DISENTANGLING THE RELATIONSHIP BETWEEN DEPRESSION, OBESITY AND CARDIOMETABOLIC DISEASE

Tahani Alshehri

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Colophon

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1

Introduction

INTRODUCTION

Obesity, depression, and cardiometabolic diseases are known as "diseases of modernity" due to the alarmingly increased prevalence since the last century [1, 2]. The first notion of the link between obesity and depression was made by Mary E. Moore in 1962 [3]. This was followed by epidemiological studies, which confirmed [4] the presence of this association. Simultaneously, epidemiological studies also reported on the link between obesity and cardiometabolic diseases [5], and depression and cardiometabolic diseases [6-9]. However, the links between these conditions appear complex and not fully understood. The comprehensive aim of this thesis is to elucidate the nature of the relationship between depression, obesity, and cardiometabolic diseases by investigating the heterogeneity of the three conditions.

Depression, obesity and cardiometabolic diseases: a complex relationship

Depression is the state of low mood and/or persistent inability to feel pleasure or reword accompanied by emotional, cognitive and somatic symptoms [10] and has been shown to be linked to obesity and cardiometabolic diseases (Table 1). The "Global burden of diseases" between 1999-2019 showed that depression, obesity and cardiometabolic diseases were among the ten leading causes of the highest absolute number of days lost for disability and premature death [11, 12]. Individuals with depression are at 58% increased risk of developing obesity [13] and 40% increased risk of premature death due to other comorbid diseases such as cardiometabolic diseases [14, 15]. To be diagnosed with depression, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria, a person should report having substantial functional impairment with five out of nine symptoms for more than two weeks; two of them should be fundamental symptoms of depressed mood and anhedonia [10]. DSM contains four emotional symptoms (depressed mood, anhedonia, feeling of worthlessness or guilt, and suicidal ideation), three neurovegetative symptoms (low energy level, increased or decreased sleep, and increased or decreased weight), and finally, two neurocognitive symptoms (ability to think or concentrate or indecisiveness, and psychomotor retardation or agitation) [10, 16]. Depression can be assessed via structured clinical diagnostic interview such as the Composite International Diagnostic Interview (CIDI, version 2.1)) (then labelled as clinical depression or major depressive disorder (MDD) or a validated self-report questionnaires with specific cut-offs used to defined participants with depressed mood. Many instruments have been developed to extensively assess depressive symptomatology [17]. For example, the Inventory of Depressive Symptomatology (IDS-SR30) assesses (via a 4-points likert scale) the presence during the last week and the severity of the core symptoms of a major depressive episodes, melancholic

(e.g., anhedonia, non-reactive mood, psychomotor retardation/agitation, appetite or weight decrease, early morning awakening and self-outlook) and atypical (e.g., mood reactivity, leaden paralysis, weight gain or increased appetite, hypersomnia, and interpersonal sensitivity) features, and commonly associated symptoms (e.g., irritability, anxiety, somatic complaints) [18].

Table 1. The association between depression and cardiometabolic diseases

	Cross-sectional studies from meta-analyses	Longitudinal studies from meta-analyses	
Cardiometabolic disease	Depression-Cardiometabolic disease	Depression-> Cardiometabolic disease	Cardiometabolic disease->Depression
Obesity [1]	Pooled OR range (1.14-1.41)	Pooled OR range (from 1.37 to 1.71)	Pooled OR range (from 1.19 - to 2.15)
Type 2 diabetes [2, 3]	OR: 2.9, 95% CI 2.3–3.7	RR: 1.60 (95% CI 1.37-1.88)	RR: 1.15 (95% CI 1.02-1.30)
Cardiovascular disease:			
Ischaemic heart disease [4,5]	OR: 1.88, 95% CI, 1.59–2.23	HR: 1.63 (95% CI 1.36-1.95)	HR: 1.79 (95% CI 1.43-2.23)
stroke [6,7]	OR: 1.53, 95% CI 1.8-1.84	HR: 1.94 (95% CI 1.63-2.30)	HR: 2.62 (95% CI 2.09-3.29)

Obesity is characterized by a shift in energy balance toward excessive storing of fat droplets in adipose tissue, which is associated with low-grade inflammation and impairment of metabolic flexibility (i.e., impairment of sensing and trafficking essential substances for cellular energy homeostasis) [19]. Obesity is defined based on body mass index, which is calculated as weight (kg) divided by squared height (m^2). The World Health Organization (WHO) standard measure for defining obesity is BMI ≥ 30) [20]. Globally, the prevalence of overweight and obesity has been continuously increasing since the 1980s, and if trends do not level off or reverse, more than half of the world's adult population could be overweight or obese by 2030 [2]. Moreover, obesity is a complex condition and is also comorbid with other complex diseases such as depression, type 2 diabetes, heart disease, and stroke (Table 1) [21].

There is compelling epidemiological evidence that confirms that obesity and depression are associated [4, 13, 22, 23] in cross-sectional (Table 1; pooled odds ratios from 6 meta-analyses ranged from 1.14-1.41) and bi-directionally in longitudinal settings (Table 1; pooled odds ratios for depression as an outcome ranged from 1.19 to 2.15, and for obesity as an outcome from 1.37 to 1.71). This association between obesity and depression is only partially explained by distal factors such as lifestyle, medication, and comorbidity [4, 13, 22]. Hence the hypothesis is that there is a high potential for an underlying biological link.

Heterogeneity of depression and obesity

Depression is a heterogeneous condition [24], as the depression diagnosis, by definition, allows for many ways for the DSM criteria to be met [25, 26]. To understand depression heterogeneity, various subtypes of depression have been described [27]. Two clinical depression subtypes, the atypical depression and the melancholic depression [28, 29], have traditionally received more attention. Atypical depression is characterized by mood reactivity (i.e., mood brightens in response to positive events), fatiguability, excessive sleepiness, hyperphagia, weight gain, and interpersonal rejection sensitivity [28]. Melancholic depressive symptoms reflect a state of the hyperarousal stress response, characterized by the inability to have pleasure or reward, pronounced feelings of worthlessness, non-reactive mood, psychomotor disturbances (agitation or retardation), insomnia, loss of appetite and weight, having the worse mood early in the morning [29]. However, this concept of distinct binary depression subtypes has been criticized as it is almost impossible for the subtypes not to overlap [27]. More recently, data-driven approaches have been used in an attempt to perform cluster analysis for depressive symptoms in relation to biomarkers and clinical features. In the top-down approach, studies [30, 31] investigators performed depressive symptom-based clustering as a first step and subsequently evaluated the clustering results via association with biomarker levels. These studies reported that a cluster of atypical energy-related depressive symptoms, such as increased weight and fatigue, were associated with metabolic and inflammatory dysregulations [30, 31]. In contrast, in bottom-up approach studies [32, 33], biomarker-based clustering was done as a first step, and subsequently, the clustering results were evaluated via association with clinical features. These studies led to reports of a cluster of participants with higher metabolic and inflammatory markers who tended to be more vulnerable to depression [32, 33].

Regardless of the differences in the definitions of the different subtypes, accumulated scientific evidence highlighted that individuals who express behavioural symptoms related to energy homeostasis (as a dimension or continuous score of symptoms and not as a binary subtype) are most likely to have increased: BMI, total body fat, proinflammatory markers, acute phase proteins (i.e., IL-6, and CRP), fasting glucose, triglycerides, blood pressure, waist circumference, insulin resistance, leptin resistance and inflammation-related tryptophan catabolites (i.e., kynurenine and quinolinic acid), and decreased HDL-cholesterol [22, 34-40]. Milaneschi et al. [24] conceptualized these findings in the "immuno-metabolic depression" hypothesis, where they postulated the existence of an "immune-metabolic depression" (IMD) dimension characterized by the clustering of depressive symptoms, namely atypical energy-related symptoms (i.e., increased sleepiness, increased appetite, increased weight, low energy level and leaden paralysis) with immuno-metabolic dysregulations such as adiposity,

hyperglycaemia, dyslipidaemia, and inflammation. This model is characterized by the presence of immuno-metabolic dysregulation linked to behavioural symptoms that favour a homeostatic shift toward positive energy balance (increased intake and decreased expenditure) [24].

