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NEW STUDY

The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection

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Abstract Obesity is a well-established risk factor for many chronic diseases. Incomplete insight exists in the causal pathways responsible for obesity-related disorders and consequently, in the identification of obese individuals at risk of these disorders. The Netherlands Epidemiology of Obesity (NEO) study is designed for extensive phenotyping to investigate pathways that lead to obesity-related diseases. The NEO study is a population-based, prospective cohort study that includes 6,673 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. At baseline, data on demography, lifestyle, and medical history have been collected by questionnaires. In addition, samples of 24-h urine, fasting and postprandial blood plasma and serum, and DNA were collected. Participants underwent an extensive physical examination, including anthropometry, electrocardiography, spirometry, and measurement of the carotid artery intima-media thickness by ultrasonography. In random subsamples of participants, magnetic resonance imaging of abdominal fat, pulse wave velocity of the aorta, heart, and brain, magnetic resonance spectroscopy of the liver, indirect calorimetry, dual-energy X-ray absorptiometry, or accelerometry measurements were performed. The collection of data started in September 2008 and completed at the end of September 2012. Participants are followed for the incidence of obesity-related diseases and mortality. The NEO study investigates pathways that lead to obesity-related diseases. A better understanding of the mechanisms underlying the development of disease in obesity may help to identify individuals who are susceptible to the detrimental metabolic, cardiovascular and other consequences of obesity and has

This study was conducted for the NEO Study Group.
Please refer the "Appendix" section for NEO Study Group members.

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implications for the development of prevention and treatment strategies.

Keywords Cardiovascular disease · Cohort · Diabetes · Epidemiology · Obesity · Study design

Introduction

The prevalence of obesity has reached epidemic proportions. Worldwide in 2008, 1.5 billion adults were overweight, defined as a body mass index (BMI) of 25 kg/m^2 or higher. Of these adults, over 200 million men and nearly 300 million women were obese (BMI $\geq$ 30 kg/m^2) [1]. In the Netherlands, the prevalence of overweight and obesity is relatively low in comparison with other European countries and the USA [2, 3], but steadily increasing [4, 5]. In the Netherlands in 2011, 48 % of the population were overweight, and 11 % were obese [2]. In comparison, the prevalence of overweight in the US in 2007–2008 was 68 %, and the prevalence of obesity was 34 % [6].

Obesity is a well-established risk factor for type 2 diabetes, cardiovascular diseases (CVD), chronic kidney disease, and certain cancers and is therefore a major public health problem [7, 8]. In addition, obesity may contribute to debilitating health problems such as osteoarthritis and pulmonary diseases [8], and is related to stress, anxiety and depression [9, 10]. Obesity-related diseases are highly prevalent [8] and related to premature death [11]. Therefore, understanding of the underlying pathofysiology of obesity-related diseases is important.

Obesity is a complex, multifactorial condition, and many endogenous genetic, endocrine and inflammatory pathways and environmental factors are involved in the development of obesity-related diseases. The hierarchy and interactions between these pathways have only been identified to a limited extent. The notion of obesity as a heterogeneous condition is underscored by the fact that the threshold for adipose tissue accumulation to ultimately result in disease apparently varies considerably among obese individuals. In order to tailor preventive strategies in obesity and to treat obesity-related diseases in terms of personalized care, it is extremely important to improve the risk-stratification of obese individuals by extensive phenotyping and dissecting causal pathways that contribute to the pathogenesis of obesity-related disorders.

We hypothesize that obesity affects major systemic responses in certain subgroups, such as dyslipidemia, inflammation, insulin resistance, hypercoagulation, oxidative stress and endothelial dysfunction, that may be causally related to the occurrence of major common diseases. In addition, we hypothesize that the extent and occurrence of these diseases is related to individual characteristics, including genetics, differential fat distribution, and environmental factors including diet and physical activity, as well as interactions between the various systemic responses, and the (subclinical) disease outcomes. The Netherlands Epidemiology of Obesity (NEO) study is designed to investigate the pathways that lead to obesity-related diseases and conditions in a population-based prospective cohort study of individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. As subclinical endpoints insulin resistance, hypertension, dyslipidemia, airways obstruction, atherosclerosis, and non-alcoholic fatty liver disease will be studied. Clinical endpoints of the NEO study include type 2 diabetes, CVD, thrombosis, chronic kidney disease, asthma, chronic obstructive pulmonary disease (COPD), osteoarthritis, cirrhosis, depression, and mortality. The interrelationships between classic established risk factors and novel determinants and between various diseases and conditions that will be studied in the NEO study are depicted in Figure 1.

Study design NEO study

Study population

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.

The study was approved by the medical ethical committee of the Leiden University Medical Center (LUMC). Eligible participants were given detailed written information on the study, in addition to an oral explanation at the study site. Participants have given written informed consent for participation in the study, for storage of urine and blood samples, and for obtaining medical records and information on vital status during follow-up.

Recruitment of participants started in September 2008 and completed at the end of September 2012. In total, 6,673 participants have been included, of whom 5,217 with a BMI of 27 kg/m^2 or higher. According to the WHO criteria, 2,880 participants had overweight (BMI of 25–30 kg/m^2) and 3,020 participants were obese (BMI of 30 kg/m^2 or higher).

