic regression models exploring associations

between each of the 92 proteins and HO were carried out applying the Benjamini-Hochberg multiple testing correction [17] (false discovery rate, FDR, < 0.05). Significant associations were carried forward to analyses according to *Model 1* (age- and sex-adjusted), and further adjusted according to *Model 2* (total cholesterol, current smoking, hypertension, BMI, prevalent diabetes of any type, and log(FPG)). Hypertension was defined as a measured systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or currently on antihypertensive medication. Finally, for associations significant in *Model 2*, a post-hoc analysis was carried out in subjects with and without diabetes using the remaining variables in *Model 2*. Lastly, to test for linearity between remaining variables with significant associations in *Model 2* and independent variables, quartile analyses were carried out. All analyses were carried out using SPSS 25.0 (IBM, Chicago, IL, USA). A nominal two-sided p-value of less than 0.05 was considered statistically significant.

Results

Study characteristics

Characteristics of the study population are presented in Table 1. HO individuals were older than NHO. Furthermore, lower levels of total cholesterol and LDL-C, as well as lower systolic and diastolic blood pressures were seen in HO when compared with NHO. However, the use of both lipid- and blood pressure lowering drugs was significantly higher in the HO group. No difference between the two groups were seen in FPG levels, prevalent diabetes, BMI, or waist circumference.

Biomarker analyses

Of 92 analyzed unadjusted associations between biomarkers and HO, increased levels of two proteins were significant at an FDR < 0.05: Galectin-4 (Gal-4) and insulin-like growth factor-binding protein 1 (IGFBP-1) (Additional file 3: Table S3). When these two proteins were included in logistic regression analyses adjusted for age and sex, Gal-4 remained significant (OR 1.76; CI 95% 1.23–2.51; p = 0.002) whereas IGFBP-1 did not (OR 1.24; CI95% 0.97–1.58; p = 0.087). Each 1 SD increase in Galectin-4 (Gal-4) levels was associated with a higher probability of being HO in the fully adjusted logistic regression model (OR 1.72; CI95% 1.16–2.54; p = 0.007) (Table 2). When further excluding external trauma (n = 38) as a determinant of being HO, the positive association for Gal-4 remained significant (p = 0.024). An interaction analysis was performed, showing no significant interaction between Gal-4 and prevalent diabetes (p = 0.16). However, given the known correlation between these two variables, [18, 19] a post-hoc stratified analysis was carried out and revealed that the association between Gal-4 and HO was only present among patients with diabetes (Table 3). To elucidate if the association between Gal-4 and the probability of being HO was linear, we carried out additional quartile analyses. In *Model 2*, p for trend was 0.009, and further analyses revealed

Table 1 Characteristics of the study population

	Total	HO	NHO	p
n	517	407	110	
Age (years)	67.2 (± 5.9)	67.7 (± 5.9)	65.4 (± 5.9)	**< 0.001**
Sex (women); n (%)	174 (33.7)	144 (35.4)	30 (27.3)	0.11
BMI (kg/m^2)	33.5 (± 3.3)	33.5(± 3.2)	33.4 (± 3.4)	0.76
Waist (cm)	110.2 (± 10.3)	110.4 (± 10.1)	109.5 (± 10.9)	0.46
Smoker; n (%)	67 (13.0)	54 (13.3)	13 (11.8)	0.67
SBP (mmHg)	149.6 (± 20.4)	148.5 (± 20.1)	153.8 (± 20.9)	**0.01**
DBP (mmHg)	86.1 (± 10.6)	85.6 (± 10.6)	88.0 (± 10.2)	**0.04**
Total cholesterol (mmol/L)	5.3 (± 1.1)	5.2 (± 1.1)	5.6 ± 1.1)	**0.001**
LDL-C (mmol/L)	3.4 (± 1.0)	3.3 (± 1.0)	3.7 (± 0.9)	**0.001**
HDL-C (mmol/L)	1.2 (± 0.3)	1.2 (± 0.3)	1.2 (± 0.4)	0.98
Triglycerides (mmol/L)	1.5 (1.0)	1.5 (1.0)	1.6 (1.2)	0.33
Fasting plasma glucose (mmol/L)	7.4 (± 2.2)	7.4 (± 2.1)	7.2 (2.3)	0.37
Lipid-lowering drugs; n (%)	159 (30.8)	142 (34.9)	17 (15.5)	**< 0.001**
Hypertension; n (%)	467 (90.3)	371 (91.2)	96 (87.3)	0.22
AHT drugs; n (%)	359 (69.4)	302 (74.2)	57 (51.8)	**< 0.001**
Prevalent diabetes; n (%)	262 (50.7)	209 (51.4)	53 (48.2)	0.56