Obesity too is a heterogenous condition, which can be defined and characterized in different ways. As stated, body mass index (BMI) is the WHO standard measure for measuring obesity (BMI ≥ 30) [20]. Studies that investigated the association between obesity and depression mainly define obesity based on BMI [41-43]. BMI has a high correlation with the amount of fat stored in the body as adipose tissue, but it is also a proxy for high fat-free mass (i.e., muscle mass). Therefore, when BMI is used alone it can be problematic, for instance for interethnic comparison [20, 44] because it has been shown that total body fat storage and distribution varies among ethnic groups. For example, people from the Asian population have lower BMI and a higher tendency for abdominal fat accumulation than the European population. Therefore, the prevalence of type 2 diabetes and cardiovascular disease in the Asian population was reported in the BMI cut-off ≤25 [45]. The amount of total body fat can be directly measured and reported utilizing bioelectrical impedance analysis [46]. The term "adiposity" is used when referring to body fat. Even when total body fat is measured accurately, the location of fat accumulation (i.e., fat distribution) in the peripheral parts of the body or in between organs in the abdominal cavity (i.e., abdominal adiposity) particularly has an additive value for understanding the link between obesity and depression. Abdominal adiposity can be measured as waist circumference; furthermore, by exploiting magnetic resonance imaging, we can more accurately assess the amount of visceral adiposity [46]. A stronger association between depression and abdominal adiposity, as compared to overall adiposity, has been confirmed in previous studies [47, 48]. Previous work has indicated that obesity can affect health and disease differently [49, 50] by showing different and sometimes opposing relationship with metabolic dysregulations. [51-53]. These opposing forms of obesity have also been described as a) metabolically unhealthy obesity, which is associated with excess body fat with the presence of inflammation and metabolic dysregulation, and b) metabolically healthy obesity with excess body fat and healthy metabolic profile (favourable metabolic profile) [49, 50].

The comorbidity of obesity and depression with cardiometabolic diseases

Besides obesity and depression, this thesis will also examine how "cardiometabolic diseases" fits into this relationship. Twenty years ago, Linda Pescatello introduced the name "cardiometabolic diseases" to include all metabolic dysregulation resulting from insulin resistance (i.e., metabolic syndrome and cardiovascular disease, stroke and type 2 diabetes) [54]. Currently, the term cardiometabolic

diseases has no clear definition. Instead, it is used to describe type 2 diabetes and cardiovascular disease and their risk factors, such as insulin resistance, hypertension, hyperglycaemia, and dyslipidaemia, without clear criteria. This implies a heterogeneous nature of cardiometabolic diseases, especially with the notion that factors that predict diabetes, such as components of metabolic syndrome (high waist circumference, triglyceride, and fasting glucose, hypertension, and low HDL cholesterol), do not (or weakly) predict cardiovascular disease [55]. Following the literature in this field, we define cardiometabolic diseases as all insulin resistance related dysregulation unless we specify a subgroup of this constellation. Large meta-analyses of longitudinal studies [56-58] indicate that depression is associated with an increased risk of cardiometabolic diseases (i.e., myocardial infarction, type 2 diabetes, and stroke). Moreover, there is evidence that diabetes, heart disease, and stroke also increase the risk of depression) [56, 58, 59]. However, the link between depression and cardiometabolic diseases is not fully understood.

Using -omics to disentangle the relationship between obesity and depression

An overlap between obesity and depression has been reported on metabolomic and genetic levels, which may indicate a shared biological mechanism between the two conditions [22, 60]. The advancement in the targeted proton nuclear magnetic resonance platform (^{1}H-NMR) spectroscopy and mass spectrometry-based (GC-MS) technologies is opening new opportunities to study obesity and depression based on their metabolic (phenotypic) signature. Metabolomics; is defined as "the study of the unique chemical fingerprints that specific cellular processes leave behind" [61]. The role of metabolic dysregulation was previously investigated in patients with depression and an animal model of depression in a few studies [62-64]. Shao et al. [63] used gas chromatography-mass spectrometry (GC-MS) to study cerebellar metabolomics in a chronic mild stress rodent model of depression. This study showed evidence that the depression model in the rodent is associated with metabolic dysregulation in glucose, lipid, and energy biosynthesis pathways. Similarly, Zheng et al. [62] found that glucose and lipid dysregulation such as polyunsaturated fatty acids, very low density lipoprotein and low density lipoprotein signalling could be potential predictors for depression. In a small sample size study (N=30), Paige et al. [64] used GC-MS to study the metabolic signature in over 60 years old patients with depression and healthy controls. They found a metabolic signature of declined gamma-aminobutyric acid (GABA), glycerol, and short-chain fatty acids such as palmitate and oleate to be linked to depression. Despite the existence of small scales of metabolomics analysis in depression, the heterogeneity of different metabolomics technologies and the heterogeneity of the depression phenotype make it hard to draw a valid conclusion about depression metabolic signature [65].

One important genetic study explored the role of metabolic dysregulation in the relationship between adiposity and depression using a Mendelian Randomization (MR) analysis [66]. Mendelian Randomization uses genetic variants for modifiable risk factors as an unconfounded instrument variable (e.g., genetic variants for obesity), leveraging the random assortment of genes from parents to offspring during gamete formation and conception [67]. Two genetic risk scores, which reflects an individual's genetic liability for a given phenotype, were created [66]. A genetic risk score is calculated as sum of number of risk alleles across all single nucleotide polymorphisms (SNPs) related to a certain trait, weighted for the SNPs' estimates derived from an independent GWAS [68]. The first genetic risk score was built to index adiposity associated with favourable metabolic profile [51], while the second was associated with adiposity associated with an unhealthy metabolic profile [66]. Results indicated that both genetic risk scores were associated with depression, leading the authors to conclude that both favorable and unfavorable adiposity are associated with depression. This study is a clear example of how treating depression as a unity and not considering its heterogenous nature might hinder our effort to understand its biological underpinning in relation to obesity. Other genetics studies reported specific and different profiles of overlap between obesity, immuno-metabolic dysregulations and depression when considering depression heterogeneity. These studies showed that depression expressing atypical energy-related symptoms was associated with the genetic risk scores (GRS) that related to a higher risk of adiposity (i.e., genetic risk scores of BMI) and its related immuno-metabolic dysregulations (e.g., GRS of C reactive protein CRP and GRS of leptin) [69]. Two large scale studies by the UK Biobank [70] and in Psychiatric Genomics Consortium (PGC) [71] found a genetic overlap between adiposity related traits such as BMI, leptin and CRP levels and MDD with atypical energy-related symptoms such as increased appetite, weight and sleep). Moreover, these metabolic dysregulations have been hypothesized to be the link between depression and cardiovascular disease. For example, genetic instruments for immuno-metabolic dysregulations traits commonly linked to CVD, such as triglyceride, IL-6, and CRP, were associated with higher risk of depression [72]. Particularly, genetic variants that predict increased IL-6 were associated with fatigue and sleep alterations [73].

Thesis objectives

In the present thesis, we aimed to disentangle the nature of the relationship between obesity, depression and cardiometabolic diseases. We characterized the association of different measures of obesity and commonly related metabolic dysregulations with depression. Furthermore, we investigated whether this association varied across different depressive symptoms profiles. We also examined the role of metabolic dysregulation as potential linking mechanism between obesity and a depressive profile characterized by atypical symptoms

reflecting energy homeostasis. Finally, we intended to study further the effect of overall depression and specific depressive symptoms profiles on the risk of developing the cardiometabolic diseases.

OUTLINE OF THIS THESIS

Figure 1 illustrates the outline of this thesis. In **chapter two** of this thesis, we aspired to gain more knowledge about the previously reported relationship between obesity and depression by studying the association of four adiposity measures (BMI and total body fat reflecting overall adiposity, and waist circumference and visceral adipose tissue reflecting the abdominal adiposity) with overall depression scores and individual symptoms of depression measured by IDS-SR30 in participants from a population-based cohort (Netherlands Epidemiology of Obesity (NEO) study). In **chapters three and four,** we aimed to identify plasma metabolites associated with depression. We did this in two large-scale studies with two different metabolomics platforms measuring more than 1000 metabolites with a limited overlap (N=18 metabolites) in nine and five Dutch and European cohorts, respectively, from the general population and clinical settings. In **chapter five,** we considered to identify depression dimensions associated with increased risk of adverse metabolic profile by combining data on metabolomics and depressive symptoms. We performed data-driven clustering based on both symptoms and metabolomics in participants diagnosed with clinical depression. In order to replicate our findings, we examined the association between the identified dimensions and the same metabolomics panel and individual cardiometabolic risk markers (e.g., fasting glucose, insulin resistance, total body fat, and visceral adipose tissue) in an independent population-based cohort. In **chapter six**, we use genetics to separate the effect of adiposity from that of metabolic dysregulations to examine whether the link between obesity and atypical energy-related depressive symptoms is dependent on the presence of metabolic dysregulations. Finally, in **chapter seven,** we examined the effect of overall depression and specific depressive symptoms profiles on the risk of eveloping the cardiometabolic diseases. We performed a time to event analysis to disentangle the risk of overall depression and atypical energy-related symptom profile and cardiometabolic diseases and their components (type 2 diabetes and cardiovascular disease) in a median follow-up of 7 years. In **chapter eight**, we discussed the results of this thesis, methodological considerations, suggestions for future work, and the clinical implication of the thesis findings.