Recruitment strategies

Participants were recruited via three recruitment strategies. First, participants were recruited by general practitioners in the area of Leiden, The Netherlands. The majority of these

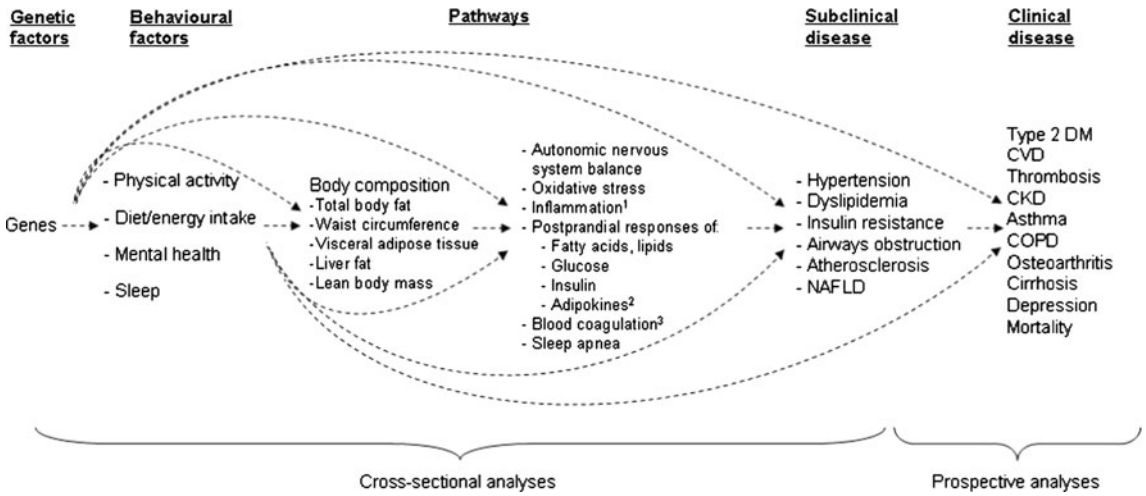

Fig. 1 Path diagram of the hypothetical pathways between genetic and behavioral factors and disease in persons with overweight or obesity. Pathways may also be interlinked, but these associations are not shown. *NAFLD* Non-alcoholic fatty liver disease, *Type 2 DM* Type 2 diabetes mellitus, *CVD* Cardiovascular diseases (angina pectoris, myocardial infarction, congestive heart failure, stroke or peripheral vascular disease), *Thrombosis* Deep venous thrombosis and/or pulmonary embolism, *CKD* Chronic kidney disease, *COPD* Chronic obstructive pulmonary disease. [1]inflammation (increased levels of CRP, TNF-a, IL-6 and leptin, and decreased levels of adiponectin), [2]Adipokines: TNF-a, IL-6, leptin, adiponectin, resistin, visfatin, RBP-4, renin, angiotensinogen. [3]Blood coagulation: thrombin generation, factor VIII, fibrinogen, von Willebrand factor, protein C, protein S, antithrombin, thrombin-antithrombin complexes (TAT), and fragment 1,2

general practitioners (GP) are part of a research network of university-affiliated primary care centers (LEON). General practitioners sent invitations to participate in the NEO study to their population aged between 45 and 65 years. Men and women within this age group and with a self-reported BMI of 27 kg/m^2 or higher were invited to contact the NEO study center by telephone or by completing a web-based form. After 2 weeks, a reminder was sent. Second, participants were recruited through advertisements in local newspapers and through posters distributed in public areas of Leiden and surroundings. Third, participants were recruited via the registries of three municipalities surrounding Leiden (Katwijk, Leiderdorp and Teylingen). All inhabitants aged between 45 and 65 years were sent an invitation to participate in the NEO study. Inhabitants of Katwijk and Teylingen were invited to participate if they had a self-reported BMI of 27 kg/m^2 or higher. All inhabitants aged between 45 and 65 years of Leiderdorp were invited to participate, irrespective of their BMI.

Table 1 gives the number of included participants and the age, sex and BMI, stratified by recruitment source. The BMI distribution of the participants from Leiderdorp is similar to the BMI distribution of the Dutch general population [12], suggesting that the Leiderdorp population may be representative for this age group.

Table 1 Number and characteristics of participants of the NEO study stratified by recruitment source (n = 6,673)

Recruitment source[b]	N	Characteristics[a]		
		Sex (% M)	Age (years)	BMI (kg/m^2)
GP	2,365	50	57 (44, 66)	30.1 (28.4, 32.6)
Advertisements	1,081	38	55 (22, 44)	31.2 (28.8, 35.0)
Mun. registries				
Katwijk	1,116	54	57 (51, 62)	30.6 (28.6, 33.2)
Teylingen	440	54	56 (45, 66)	30.1 (28.5, 32.4)
Leiderdorp	1,671	44	56 (45, 66)	25.7 (23.3, 28.4)[c]

M men, *GP* general practitioner, *Mun. registries* registries of municipalities of Leiderdorp, Katwijk, and Teylingen

[a] Age and BMI are reported as median (25th, 75th percentiles)

[b] A response rate can only be calculated from recruitment in the municipality Leiderdorp, where all inhabitants within the age range of 45–65 years were invited, irrespective of their BMI. Of the 8,229 inhabitants within the age range of 45–65 years who were sent an invitation to participate, 1,671 participated in the NEO study (20.3 %). Response rates of other recruitment sources can not be calculated because the proportion of the population with a BMI of 27 kg/m^2 or higher was unknown

[c] In the municipality Leiderdorp all inhabitants within the age range of 45–65 years were invited, irrespective of their BMI. The BMI distribution of participants from Leiderdorp is similar to the BMI distribution of the Dutch general population within the age range of 45–65 years [12]

Cross-sectional analyses with the baseline data will be weighted to the BMI distribution of the Leiderdorp population, so that results apply to the general population.

Study design

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. Participants were invited to come to the NEO study center of the LUMC for one baseline study visit after an overnight fast. Prior to this study visit, participants collected their urine over 24 h and completed a general questionnaire at home to report demographic, lifestyle, and clinical data in addition to questions on diet, physical activity, symptoms of osteoarthritis, sleep and anxiety, quality of life and depression. The participants were asked to bring all medication they were using in the month preceding the study visit to the NEO study site, both prescribed medication, as well as self-medication such as vitamin supplements. Names and dosages of all medication were recorded in a database by research nurses. At the NEO study center several measurements have been performed including an extensive physical examination and blood sampling. Magnetic resonance imaging (MRI) of abdominal fat, pulse wave velocity, heart, and brain, magnetic resonance spectroscopy (MRS) of the liver, indirect calorimetry, dual-energy X-ray absorptiometry, accelerometry, and heart rate variability measurements were performed in random subsets of participants. The measurements are listed in Table 2 and described in detail below.

