Adult weight change and cardiometabolic disease: studies into underlying pathways

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CHAPTER 7

Preventive factors and underlying pathways of incident cardiometabolic disease in obesity

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ABSTRACT

Introduction: Not all individuals with obesity develop chronic cardiometabolic disease. We aimed to identify risk factors that may help to prevent cardiometabolic disease in people with obesity.

Methods: The Netherlands Epidemiology of Obesity study is a prospective cohort study including 6671 middle-aged men and women. Incident diagnoses of diabetes and cardiovascular disease were collected through medical records from general practitioners during ten years of follow-up. With Cox proportional hazards models, we first calculated hazard ratios (HR) with 95% confidence intervals (CI) for incident cardiometabolic disease (including type 2 diabetes mellitus, myocardial infarction and cerebrovascular events) in individuals with body mass index (BMI) ≥27 kg/m^2, related to measures of body fat distribution, metabolic syndrome, and lifestyle factors, adjusted for appropriate confounding factors. Second, we calculated the synergy index (SI) to investigate interaction between risk factors and obesity on an additive scale.

Results: After exclusion of participants with a history of cardiometabolic disease at baseline (n=860), who were not in a fasting state (n=21) or who were lost to follow-up (n=120), 5670 participants (54% women, 42% obesity) were analysed. During a total of 37,034 person-years follow-up, 383 participants were diagnosed with incident cardiometabolic disease. In participants with BMI ≥27 kg/m^2, all studied risk factors related to body fat distribution and metabolic syndrome were associated with increased risk of incident cardiometabolic disease. High fasting plasma glucose was particularly harmful in those with obesity (HR 7.62 [5.63 – 10.31]) as was smoking (being a smoker with obesity was associated with a 3-fold increased risk of cardiometabolic disease compared with non-smokers without obesity (HR 2.89 [2.12 – 3.95])

Discussion: We observed that abdominal adiposity, smoking and hyperglycaemia were particularly harmful to cardiometabolic disease risk in individuals with obesity. A healthy lifestyle, preventing or reducing abdominal obesity, and treatment of metabolic risk factors may help to prevent cardiometabolic disease in people with obesity.

INTRODUCTION

Obesity, as defined by a body mass index (BMI) of 30 kg/m^2 or higher, is a well-established causal risk factor for type 2 diabetes and cardiovascular diseases (1). However, importantly, many individuals with obesity remain free of cardiometabolic disease (2). Body fat distribution, metabolic consequences of obesity, and lifestyle behaviour may differ considerably between individuals with obesity and may in part explain why some people with obesity develop cardiometabolic disease and others do not.

It is well-established that abdominal obesity is driving the increased cardiometabolic risk associated with obesity (1, 3-5). Within the abdomen, fat is stored subcutaneously or in the visceral area. According to the so-called 'lipid overflow' hypothesis, when the capacity of hypertrophic adipocytes to expand is exceeded, lipids 'overflow' and accumulate in the visceral area and in normally lean organs as the heart or the liver (ectopic fat) (3, 6, 7). Excess visceral adipose tissue is associated with a systemic low-grade inflammatory state (3, 8-11), insulin resistance (11), dyslipidaemia, hepatic steatosis, and thereby an increased risk of cardiometabolic disease (12).

Next to body fat distribution and metabolic risk factors, lifestyle factors including smoking, physical activity, alcohol intake, and diet quality have been associated with incidence of chronic diseases and disease-free life expectancy (13). Insights in factors that increase the risk of cardiometabolic disease in individuals with obesity may yield particular targets for interventions, which show the largest reduction in risk in this high-risk population to develop cardiometabolic disease.

The present study aimed to investigate the association between a wide range of risk factors related to body fat distribution, metabolic syndrome, and lifestyle with incident cardiometabolic disease in people with obesity. Thereby, we aimed to identify risk factors that may help to prevent cardiometabolic disease in individuals with obesity.

METHODS

Study design and study population

The NEO study is a population-based, prospective cohort study of individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. Men and women aged between 45 and 65 years with a self-reported BMI of 27 kg/m^2 or higher, living in the greater area of Leiden (in the West of the Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited, irrespective of their BMI.

Recruitment of participants started in September 2008 and completed at the end of September 2012. In total, 6671 participants have been included, of whom 5217 with a BMI of 27 kg/m^2 or higher. The study design and population are described in detail elsewhere (14).

Participants were invited to visit the NEO study center of the LUMC for a baseline study visit after an overnight fast and were asked to bring all medication they were using in the month preceding the study visit. Prior to the study visit, participants completed a general questionnaire at home to report demographic, lifestyle and clinical information.

At the study center, participants underwent an extensive physical examination, including anthropometry and blood sampling. In addition, participants completed a screening form, asking about anything that might create a health risk or interfere with magnetic resonance imaging, e.g., presence of metallic devices, claustrophobia and a body circumference > 1.70 m. Of the eligible participants, 2580 participants were randomly selected to undergo magnetic resonance imaging (MRI).

During ten years of follow-up, new diagnoses of cardiometabolic diseases have been collected by extraction of medical data from the electronic health records of general practitioners. Time of follow-up was defined as the number of days between the baseline of the study and the date of diagnosis, or censoring due to death, loss to follow-up, or the end of the follow-up (extraction date at the GP in 2018), whichever came first.

For the present study, participants with a medical history of myocardial infarction (n=114), cerebrovascular accident (n=128) or type 2 diabetes at baseline (n=618) were excluded from the analyses. In addition, individuals who did not come to the study center in a fasting state (n=21) were excluded, as well as 120 participants who were lost to follow-up.

The Medical Ethical Committee of the LUMC approved the NEO study and all participants provided written informed consent.

Risk factors for cardiometabolic disease in a population with obesity

Risk factors related to body fat distribution

Body height without shoes was measured with a vertically fixed, calibrated tape measure. Body weight was measured and percent body fat was estimated by the Tanita bio impedance balance (TBF-310, Tanita International Division, United Kingdom) without shoes and 1 kg was subtracted to correct for weight of clothing. BMI at baseline was calculated by dividing the weight in kg by the height in meters squared. Weight gain during adulthood was calculated by subtracting recalled BMI at age 20 years from BMI at middle age, as measured during the baseline visit of our study. We calculated relative weight gain as (body weight at middle age (kg) – body weight at age 20 years (kg)) / body weight at age 20 years (kg) * 100. Waist circumference was measured between the border of the lower costal margin and the iliac crest with the precision of 0.1 cm.

Visceral adipose tissue was directly assessed by MRI (1.5 Tesla MR imaging, Philips Medical Systems, Best, Netherlands) using a turbo spin echo imaging protocol (300/20; flip angle, 90º; section thickness, 10 mm, section gap, 2 mm). At the level of the fifth lumbar vertebra, three transverse slices were obtained during one breath-hold. Visceral fat areas were quantified by converting the number of pixels to centimetres squared for all three slices and totalling the areas of the three sections, using in-house-developed software (MASS; Leiden

University Medical Center, Leiden, the Netherlands). The mean of visceral adipose tissue content was used in the analyses.