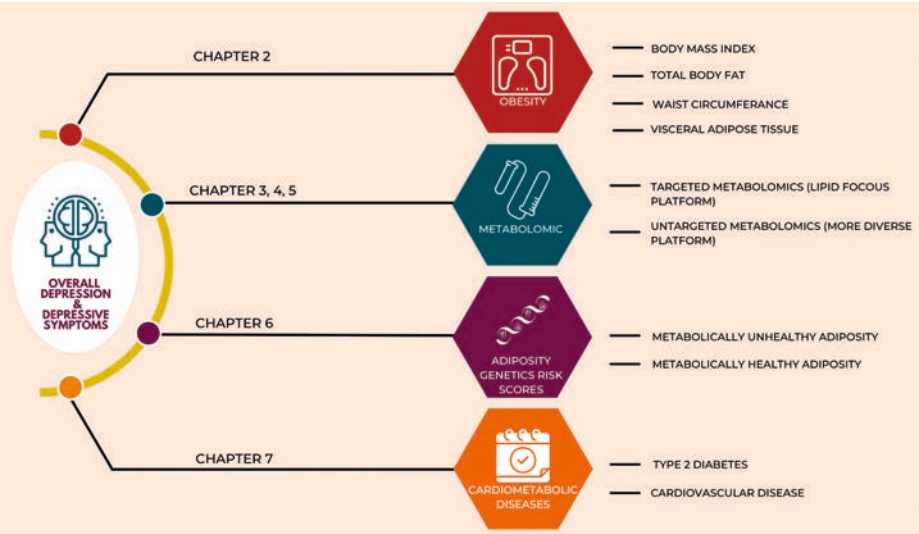

Figure 1. Outline of the thesis

Overview of the used data sources

The Netherlands Epidemiology of Obesity (NEO) study

In chapters two to seven we analysed data from *The Netherlands Epidemiology of Obesity* (NEO) study, a population-based cohort study including 6671 men and women aged 45 to 65 years [45]. All inhabitants with a self-reported body mass index (BMI) of 27 kg/m^2 or higher and living in the greater area of Leiden, the Netherlands were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one adjacent municipality (Leiderdorp, the Netherlands) were invited to participate irrespective of their BMI, allowing for a reference distribution of BMI. Prior to the study visit, participants completed questionnaires at home with respect to demographic, lifestyle, and clinical information. Participants visited the NEO study center after an overnight fast for an extensive physical examination including anthropometry. The present analyses are cross-sectional analyses (i.e., chapter two to six) of the baseline measurements of the NEO study and longitudinal analysis (chapter seven) of the baseline measurement of NEO study and the developing of cardiometabolic diseases extracted from GP registration in 2018. The NEO study was approved by the medical ethics committee of Leiden University Medical Center (LUMC) and all participants gave written informed consent.

Netherlands Study of Depression and Anxiety

In chapters, three, five, and six, we analysed data from *Netherlands Study of Depression and Anxiety* (NESDA), which is an ongoing longitudinal cohort study

that aims to describe the long-term course and consequences of depression and to examine its interaction with biological and psychosocial factors [82]. At baseline (n = 2981) individuals aged 18 through 65 years with depressive and/or anxiety disorders and healthy controls were included from the community, primary care, and secondary care settings between 2004 and 2007. The assessment included a diagnostic interview to assess the presence of depressive and anxiety disorders, a medical exam, and several questionnaires on symptom severity, other clinical characteristics and lifestyle. Participants were followed-up during four biannual assessments. The research protocol of NESDA was approved by the medical ethical committees of the following participating universities: Leiden University Medical Center (LUMC), Vrije University Medical Center (VUMC), and University Medical Center Groningen (UMCG).

BBMRI-NL Metabolomics Consortium

In chapter three, we analysed data from Biobanking and BioMolecular resources Research Infrastructure-The Netherlands (BBMRI-NL) with data on depression and metabolites for over 25,000 people. In addition to the described above NEO study and NESDA, data from Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) [74], The Maastricht Study [84], Erasmus Rotterdam Family study (ERF) [75], Leiden University Migraine Neuro-Analysis (LUMINA) [76], Netherlands Twin Register (NTR) [77], the Rotterdam Study (RS) [78], and Lifelines Deep (LLD) [79-81] was also included. Detailed information on these cohorts is provided in the Supplementary Materials of chapter three. All participants provided written informed consent. Studies were approved by local ethics committees.

Additional study cohorts

In chapter four, the association analysis of metabolite levels with depression was estimated in more than 13000 participants separately recruited in five different cohort studies. The following cohort studies were included: the Rotterdam Study (RS) [82], the Study of Health in Pomerania (SHIP-TREND) [83], the Cooperative Health Research in the Region of Augsburg (KORA) study [84], the European Prospective Investigation into Cancer (EPIC)-Norfolk Study [85], in addition to the Netherlands Epidemiology of Obesity (NEO) study described above. Detailed information on these cohorts is provided in the Supplementary Materials of chapter four. All participants provided written informed consent, studies were approved by their local ethics committees and conformed to the principles of the declaration of Helsinki.

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2

The Association Between Overall and Abdominal Adiposity and Depressive Mood: A Cross-Sectional Analysis in 6459 Participants

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ABSTRACT

Objective
We aimed to evaluate the association between measures of adiposity with depressive mood and specific depressive symptoms.

Methods
This study was performed in the Netherlands Epidemiology of Obesity (NEO) study, a population-based study that consists of 6671 middle-aged individuals. We examined the association between measures of overall adiposity (BMI and total body fat), and abdominal adiposity (waist circumference and visceral adipose tissue), with depressive mood severity subgroups and 30 depressive symptoms. Multinomial logistic regression was performed adjusting for potential confounding.

Results
Measures of adiposity were associated with depressive mood in a graded fashion. Total body fat showed the strongest association with mild (Odds Ratio (OR): 1.59 per standard deviation, 95% Confidence Interval (95% CI): 1.41-1.80) and moderate to very severe (OR: 1.97, 95% CI: 1.59-2.44) depressive mood. Regarding individual symptoms of depressive mood, total body fat was associated with most depressive symptoms (strongest associations for hyperphagia and fatigability).

Conclusions
In the general population, overall and abdominal adiposity measures were associated with depressive mood. This association encompasses most of the depressive symptoms and appeared to be the strongest with specific “atypical” neurovegetative symptoms, which may be an indication of an alteration in the energy homeostasis.

INTRODUCTION

Obesity and depression are serious health conditions that both constitute major economic and social burdens worldwide [1]. Although there is an abundance of research that examined the complex association between both conditions, the conclusions are inconsistent [2]. Where the larger body of evidence is leaning toward the presence of a link between obesity and major depressive disorder (MDD) [3], there are studies that reported that both conditions are unrelated [4] or only reported the presence of an association in sub-groups, for example in women [5].

A recent review [3] summarized the epidemiological evidence of the interconnection between obesity and MDD from large meta-analyses: overall, evidence suggests that obesity and depression are bidirectionally associated, with the presence of one increasing the risk of developing the other. Nevertheless, several important aspects of the relationship between obesity and depression need to be clarified. First of all, the majority of previous work in this field define obesity according to body mass index (BMI=body weight in kg/(height in m^2)) [6]. However, BMI is an approximation of total body fat and does not distinguish between high muscle or fat mass [7]. Furthermore, BMI value does not inform us about the distribution of the fat in the body [7, 8]. This could be of importance, because it is known that especially abdominal adiposity is associated with inflammation, insulin resistance and metabolic syndrome [9].

Depression is also a heterogeneous condition: patients with a diagnosis of the same depressive disorder may endorse very different symptoms. This heterogeneity may have contributed to the inconsistency and variability observed in the reported association between adiposity and depression. This association appears to be stronger in certain subgroups of patients. Emerging evidence suggests that the MDD link with obesity measures, and related metabolic and inflammatory dysregulations (i.e. high lipid and glucose levels, low HDL-cholesterol and high inflammation markers), is stronger for patients with a symptom profile often labeled as “atypical”, including neurovegetative symptoms related to energy metabolism such as hyperphagia, hypersomnia, fatigability and physical exhaustion [10]. Results from the Netherlands Study of Depression and Anxiety (NESDA) cohort showed for instance that among patients with Major Depressive Disorder (MDD) appetite upregulation and ’leaden paralysis’ (described as the feeling of being physically weighted down) during an active depressive episode were the symptoms most strongly associated with BMI and obesity-related inflammatory (high C-reactive protein (CRP) and tumor necrosis-α (TN-α)) [11] and endocrine (high leptin) alterations [12]. Whether this link between obesity

correlates and specific depressive symptoms exists also in the general population is unknown.

We set out to coherently interrogate the relationship between overall and abdominal adiposity and depressive mood and its individual symptoms in 6459 participants from a population-based cohort (Netherlands Epidemiology of Obesity (NEO) study). Several measures of adiposity were examined, including overall (BMI and total body fat) and abdominal or central (waist circumference and visceral adipose tissue) adiposity. Among these measures, total body fat and visceral adipose tissue are accurate measures for overall and abdominal adiposity, respectively. Furthermore, we examined the specific associations between the measures of adiposity with 30 depression-related symptoms (assessed by Inventory of Depressive Symptomatology-Self Report 30 questionnaire (IDS-SR30)).