The results of all laboratory analyses and the ECGs and MRI scans were screened by specialists on incidental findings. Incidental abnormal results that are likely to have serious health consequences if left undiagnosed, have been disclosed to the participants and their general practitioners, accompanied by an advice for further work-up. The number (% of screened) of incidental findings were 6 (0.1) for the laboratory analyses, 25 (0.4) for ECGs, 48 (1.9) for MRI of the body, 8 (0.7) for MRI of the brain, and 11 (0.9) of MRI of the knee.

Within 2 weeks after the study visit, participants received feedback on the results of tests for which, according to national guidelines, advice or testing is recommended (e.g. blood pressure, renal function, lung function, and blood concentrations of glucose, triglycerides, and total and HDL-cholesterol). Abnormal results were marked and accompanied with an advice to consult the general practitioner. Results of other measurements have not been disclosed.

Participants are followed for the incidence of obesity-related diseases and mortality.

Data collection

Questionnaires

Questionnaires were sent to all participants to be completed at home. The general questionnaire included questions about demographic, lifestyle, and clinical data such as family history of type 2 diabetes, CVD, stroke, colon cancer and breast cancer, asthma and allergies in first-degree relatives and personal medical history, and about current weight, weight change in the past 5 years, weight at the age of 20 years, and birth weight. In addition, validated questionnaires on diet, physical activity, symptoms of osteoarthritis, sleep, anxiety, and quality of life were completed, as described below.

Dietary intake

Dietary intake was assessed using a semi-quantitative food frequency questionnaire (FFQ), originally validated in the Dutch general population [13]. For calibration purposes in our study population, two 24-h dietary recalls have been performed by telephone in a random subsample of 110 men and 119 women.

Physical activity

Participants reported their usual physical activity over the past 4 weeks on the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH), a previously validated tool to assess physical activity in the Dutch population [14, 15].

Symptoms of osteoarthritis

An osteoarthritis phenotype was determined based on the clinical criteria sets from the American College of Rheumatology (ACR), using the Knee injury and Osteoarthritis Outcome Score (KOOS) [16, 17] and the Australian/Canadian (AUSCAN) Osteoarthritis Hand Index [18, 19].

Sleep

Several questionnaires were completed to assess daytime sleepiness (Epworth sleepiness scale) [20], quality of sleep (Pittsburg sleep quality index) [21], and the presence of obstructive sleep apnea syndrome (Berlin questionnaire) [22].

Depression and anxiety

Several validated subjective stress indicators were collected including the Inventory of Depressive Symptoms

Table 2 List of baseline measurements of the NEO study

Measurement	Assessment
Total population (n = 6,673)	
Questionnaires	
General	Demography, medical history
Food frequency questionnaire [13]	Dietary intake
SQUASH [14]	Physical activity
KOOS [17], AUSCAN [18]	Symptoms of osteoarthritis
Epworth sleepiness scale [20], Pittsburg	Sleep
Sleep quality index, [21], Berlin [22]	
IDS, [23] BAI, [24], Brugha [26]	Depression and anxiety
SF-36 health survey [29]	Quality of life
Urine collection	
Morning spot	Albumine, creatinine
24-h urine sample	Storage in biobank
Fasting blood sampling	Glucose, insulin, HbA1c, total and HDL cholesterol, triglycerides, albumin, creatinine, 25-hydroxyvitamin D, ALT, AST, calcium, sodium, potassium, uric acid, routine hematology
	Storage of plasma, serum, DNA in biobank
Postprandial blood sampling (t = 30 min, 150 min)	Glucose, insulin, total cholesterol, HDL cholesterol, triglycerides
Physical examination	Weight, height, waist circumference, hip circumference, blood pressure, examination of joints for symptoms of osteoarthritis
Bioelectrical impedance analysis	Percentage of body fat
Resting ECG	Minnesota coding, silent ischemia, AMI, left ventricular hypertrophy
Ultrasonography	Carotid artery intima-media thickness
Pulmonary function tests	FEV1, peakflow, forced vital capacity, exhaled NO
Random subsets of participants	
MRI	Abdominal subcutaneous and visceral fat, and pulse wave velocity (n = 2,580), cardiac function (n = 1,207), brain (n = 1,212), osteoarthritis of the knee (n = 1,285)
MRS	Hepatic triglyceride content (n = 2,580)
Indirect calorimetry	Resting metabolic rate (n = 1,452)
DXA	Bone mass density at the hip and lumbar spine, total fat mass, abdominal fat mass, lean body mass, abdominal aortic calcifications (n = 916)
Actigraphy for 7 days	Objective assessment of sleep schedule variability, sleep quantity statistics and daytime activity patterns (n = 360)
Combined accelerometer and continuous heart rate measurement for 4 days	Objective assessment of physical activity and energy expenditure, heart rate variability (n = 955)

SQUASH short questionnaire to assess health-enhancing physical activity, *KOOS* knee injury and osteoarthritis outcome score, *AUSCAN* Australian/Canadian Osteoarthritis Hand Index, *IDS* inventory of depressive symptomatology, *BAI* beck anxiety inventory; *HbA1c* glycated hemoglobin, *ALT* alanine transaminase, *AST* aspartate transaminase, *HDL* high-density lipoprotein, *ECG* electrocardiogram, *AMI* acute myocardial infarction, FEV_1 forced expiratory volume in 1 s, *MRI* magnetic resonance imaging, *MRS* magnetic resonance spectroscopy, *DXA* dual-energy X-ray absorptiometry

[23], Beck Anxiety Inventory [24, 25], and the Brugha questionnaire of negative life events [26].