Values are means (± standard deviation), medians (IQR) or numbers (%). *AHT* antihypertensive, *BMI* body mass index, *DBP* diastolic blood pressure, *HDL-C* high density lipoprotein concentration, *HO* hospitalized subjects with obesity, *LDL-C* low density lipoprotein concentration, *NHO* non hospitalized subjects with obesity. Bold values denote statistical significance at the p<0.05

Table 2 Logistic regression models displaying associations of Galectin-4 levels and probability of being HO

	HO (n = 407) vs. NHO (n = 110)	
	OR (CI95%)	**p**
Unadjusted		
Galectin-4	2.03 (1.42–2.90)	**< 0.001**
Model 1		
Galectin-4	1.85 (1.28–2.67)	**0.001**
Age	1.05 (1.01–1.09)	**0.013**
Sex	0.93 (0.56–1.53)	0.765
Model 2		
Galectin-4	1.72 (1.16–2.54)	**0.007**
Age	1.05 (1.00–1.09)	**0.030**
Sex	0.73 (0.42–1.25)	0.246
Diabetes	0.60 (0.33–1.10)	0.098
Total cholesterol	0.71 (0.56–0.86)	**< 0.001**
Smoking	1.34 (0.67–2.65)	0.407
Hypertension	1.03 (0.50–2.11)	0.938
BMI	1.00 (0.93–1.07)	0.885
FPG	1.27 (0.92–1.75)	0.140

Values are odds ratios (OR) and 95% confidence intervals. Bold values denote statistical significance at the p<0.05

BMI body mass index, *FPG* fasting plasma glucose, *HO* hospitalized subjects with obesity

that the risk of being HO was found to be strongest in the upper quartile (Additional file 4: Table S4). Finally, we explored how diabetes prevalence and glucose levels differed across quartiles of Gal-4 levels. The highest proportion of subjects with diabetes was found in the upper quartile (Q4) of Gal-4 (65.9%), compared to 27.9% in the lowest quartile of Gal-4 (p for difference between groups $= 9.6 \times 10^{-9}$). Similarly, glucose levels were higher in the upper quartile (Q4) of Gal-4 (p for difference between Q1 and Q4 $= 6.1 \times 10^{-7}$) as compared with Q1.

.

Discussion

By using a newly adopted definition of metabolic health in obesity, based on history of hospitalization for somatic disorders up until late mid-life, [9–11, 20] we found that increased levels of Gal-4 were independently associated with a higher probability of having been hospitalized in a cohort of middle-aged and older obese subjects. Descriptive data at baseline examination did not reveal any differences in neither BMI nor waist circumference between HO and NHO, suggesting a similar fat distribution. However, plasma total cholesterol, LDL-C and blood pressure were significantly lower among HO, likely because of a higher prevalence of medical treatment with both antihypertensive and lipid-lowering drugs. Finally, the positive association between Gal-4 and the HO phenotype was significant only in subjects with diabetes.

We have previously carried out cross-sectional studies in the Malmö Diet and Cancer Study cohort, where NHO was defined by using a novel approach of a history of

Table 3 Post-hoc analysis comparing levels of Gal-4 in obese subjects with or without prevalent diabetes

	Subjects without diabetes		**Subjects with diabetes**	
	n = 255		**n = 262**	
	HO n = 198; NHO n = 57		**HO n = 209; NHO n = 53**	
Model 1	**OR (CI95%)**	**p**	**OR (CI95%)**	**p**
Galectin-4	1.52 (0.99–2.53)	0.111	2.45 (1.38–4.35)	**0.002**
Age	1.06 (1.01–1.12)	**0.024**	1.07 (1.01–1.13)	**0.016**
Sex	1.08 (0.56–2.09)	0.824	0.62 (0.29–1.35)	0.228
Model 2	**OR (CI95%)**	**p**	**OR (CI95%)**	**p**
Galectin-4	1.45 (0.84–2.49)	0.172	2.26 (1.25–4.07)	**0.007**
Age	1.06 (1.00-1.12)	**0.039**	1.03 (0.97–1.10)	0.279
Sex	0.93 (0.44–1.96)	0.843	0.41 (0.18–0.97)	**0.043**
Total choles§terol	0.92 (0.67–1.25)	0.574	0.60 (0.44–0.81)	**0.001**
Current smoker	1.97 (0.74–5.29)	0.177	1.01 (0.38–2.68)	0.991
Hypertension	1.09 (0.46–2.57)	0.849	0.84 (0.23–3.03)	0.784
BMI	1.08 (0.95–1.23)	0.250	0.94 (0.86–1.03)	0.200
FPG	1.89 (0.88–3.99)	0.063	1.15 (0.80–1.63)	0.454