Hepatic triglyceride content was quantified using ^{1}H-magnetic resonance spectroscopy of the liver. An 8 mL voxel was positioned in the right lobe of the liver, avoiding gross vascular structures and adipose tissue depots. Sixty-four averages were collected with water suppression (repetition time = 2900 ms; echo time = 23 ms [2900/23]). Without changing any parameters, spectra without water suppression, with a repetition time of 10 s and with four averages were obtained as internal reference. Hepatic triglyceride content relative to water was calculated as (signal amplitude of triglyceride)/(signal amplitude of water) × 100. Fatty liver was defined as a hepatic triglyceride content of ≥5.56% (15).

Metabolic risk factors
Systolic and diastolic blood pressure were obtained by an OMRON™ digital sphygmomanometer at the left arm. Fasting blood samples were drawn from the antecubal vein after 5 min rest of the participant, after an overnight fast of at least 10 h. Fasting plasma glucose and serum cholesterol and triglyceride concentrations were determined using standard clinical chemistry methods (Roche Modular P800 Analyzer, Roche Diagnostics, Mannheim, Germany) in the central clinical chemistry laboratory of the LUMC (14). Serum LDL cholesterol was calculated using the Friedewald formula (16).

Concentrations of CRP were determined using a high sensitivity CRP assay (TINA-Quant CRP HS system, Roche, Germany and Modular P800, Roche, Germany).

Lifestyle risk factors
Habitual dietary intake of all participants was estimated using a semiquantitative self-administered 125-item Food Frequency Questionnaire (FFQ) (17, 18). In this questionnaire, participants reported their frequency of intake of foods during the past month (times per day, week, month, never). This was combined with the assessment of serving size (spoons of potatoes, pieces of fruit, etc). Dietary intake of nutrients and total energy was estimated using the Dutch Food Composition Table (NEVO-2011). Based on the FFQ, we calculated the adapted version of the Dutch Healthy Diet(DHD)-index for each participant, which is a continuous score ranging from 0 and 130 and represents the adherence to the Dutch Guidelines for Healthy Diet of 2015 as described by the Health Council of the Netherlands and originally consists of fifteen components (19). A higher score means a better adherence to the 2015 Dutch Guidelines for a Healthy Diet. Alcohol consumption was reported in the FFQ and expressed as grams of alcohol consumed per day.

Tobacco smoking habits were reported and divided into three categories: current smoker, former smoker, and never smoker. Participants reported the frequency and duration of their physical activity during leisure time on the Short Questionnaire to Assess Health-enhancing activity (SQUASH), which we expressed in MET-hours per week

Incident cardiometabolic disease
New diagnoses of cardiometabolic disease, including type 2 diabetes mellitus, myocardial infarction and cerebrovascular accident, were extracted from electronic health records of the participants. Extraction was based on 1) the International Classification of Primary

Care (ICPC), coding used by GPs to indicate health problems, 2) screening of predefined key words in the descriptions in the GP database, and 3) prescription of specific medication, registered according to the Anatomical Therapeutic Chemical (ATC) codes or by screening medication names (only for type 2 diabetes and cerebrovascular accident). The index date was defined as the first date of an ICPC-coded diagnosis, strong indication for the diagnosis based on key words or prescription of relevant medication.

Diagnosis of type 2 diabetes mellitus was indicated by T90 (diabetes mellitus), T90.02 (diabetes mellitus type 2) or the presence of any keywords (e.g., synonyms of (type 2) diabetes mellitus). In addition, the medication list of participants was checked for the use of insulin, metformin and sulfonylurea derivative; participants using these medications were considered to have diabetes mellitus type 2.
Diagnosis of myocardial infarction was indicated by ICPC K75 (recent myocardial infarction) or K76.02 (myocardial infarction > 4 weeks ago) or presence of any keywords. Keywords included synonyms of myocardial infarction, presence of chest pain, or mention of cardiovascular surgery procedures such as coronary artery bypass grafting (CABG) and angioplasty. In case only a key word was found and an ICPC was lacking in the search, the electronic health record was manually checked to confirm the diagnosis.

Diagnosis of cerebrovascular accident was indicated by K90 (cerebrovascular accident), or one of its subtypes K90.01 (subarachnoid haemorrhage), K90.02 (intracerebellar haemorrhage) or K90.03 (cerebral infarction), or presence of keywords. Keywords included synonyms of cerebrovascular accident or haemorrhage. The medication list of participants was checked for the use of specific anticoagulants.

In case of uncertainty, the general practitioner of the participants was contacted to confirm the (date of) diagnosis.

Statistical analyses
The characteristics of the study population were expressed as percentages, mean and standard deviation or median and interquartile range, stratified by BMI <27 kg/m^2 and BMI ≥27 kg/m^2 based on the inclusion criteria of the NEO study. We first examined risk factors in association with cardiometabolic disease in individuals with BMI≥27 kg/m^2, and subsequently examined the interactions between the risk factors and obesity (BMI>30 kg/m^2) in relation to risk of cardiometabolic disease.

We calculated the incident rates with 95% confidence interval of type 2 diabetes, myocardial infarction, cerebrovascular accident and the composite outcome cardiometabolic disease. Second, we tested for the proportional hazard assumption by making log-log plots. Cox proportional hazards models were used to calculate hazard ratios (HRs) with 95% confidence intervals to examine the association between the risk factors and the composite outcome in the population with BMI 27 kg/m^2 or higher. Results were presented as the hazard ratios with the accompanying 95% confidence intervals, with the reference category coded as the category with the lowest risk.

Because some individuals might experience more than one event, we first analysed the oc-

currence of first ever cardiometabolic disease event. In addition, we analysed the separate outcomes using cause-specific hazards (20), where cases were censored at the development of the first event. For example, some participants might be diagnosed with type 2 diabetes, and later during follow-up suffer from a myocardial infarction. In this example, the participants were counted as cases in the cause-specific hazard of type 2 diabetes (first event), but were censored in the cause-specific hazard of myocardial infarction.

Cox proportional hazards models were used to examine the presence of interaction between the relation between obesity and risk factors in relation to the risk of cardiometabolic disease on an additive scale. Continuous exposures were standardised using the population mean and standard deviation. Results were presented as the hazard ratios with the accompanying 95% confidence intervals, with the reference category coded as the category with the lowest. By this means, the reference category was the exposure to both preventive factors. The cut-offs (either clinical cut-off or median-based) of the risk factors can be found in **Supplementary Table 1**.

Based on these analyses, we calculated the synergy index (SI), which can be interpreted as the excess risk from exposure to both exposures jointly, relative to the risk from exposure without presence of interaction (21, 22). Synergy index can then be calculated as follows:
$S= [HR^{++}] - 1]/[HR^{+-} - 1)+(HR^{-+} - 1)]$.

As a sensitivity analysis, we excluded all participants with missing data on lifestyle factors or metabolic risk factors, and repeated the interaction analyses for all exposures, adjusted for sex and age. In all analyses, we adjusted the first model for sex and age, and the second model was additionally adjusted for BMI. In our multivariable-adjusted model, we additionally adjusted the analyses with exposures related to body fat distribution for lifestyle factors included in our analyses (Dutch Healthy Diet Index, physical inactivity, alcohol consumption and smoking). The analyses with exposures related to metabolic syndrome were additionally adjusted for lifestyle factors and measures of body fat distribution (BMI, adult weight gain, total body fat, waist circumference, visceral fat and liver fat) (see **Figure 1**).

Analyses were performed with STATA Statistical Software version 14.1 (Statacorp, College Station, TX, USA). Interactions and corresponding Synergy indices and 95% confidence intervals were calculated using the icp tool in STATA.