Quality of life

The SF-36® Health Survey was used to assess quality of life. The SF-36 is a multipurpose, 36-item survey that measures eight domains of health: physical functioning, role limitations due to physical health, bodily pain, general health perceptions, vitality, social functioning, role limitations due to emotional problems, and mental health. It yields scale scores for each of these eight health domains, and two summary measures of physical and mental health: the Physical Component Summary (PCS) and Mental Component Summary (MCS) [27–29].

Urine sampling

Containers together with a detailed instruction were distributed to all participants for collection of 24-h urine samples prior to the study visit. In addition, a separate early morning urine sample was collected. This morning urine sample was analyzed for albumin and creatinine in the central clinical chemical laboratory of the LUMC (Table 2). The 24-h urine sample was aliquoted and 1.5 mL aliquots were stored at −80 °C for future analyses of urinary concentrations of nitrogen, creatinine, cortisol, catecholamines, and other research questions.

Fasting and postprandial blood sampling

A first blood sample of 108 mL was taken after an overnight fast of at least 10 h. Within 5 min after the first blood sample, participants drank a liquid mixed meal. This meal (total 400 mL) contained 600 kCal, with 16 percent of energy (En%) derived from protein, 50 En% carbohydrates, and 34 En% fat. Two postprandial blood samples of 63 mL were drawn 30 and 150 min after the mixed meal. Subjective ratings of hunger, satiety, fullness and prospective food consumption were made on 100 mm visual analogue scales (VAS) immediately before the meal and before drawing the two postprandial blood samples.

Serum, heparin-plasma, citrated-plasma and EDTA-plasma were collected during each of the threeblood draws. Serum or plasma concentrations of glucose, insulin, HbA1c, total cholesterol, high-density lipoprotein cholesterol, triglycerides, albumin, creatinine, 25-hydroxyvitamin D, alanine transaminase, aspartate transaminase, calcium, sodium, potassium, and uric acid were determined in the central clinical chemistry laboratory of the LUMC by using standard methods (Table 3). Hemoglobin, hematocrit, white blood cell count, mean corpuscular volume, platelet count, platelet distribution width, and mean platelet volume were determined in the central clinical hematology laboratory of the LUMC by using standard methods (Table 3). Throughout the study biases were below the maximum allowable biases based on biological variation [30].

The remaining blood was separated into plasma and serum, and aliquots were stored at −80 °C for future analyses of inflammatory markers, incretines, adipokines, markers of oxidative stress, haemostatic factors and markers related to other research questions. Genomic DNA was isolated and stored for SNP genotyping analyses.

Physical examination

All participants underwent an extensive physical examination, including anthropometry, blood pressure measurements, electrocardiography (ECG), and measurements of the carotid intima-media thickness (cIMT) and of the pulmonary function. Height was measured with a vertically fixed, calibrated tape measure. Body weight and percent body fat were measured by the Tanita bio impedance balance (TBF-310, Tanita International Division, UK) without shoes and one kilogram was subtracted to correct for the weight of clothing. BMI was calculated by dividing the weight in kilograms by the height in meters squared. Waist circumference was measured with a horizontally placed tape measure mid-way between the lower costal margin and the iliac crest with a precision of 0.1 cm. Hip circumference was measured at the maximum circumference of the buttocks.

Brachial blood pressure was measured in a seated position on the right arm using a validated automatic oscillometric device (OMRON, Model M10-IT, Omron Health Care Inc, IL, USA). Blood pressure was measured three times with 5 min rest between consecutive measurements. The mean systolic and diastolic blood pressure was calculated.

All participants were examined on symptoms of osteoarthritis by examination of the presence of swelling and hard tissue enlargement of the interphalangeal joints of the hands and knees, and crepitations of the knees.

A resting 12-lead ECG was obtained using a Mortara Eli-350 (Mortara Instrument Inc., Milwaukee, WI, USA). The raw data were extracted and transferred to the University of Glasgow ECG core lab where ECGs were automatically processed and Minnesota codes assigned using the University of Glasgow ECG analysis program.

Carotid intima-media thickness

The cIMT was assessed by ultrasonography of the far wall of the left and right common carotid arteries (CCA) along a 15 mm long section 10 mm proximal of the bifurcation in recumbent position. A 7.5–10 MHz linear-array transducer (Art.Lab version 2.1, Esaote, Maastricht, The Netherlands) in B-mode setting was used to visualize the distal CCA and a wall track system was used to detect the lumen-intima and media-adventitia boundaries. The cIMT was measured in three predefined angles per side (180, 135 and 90 degrees for the right CCA and 180, 225 and 270 degrees for the left CCA).

Pulmonary function

Forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were determined by spirometry. FEV_1 is a volumetric measurement, a reflection of the speed of emptying of the lung, and therefore, indirectly of airflow. The FVC is the maximum volume obtained by a forced exhalation from a maximal inspiration. Participants were required to perform at least three forced expiratory maneuvers. The highest FEV_1 value of the minimum of