METHODS

Study design and population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based cohort study including 6671 men and women aged 45 to 65 years [13]. All inhabitants with a self-reported body mass index (BMI) of 27 kg/m^2 or higher and living in the greater area of Leiden, the Netherlands were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one adjacent municipality (Leiderdorp, the Netherlands) were invited to participate irrespective of their BMI, allowing for a reference distribution of BMI. Prior to the study visit, participants completed questionnaires at home with respect to demographic, lifestyle, and clinical information. Participants visited the NEO study center after an overnight fast for an extensive physical examination including anthropometry. In a random subgroup of participants without contraindications (i.e., body circumference ≥ 170 cm, implanted metallic devices, or claustrophobia) magnetic resonance imaging (MRI) of abdominal fat was performed. The present analysis is a cross-sectional analysis of the baseline measurements of the NEO study. The NEO study was approved by the medical ethics committee of Leiden University Medical Center (LUMC) and all participants gave written informed consent. We selected 6459 participants with complete measures of body mass index (BMI), depressive symptoms via IDS-SR30 and relevant covariates. Among these participants, 6428 were available for analyses based on total body fat, 6420 for waist circumference and 2475 for visceral adipose tissue.

Measures of adiposity

For this analysis, we assessed four adiposity measures: body mass index (BMI), total body fat, waist circumference and visceral adipose tissue. We used BMI

and the percent of total body fat as measures of overall adiposity; and waist circumference and visceral adipose tissue as measures of abdominal adiposity. Body height was measured with a vertically fixed, calibrated tape measure. Body weight and total body fat were measured by Tanita bioelectrical impedance balance (TBF-310, Tanita International Division, UK). BMI was calculated by dividing the weight by the height squared (kg/m^2). For abdominal fat, waist circumference was measured with a measuring tape placed midway horizontally between the lower costal margin and the iliac crest. For visceral adipose tissue, analyses were performed in a random subgroup of participants without contraindications. Visceral adipose tissue was assessed by a turbo spin echo imaging protocol using MRI. Imaging was performed on a 1.5 Tesla MR system (Philips Medical Systems, Best, The Netherlands). At the level of the fifth lumbar vertebra, three transverse images each with a slice thickness of 10 mm were obtained during a breath hold. The fat depots were converted from the number of pixels to squared centimeters for all three slides, using in-house-developed software (MASS, Medis, the Netherlands). In the analysis, the average of the three slices was used [14].

Assessment of depressive mood

We asked all participants to complete the Dutch translation of the IDS-SR30 questionnaire, which assesses specific depressive symptoms during the last week and their severity. The IDS-SR30 rates (via a 4-level response system) the presence of a wide array of depressive symptoms, including core symptoms of major depressive episodes, melancholic (e.g., anhedonia, nonreactive mood, psychomotor retardation/agitation, appetite or weight decrease, early morning awakening, and self-outlook) and atypical (e.g., mood reactivity, leaden paralysis (physical exhaustion), weight gain or increased appetite, hypersomnia, and interpersonal sensitivity) features, and commonly associated symptoms (e.g., irritability, anxiety, somatic complaints). The total score ranges from 0 to 84, with higher scores indicating higher severity.

We regarded the participants as having clinically relevant depressive mood when their IDS-SR30 total score was ≥ 14. Furthermore, we grouped the participant according to the clinically predefined severity cut-offs as follow: score ≤ 13 as "no depressive mood" status (n=4540, reference), 14-25 as "mild depressive mood" (n=1397), 26-38 as "moderate depressive mood" (n=428), 39-48 is "severe depressive mood" (n=68) and 49-84 is "very severe depressive mood" (n=26) [15]. For analysis purposes and due to the relatively small sample size in moderate, severe and very severe sub-categories, they have been merged into "moderate to very severe".

Covariates

By a self-reported questionnaire, participants were asked to report their date of birth, ethnicity, educational level (as a proxy for the socioeconomic status), tobacco smoking status and alcohol consumption. Participants reported the frequency, duration and intensity of their physical activity during leisure time, which was expressed in metabolic equivalents of tasks in hours per week [16]. Caloric intake (KJ/day) was estimated by a food frequency questionnaire [17]. For the antidepressants N06AA and N06A, participants were asked to bring all the medications that they have been using for the last month to the NEO study centre. Then, all prescribed and self-medication were recorded by research nurses based on Anatomical Therapeutic Chemical Classification System (ATC).

Statistical analysis

In the NEO study, individuals with a BMI of 27 kg/m^2 or higher were oversampled. To correctly represent associations in the general population adjustments for the oversampling of individuals with high BMI were made [18]. This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality [19], whose BMI distribution was similar to the BMI distribution of the general Dutch population. All results are based on weighted analyses. Consequently, the results apply to a population-based study without oversampling of individuals with a BMI ≥ 27 kg/m^2. Characteristics of the study population were expressed as a mean with standard deviation (SD), a median (25th and 75th) or as percentages (%). We standardized all measures of adiposity to a mean of zero and a standard deviation of one to allow comparison across different measures.

First, we examined the association between each measure of adiposity with the IDS-SR30 clinical groups using multinomial logistic regression models; the "no depressive mood" groups was set as the reference group. The first model was adjusted for age and sex; the second model was adjusted for age, sex, education level, tobacco smoking, alcohol consumption, physical activity, caloric intake, and ethnicity. Additionally, since abdominal adiposity is strongly related to overall adiposity (Table S 1), all abdominal adiposity analyses were adjusted for total body fat [20]. Subsequently, we repeated these analyses after excluding participants who were using N06AA and N06A antidepressants. Finally, we stratified our main analysis (i.e., the multinomial logistic regression between adiposity measures and depressive mood) by sex.

Second, we used logistic regression to examine the relationship between the overall and abdominal adiposity measurements and the 30 individual items from the IDS-SR30. For each item, the four-level answer system was dichotomized to code for low (reference: levels 0) versus medium-high (levels 1,2,3) symptoms.

Likewise, this analyses were adjusted for age and sex in the first model, and the confounding factors in model 2. Additionally, in order to account for the average depressive symptoms severity, adjustment for the IDS-SR30 total score was done (model 3 and 4). Analyses that included abdominal adiposity were additionally adjusted for total body fat. All statistical analysis were performed with STATA statistical software (StataCorp, College Stations, Texas, USA), version 14.0).

RESULTS

Baseline characteristics for all 6459 participants included in this analysis of NEO cohort are shown in Table 1. The mean age in the NEO population was 55.7 years (standard deviation (SD)): 6.0 years), 56.4% of participants were women and 95.0% were of Caucasian ethnicity. There are large differences in the total body fat and visceral adipose tissue between men and women. Out of the total NEO population 24.3% participants had depressive mood problems. Finally, in the IDS-SR30 questionnaire women reported more depressive symptoms than men (9 points (25^{th}-75^{th} percentiles): 6-15)) versus (6 points (25^{th}-75^{th} percentiles): 3-11)).

Measures of adiposity and depressive mood

The percentage of participants with depressive mood in each quartile of adiposity measures are illustrated in Figure 1. For all adiposity measures the proportion of individuals with mild and moderate to very severe depressive mood is largest in the highest adiposity measure quartile. Odds ratios (OR) and 95% confidence intervals from adjusted multinomial logistic regression for the association between overall and abdominal adiposity measures and the severity of the depressive mood are shown in Table 2. Overall and abdominal adiposity measures were positively associated with mild and moderate to very severe depressive mood in a graded fashion, with higher ORs for the moderate to very severe depressive mood than mild depressive mood. In general, ORs of total body fat were relatively higher than those obtained from other adiposity measures. For example, increased total body fat was associated with mild and moderate to very severe depressive mood (OR: 1.59 (95% CI: 1.41-1.80)), (OR: 1.97 (95% CI: 1.59-2.44)) respectively. In covariate-adjusted models, measures of abdominal adiposity were also associated with depressed mood (waist circumference: mild depressed mood (OR: 1.45 (95% CI: 1.33 -1.59)) and moderate to very severe depressive mood (OR: 1.82 (95% CI: 1.59-2.08)); visceral adipose tissue, mild depressed mood (OR: 1.36 (95% CI: 1.19-1.54)) and moderate to very severe depressive mood (OR: 1.57 (95% CI: 1.25-1.97)). Nevertheless, further adjustment for total body fat substantially reduced the magnitude of these estimates (Table 2), suggesting that the association between abdominal adiposity and depression may largely explained by total body fat (i.e., the association between visceral adipose tissue and mild and moderate to very severe depressive mood was (OR: 1.08 (95% CI: 0.90-1.29)), (OR: 1.23 (95% CI: 0.87-1.73)) respectively).