Figure 1. Hypothesis path diagram depicting the associations between lifestyle factors, body fat distribution, metabolic syndrome and cardiometabolic disease. Analyses with one of the measures of body fat distribution as exposure and cardiometabolic disease as outcome were adjusted for all lifestyle factors. Analyses with one of the metabolic risk factors as exposure and cardiometabolic disease as outcome were adjusted for all lifestyle factors and body fat distribution measures. All analyses will be adjusted for sex and age.

RESULTS

Characteristics of the study population

The characteristics of the study population at risk stratified by BMI<27 kg/m^2 and BMI≥27 kg/m^2 are presented in **Table 1**. In the present study, 1360 participants had a BMI lower than 27 kg/m^2 (59% women) and 4310 had a BMI of 27 kg/m^2 or higher (53% women). In both groups, the mean (SD) age was 56 (6) year.

The mean (SD) body mass index in the group with BMI<27 kg/m^2 was 24.3 (17.2 – 26.9) kg/m^2, and 31.7 (27.0 – 61.2) kg/m^2 in the group with BMI≥27 kg/m^2. Overall, participants with BMI<27 kg/m^2 adhered more to the dietary guidelines, were more physically active, but consumed more alcohol. Metabolic factors were more favourable in individuals with BMI<27 kg/m^2 than in individuals with BMI≥27 kg/m^2, for example fasting glucose was lower (5.3 [0.8] mmol/l and 5.8 [1.2] mmol/l), as was the mean systolic blood pressure (128.8 [17.0] mmHg and 133.6 [16.9] mmHg).

Table 1. Characteristics of the Netherlands Epidemiology of Obesity study, stratified by body mass index (BMI)<27 kg/m^2 and BMI≥27 kg/m^2 (n=5670)

Characteristic	**BMI<27.0 kg/m^2**	**BMI≥27.0 kg/m^2**
	n=1360	n=4310
Sex (% men)	41	47
Age (years)	55.6 (6.1)	55.5 (6.0)
Ethnicity (% white)	95	95
Education (% high)	50	35
Body fat distribution		
Body Mass Index (kg/m^2)	24.3 (2.2)	31.5 (3.9)
Relative weight gain since age 20 years (%)	15.5 (12.9)	34.6 (19.0)
Body fat (%)	M: 22.3 (3.6) / W: 34.4 (5.1)	M: 30.2 (5.1) / W: 44.3 (3.9)
Waist circumference (cm)	M: 93.2 (6.7) / W: 82.2 (8.5)	M: 108.5 (9.2) / 102.0 (10.5)
Visceral adipose tissue (cm^2)[a]	M: 95.1 (44.5) / W: 54.5 (29.5)	M: 151.1 (57.6) / W: 109.9 (48.0)
Hepatic triglyceride content (%)[b]	M: 2.8 (1.8 – 5.3) / W: 1.5 (1.0 – 3.2)	M: 7.4 (3.7 – 15.0) / W: 4.4 (1.3 – 10.5)
NAFLD (>5.56% HTGC)	M: 8 W: 5	M: 22 W: 12
Metabolic factors		
Mean systolic blood pressure (mmHg)	128.5 (17.0)	133.2 (16.7)
Mean diastolic blood pressure (mmHg)	82.0 (10.2)	85.8 (10.1)
Hypertension (% yes)	30	43
Waist circumference (cm)	M: 8 W: 5	M: 8 W: 5
Blood pressure-lowering medication (%)	17	29
Triglycerides (mmol/L)	0.9 (0.7 – 1.3)	1.4 (1.0 – 1.9)
HDL-C (mmol/L)	1.6 (0.5)	1.4 (0.4)
LDL-C (mmol/L)	4.5 (1.2)	5.1 (1.3)
Lipid-lowering medication (%)[c]	6	11
Fasting glucose (mmol/L)	5.3 (0.7)	5.6 (0.7)
hsCRP (mg/L)	0.9 (0.5 – 1.8)	1.9 (1.1 – 3.8)
Triglycerides (mmol/L)	0.9 (0.7 – 1.3)	1.4 (1.0 – 1.9)
Lifestyle factors		
Dutch Healthy Diet Index (score)	71.7 (15.0)	68.4 (14.5)
Physical activity (MET/week)[d]	30.5 (16.8 – 50.8)	26.3 (12.5 – 46.0)
Alcohol consumption (grams per day)	10.0 (3.2 – 21.0)	9.1 (1.7 – 22.2)
Smoking (% current)	16	16

[a] n=2,258. [b] n=1,854 [c] Use of lipid-lowering medication included fibrates and statins. [d] Physical activity during leisure time. Abbreviations: BMI, body mass index; HOMA-IR, homeostatic model assessment insulin resistance; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol. Data are presented as mean (SD or range), median (25th–75th percentile) or percentage.

Associations of risk factors with incident cardiometabolic disease in a population with BMI≥27 kg/m²

During a median of 6.7 years of follow-up, 383 participants experienced a first event of cardiometabolic disease. The population at risk was n=5670 with 37034 person-years, resulting in an incidence rate of 1.034 per 100 person years. Of this population, the incidence rate was 0.48 per 100 person-years in individuals with BMI<27 kg/m², and 1.19 in individuals with BMI≥27 kg/m² per 100 person years (**Table 2**).

Table 2. Population at risk, their time at risk, the number of events and incidence of cardiometabolic disease (type 2 diabetes mellitus, myocardial infarction and cerebrovascular accidents) in ten years of follow-up in the NEO study.

Event	Population at risk (n)	Follow-up time (person-years)	Number of events	Incidence rate (per 100 py)
Cardiometabolic disease	5670	37034.2	383	1.03
BMI<27 kg/m²	1360	8268.6	40	0.48
BMI≥27 kg/m²	4310	28765.6	343	1.19

Abbreviations: BMI, body mass index.

Table 3 shows the association between the standardised risk factors and incident cardiometabolic disease in the population with BMI≥27 kg/m². Overall, we observed that all risk factors related to body fat distribution were associated with the risk of incident cardiometabolic disease, also after taking into account BMI at baseline. For example, one standard deviation (57.2 cm²) in visceral adipose tissue was associated with 1.45 (95% CI 1.21 – 1.75) fold increased risk of cardiometabolic disease. In addition, metabolic factors were associated with the risk of incident cardiometabolic disease. For example, one standard deviation (0.9 mmol/L) in fasting glucose was associated with 2.24 (95% CI 2.09 – 2.39) fold increased risk of cardiometabolic disease. Lifestyle was not or weakly associated with the risk of cardiometabolic disease (HR 0.90 [95% CI 0.81 – 1.00]) per standard deviation (14.5 points) in Dutch Healthy Diet index.

After adjustment for lifestyle factors and measures of body fat distribution, results were similar. The results for the cause-specific analyses were similar (data not shown).