Table 3 Laboratory analyses of blood and urine samples in the NEO study

Serum/plasma concentrations	Analysis method	Overall maximum CVa (%)
Fasting concentrations		
HbA1c[1]	HPLC boronate affinity chromatography[a]	<3
Vitamin D ($25(OH)D_3$)[2]	RIA method (Sept 1st, 2008–Oct 4th, 2010)[b]	<10
	Chemoluminescent Immunoassay (Oct 5th, 2010–Sept 29th, 2011)[c]	
	LC-MSMS calibrated 2nd generation Electrochemoluminescence Immunoassay (ECLIA) (since Sept 30th, 2011)[d]	
Albumin	Colorimetric assay (Bromocresol Green)[e]	<1
Creatinine[3]	1) Jaffe kinetic compensated method (Sept 1st, 2008–Nov 30th, 2010)[e]	<3
	2) Enzymatic (IDMS calibrated against SRM 967) (since Dec 1st, 2010)[e]	
Calcium	Colorimetric assay[e]	<1
Sodium	Ion-selective electrode method[e]	<1
Potassium	Ion-selective electrode method[e]	<3
Uric acid	Enzymatic colorimetric[e]	<1
ALT	UV test[g]	<2
AST	UV test[g]	<2
WBC	Flow cytometry method using semiconductor laser[h]	<3
Hemoglobin	SLS hemoglobin detection method[h]	<1
Hematocrit	RBC cumulative pulse height detection method[h]	<1.5
MCV[4]	Calculation with RBC and HCT[h]	<1
PLT, PDW[5], MPV[6]	Hydro dynamic focusing (DC detection) or Flow cytometry method using semiconductor laser[h]	PLT < 4, PDW < 10, MPV < 3
Fasting and postprandial concentrations (0, 30, 150 min)		
Glucose[7]	Enzymatic colorimetric[e]	<2
Insulin	Two-site chemiluminescent immunometric assay[f]	<5
Total cholesterol	Enzymatic colorimetric[e]	<2
HDL-cholesterol	Homogenous HDLc method, 3rd generation[e]	<3
Triglycerides	Enzymatic colorimetric[e]	<3
Urine (morning spot)		
Albumin	Immunoturbidimetric[g]	<15 at 10 mg/L
		<4 at 58 mg/L
Creatinine[8]	1) Jaffe kinetic compensated method (Sept 1st, 2008–Nov 30th, 2010)[e]	<5
	2) Enzymatic (IDMS calibrated against SRM 967) (since Dec 1st, 2010)[e]	

Overall maximum CVa overall maximum analytical coefficient of variation, *HbA1c* glycolated hemoglobin, *HPLC* high-performance liquid chromatography, *LC-MSMS* liquid chromatography-tandem mass spectrometry, *IDMS* isotope dilution mass spectrometry reference measurement procedure, *WBC* white blood cell count, *MCV* mean corpuscular volume, *RBC* red blood cell count, *HCT* hematocrit, *PLT* platelet count, *PDW* platelet distribution width, *MPV* mean platelet volume, *HDLc* high-density lipoprotein cholesterol, *IRMA* immunoradiometric assay, *SRM 967* a standard reference material for creatinine in serum

[1] HbA1c[IFCC] (mmol/mol) = (10.93 × HbA1c[NGSP %]) − 23.5

[2] Vitamin D ($25(OH)D_3$) concentrations are interchangeable across the three methods used

[3] Serum Jaffe results were corrected with a fixed compensation factor of −26 μmol/L to compensate for assay non-specificity

[4] MCV (fL) = (HCT (%)/RBC ($\times 10^6$/μL))×10

[5] PDW (fL): Distribution width at the 20 % frequency level

[6] MPV (fL) = PCT (%)/PLT ($\times 10^3$/μL)) × 1,000; PCT = platelet hematocrit or platelet volume ratio

[7] Since January 2011 NaF tubes were used in the NEO study for the analysis of glucose concentrations. Serum glucose concentrations (G) of the first 3,500 participants have been converted on the basis of 295 samples that have been analyzed with both methods (fasting: G(plasma) = 0.846 +0.947 × G(serum), R^2 0.95; 30 min: G(plasma) = 1.165 + 0.931 × G(serum), $R^2 = 0.93$; 150 min: G(plasma) = 0.674 + 0.943 × G(serum), $R^2 = 0.97$)

[8] Urinary creatinine concentrations are not affected by pseudochromogens and hence exchangeable using either a Jaffe or an enzymatic method

Analyzers and suppliers

[a] Primus Ultra, Siemens Healthcare Diagnostics, Breda, Netherlands

[b] Diasorin, Inc., Italy

[c] iSYS analyzer, ImmunoDiagnostic Systems Inc., Boldon, UK

[d] Modular Analytics E170 analyzers, Roche Diagnostics, Mannheim, Germany

[e] Roche Modular P800 Analyzer, Roche Diagnostics, Mannheim, Germany

[f] Siemens Immulite 2500, Siemens Healthcare Diagnostics, Breda

[g] Cobas Integra 800 analyzer, Roche Diagnostics, Mannheim, Germany

[h] XE-2100, Sysmex, UK

three tests with acceptable curves was used in analyses. The lung function results are expressed as percentage of the predicted values of individuals with similar characteristics (height, age, sex).

The following paragraphs describe the protocols for MRI and MRS, indirect calorimetry, dual-energy X-ray absorptiometry, accelerometry, and heart rate variability measurements, which were performed in subsets of participants. Approximately fifty percent of the participants without contraindications for MRI were appointed to different compositions of MRI studies according to standardized protocols. The other measurements were randomly distributed over all participants.

MRI and MRS measurements

A screening form was completed by all participants at the time of inclusion in the NEO study, asking about anything that might create a health risk or interfere with imaging (most notably metallic devices, or claustrophobia). A body circumference of more than 1.70 m was an additional contraindication. Abdominal subcutaneous and visceral fat was assessed in 2,580 participants, in combination with imaging of the pulse wave velocity of the aorta, and proton (^{1}H)-MRS of the liver to assess hepatic triglyceride content. In addition, either the heart was imaged (1,207 participants), or the brain (1,212 participants).

Abdominal subcutaneous and visceral fat depots were quantified by a turbo spin echo imaging protocol. At the level of the 5th lumbar vertebra 3 transverse images each with a slice thickness of 10 mm were obtained during a breath-hold [31].

Velocity-encoded MRI was used for assessment of transmitral flow for evaluation of LV diastolic function and pulse wave velocity (PWV) of the aorta. Data were analysed using MASS and FLOW software.