Table 1. Baseline characteristics for 6459 men and women aged 45 to 65 years included in the analysis from Netherlands Epidemiology of Obesity study.

Characteristics	Total population	Men (43.6%)	Women (56.4%)
Age (years)	55.7 (6.0)	56.1 (6.1)	55.5 (6.0)
Educational level (% high)	45.9	48.0	44.3
Tobacco smoking (%)			
Never	38.5	34.4	41.7
Former	45.4	47.0	44.1
Current	16.1	18.6	14.2
Alcohol consumption (g/day)	14.7 (16.3)	20.5 (19.2)	10.3 (11.9)
Physical activity (metabolic equivalent of task (MET)-hours per week)	120.1 (59.5)	118.3 (62.4)	121.5 (57.1)
Ethnicity (% Caucasian)	94.9	95.1	94.8
Depressive mood characterization			
Current depressive mood (%)	24.3	16.6	30.2
IDS-SR30 total score	8 (4, 13)	6 (3, 11)	9 (6, 15)
None (%)	75.7	83.4	69.7
Mild (%)	18.5	12.4	23.3
Moderate to very severe (%)	5.8	4.2	7.0
Use of antidepressants (%)	6.6	4.5	8.2
Measures of adiposity			
Overall adiposity			
BMI (Kg/m^2)	26.3 (4.5)	26.9 (3.7)	25.9 (4.9)
Total body fat (%)	31.6 (24.8, 38.3)	24.5 (21.2, 28.1)	37.0 (32.3, 41.4)
Abdominal adiposity			
Waist circumference (cm)	92.2 (13.4)	98.5 (10.9)	87.3 (13.1)
Visceral adipose tissue (cm^2)	89.8 (56.1)	115.8 (57.7)	66.7 (42.9)

Normally distributed data shown as mean and standard deviation (SD), skewed distributed data shown as median (25th ,75th percentiles) and categorical data are shown as percentage. High education level: university or college education, while other education level: none, primary school or lower vocational education. IDS-SR30: Inventory of Depressive Symptomatology (self-report). BMI: body mass index. Number of individual with available data for each adiposity measures (BMI=6459, total body fat n=6428, waist circumference=6420, visceral adipose tissue n=2475).

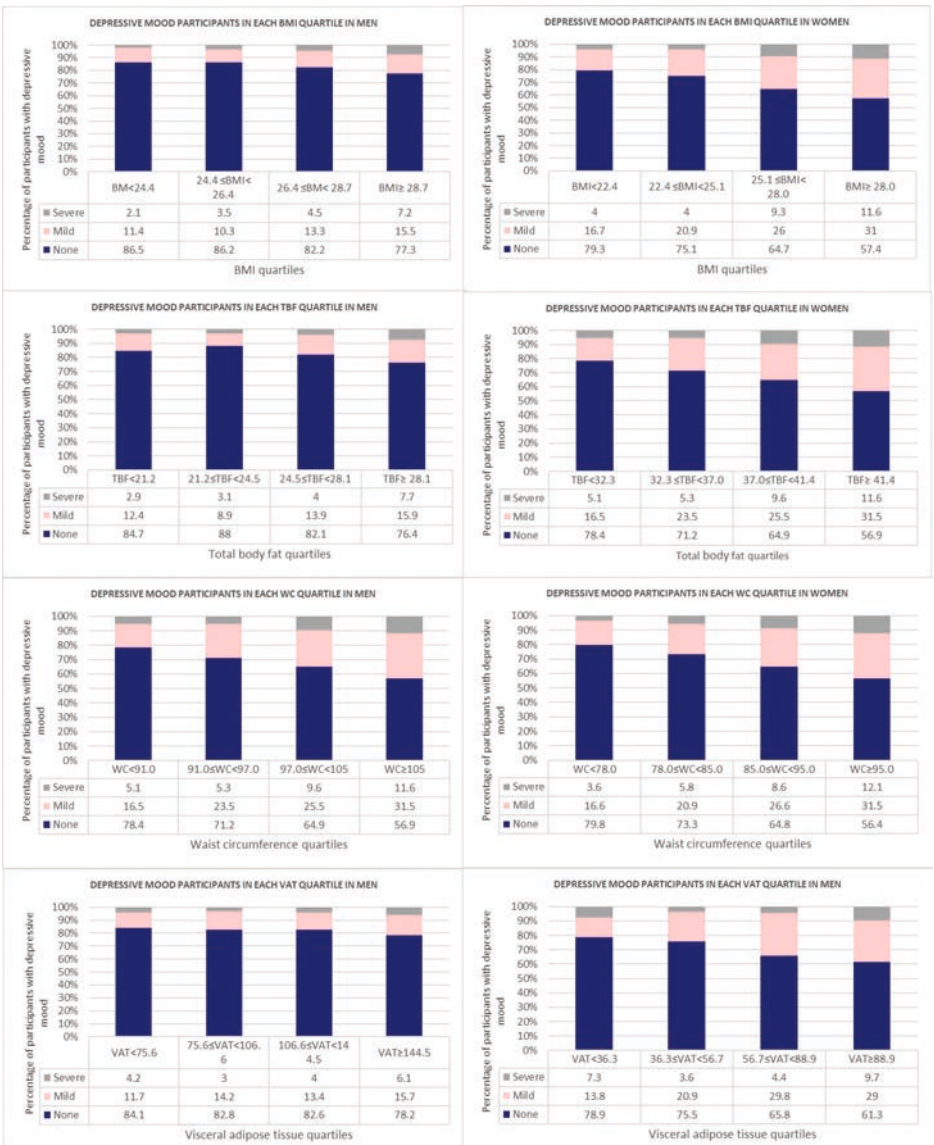

Figure 1. The percentage of participants with depressive in each quartile of adiposity measures

When we repeated the analyses of multinomial logistic regression between overall and abdominal adiposity and depressive mood categories after exclusion of participants who were using antidepressants (6.6%) for any reason, results did not materially change (Table S 2). We also excluded individuals with type 2 diabetes, cardiovascular disease and hypertension and the effect estimates again did not

materially change (Table S 3). The sex-stratified analyses are shown in Table S 4. Overall, direction and strength of effect sizes were similar between sexes.

Table 2. Results of the multinomial logistic regression analysis of the association between overall and abdominal adiposity measures and the severity of depressive mood.

	1 SD	OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Overall adiposity						
		None (75.7%)	Mild (18.5%)		Moderate to very severe (5.8%)	
BMI (kg/m^2)	4.5	Reference	1.36 (1.27-1.47)	1.35 (1.25-1.46)	1.63 (1.48-1.81)	1.58 (1.42-1.75)
Total body fat (%)	8.7	Reference	1.61 (1.43-1.81)	1.59 (1.41-1.80)	2.06 (1.66-2.56)	1.97 (1.59-2.44)
Abdominal adiposity						
Waist circumference (cm)	13.4	Reference	1.28 (1.08-1.52)	1.25 (1.05-1.49)	1.90 (1.44-2.51)	1.82 (1.37-2.43)
Visceral adipose tissue (cm^2)	56.1	Reference	1.09 (0.92-1.30)	1.08 (0.90-1.29)	1.27 (0.89-1.81)	1.23 (0.87-1.73)

OR: odds ratio per standard deviation. IDS-SR30: Inventory of depressive symptomatology (self-report). None: score (0-13). Mild: score (14-25). Moderate to very severe: (26-84). BMI: body mass index. For analysis purposes moderate, severe and very severe IDS-SR30 groups have been merged into (moderate to very severe). Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, educational level, smoking, alcohol consumption, physical activity, caloric intake and ethnicity. Models for waist circumference and visceral adipose tissue were additionally adjusted for total body fat. Number of individual with available data for each adiposity measures (BMI=6459, total body fat n=6428, waist circumference=6420, visceral adipose tissue n=2475).

Body fat measurements and depressive mood symptoms

The logistic regression analysis results of overall and abdominal adiposity measures and the individual 30 items of IDS-SR30 are shown in Figure 2 and fully reported Table S 5. We found that overall and abdominal adiposity measurements were significantly associated with 27 (BMI), 26 (total body fat), 14 (waist circumference), and 2 (visceral adipose tissue) of the 30 depressive mood symptoms. We ranked the ORs of the fully adjusted model (i.e., model2) of logistic regression of overall and abdominal measures and the individual items of IDS-SR30 from high to low (Table S 6). "Atypical" neurovegetative symptoms, such as hyperphagia, low energy level and physical exhaustion were consistently among top ranked symptoms across different measures of adiposity. Symptoms of problems falling asleep and early morning awakening showed no association with adiposity measures.