Values are odds ratios (OR) and 95% confidence intervals. Bold values denote statistical significance at the p<0.05

BMI body mass index, *FPG* fasting plasma glucose, *HO* hospitalized subjects with obesity, *NHO* non hospitalized subjects with obesity

non-hospitalization for somatic disorders up until midlife [9–11]. In those studies we found that NHO had a decreased risk of both total mortality and incident CVD compared with HO during a 20-year follow-up period. When comparing NHO with non-obese controls, there were no significant differences in terms of mortality or CVD risk [9]. Potential protective factors included a more favorable lipid and glucose profile, downregulation of potentially harmful proteomic biomarkers and a less sedentary lifestyle [10]. Moreover, lower plasma levels of antibodies against anti-phosphorylcholine, which possess anti-inflammatory properties and is coupled with lower CVD risk, were associated with a higher risk of being HO [11]. This is in line with previous research focusing on obesity phenotypes with different cardiometabolic disease risk but with a different terminology, namely metabolically healthy obesity (MHO) [20, 21].

Metabolically healthy obesity (MHO)

The evolving concept of MHO describes obese individuals that through proposed protective mechanisms, such as peripheral body fat distribution, lower grade of chronic inflammation and higher insulin sensitivity, seem to escape metabolic or cardiovascular complications [20–22]. This description could be considered controversial, since increasing evidence suggests that MHO is not a steady state and can transform into metabolically unhealthy obesity over time. Moreover, when compared with metabolically healthy individuals with normal weight, there is a significantly increased risk for incident CVD and metabolic complications linked to MHO [23–26]. One major concern about the conflicting results lies in the definition of MHO which differs substantially between different studies, but mainly focuses on the absence of risk variables included in the metabolic syndrome [27]. There is now an ongoing debate as to whether the term MHO should be avoided and instead be treated as a conceptual model to study mechanisms linking obesity to risk for or protection from cardiometabolic complications [28].

Galectin-4

Being part of the galectin family (consisting of 15 small leptin peptides), Gal-4 is expressed almost exclusively in the gastrointestinal tract of healthy individuals, where it plays a role in controlling intestinal inflammation. It reduces proinflammatory cytokine production in the intestinal mucosa, and knockdown of the Gal-4 peptide promotes colorectal cancerogenesis. This suggests that Gal-4 plays a significant role in the pathophysiology of the development of both inflammatory bowel disease and colorectal cancers [29]. However, the physiological role of Gal-4 is multifaceted and further include apical protein trafficking, lipid raft stabilization, intestinal wound healing and bacterial pathogen fighting [30]. Epidemiological data also strongly propose an involvement of Gal-4 in cardiometabolic diseases, suggesting it may be considered as a predictive biomarker for the development of CVD and diabetes [18]. Still, the causal pathway is poorly understood [13, 19]. One theory might lie at the cellular level, where Gal-4 is part of the apical protein transport from the Golgi-apparatus to the apical cell membrane of the enterocyte, including the well-known protease dipeptidyl peptidase-4 (DPP-4) [31]. In mice, DPP-4 seems to be misguided and accumulates intracellularly when Gal-4 is depleted [31]. DPP-4 plays a major role in promoting cardiometabolic disease by cleaving and thus inactivating glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1 (GLP-1), i.e., two of our most common incretins [32]. Modern anti-diabetic drugs such as DPP4-inhibitors and GLP-1 agonists are incretin-based and part of the standard treatment of DM2 as second-line drugs in most patients [33]. Incretins are involved in appetite control and delaying gastric emptying actions that are dependent on GLP-1 receptor activation within the central nervous system, thus having the potential to regulate body weight [34]. Furthermore, another study of women with gestational diabetes found an overexpression of Gal-4 in the placental syncytiotrophoblast cells, compared to healthy controls [35]. Thus, one proposed explanation for our main finding may be Gal-4's involvement in the development of diabetes, which also has been suggested in a previous publication with a similar approach of proteomic exploration [36]. To elucidate this, we carried out a post-hoc analysis, suggesting that elevation in Gal-4 was associated with higher probability of being HO only in those with prevalent diabetes.