The heart was imaged in short-axis view using a ECG-triggered balanced turbo-field-echo sequence. Imaging of the brain was performed with T2-weighted and FLAIR (white matter lesions), diffusion weighted MRI (ischemic changes) and susceptibility weighted scans (microbleeds) with 3 mm contiguous slices covering the entire brain. In addition, isotropic 3D-T1-weighted MRI was performed (atrophy) with a 1.5 mm resolution also covering the entire brain. Data were analysed using FSL and Q-BRAIN software.

In another 1,285 participants a knee osteoarthritis phenotype was determined based on structural osteoarthritic damage in the right knee as detected at MRI and assessed by the Knee Osteoarthritis Score System (KOSS).

All imaging was performed on an MR system operating at a field strength of 1.5 Tesla (Philips Medical Systems, Best, Netherlands).

Indirect calorimetry

Indirect calorimetry was performed in 1,452 participants. After an overnight fast and a period of resting for 15 min, participants were placed under a ventilated hood while lying on a bed, awake, in a quiet room for 30 min. The volume of oxygen inspired ($\dot{V}O_2$) and the expired volume of carbon dioxide ($\dot{V}CO_2$) were measured every minute (Oxycon Pro). Resting metabolic rate and substrate oxidation rates were calculated using standard formulas [32].

Dual-energy X-ray absorptiometry

Dual-energy X-ray absorptiometry (DXA) was used to asses bone mineral densitometry (BMD), total fat mass, lean body mass, abdominal fat mass, and abdominal aortic calcifications in 916 participants (Hologic Discovery A, Tromp Medical BV, Castricum, The Netherlands). Absolute BMD values, Z-scores and T-scores (number of standard deviations below the BMD of a younger reference group) for lumbar spine (L1–L4) and proximal femur were reported. Total fat mass (kg) was assessed from the whole-body DXA scans. The androidal or abdominal region of interest was defined as 20 % from pelvis cut to neck cut. Abdominal fat mass is the weight of fat tissue in this region. Lateral images of the thoraco-lumbar spine were obtained to assess vertebral deformities. Abdominal aortic calcification on lateral DXA images was assessed using a previously validated 8-point aortic calcification scale [33].

Accelerometry, heart rate variability and sleep patterns

Fifteen percent of the participants (n = 955) were equipped with an accelerometer combined with 2 ECG electrodes (ActiHeart®, CamNtech Ltd, UK) for four consecutive days. The Actiheart was placed on the chest of the participants at the level of the third intercostal space. This combined heart rate monitor and accelerometer simultaneously measures heart rate and uniaxial (vertical when standing up) acceleration of the torso. In a subgroup of participants who were equipped with an Actiheart, an 8-min ramped step test was performed to assess the individual relationship between heart rate and workload. Using a branched equation algorithm the acceleration and heart rate information was translated into calibrated estimates of physical activity energy expenditure and of minutes per day spent in several MET-categories [34, 35]. In addition, measures of heart rate variability (HRV) are calculated for assessing activity of the cardiac autonomic nervous system [36].

A small subgroup (n = 360) not allocated to the Actiheart were equipped with a single accelerometer (ActiWatch®, CamNtech Ltd, UK) for a period of 7 days to

collect objective data on sleep patterns for multiple days in a person's ambulatory environment. This noninvasive approach enables to objectively assess sleep onset latency, number of awakenings, total sleep time, wake after sleep onset, and sleep efficiency [37].

Ascertainment of incident diseases during follow-up

Study endpoints of the NEO study include clinical diagnoses of type 2 diabetes, CVD (angina pectoris, myocardial infarction, congestive heart failure, stroke or peripheral vascular disease, deep venous thrombosis and/or pulmonary embolism), COPD, asthma, chronic kidney disease, osteoarthritis, cirrhosis, depression, and mortality (Fig. 1).

Participants are followed for the incidence of these obesity-related diseases via postal follow-up questionnaires, through the medical records of their general practitioner, and by linkage with hospital discharge databases. Information on vital status and causes of death are obtained from the Central Bureau of Statistics Netherlands, which stores all Dutch death certificates.

In the Netherlands, general practitioners keep an electronic patient record (EPR) of each patient that covers all medical information concerning the patient including information on prescriptions and reports from laboratories and specialists. Drug prescriptions in the EPR are coded according to the Anatomical Therapeutic Chemical (ATC) codes [38]. Confirmed diagnoses are coded according to the International Classification of Primary Care (ICPC) [39]. NEO participants are marked as such in the electronic information system of the general practitioner. At each follow-up moment, incident diagnoses of the study endpoints, laboratory results and medication prescriptions are retrieved from the EPR's of the NEO participants. Four years after the start of the study, data is extracted for the first time, which will be repeated with three-year intervals.

Statistical considerations

Sample size

Each of the study endpoints has an incidence of 1 per 1,000 person-years (ischaemic stroke) to 10 per 1,000 person-years (osteoarthritis) in the general population [40–44]. For obese individuals the incidences will be 1.3 (asthma, myocardial infarction) to 10-fold (osteoarthritis) higher [8, 43, 45–48]. As an example, we estimated the number of cases and incidence rates for type 2 diabetes [49] and coronary heart disease in our study population after a mean follow-up of 4 years, on the basis of age-standardized incidence rates of type 2 diabetes and coronary heart disease [40] in the Dutch population aged 45–65 years. For type 2 diabetes, we estimated to detect at least 343 cases with a corresponding incidence rate with 95 % confidence interval of 1.43 (1.28, 1.59) per 100 person-years. For coronary heart disease at least 322 cases are expected with an incidence rate of 1.33 (1.19, 1.49) per 100 person-years.