Figure 2. Results of logistics regression between adiposity measures and individual items of IDS-SR30 questionnaire (model 2)

DISCUSSION

This study examined the nature of the association between accurate measures of adiposity (i.e., total body fat and visceral adipose tissue) and depressive mood in a population-based study that consisted of 6459 middle-aged individuals. We found that especially total body fat, and to a lesser extent other measures of overall and abdominal adiposity, was positively associated with the depressive mood in a graded fashion; as the severity of obesity increases, the severity of depressive mood increases.

In this study, we were able to replicate the previously reported positive association between BMI and depressive mood [2, 3, 21]. However, the question remained whether this positive association is due to high body fat or high muscle mass. To answer this question, we investigated the association between total body fat as estimated by bio-impedance analysis and depressive mood. Previous studies that investigated the association between total body fat and depression were small. The presence of a positive association between total body fat and depression was observed only in women in a previous work that aimed to determine the sex-specific relationship between obesity and depression (n=67) [22]. In the current study, we were able to detect a positive association between total body fat and depressive mood both in men and women, which may imply that total body fat specifically plays a crucial role in relation to depression.

We also set out to examine whether abdominal adiposity contributes to the previously reported association between adiposity and depressive mood. Compiled evidence has indicated that waist circumference, which has been used as a proxy for visceral adiposity, is positively associated with depression [23]. Nonetheless, waist circumference does not discriminate between visceral adipose tissue and abdominal subcutaneous fat [23, 24]. A population-based study of well-functioning older participants [25] showed that depressive mood at baseline predicted an elevation of the visceral adipose tissue measured by the computed tomographic (CT) scanning after five years follow-up. In our analysis, we found a positive association between the measures of abdominal adiposity (both waist circumference and visceral adipose tissue) and depressive mood. Nonetheless, since abdominal adiposity can be an indicator for overall adiposity we adjusted the analysis for total body fat to estimate the specific association of abdominal fat. As it has been reported previously [24], we found that the association between visceral adipose tissue and depressive mood attenuated after taking into account the total body fat adjustment, which may indicate that total body fat is a large contributor to the association between adiposity and depression. Interestingly, we found that the pattern of the main results were similar when stratifying the analyses by sex. This suggests that, despite the established differences in adiposity and depression

prevalence across sex, the association between adiposity and depressive mood is consistent in men and women.

Depressive mood is a heterogeneous condition [26]. It has previously been suggested [10, 27] that adiposity related immune-metabolic dysregulations such as abnormal glucose, triglyceride, C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis-α (TN-α) concentrations are mainly associated with "atypical" neurovegetative symptoms of depression [28]. Using data from the NESDA cohort [12], it has been shown that among patients with a current diagnosis of MDD, higher leptin concentration in the blood (which directly associated with the adiposity level in the body) is associated with symptoms related to energy metabolism like hyperphagia, fatigability and physical exhaustion, independently from BMI. More recent evidence confirmed that the association between this phenotypic constellation, and adiposity and immuno-metabolic dysregulation markers (i.e., C-reactive protein (CRP) and leptin) extended down to the genetic level. Large collaborative genetic studies [29, 30] reported that subjects with a MDD diagnosis reporting hyperphagia or weight gain during the most severe depressive episode in their lifetime, carried a higher number of risk variants for immuno-metabolic traits such as obesity, C-reactive protein (CRP), leptin, and triglycerides dysregulation. In the present study, we demonstrated that both overall and abdominal adiposity were most strongly associated with the same cluster of depressive mood symptoms that relate to energy metabolism (i.e. hyperphagia, low energy level, and increased physical exhaustion) in addition to the more typical symptoms of depressive mood.

Biologically, depression is associated with imbalances in either the hypothalamic-pituitary-adrenal (HPA) axis, the immune system (inflammation), or the regulation of the metabolic pathways. Since these physiological systems are also highly interconnected, it is a challenging process to look at each one of them individually [3, 31]. Accumulation of adipose tissue above the normal levels is associated with low-grade inflammation, insulin resistance [32], leptin resistance [33], and imbalanced activity of the hypothalamic-pituitary-adrenal (HPA) axis [34] which are known to be directly or indirectly associated with depressive mood [35]. Previous studies suggested that the neuroendocrine signaling processes that regulate both mood and energy metabolism are strongly interconnected [36]. Leptin hormone stimulates the proopiomelanocortin (POMC) neuron in the nucleus of the hypothalamus that activates the transcription of the melanocortin peptides (i.e. α, β, and γ MSH, and Mc3r and Mc4r) [37]. These peptides have been suggested to be responsible for regulating energy intake and energy expenditure [38]. Common forms of obesity are thought to be associated with leptin resistance in the brain, blunting its anorexigenic effect and consequently disinhibiting feeding and energy storage despite increasing circulating leptin [39]. An impact of leptin on depression has been suggested by research on animal models [40,

41] indicating antidepressant-like effects of leptin, although exact underlying mechanisms remain unknown. It has been proposed [42] that alterations of the leptin–melanocortin pathway may impair not only its anorexigenic effect, leading to obesity, but also its effect on mood regulation, potentially leading to the development of depression. Furthermore, genome-wide association studies for both obesity and depression show an intersectional association between genes that show strong hits in both conditions, such as neural growth regulator 1 (*NEGR1*) and olfactomedin 4 (*OLFM4*). Noteworthy, these genes play a role in energy regulating mechanism by modulating the synaptic plasticity in brain areas essential for regulating both mood and appetite [3]. We could hypothesize that the impairment of energy homeostasis systems may represent the link that mechanistically connect adiposity with depressive mood. This mechanism may act in two, non-mutually exclusive, ways: as common underlying factor influencing the liability to both depression and obesity, or as mediating mechanisms in causal relationships between the two conditions.

Several additional mechanisms may explain the association between adiposity and depressive mood, including social and behavioral factors such as social rejection, exclusion and/or stigma [43]. An agent-based approach to study the effect of social rejection on depression found that individuals with obesity are more vulnerable to develop depression when obesity is less common in their social networks [44]. It is also possible that behavioral factors that define depressive mood such as low motivation, low energy level, physical inactivity and overconsumption of energy-dense food disturb the body homeostasis and lead to an accumulation of adiposity [36].

Some methodological aspects should be considered. The NEO study is a population-based study in which adiposity measures and depressive mood along with potential confounding factors where thoroughly phenotyped. However, the cross-sectional design of this study does not allow us to draw a conclusion about the directionality of associations. Second, although we adjusted for a large number of covariates in the models, based on the nature of observational studies, residual confounding may still be present. Third, the question of whether total body fat or abdominal fat is more important cannot be answered from this data. Fourth, the depressive mood was assessed only via the self-report IDS-SR30 that may introduce a misclassification of the participants with depressive mood. Nevertheless, this instruments has been extensively validated and used in previous research and the proportion of identified patients with depressive mood in the present study (~30%) is similar to the previous report in populations with obesity [45].

In conclusion, in this study we showed that in the general population overall and abdominal adiposity measures were positively associated with the depressive

mood. This association encompasses almost all depressive symptoms but was strongest for a specific cluster of ''atypical'' neurovegetative depressive symptoms that indicate a deformity in the energy metabolism and homeostasis pathways. Our results suggests that the energy homeostasis dysfunction could connect the mechanisms responsible for developing both adiposity and depressive mood, either as a common cause or in a mediating role. Future longitudinal and experimental studies that exploit the available '-omics' technologies, such as metabolomics and proteomics, are needed to fully elucidate the pathophysiological links that may connect adiposity and depression.

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SUPPLEMENTARY MATERIAL

Full version of supplementary materials can be found through the following link: https://ars.els-cdn.com/content/image/1-s2.0-S0306453019304147-mmc1.docx

Table S 5. Results of logistic regression between the adiposity measures and the individual items from the IDS-SR30 ranked based on their ORs from high to low. (Model 2)

	BMI	Total body fat	Waist circumference	Visceral adipose tissue
1	12. Increase in appetite (Hyperphagia)	12. Increase in appetite (Hyperphagia)	18. Thought of death or suicide	20. Low energy level (Fatigability)
2	20. Low energy level (Fatigability)	30. Physical exhaustion	23. Psychomotor retardation (Feeling slowed down)	30. Physical exhaustion
3	30. Physical exhaustion	20. Low energy level (Fatigability)	20. Low energy level (Fatigability)	18. Thought of death or suicide
4	14. Increased weight (Within the last two weeks)	14. Increased weight (Within the last two weeks)	12. Increase in appetite (Hyperphagia)	10. Diminished quality of mood
5	25. Having Aches and pains	25. Having Aches and pains	10. Diminished quality of mood	16. Self-criticism or blame
6	13. Decreased weight (Within the last two weeks)	13. Decreased weight (Within the last two weeks)	30. Physical exhaustion	23. Psychomotor retardation (Feeling slowed down)
7	21.Diminished capacity of pleasure or enjoyment	21.Diminished capacity of pleasure or enjoyment	08. Diminished reactivity of mood	15. Concentration / decision-making problems
8	19. Diminished interest in people and activity	19. Diminished interest in people and activity	16. Self-criticism or blame	17.Future pessimism
9	26. Having other bodily symptoms	26. Having other bodily symptoms	05. Feeling sad	12. Increase in appetite (Hyperphagia)
10	10. Diminished quality of mood	10. Diminished quality of mood	19. Diminished interest in people and activity	19. Diminished interest in people and activity

3

Metabolomics profile in depression: a pooled analysis of 230 metabolic markers in 5,283 cases with depression and 10,145 controls

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ABSTRACT

Background
Depression has been associated with metabolic alterations, which adversely impact cardiometabolic health. Here, a comprehensive set of metabolic markers, predominantly lipids, was compared between depressed and non-depressed persons.