Table 3. The association between the risk factors (standardised using the population standard deviation) and incident cardiometabolic disease in an population with BMI ≥27.0 kg/m^2, n=4310

Exposure	Standard deviation (SD)	Hazard ratio (all events)[1]	Hazard ratio (all events, adjusted for sex, age, BMI at baseline)	Hazard ratio (all events, multivariable-adjusted analysis)[2]
Body fat distribution				
Body mass index (kg/m^2)	3.9	1.63 (1.49 – 1.79)	-	1.60 (1.46 – 1.76)
Adult weight gain (%)	19.0	1.40 (1.28 – 1.52)	1.11 (1.00 – 1.24)	1.15 (0.93 – 1.43)
Total body fat (%)	8.3	2.16 (1.83 – 2.55)	1.41 (1.09 – 1.82)	1.32 (1.01 – 1.73)
Waist circumference (cm)	10.5	1.76 (1.57 – 1.96)	1.37 (1.11 – 1.70)	1.28 (1.03 – 1.59)
Change in weight (%, range)	−7.6 (−32.2; −5.8)	1.9 (−4.9; 4.8)	14.9 (5.0; 24.9)	32.3(25.0; 49.8)
Visceral adipose tissue (cm^2)	57.2	1.73 (1.48 – 2.02)	1.45 (1.21 – 1.75)	1.42 (1.18 – 1.72)
Hepatic triglyceride content (%)	9.9	1.63 (1.43 – 1.86)	1.54 (1.34 – 1.77)	1.48 (1.30 – 1.70)
Metabolic risk factors				
Systolic blood pressure (mmHg)	16.7	1.28 (1.15 – 1.41)	1.23 (1.11 - 1.37)	1.26 (1.02 – 1.56)
Diastolic blood pressure (mmHg)	10.1	1.19 (1.08 – 1.32)	1.15 (1.04 – 1.28)	1.12 (0.91 – 1.39)
Triglycerides (mmol/L)	1.0	1.20 (1.14 – 1.26)	1.19 (1.13 – 1.25)	1.13 (0.98 – 1.30)
HDL-c (mmol/L)	0.4	0.58 (0.50 – 0.67)	0.63 (0.54 – 0.73)	0.68 (0.50 – 0.92)
LDL-c (mmol/L)	0.9	1.27 (1.15 – 1.40)	1.28 (1.16 – 1.41)	1.20 (1.00 – 1.44)
Glucose (mmol/L)	0.7	2.40 (2.26 – 2.56)	2.24 (2.09 – 2.39)	1.78 (1.58 – 2.01)
hsCRP (mg/L)	3.4	1.41 (1.31 – 1.51)	1.32 (1.22 – 1.43)	1.36 (1.18 – 1.57)
Lifestyle factors				
Smoking (yes)	-	1.38 (1.09 – 1.76)	1.40 (1.10 – 1.78)	-
Dutch Healthy Diet Index (score)	14.5	0.89 (0.80 – 0.99)	0.90 (0.81 – 1.00)	-
Physical inactivity (MET/h)	33.1	0.87 (0.78 – 0.98)	0.90 (0.80 – 1.00)	-
Alcohol consumption (grams per day)	17.8	1.03 (0.93 – 1.14)	1.06 (0.97 – 1.17)	-

[1]All analyses were adjusted for sex and age; [2]The analyses with exposures related to body fat distribution were adjusted for lifestyle factors (Dutch Healthy Diet Index, physical inactivity, alcohol consumption and smoking); the analyses with exposures related to metabolic syndrome were adjusted for lifestyle factors and body fat distribution (BMI, adult weight gain, body fat percentage, waist circumference, visceral fat and liver fat

Interaction between obesity and risk factors in relation to incident cardiometabolic disease
Table 4A to **4C** show the interaction analyses and corresponding Synergy Indices for the risk factors related to body fat distribution, metabolic syndrome and lifestyle, and obesity defined as BMI≥30 kg/m^2. Of this study population, 42% had a BMI of 30 kg/m^2 or higher.

Compared with non-smokers with a BMI below 30 kg/m^2, the combination of obesity and smoking was associated with an increased risk of cardiometabolic disease (HR; 95% CI 2.93; 2.16 – 3.98) with corresponding Synergy Index of 2.29 (1.04 – 5.04). Individuals with obesity and with a fasting plasma glucose concentration of 7.0 mmol/L or higher had an 8.32 (6.25 – 11.35) fold increased risk of cardiometabolic disease compared with individuals without obesity and with normal glucose concentrations. For obesity and glucose concentrations we observed additive interaction as well, based on the Synergy Index of 2.28 (1.63 – 3.18). Finally, individuals with obesity and excess visceral fat (based on sex-specific median levels) had an increased risk of cardiometabolic disease. In addition, excess visceral adipose tissue showed additive interaction with obesity on the risk of cardiometabolic disease compared with individuals without obesity and without excess visceral adipose tissue (HR: 4.16 [2.75 – 6.30] Synergy Index 2.66 [0.94 – 7.58]).

After adjustment for lifestyle factors and measures of body fat distribution, results were similar, as well as for the complete case analyses (**Supplementary Table 2A-2C**).

Table 4A. Interaction analysis between BMI lower or higher than 30 kg/m^2 and protective factors related to body fat distribution (reference category = BMI<30 g/m^2 and lowest risk category of protective factor), n=5697.

Exposure	Hazard ratio (all events)	Hazard ratio (all events, adjusted for lifestyle factors)[1]
Adult weight gain (%, ref = no excess weight gain)	1	1
Synergy Index	1.51 (0.85 – 2.69)	1.46 (0.83 – 2.57)
No excess weight gain and BMI≥30.0 kg/m^2	2.08 (1.45 – 2.98)	2.11 (1.47 – 3.05)
Excess weight gain and BMI<30.0 kg/m^2	1.34 (0.94 – 1.90)	1.38 (0.96 – 1.96)
Excess weight gain and BMI≥30.0 kg/m^2	3.14 (2.42 – 4.07)	3.18 (2.43 – 4.15)
Body fat percentage (ref = low body fat)	1	1
Synergy Index	1.03 (0.19 – 5.45)	0.76 (0.15 – 3.76)
Low body fat and BMI≥30 kg/m^2	2.96 (0.71 – 12.41)	3.84 (0.91 – 16.12)
High body fat and BMI<30 kg/m^2	1.54 (1.03 – 2.30)	1.51 (1.01 – 2.32)
High body fat and BMI≥30 kg/m^2	3.57 (2.45 – 5.21)	3.57 (2.41 – 5.27)
Waist circumference (ref = high waist circumference)	1	1
Synergy Index	1.07 (0.71 – 1.61)	1.09 (0.71 – 1.67)
Low waist circumference and BMI≥30.0 kg/m^2	2.58 (1.94 – 3.43)	2.55 (1.91 – 3.41)
High waist circumference and BMI<30.0 kg/m^2	1.83 (1.16 – 2.86)	1.69 (1.07 – 2.66)
High waist circumference and BMI≥30.0 kg/m^2	3.56 (2.59 – 4.89)	3.44 (2.49 – 4.74)
Visceral adipose tissue (cm^2, ref = low VAT)	1	1
Synergy Index	3.06 (0.79 – 11.89)	3.15 (0.74 – 13.32)
Low VAT and BMI≥30.0 kg/m^2	1.45 (0.72 – 2.91)	1.45 (0.72 – 2.92)
High VAT and BMI<30.0 kg/m^2	1.45 (0.85 – 2.48)	1.40 (0.81 – 2.43)
High VAT and BMI≥30.0 kg/m^2	3.76 (2.46 – 5.76)	3.68 (2.38 – 5.69)
Non-alcoholic fatty liver disease (%, ref = no NAFLD)	1	1
Synergy Index	1.77 (1.00 – 3.13)	1.75 (1.00 – 3.08)
No NAFLD and BMI≥30.0 kg/m^2	2.39 (1.89 – 3.01)	2.39 (1.89 – 3.03)
NAFLD and BMI<30.0 kg/m^2	1.04 (0.63 – 1.71)	1.11 (0.67 – 1.83)
NAFLD and BMI ≥30.0 kg/m^2	3.53 (2.60 – 4.79)	3.63 (2.67 – 4.95)