Imputation

MRI techniques, indirect calorimetry, dual-energy X-ray absorptiometry, and accelerometry are too time-consuming and expensive to be performed in all participants. We will use imputation techniques which allow performing these measurements in random subsets of the participants and imputing them in the remainder of the study population, based on their relation to other more feasible measurements that are performed in the total study population [50].

Reliability

The short-term and long-term reliability of the measurements in the NEO study is evaluated by assessment of the magnitude of measurement error and intra individual variation in all measurements by estimation of the intraclass correlation coefficient (ICC) of two repeated measurements [51]. To that extent, repeated measurements have been performed in 183 participants who have been invited to come to the LUMC for a second study visit. For the evaluation of the short-term reliability, the second visit occurred approximately 3 months after the first visit ($n = 100$). For the long-term reliability, the second visit occurred approximately 2.5 years after the first visit ($n = 83$).

Conclusion

The NEO study investigates pathways that lead to obesity-related diseases. The collection of data started in September 2008 and completed at the end of September 2012. Cross-sectional analyses with baseline measurements are currently ongoing. Participants are followed for the incidence of obesity-related diseases and mortality. A better understanding of the mechanisms underlying the development of disease in obesity may help to identify individuals who are susceptible to the detrimental metabolic, cardiovascular and other consequences of obesity and has implications for the development of prevention and treatment strategies.

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Conflict of interest The authors declare that they have no conflict of interest.

Appendix

NEO Study Group

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References

1. WHO: Obesity and overweight. In: Fact sheet No 311. http://www.who.int/mediacentre/factsheets/fs311/en/ (2012). Accessed 1 Nov 2012.
2. Nationaal Kompas Volksgezondheid: http://www.nationaalkompas.nl/gezondheidsdeterminanten/persoonsgebonden/lichaamsgewicht/trend/ (2012). Accessed 1 Nov 2012.
3. International Obesity TaskForce Prevalence Data: http://www.iaso.org/iotf/obesity/ (2010). Accessed 1 Nov 2012.
4. Visscher TL, Kromhout D, Seidell JC. Long-term and recent time trends in the prevalence of obesity among Dutch men and women. Int J Obes Relat Metab Disord. 2002;26:1218–24.
5. Gast GC, Frenken FJ, van Leest LA, Wendel-Vos GC, Bemelmans WJ. Intra-national variation in trends in overweight and leisure time physical activities in The Netherlands since 1980: stratification according to sex, age and urbanisation degree. Int J Obes (Lond). 2007;31:515–20.
6. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999–2008. JAMA. 2010;303:235–41.
7. James WPT, Jackson-Leach R, Mhurchu CN, Kalamara E, Shayeghi M, Rigby NJ, Nishida C, and Rodgers A: Overweight and obesity (high body mass index). In: Ezzati M, Lopez AD Rodgers A Murray CJL, editors. Comparative quantification of health risks: Global and regional burden of disease attributable to selected major risk factors. Geneva: World Health Organization; 2004. pp 497–596.
8. WHO Technical Report Series 894. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation. Geneva: World Health Organization; 1999.
9. Vogelzangs N, Kritchevsky SB, Beekman AT, Brenes GA, Newman AB, Satterfield S, Yaffe K, Harris TB, Penninx BW. Obesity and onset of significant depressive symptoms: results from a prospective community-based cohort study of older men and women. J Clin Psychiatry. 2010;71:391–9.
10. Simon GE, Von KM, Saunders K, Miglioretti DL, Crane PK, van BG, Kessler RC. Association between obesity and psychiatric disorders in the US adult population. Arch Gen Psychiatry. 2006;63:824–30.
11. Stewart ST, Cutler DM, Rosen AB. Forecasting the effects of obesity and smoking on U.S. life expectancy. N Engl J Med. 2009;361:2252–60.
12. http://www.rivm.nl/nldemaat. Accessed 20 March 2013
13. 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;61:610–5.
14. Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. J Clin Epidemiol. 2003;56:1163–9.
15. de Hollander EL, Zwart L, de Vries SI, Wendel-Vos W. The SQUASH was a more valid tool than the OBiN for categorizing adults according to the Dutch physical activity and the combined guideline. J Clin Epidemiol. 2012;65:73–81.
16. Roos EM, Roos HP, Lohmander LS, Ekdahl C, Beynnon BD. Knee injury and osteoarthritis outcome score (KOOS)–development of a self-administered outcome measure. J Orthop Sports Phys Ther. 1998;28:88–96.
17. de G, I, Favejee MM, Reijman M, Verhaar JA, Terwee CB: The Dutch version of the knee injury and osteoarthritis outcome score: a validation study. Health Qual Life Outcomes 2008;6:16.
18. Bellamy N, Campbell J, Haraoui B, Gerecz-Simon E, Buchbinder R, Hobby K, MacDermid JC. Clinimetric properties of the AUSCAN osteoarthritis hand index: an evaluation of reliability, validity and responsiveness. Osteoarthritis Cartilage. 2002;10:863–9.
19. Bellamy N, Campbell J, Haraoui B, Buchbinder R, Hobby K, Roth JH, MacDermid JC. Dimensionality and clinical importance of pain and disability in hand osteoarthritis: development of the