Methods
Nine Dutch cohorts were included, comprising 10,145 controls and 5,283 persons with depression, established with diagnostic interviews or questionnaires. A proton nuclear magnetic resonance metabolomics platform provided 230 metabolite measures: 51 lipids, fatty acids and low-molecular-weight metabolites, 98 lipid composition and particle concentration measures of lipoprotein subclasses and 81 lipid and fatty acids ratios. For each metabolite measure logistic regression analyses adjusted for sex, age, smoking, fasting status and lipid-modifying medication were performed within cohort, followed by random-effects meta-analyses.

Results
Of the 51 lipids, fatty acids and low-molecular-weight metabolites, 21 were significantly related to depression (false discovery rate $q<0.05$). Higher levels of apolipoprotein B, very-low density lipoprotein cholesterol, triglycerides, diglycerides, total and mono-unsaturated fatty acids, fatty acid chain length, glycoprotein acetyls, tyrosine, and isoleucine, and lower levels of high-density lipoprotein cholesterol, acetate, and apolipoprotein A1 were associated with increased odds of depression. Analyses of lipid composition indicators confirmed a shift towards less high-density lipoprotein cholesterol and more very-low density lipoprotein cholesterol and triglycerides particles in depression. Associations appeared generally consistent across sex, age and body mass index strata, and across cohorts with depressive diagnoses versus symptoms.

Conclusions
This large-scale meta-analysis indicates a clear distinctive profile of circulating lipid metabolites associated with depression, potentially opening new prevention or treatment avenues for depression and its associated cardiometabolic comorbidity.

INTRODUCTION

Depression imposes a huge burden on individuals and society [1]. With a high annual (6%) and lifetime (19%) prevalence, depression is among the leading contributors to global disease burden [1, 2]. It has been associated with an increased risk of somatic disease, including cardiometabolic conditions such as metabolic syndrome [3], obesity [4], diabetes mellitus [5], stroke [6], and cardiovascular disease [7], as well as an increased risk of all-cause mortality [8].

Depression is correlated with metabolic alterations in peripheral bodily systems [1]. A systematic review [9] summarizing metabolomics analyses of urine, cerebrospinal fluid, and blood samples of patients with depression highlighted a set of altered metabolites implicated in energy metabolism, neuronal integrity and transmission. Meta-analyses showed that depression was associated with increased blood levels of total cholesterol [10] and triglycerides (TG) [3], and decreased low density lipoprotein (LDL) cholesterol [11], high density lipoprotein (HDL) cholesterol [3], and omega-3 polyunsaturated fatty acids [12]. However, considerable heterogeneity was noted between studies, which was partly explained by differential lipid classifications [11].

Alterations in circulating lipid concentrations may be linked to pathophysiological pathways related to depression, such as chronic activation of the hypothalamic-pituitary-adrenal (HPA) axis or chronic low-grade inflammation [1]. Glucocorticoid-induced hypercortisolemia is known to result in lipolysis, the release of fatty acids and synthesis of very-low density lipoprotein (VLDL) [13]. Similarly, activation of the pro-inflammatory response leads to a reduction in HDL cholesterol and phospholipids, and an increase in TG, caused by the compensatory production and accumulation of phospholipid-rich VLDL [14]. In addition, omega-3 fatty acids have anti-inflammatory properties, impact HPA-axis functioning, promote cell membrane fluidity, and are involved in the regulation of dopaminergic and serotonergic neurotransmission, which can be altered in depression [15]. Alterations of circulating concentrations of lipids may also represent a consequence of depression. Patients with depression are more likely to engage in unhealthy behaviors, such as sedentariness, excessive alcohol use and poor nutrition (with preference for high palatable food rich in saturated fats), which may lead to dyslipidemia [16], that can result in metabolic syndrome and cardiovascular disease.

Emerging technologies allow high-throughput profiling of lipids and other metabolites, which has led to efforts of determining metabolic signatures of various diseases [17, 18]. A few studies have applied this to depression [19, 20], but the results remain inconsistent [21, 22]; this is partly due to different

methodologies used and different metabolites (lipids, amino acids and other small molecules) analyzed [23].

This study aims to identify plasma lipids, fatty acids and low-molecular-weight metabolites associated with depression by analyzing data from nine Dutch clinical- and population-based studies, and to assess consistency of findings across studies. A strength of the study is that all metabolites were measured around the same time with the same targeted proton nuclear magnetic resonance platform that quantifies lipids, fatty acids and low-molecular-weight metabolites, including those that have been related to consequences of depression (e.g., insulin resistance [24], onset of cardiovascular events [25], and mortality [26]).

METHODS AND MATERIALS

Sample description

Eleven datasets from nine cohorts participating in the Biobanking and BioMolecular resources Research Infrastructure-The Netherlands (BBMRI-NL) were included: Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) [27], The Maastricht Study [28], Erasmus Rucphen Family study (ERF) [29], Leiden University Migraine Neuro-Analysis (LUMINA) [30], Netherlands Epidemiology of Obesity study (NEO), Netherlands Study of Depression and Anxiety (NESDA), Netherlands Twin Register (NTR) [31], the Rotterdam Study (RS), and Lifelines-DEEP (LLD) [32-34]. Both CODAM and The Maastricht Study contributed two datasets stratified by diabetes mellitus status. In total, we included 5,283 persons with depression and 10,145 control subjects (see Supplement 1 for detailed cohort descriptions). All participants provided written informed consent. Studies were approved by local ethics committees.

Measurements

Depression

The presence of depression was measured either before blood sampling or up to a maximum of one month after blood sampling. Subjects were defined as cases when meeting all the criteria required for a diagnosis of major depressive disorder (MDD) in clinical structured interviews in four cohorts, or when scoring above validated clinical cut-off score in depression questionnaires in five cohorts (see Table S1 in Supplement 1 for all instruments and definitions). In the main analyses, cases included subjects with any history of depression in lifetime.

Metabolites

Supplement 1 shows details on blood collection (for each cohort), measurement and processing of metabolite measurements. Using targeted high-throughput

proton Nuclear Magnetic Resonance metabolomics (Nightingale Health Ltd, Helsinki, Finland), 230 metabolites or metabolite ratios were reliably quantified from ethylenediamine tetraacetate plasma samples [35]. This metabolomics platform has been used in large-scaled epidemiological studies of diabetes [24], cardiovascular disease [25], mortality [26] and alcohol intake [36]. To enhance interpretation, metabolites were classified into three clusters curated by Nightingale Health [37]: 1) lipids, fatty acids and low-molecular-weight metabolites (N=51); 2) lipid composition and particle concentration measures of lipoprotein subclasses (N=98); 3) metabolite ratios (N=81). Data were processed according to a shared protocol applied also in other studies of BBMRI-NL [38]. In each cohort, values of metabolites that could not be quantified (≤5 metabolites per cohort) were set as missing for all subjects. Furthermore, metabolites values in subjects with outlying concentrations (±5 SDs) were additionally set as missing. A value of 1 was added to all metabolite values (Supplement 1 includes extensive analyses indicating that the degree of bias potentially introduced by this transformation is likely negligible) that were subsequently natural log-transformed to approximate normality. The obtained values were scaled to standard deviations units in each cohort to enable comparison.

Statistical analyses

Per-metabolite logistic regression analyses were initially performed in each dataset. The dependent variable was depression, and independent variables were the 230 metabolite measurements. For the Netherlands Twin Register cohort, logistic regression using generalized estimating equations were conducted, accounting for family-relatedness. All models were adjusted for age, sex, fasting status, use of lipid-modifying drugs listed under ATC (Anatomical Therapeutic Chemical Classification System) code C10 and smoking (Supplement 1 for measurements). All analyses were based on available data per metabolite (pair-wise deletion). Dataset-specific estimates were combined using random-effects meta-analyses (restricted maximum-likelihood estimator) to obtain pooled odds ratios (ORs). Heterogeneity of results between datasets was quantified by I^2 [39] along with 95% confidence intervals (CI) as recommended [40, 41].