All analyses were adjusted for sex and age, where applicable. [1]Additionally adjusted for lifestyle factors (Dutch Healthy Diet Index, physical inactivity, alcohol consumption and smoking)

Table 4B. Interaction analysis between BMI lower or higher than 30.0 kg/m^2 and metabolic factors (reference category = BMI<30.0 kg/m^2 and lowest risk category of protective factor), n=5697

Exposure	Hazard ratio (all events)	Hazard ratio (all events, adjusted for lifestyle factors)[1]
Blood pressure (mmHg, ref = low blood pressure)	1	1
Synergy Index	0.98 (0.63 – 1.51)	0.57 (0.21 – 1.58)
Low blood pressure and BMI≥30.0 kg/m^2	3.21 (2.19 – 4.70)	2.82 (1.08 – 7.35)
High blood pressure and BMI<27 kg/m^2	2.52 (1.60 – 3.97)	2.84 (1.08 – 7.46)
High blood pressure and BMI≥30.0 kg/m^2	4.64 (3.17 – 6.79)	3.10 (1.15 – 8.39)
Triglycerides (mmol/L, ref = low triglycerides)	1	1
Synergy Index	1.72 (0.75 – 3.97)	0.58 (0.11 – 3.15)
Low triglycerides and BMI≥30.0 kg/m^2	2.74 (2.04 – 3.67)	2.27 (1.00 – 5.15)
High triglycerides and BMI<30.0 kg/m^2	1.57 (0.68 – 3.61)	3.50 (0.99 – 12.39)
High triglycerides and BMI≥30.0 kg/m^2	4.96 (2.96 – 8.31)	3.18 (0.98 – 10.29)
HDL-c (mmol/L, ref = high HDL-c)	1	1
Synergy Index	1.63 (1.07 – 2.50)	1.80 (0.38 – 8.43)
High HDL-c and BMI≥30.0 kg/m^2	2.34 (1.81 – 3.02)	1.67 (0.91 – 3.05)
Low HDL-c and BMI<30.0 kg/m^2	1.95 (1.32 – 2.86)	1.13 (0.48 – 2.63)
Low HDL-c and BMI≥30.0 kg/m^2	4.74 (3.59 – 6.25)	2.43 (1.25 – 4.72)
LDL-c (mmol/L, ref = low LDL-c)	1	1
Synergy Index	1.20 (0.82 – 1.76)	1.67 (1.14 – 5.04)
Low LDL-c and BMI≥30.0 kg/m^2	2.71 (1.94 – 3.78)	1.58 (0.70 – 3.55)
High LDL-c and BMI<30.0 kg/m^2	1.51 (1.08 – 2.13)	1.25 (0.61 – 2.57)
High LDL-c and BMI≥30.0 kg/m^2	3.68 (2.71 – 5.00)	2.39 (1.14 – 5.04)
Glucose (mmol/L, ref = low glucose)	1	1
Synergy Index	2.39 (1.66 – 3.46)	1.88 (0.94 – 3.74)
Low glucose and BMI≥30.0 kg/m^2	1.66 (1.13 – 2.42)	1.76 (0.75 – 4.12)
High glucose and BMI<30.0 kg/m^2	3.11 (2.20 – 4.40)	3.58 (1.72 – 7.46)
High glucose and BMI≥30.0 kg/m^2	7.62 (5.63 – 10.31)	7.27 (3.33 – 15.88)
hsCRP (mg/L, ref = low hsCRP	1	1
Synergy Index	1.60 (1.04 – 2.46)	1.20 (0.47 – 3.05)
Low hsCRP and BMI≥30.0 kg/m^2	2.17 (1.58 – 2.97)	1.83 (0.90 – 3.70)
High hsCRP and BMI<30.0 kg/m^2	1.77 (1.26 – 2.49)	1.73 (0.87 – 3.44)
High hsCRP and BMI≥30.0 kg/m^2	4.10 (3.13 – 5.38)	2.86 (1.44 – 5.68)

All analyses were adjusted for sex and age, where applicable. [1]Additionally adjusted for lifestyle factors and body fat distribution (BMI, adult weight gain, body fat percentage, waist circumference, visceral fat and liver fat).

Table 4C. Interaction analysis between BMI lower or higher than 30.0 kg/m^2 and lifestyle factors (reference category = BMI<30.0 kg/m^2 and lowest risk category of protective factor), n=5697

Exposure	Hazard ratio (all events)
Dutch Healthy Diet Index (score, ref = high DHDI)	1
Synergy Index	1.36 (0.80 – 2.30)
High DHDI and BMI≥30.0 kg/m^2	2.32 (1.71 – 3.14)
Low DHDI and BMI<30.0 kg/m^2	0.96 (0.69 – 1.35)
Low DHDI and BMI≥30.0 kg/m^2	2.74 (2.06 – 3.64)
Physical activity (MET/h, ref = physically active)	1
Synergy Index	1.48 (0.91 – 2.41)
Physically active and BMI≥30.0 kg/m^2	2.37 (1.74 – 3.22)
Physically inactive and BMI<30.0 kg/m^2	1.09 (0.77 – 1.53)
Physically inactive and BMI≥30.0 kg/m^2	3.16 (2.36 – 4.22)
Alcohol consumption (ref= low alcohol consumption)	1
Synergy Index	1.08 (0.61 – 1.92)
Low alcohol consumption and BMI ≥30.0 kg/m^2	2.33 (1.75 – 3.11)
High alcohol consumption and BMI<30.0 kg/m^2	0.76 (0.54 – 1.06)
High alcohol consumption and BMI≥30.0 kg/m^2	2.17 (1.61 – 2.93)
Smoking (ref = smoking no)	1
Synergy Index	2.52 (1.00 – 6.34)
Not smoking and BMI≥30.0 kg/m^2	1.79 (1.22 – 2.61)
Smoking and BMI<30.0 kg/m^2	0.96 (0.68 – 1.36)
Smoking and BMI≥30.0 kg/m^2	2.89 (2.12 – 3.95)

All analyses were adjusted for sex and age, where applicable

DISCUSSION

The aim of the present study was to identify risk factor that may help to prevent cardiometabolic disease in people with obesity. To that extent, we investigated a wide range of established risk factors related to body fat distribution, metabolic factors, and lifestyle factors in relation to incident cardiometabolic diseases in middle-aged people with obesity during a maximum of 10 years of follow-up. In a population with BMI≥27 kg/m^2, a favourable body fat distribution and healthy metabolic profile were strongly associated with a decreased risk of cardiometabolic disease, whereas healthy lifestyle factors were not or only weakly associated with an increased risk of developing cardiometabolic disease. When considering the joint effect of the risk factors and obesity, defined as BMI≥30 kg/m^2, we observed that fasting glucose and smoking showed additive interaction with obesity on the risk of cardiometabolic disease, as well excess visceral fat. These results suggest that that preserving low glucose levels and being a non-smoker, as well as maintaining low visceral adipose tissue will largely reduce the risk of incident cardiometabolic disease in individuals in obesity.