Australian/Canadian (AUSCAN) Osteoarthritis Hand Index. Osteoarthritis Cartilage. 2002;10:855–62.
20. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep. 1991;14:540–5.
21. Buysse DJ, Reynolds CF III, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28:193–213.
22. Netzer NC, Stoohs RA, Netzer CM, Clark K, Strohl KP. Using the Berlin questionnaire to identify patients at risk for the sleep apnea syndrome. Ann Intern Med. 1999;131:485–91.
23. Rush AJ, Gullion CM, Basco MR, Jarrett RB, Trivedi MH. The inventory of depressive symptomatology (IDS): psychometric properties. Psychol Med. 1996;26:477–86.
24. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. J Consult Clin Psychol. 1988;56:893–7.
25. Fydrich T, Dowdall D, Chambless DL: Reliability and validity of the beck anxiety inventory. J Anxiety Disorders 1992;55–61.
26. Brugha TS, Cragg D. The list of threatening experiences: the reliability and validity of a brief life events questionnaire. Acta Psychiatr Scand. 1990;82:77–81.
27. McHorney CA, Ware JE Jr, Lu JF, Sherbourne CD. The MOS 36-item short-form health survey (SF-36): III. Tests of data quality, scaling assumptions, and reliability across diverse patient groups. Med Care. 1994;32:40–66.
28. McHorney CA, Ware JE Jr, Raczek AE. The MOS 36-item short-form health survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs. Med Care. 1993;31:247–63.
29. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992;30:473–83.
30. Fraser CG. Biological variation: from principles to practice. Washington, DC: AACC Press; 2001.
31. van der Meer RW, Rijzewijk LJ, de Jong HW, Lamb HJ, Lubberink M, Romijn JA, Bax JJ, de RA, Kamp O, Paulus WJ, Heine RJ, Lammertsma AA, Smit JW, Diamant M: Pioglitazone improves cardiac function and alters myocardial substrate metabolism without affecting cardiac triglyceride accumulation and high-energy phosphate metabolism in patients with well-controlled type 2 diabetes mellitus. Circulation 2009;119:2069–77.
32. Livesey G, Elia M. Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. Am J Clin Nutr. 1988;47:608–28.
33. Schousboe JT, Wilson KE, Hangartner TN. Detection of aortic calcification during vertebral fracture assessment (VFA) compared to digital radiography. PLoS ONE. 2007;. doi:10.1371/journal.pone.0000715.
34. Brage S, Brage N, Franks PW, Ekelund U, Wong MY, Andersen LB, Froberg K, Wareham NJ. Branched equation modeling of simultaneous accelerometry and heart rate monitoring improves estimate of directly measured physical activity energy expenditure. J Appl Physiol. 2004;96:343–51.
35. Brage S, Brage N, Franks PW, Ekelund U, Wareham NJ. Reliability and validity of the combined heart rate and movement sensor Actiheart. Eur J Clin Nutr. 2005;59:561–70.
36. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996;93:1043–65.
37. Chae KY, Kripke DF, Poceta JS, Shadan F, Jamil SM, Cronin JW, Kline LE. Evaluation of immobility time for sleep latency in actigraphy. Sleep Med. 2009;10:621–5.
38. WHO Collaboration Centre for Drug Statistics Methodology. Anatomical Therapeutic Chemical classification system. http://www.whocc.no/atc/ (2012). Accessed 1 Nov 2012.
39. Wonca International Classification Committee. The international classification of primary care. http://www.ph3c.org/4daction/w3_CatVisu/en/icpc.html?wCatIDAdmin=1106 (2012). Accessed 1 Nov 2012.
40. Vaartjes I, van Dis I, Visseren FLJ, Bots ML. Incidentie en prevalentie van hart- en vaatziekten in Nederland. In: Vaartjes I, van Dis I, Visseren FLJ, Bots ML, editors. Hart- en vaatziekten in Nederland 2010, cijfers over leefstijl- en risicofactoren, ziekte en sterfte. Den Haag: Nederlandse Hartstichting; 2010. p. 29–52.
41. Kolominsky-Rabas PL, Weber M, Gefeller O, Neundoerfer B, Heuschmann PU. Epidemiology of ischemic stroke subtypes according to TOAST criteria: incidence, recurrence, and long-term survival in ischemic stroke subtypes: a population-based study. Stroke. 2001;32:2735–40.
42. Fowkes FG. Epidemiological research on peripheral vascular disease. J Clin Epidemiol. 2001;54:863–8.
43. Felson DT, Zhang Y, Hannan MT, Naimark A, Weissman B, Aliabadi P, Levy D. Risk factors for incident radiographic knee osteoarthritis in the elderly: the Framingham Study. Arthritis Rheum. 1997;40:728–33.
44. Murphy JM, Olivier DC, Monson RR, Sobol AM, Leighton AH. Incidence of depression and anxiety: the Stirling County Study. Am J Public Health. 1988;78:534–40.
45. Yusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, Lang CC, Rumboldt Z, Onen CL, Lisheng L, Tanomsup S, Wangai P Jr, Razak F, Sharma AM, Anand SS. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. Lancet. 2005;366:1640–9.
46. Hu G, Tuomilehto J, Silventoinen K, Sarti C, Mannisto S, Jousilahti P. Body mass index, waist circumference, and waist-hip ratio on the risk of total and type-specific stroke. Arch Intern Med. 2007;167:1420–7.
47. Nystad W, Meyer HE, Nafstad P, Tverdal A, Engeland A. Body mass index in relation to adult asthma among 135,000 Norwegian men and women. Am J Epidemiol. 2004;160:969–76.
48. Meisinger C, Doring A, Thorand B, Heier M, Lowel H. Body fat distribution and risk of type 2 diabetes in the general population: are there differences between men and women? The MONICA/KORA Augsburg cohort study. Am J Clin Nutr. 2006;84:483–9.
49. http://www.nationaalkompas.nl/gezondheid-en-ziekte/ziekten-en-aandoeningen/endocriene-voedings-en-stofwisselingsziekten-en-immuniteitsstoornissen/diabetes-mellitus/prevalentie-en-incidentie-naar-leeftijd-en-geslacht. Accessed 20 March 2013
50. Donders AR, van der Heijden GJ, Stijnen T, Moons KG. Review: a gentle introduction to imputation of missing values. J Clin Epidemiol. 2006;59:1087–91.
51. Shoukri MM, Asyali MH, Donner A. Sample size requirements for the design of reliability study: review and new results. Stat Methods Med Res. 2004;13:251–71.