Gal-4 has a potential inflammatory role in the intestinal mucosa. Previous studies have linked obesity and diabetes to altered composition of the gut microbiota [37, 38]. Changes in gut microbiota, i.e., through an unhealthy diet, lead to damage of the intestinal barrier, promote leakage and thus endotoxemia through higher levels of lipopolysaccharides systemically, which in turn stimulates the development of low-grade systemic inflammation associated with the negative impact of both obesity and metabolic disorders [37]. Therefore, Gal-4 might, at least in theory, aggravate the pathological processes induced by the obese-diabetic microbiota.

Study strengths and limitations

By using a definition of individuals with obesity with a more favorable metabolic health as not having been hospitalized for somatic disease up until late midlife, we were able get an objectively defined and more stable phenotype which could serve as an alternative to the

conventional way of defining metabolic health within the population with obesity, commonly called MHO. Previous definitions focus on the absence of criteria for the metabolic syndrome, which could shift intra-individually during repeated measurements at different occasions. Moreover, by renaming metabolic health in obesity as non-hospitalized versus hospitalized individuals with obesity instead of MHO, we avoid the perception of certain phenotypes of obesity labeled as healthy.

There are limitations to this study. Its cross-sectional nature precludes any conclusions about causality. However, the study subjects come from a well-characterized, retrospective cohort with excellent national, and well-validated, register data on hospitalization, which is why it was possible to apply our approach to define NHO and HO. This study only covers individual data collected at one regional center. A multicenter study to replicate the findings would be preferable, but to reduce false positive findings, the use of FDR analysis was carried out. Furthermore, because our subjects were of European descent, these findings might not be generalizable to other populations. Similarly, the population selection based on glucometabolic disturbances could raise concerns of how well this cohort represents the general population. However, when compared with similar cohorts, the incidence rate of diabetes was proportionate [39, 40]. The Olink CVD III panel is partially restricted to proteins associated with CVD and inflammation, and an extended analysis including biomarkers related to diabetes and/or metabolism would most likely add information about the pathophysiology in HO. Lastly, another limitation of this study was that subjects with a non-hospitalization status prior to baseline could still suffer from cardiometabolic disturbances, since no pre-defined diagnoses of hospitalization were decided upon, and many individuals could be treated for chronic illnesses within a primary health care unit. On the other hand, these conditions could have been milder or counterbalanced by protective mechanisms in the affected subjects leading to a status of non-hospitalization in our analyses.

Conclusions

In obese subjects during late mid-life, increased Galectin-4 levels were associated with a higher probability of being an individual with a history of HO. This association was only significant in subjects with diabetes, implying a role for Galectin-4 in diabetes and its complications.

Abbreviations

BMI: Body mass index; CVD: Cardiovascular disease; DM2: Type-2 diabetes; Gal-4: Galectin-4; GLP-1: Glucagon-like peptide 1; HO: Hospitalized subjects with obesity; IFG: Impaired fasting glucose; MHO: Metabolically healthy obesity; MPP: Malmö Preventive Project; MPP-RES: MPP-Re-Examination Study; MUO: Metabolically unhealthy obesity; NHO: Non-hospitalized subject with obesity; NOC: Non-obese controls; NT-proBNP: N-terminal pro-b-type natriuretic peptide; SD: Standard deviation.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12933-022-01559-9.

Additional file 1: Table S1. List of all causes of hospitalization in the HO subgroup

Additional file 2: Table S2. List of all 92 proteins included in analyses.

Additional file 3: Table S3. False discovery rate (FDR) detection of all 92 proteins included in the analyses.

Additional file 4: Table S4. Quartile analyses of the association between Gal-4 and the probability of being HO.

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Author contributions

JK, AJ, HH and MM analyzed and interpreted the patient data, as well as wrote the majority of the manuscript. PMN helped with the design of the work and contributed revision of the work. OM, MP, JM, LR, UL, BD, ML, PMN, EB, MHO contributed substantially in the multiple revisions of the manuscript with useful comments, manuscript changes and interpretation of the results. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Regional Ethical Review Board at Lund University, Sweden (LU 244-02) and complied with the Helsinki Declaration.

Consent for publication

Not applicable.

Competing interests

EB is an employee of AstraZeneca. The remaining authors report no conflicts of interest.

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