The results of our study demonstrate that within the population with obesity having a favourable body fat distribution, including low body fat, low waist circumference, and low levels of visceral adipose tissue and liver fat, is associated with a decreased risk of cardiometabolic disease. In a previous study using data from the NEO study, we demonstrated that weight gain during adulthood is associated with more visceral adipose tissue at middle age, compared with weight maintenance (23). This suggests that weight maintenance during adulthood plays an important role in preventing accumulation of excess visceral fat and eventually, cardiometabolic disease at middle age and older age. This finding can be explained by the association of excess visceral fat with an array of metabolic abnormalities (3, 8, 9), including systemic low-grade inflammation, insulin resistance, and dyslipidaemia, which might eventually result in an increased risk of cardiometabolic disease (3). In conclusion, our results suggest that both a favourable body fat distribution and metabolic profile contribute to a decreased risk of incident cardiometabolic disease in a population with obesity. These goals could be reached by lifestyle changes, e.g., healthy eating habits and physical exercise, that go hand in hand with weight loss (24). However, lifestyle change has been proven difficult, especially in the modern obesogenic environment (25). This underscores the need for health-promoting population-level interventions and governmental policies promoting a healthy lifestyle (25-27).

From previous literature, it is known that not everyone with obesity will develop cardiometabolic disease, and that metabolic factors, such as blood pressure, cholesterol, triglycerides, glucose concentrations and inflammatory markers likely contribute to this observation (28). This is in line with our results, as these indicate that within the population with obesity having a beneficial metabolic profile, such as low blood pressure, low triglyceride levels, low glucose levels, low high-sensitivity CRP levels and high HDL-cholesterol levels is associated with a decreased risk of cardiometabolic disease. Preserving low glucose levels will even be associated with an additive decrease in the risk of incident cardiometabolic disease in individuals in obesity, compared with individuals with both risk factors present. This might be driven by the large number of type 2 diabetes mellitus cases in the outcome of cardiometabolic disease. However, in addition to type 2 diabetes itself, glucose levels are known

to be robustly and causally associated with the risk of cardiovascular disease, as shown by Mendelian randomization analyses (29, 30). Because the number of events for myocardial infarction and cerebrovascular accident were small, we were not able to investigate the associations between risk factors and the separate outcomes of cardiometabolic disease.

In our study population of individuals with obesity, we did not observe robust associations of lifestyle factors such as dietary intake, physical activity and alcohol consumption with the risk of cardiometabolic disease. This was also the case when the analyses were not adjusted for BMI. Dietary intake, physical activity, and alcohol consumption were all based on self-report. The results of our interaction analyses indicate that being a non-smoker is associated with an additive decrease in the risk of incident cardiometabolic disease in individuals with obesity. This result might be due to an increased propensity of storing body fat centrally in smokers with obesity. It has been shown previously that smoking is associated with a decrease in overall body weight, however not with a decrease in waist circumference (31). In addition, smoking causes higher central adiposity as measured by waist circumference (32), a well-known risk factor of cardiometabolic disease. In addition to an effect on body fat distribution, smoking also has a vascular effect: in Mendelian randomization studies, smoking was associated with risk of type 2 diabetes, ischemic stroke, myocardial infarction, and heart failure (33-36).

Strengths of our study include data on many risk factors and confounding factors, and the availability of a range of directly assessed and accurate measures of body fat distribution such as visceral adipose tissue and hepatic triglyceride content. In addition, follow-up data of ten years on the development of cardiometabolic disease was available. A limitation that needs to be considered is that diagnoses of cardiovascular disease were collected by extraction of medical data from the electronic health records of general practitioners. Although we used a wide range of international codes and keywords to define diagnoses, and we checked uncertain diagnoses with the general practitioner of the study participants to ensure data quality, we might have missed or misclassified some diagnoses or dates of diagnosis. However, in general, extraction of diagnoses of type 2 diabetes mellitus and cardiovascular disease from electronic health records of general practitioners is a valid method to define cases, and is more reliable than self-report (37-39). Due to the small numbers, we were not able to perform analyses stratified by sex. Lastly, the majority of our study population was Caucasian, therefore the results of our study need to be confirmed in other ethnic groups.

In conclusion, our results suggest that both a favourable body fat distribution and metabolic profile contribute to a decreased risk of incident cardiometabolic disease in a population with obesity. Preserving low glucose levels and being a non-smoker, as well as maintaining low visceral fat, showed the largest reduction in cardiometabolic risk in this high-risk population to develop obesity-related disease. Future prospective studies need to investigate how to integrate interventions to improve body fat distribution, metabolic factors and lifestyle in the population in obesity to reduce the risk of future cardiometabolic disease.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest associated with this manuscript.

REFERENCES

1. Dale CE, Fatemifar G, Palmer TM, White J, Prieto-Merino D, Zabaneh D, et al. Causal Associations of Adiposity and Body Fat Distribution With Coronary Heart Disease, Stroke Subtypes, and Type 2 Diabetes Mellitus: A Mendelian Randomization Analysis. Circulation. 2017;135(24):2373-88.
2. Wildman RP, Muntner P, Reynolds K, McGinn AP, Rajpathak S, Wylie-Rosett J, et al. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). Arch Intern Med. 2008;168(15):1617-24.
3. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006;444(7121):881-7.
4. Neeland IJ, Poirier P, Despres JP. Cardiovascular and Metabolic Heterogeneity of Obesity: Clinical Challenges and Implications for Management. Circulation. 2018;137(13):1391-406.
5. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014;384(9945):766-81.
6. Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. Physiol Rev. 2013;93(1):359-404.
7. Virtue S, Vidal-Puig A. Adipose tissue expandability, lipotoxicity and the Metabolic Syndrome--an allostatic perspective. Biochim Biophys Acta. 2010;1801(3):338-49.
8. Bergman RN, Kim SP, Catalano KJ, Hsu IR, Chiu JD, Kabir M, et al. Why visceral fat is bad: mechanisms of the metabolic syndrome. Obesity (Silver Spring). 2006;14 Suppl 1:16S-9S.
9. Jensen MD. Is visceral fat involved in the pathogenesis of the metabolic syndrome? Human model. Obesity (Silver Spring). 2006;14 Suppl 1:20S-4S.
10. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. 2006;444(7121):840-6.
11. Frayn KN. Adipose tissue as a buffer for daily lipid flux. Diabetologia. 2002;45(9):1201-10.

12. Neeland IJ, Ross R, Despres JP, Matsuzawa Y, Yamashita S, Shai I, et al. Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement. Lancet Diabetes Endocrinol. 2019;7(9):715-25.
13. Li Y, Schoufour J, Wang DD, Dhana K, Pan A, Liu X, et al. Healthy lifestyle and life expectancy free of cancer, cardiovascular disease, and type 2 diabetes: prospective cohort study. BMJ. 2020;368:l6669.
14. de Mutsert R, den Heijer M, Rabelink TJ, Smit JW, Romijn JA, Jukema JW, et al. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. Eur J Epidemiol. 2013;28(6):513-23.
15. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab. 2005;288(2):E462-8.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.
17. Siebelink E, Geelen A, de Vries JH. Self-reported energy intake by FFQ compared with actual energy intake to maintain body weight in 516 adults. Br J Nutr. 2011;106(2):274-81.
18. Verkleij-Hagoort AC, de Vries JH, Stegers MP, Lindemans J, Ursem NT, Steegers-Theunissen RP. Validation of the assessment of folate and vitamin B12 intake in women of reproductive age: the method of triads. Eur J Clin Nutr. 2007;61(5):610-5.
19. Looman M, Feskens EJ, de Rijk M, Meijboom S, Biesbroek S, Temme EH, et al. Development and evaluation of the Dutch Healthy Diet index 2015. Public Health Nutr. 2017;20(13):2289-99.
20. Koller MT, Raatz H, Steyerberg EW, Wolbers M. Competing risks and the clinical community: irrelevance or ignorance? Stat Med. 2012;31(11-12):1089-97.
21. de Mutsert R, Jager KJ, Zoccali C, Dekker FW. The effect of joint exposures: examining the presence of interaction. Kidney Int. 2009;75(7):677-81.
22. Knol MJ, VanderWeele TJ, Groenwold RH, Klungel OH, Rovers MM, Grobbee DE. Estimating measures of interaction on an additive scale for preventive exposures. Eur J Epidemiol. 2011;26(6):433-8.
23. Verkouter I, Noordam R, de Roos A, Lamb HJ, Rosendaal FR, van Heemst D, et al. Adult weight change in relation to visceral fat and liver fat at middle age: The Netherlands epidemiology of obesity study. Int J Obes (Lond). 2019;43(4):790-9.
24. Lingvay I, Sumithran P, Cohen RV, le Roux CW. Obesity management as a primary treatment goal for type 2 diabetes: time to reframe the conversation. Lancet. 2021.
25. van Trier TJ, Mohammadnia N, Snaterse M, Peters RJG, Jorstad HT, Bax WA, et al. An appeal to our government for nationwide policies in the prevention of cardiovascular disease. Neth Heart J. 2021.
26. Seidell JC, Halberstadt J. The global burden of obesity and the challenges of prevention. Ann Nutr Metab. 2015;66 Suppl 2:7-12.
27. Swinburn B, Egger G, Raza F. Dissecting obesogenic environments: the development and application of a framework for identifying and prioritizing environmental interventions for obesity. Prev Med. 1999;29(6 Pt 1):563-70.
28. Appleton SL, Seaborn CJ, Visvanathan R, Hill CL, Gill TK, Taylor AW, et al. Diabetes and cardiovascular disease outcomes in the metabolically healthy obese phenotype: a co-

hort study. Diabetes Care. 2013;36(8):2388-94.
29. Ross S, Gerstein HC, Eikelboom J, Anand SS, Yusuf S, Pare G. Mendelian randomization analysis supports the causal role of dysglycaemia and diabetes in the risk of coronary artery disease. Eur Heart J. 2015;36(23):1454-62.
30. Benn M, Tybjaerg-Hansen A, McCarthy MI, Jensen GB, Grande P, Nordestgaard BG. Nonfasting glucose, ischemic heart disease, and myocardial infarction: a Mendelian randomization study. J Am Coll Cardiol. 2012;59(25):2356-65.
31. Winslow UC, Rode L, Nordestgaard BG. High tobacco consumption lowers body weight: a Mendelian randomization study of the Copenhagen General Population Study. Int J Epidemiol. 2015;44(2):540-50.
32. Morris RW, Taylor AE, Fluharty ME, Bjorngaard JH, Asvold BO, Elvestad Gabrielsen M, et al. Heavier smoking may lead to a relative increase in waist circumference: evidence for a causal relationship from a Mendelian randomisation meta-analysis. The CARTA consortium. BMJ Open. 2015;5(8):e008808.
33. Yuan S, Larsson SC. A causal relationship between cigarette smoking and type 2 diabetes mellitus: A Mendelian randomization study. Sci Rep. 2019;9(1):19342.
34. Larsson SC, Burgess S, Michaelsson K. Smoking and stroke: A mendelian randomization study. Ann Neurol. 2019;86(3):468-71.
35. van Oort S, Beulens JWJ, van Ballegooijen AJ, Handoko ML, Larsson SC. Modifiable lifestyle factors and heart failure: A Mendelian randomization study. Am Heart J. 2020;227:64-73.
36. Rosoff DB, Davey Smith G, Mehta N, Clarke TK, Lohoff FW. Evaluating the relationship between alcohol consumption, tobacco use, and cardiovascular disease: A multivariable Mendelian randomization study. PLoS Med. 2020;17(12):e1003410.
37. de Boer AW, Blom JW, de Waal MWM, Rippe RCA, de Koning EJP, Jazet IM, et al. Coded diagnoses from general practice electronic health records are a feasible and valid alternative to self-report to define diabetes cases in research. Prim Care Diabetes. 2021;15(2):234-9.
38. Sluijs I, van der AD, Beulens JW, Spijkerman AM, Ros MM, Grobbee DE, et al. Ascertainment and verification of diabetes in the EPIC-NL study. Neth J Med. 2010;68(1):333-9.
39. Coloma PM, Valkhoff VE, Mazzaglia G, Nielsson MS, Pedersen L, Molokhia M, et al. Identification of acute myocardial infarction from electronic healthcare records using different disease coding systems: a validation study in three European countries. BMJ Open. 2013;3(6).

Supplementary Table 1. Coding of independent variables in binaries for interaction analyses.

Primary exposure	Definition	Coding
Body fat distribution		
Obesity	<30.0 kg/m^2	0 (ref)
	≥30.0 kg/m^2	1
Relative weight change	Below median in NEO study (<27.4%)	0 (ref)
	Above median in NEO study ≥27.4%	1
Total body fat	Men ≤25%, women ≤35%	0 (ref)
	Men >25%, women >35%	1
Normal waist circumference	Men <102 cm, women <88 cm	0 (ref)
	Men ≥102 cm, women ≥88 cm	1
Visceral adipose tissue	Below median in NEO study (men<132 cm^2 and women <86 cm^2)	0 (ref)
	Above median in NEO study (men≥132 cm^2 and women≥86 cm^2)	1
Non-alcoholic fatty liver disease	<5.56% liver fat content	0 (ref)
	≥5.56% liver fat content	1
Metabolic syndrome		
Blood pressure	Systolic <130 mmHg or diastolic <85 mmHg and no use of anti-hypertensive agents	0 (ref)
	Systolic ≥130 mmHg or diastolic ≥85 mmHg or use of anti-hypertensive agents	1
Triglyceride levels	<1.7 mmol/L and no use of lipid-lowering agents	0 (ref)
	≥1.7 mmol/L or use of lipid-lowering agents	1
HDL-cholesterol	≥1.0 mmol/L in men and ≥1.3 mmol/L in women and no use of medication for reduced-HDL	0 (ref)
	<1.0 mmol/L in men and <1.3 mmol/L in women or use of medication for reduced-HDL	1
LDL-cholesterol	Below median in NEO study (<4.9 mmol/L)	0 (ref)
	Above median in NEO study (≥4.9 mmol/L)	1
Normal fasting glucose	≤5.6 mmol/L	0 (ref)
	>5.6 mmol/L	1
High sensitivity (hs)-CRP	≥2.0 mg/L	1
Lifestyle factors		
Dutch Healthy Diet Index (score)	Above median in NEO study (≥69)	0 (ref)
	Below median in NEO study (<69)	1
Alcohol consumption	Below median in NEO study (men<16.6 g/day, women<5.2 g/day)	0 (ref)
	Above median in NEO study (men≥16.6 g/day, women≥5.2 g/day)	1
Smoking	Never smoking	0 (ref)
	Current smoking or former smoking	1

Abbreviations: NEO, Netherlands Epidemiology of Obesity; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, high sensitivity C-reactive protein; MET, metabolic equivalent of task.

Supplementary Table 2A. Complete case interaction analysis between BMI lower or higher than 30.0 kg/m^2 and body fat distribution (reference category = BMI<30.0 kg/m^2 and lowest risk category of risk factor), n=1737, 111 events.

Exposure	Hazard ratio (all events)
Adult weight gain (%, ref = no excess weight gain)	1
Synergy Index	1.72 (0.66 – 4.50)
No excess weight gain and BMI≥30.0 kg/m^2	2.88 (1.53 – 5.43)
Excess weight gain and BMI<30.0 kg/m^2	0.94 (0.44 – 2.03)
Excess weight gain and BMI≥30.0 kg/m^2	4.13 (2.57 – 6.65)
Body fat percentage (ref = low body fat)	1
Synergy Index	0.44 (0.06 – 3.11)
Low body fat and BMI≥30 kg/m^2	13.3 (1.59 – 111.27)
High body fat and BMI<30 kg/m^2	2.18 (0.90 – 5.30)
High body fat and BMI≥30 kg/m^2	6.89 (2.99 – 15.87)
Waist circumference (ref = high waist circumference)	1
Synergy Index	1.28 (0.61 – 2.68)
Low waist circumference and BMI≥30.0 kg/m^2	3.63 (2.18 – 6.02)
High waist circumference and BMI<30.0 kg/m^2	1.44 (0.54 – 3.85)
High waist circumference and BMI≥30.0 kg/m^2	4.94 (2.64 – 9.23)
Visceral adipose tissue (cm^2, ref = low VAT)	1
Synergy Index	3.32 (0.92 – 11.99)
Low VAT and BMI≥30.0 kg/m^2	1.91 (0.86 – 4.26)
High VAT and BMI<30.0 kg/m^2	1.40 (0.70 – 2.78)
High VAT and BMI≥30.0 kg/m^2	5.34 (3.17 – 8.98)
Non-alcoholic fatty liver disease (%, ref = no NAFLD)	1
Synergy Index	2.46 (1.10 – 5.51)
No NAFLD and BMI≥30.0 kg/m^2	2.43 (1.20 – 4.94)
NAFLD and BMI<30.0 kg/m^2	1.83 (0.92 – 3.63)
NAFLD and BMI≥30.0 kg/m^2	6.55 (3.78 – 11.36)

All analyses were adjusted for sex and age.

Supplementary Table 2B. Complete case interaction analysis between BMI lower or higher than 30.0 kg/m^2 and metabolic risk factors (reference category = BMI<30.0 kg/m^2 and lowest risk category of risk factor), n=1737, 111 events.

Exposure	Hazard ratio (all events)
Blood pressure (mmHg, ref = low blood pressure)	1
Synergy Index	0.83 (0.40 – 1.72)
Low blood pressure and BMI≥30.0 kg/m^2	5.89 (2.72 – 12.78)
High blood pressure and BMI<27 kg/m^2	3.22 (1.23 – 8.42)
High blood pressure and BMI≥30.0 kg/m^2	6.93 (3.08 – 15.59)
Triglycerides (mmol/l, ref = low triglycerides)	1
Synergy Index	1.10 (0.33 – 3.68)
Low triglycerides and BMI≥30.0 kg/m^2	4.46 (2.29 – 7.91)
High triglycerides and BMI<30.0 kg/m^2	4.30 (1.24 – 14.98)
High triglycerides and BMI≥30.0 kg/m^2	8.24 (3.17 – 21.41)
HDL-c (mmol/l, ref = high HDL-c)	1
Synergy Index	1.77 (0.89 – 3.53)
High HDL-c and BMI≥30.0 kg/m^2	3.40 (2.10 – 5.51)
Low HDL-c and BMI<30.0 kg/m^2	1.55 (0.67 – 3.57)
Low HDL-c and BMI≥30.0 kg/m^2	6.23 (3.69 – 10.51)
LDL-c (mmol/l, ref = low LDL-c)	1
Synergy Index	1.52 (0.81 – 2.85)
Low LDL-c and BMI≥30.0 kg/m^2	3.71 (1.85 – 7.42)
High LDL-c and BMI<30.0 kg/m^2	1.62 (0.80 – 3.29)
High LDL-c and BMI≥30.0 kg/m^2	6.05 (3.24 – 11.31)
Glucose (mmol/l, ref = low glucose)	1
Synergy Index	2.68 (1.54 – 4.68)
Low glucose and BMI≥30.0 kg/m^2	2.77 (1.28 – 5.97)
High glucose and BMI<30.0 kg/m^2	4.21 (2.04 – 8.73)
High glucose and BMI≥30.0 kg/m^2	14.39 (7.56 – 27.37)
hsCRP (mg/l, ref = low hsCRP	1
Synergy Index	1.66 (0.89 – 3.08)
Low hsCRP and BMI≥30.0 kg/m^2	3.41 (1.87 – 6.22)
High hsCRP and BMI<30.0 kg/m^2	2.02 (1.02 – 4.010
High hsCRP and BMI≥30.0 kg/m^2	6.68 (3.88 – 11.50)

All analyses were adjusted for sex and age.

Supplementary Table 2C. Complete case interaction analysis between BMI lower or higher than 30.0 kg/m^2 and lifestyle factors (reference category = BMI<30.0 kg/m^2 and lowest risk category of risk factor), n=1737, 11 events.

Exposure	Hazard ratio (all events)
Dutch Healthy Diet Index (score, ref = high DHDI)	1
Synergy Index	1.19 (0.59 – 2.39)
High DHDI and BMI≥30.0 kg/m^2	3.53 (1.98 – 6.31)
Low DHDI and BMI<30.0 kg/m^2	0.96 (0.48 – 1.91)
Low DHDI and BMI≥30.0 kg/m^2	3.97 (2.29 – 6.85)
Physical activity (MET/h, ref = physically active)	1
Synergy Index	1.67 (0.80 – 3.50)
Physically active and BMI≥30.0 kg/m^2	3.14 (1.75 – 5.62)
Physically inactive and BMI <30.0 kg/m^2	1.02 (0.51 – 2.030
Physically inactive and BMI ≥30.0 kg/m^2	4.61 (2.67 – 7.97)
Alcohol consumption (ref= low alcohol consumption)	1
Synergy Index	1.28 (0.61 – 2.68)
Low alcohol consumption and BMI ≥30.0 kg/m^2	3.23 (1.79 – 5.82)
High alcohol consumption and BMI <30.0 kg/m^2	0.90 (0.46 – 1.79)
High alcohol consumption and BMI ≥30.0 kg/m^2	4.13 (2.31 – 7.38)
Smoking (ref = smoking no)	1
Synergy Index	1.29 (0.65 – 2.59)
Not smoking and BMI≥30.0 kg/m^2	3.74 (1.79 – 7.82)
Smoking and BMI <30.0 kg/m^2	1.32 (0.64 – 2.72)
Smoking and BMI ≥30.0 kg/m^2	4.96 (2.60 – 9.46)

All analyses were adjusted for sex and age.