As body mass index (BMI) has been shown to be associated with depression [4] and metabolites [42], we reran the main analyses adjusting for BMI. Furthermore, to investigate whether metabolic profiles were dependent on recent presence of depression, additional analyses were conducted comparing current depressed cases (depression present ±1 month around blood sampling) and controls. We conducted sensitivity analyses in which we excluded subjects using antidepressant medication (ATC code N06A), to study the impact of depression apart from its treatment. Here, we a priori expected to find a less distinctive metabolomics profile, given that antidepressant medication prescriptions are more likely in

individuals with higher depression severity. Correlations between estimates obtained from these sensitivity analyses and estimates obtained in the main analyses were computed to measure the impact of the factors considered.

Four additional sets of stratified analysis were performed to explore whether associations between metabolites and depression were different as a function of (1) depression assessment (diagnosis vs. self-report instrument), (2) sex (men vs. women), (3) age (<50 years vs. ≥50 years) and (4) BMI (normal (18.50-24.9) vs. overweight (25.0-29.9) and vs. obesity (≥30)). A Wald-test was performed to test differences in effect sizes across these strata [43], and correlations between estimates obtained across strata were estimated.

The False Discovery Rate (FDR) method [44] was applied to address multiple testing at the meta-analysis level for 230 metabolites. Meta-analyses were conducted with the 'metafor' package (version 2.0.0) in R v3.4.2-3.4.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Overview of cohorts

The study population comprised 15,428 adults from 11 datasets of 9 cohorts. There were 10,145 controls, and 5,283 participants with depression. Table 1 shows the characteristics of the 11 datasets. Across the cohorts, the average age ranged from 40.4-64.8 years, the proportion of women ranged from 32% to 70%, and the median prevalence of depression was 29.5%.

Table 1. Characteristics of the study populations (N=15,428)

	CODAM DM	CODAM noDM	TMS DM	TMS noDM	ERF	LUMINA	NEO	NESDA	NTR	RS	LLD
Total N	139	416	775	723	346	231	6554	2509	1523	1188	1024
Female, n (%)	46 (33.1)	168 (40.4)	248 (32.0)	455 (62.9)	198 (57.2)	136 (58.9)	3433 (52.4)	1680 (67.0)	1072 (70.4)	755 (63.6)	596 (58.2)
Age (years), mean (SD)	61.2 (6.2)	59.0 (7.1)	62.7 (7.5)	58.8 (8.0)	48.0 (14.0)	41.2 (12.2)	55.8 (6.0)	41.8 (13.0)	40.4 (13.2)	64.8 (5.8)	44.9 (13.2)
Current smoker, n (%)	26 (18.7)	86 (20.7)	122 (15.7)	94 (13.0)	127 (36.7)	25 (10.8)	1071 (16.3)	978 (39.0)	74 (4.9)	161 (13.6)	204 (19.9)
Use of lipid-modifying medications n (%)	35 (25.2)	69 (16.6)	578 (74.6)	162 (22.4)	31 (8.95)	2 (0.9)	1024 (15.6)	177 (7.0)	77 (5.1)	257 (21.6)	45 (4.4)
Fasting, n (%)	139 (100)	416 (100)	775 (100)	723 (100)	344 (99.4)	230 (99.5)	6554 (100)	2403 (95.8)	1441 (94.6)	1113 (93.7)	1013 (98.9)
BMI (kg/m^2), mean (SD)	30.3 (4.7)	28.0 (4.1)	29.8 (4.9)	29.3 (3.6)	27.2 (4.5)	23.6 (2.4)	30.1 (4.8)	25.6 (5.0)	24.7 (4.1)	27.4 (4.3)	25.2 (4.1)
No depression, n (%)	105 (75.5)	338 (81.3)	503 (64.9)	480 (66.4)	193 (55.8)	172 (74.5)	4620 (70.5)	634 (44.8)	1353 (88.8)	737 (62.0)	1010 (98.6)
Depression, n (%)	34 (24.5)	78 (18.8)	272 (35.1)	243 (33.6)	153 (44.2)	59 (25.5)	1934 (29.5)	1875 (74.7)	170 (11.2)	451 (38.0)	14 (1.4)
Of which current depression, n (%)	34 (24.5)	78 (18.8)	46 (8.4)	24 (4.8)	25 (7.2)	14 (6.1)	1934 (29.5)	782 (55.2)	N.A.	314 (26.4)	14 (1.4)
Antidepressant use, n (%)	10 (7.2)	20 (4.8)	63 (8.1)	64 (8.9)	24 (6.9)	3 (1.3)	534 (8.1)	683 (27.2)	73 (4.8)	77 (6.5)	46 (4.5)

Abbreviations: BMI = Body mass index, N.A.=not available.

Associations of 51 lipids, fatty acids and low-molecular-weight metabolites with depression

Figure 1 shows a polar plot with ORs of meta-analyses investigating associations between depression and the 51 metabolites, after adjustment for sex, age, smoking, lipid modifying drugs, and fasting status. Of these, 21 metabolites were associated with depression at FDR q<0.05 (Table 2; Figure S1 in Supplement 1). Metabolites associated with a higher odds for depression were apolipoprotein B; remnant (non-HDL and non-LDL) cholesterol, VLDL cholesterol, and mean diameter of VLDL; the glycerides and phospholipid markers diglycerides; TG in LDL, serum TG, TG in HDL, TG in VLDL, the fatty acid measures total fatty acids, monounsaturated fatty acid, and estimated fatty acid chain length; the inflammation marker glycoprotein acetyls; and the amino acids tyrosine and isoleucine. Higher levels of metabolites that were associated with a lower odds for depression were apolipoprotein A1, cholesterol content for HDL (in particular HDL_2- and HDL_3- cholesterol), and mean diameter of HDL, and ketone body acetate.

Table 2. Overview of the 21 lipids, fatty acids and various low-molecular-weight metabolites that are significantly related to depression in the pooled analysis at FDR q<0.05

Metabolite	Model 1			Model 2*		
	Pooled OR	p-value	FDR q-value	Pooled OR	p-value	FDR q-value
Apolipoproteins						
ApolipoproteinA1	0.90	2.71×10^{-7}	2.50×10^{-6}	0.94	0.007	0.021
ApolipoproteinB	1.08	2.40×10^{-4}	6.90×10^{-4}	1.05	0.014	0.040
Cholesterol						
Remnant cholesterol	1.07	0.003	0.006	1.05	0.014	0.038
VLDL cholesterol	1.10	1.68×10^{-4}	5.03×10^{-4}	1.07	0.001	0.002
HDL cholesterol	0.86	1.24×10^{-12}	9.47×10^{-11}	0.91	2.03×10^{-5}	2.59×10^{-4}
HDL_2 cholesterol	0.89	5.78×10^{-6}	2.79×10^{-5}	0.93	0.001	0.003
HDL_3 cholesterol	0.90	2.18×10^{-5}	8.37×10^{-5}	0.93	4.91×10^{-4}	0.002
Mean diameter of VLDL	1.13	1.30×10^{-6}	8.82×10^{-6}	1.08	2.39×10^{-4}	0.001
Mean diameter of HDL	0.91	2.10×10^{-4}	6.10×10^{-4}	0.96	0.104	0.222
Di- and triglycerides						
Diglycerides	1.09	2.56×10^{-5}	9.65×10^{-5}	1.07	0.003	0.008
Serum total TG	1.11	3.29×10^{-5}	1.15×10^{-4}	1.08	1.92×10^{-4}	0.001
VLDL TG	1.11	8.68×10^{-5}	2.77×10^{-4}	1.08	1.76×10^{-4}	0.001
LDL TG	1.05	0.015	0.032	1.04	0.101	0.218
HDL TG	1.09	0.007	0.015	1.07	0.029	0.072

Table 2. Continued.

Metabolite	Model 1			Model 2*		
	Pooled OR	p-value	FDR q-value	Pooled OR	p-value	FDR q-value
Fatty acids						
Mono Unsaturated FA	1.09	7.13×10^{-6}	3.35×10^{-5}	1.06	0.004	0.012
Total FA	1.05	0.013	0.027	1.03	0.102	0.219
Estimated FA chain length	1.10	0.020	0.043	1.08	0.060	0.140
Inflammation						
Glycoprotein acetyls	1.13	0.003	0.007	1.09	0.028	0.071
Ketone bodies						
Acetate	0.91	0.003	0.006	0.93	0.038	0.092
Amino acids						
Tyrosine	1.07	0.013	0.028	1.02	0.552	0.760
Isoleucine	1.14	8.26×10^{-6}	3.71×10^{-5}	1.08	0.001	0.004

Model 1: adjusted for sex, age, smoking, lipid modifying drugs, fasting status; Model 2: adjusted for model 1 and body mass index; Abbreviations: FDR=false discovery rate, FA=fatty acids, HDL=high-density lipoprotein, LDL=low-density lipoprotein, OR=odds ratio, TG=triglycerides, VLDL=very-low-density lipoprotein.

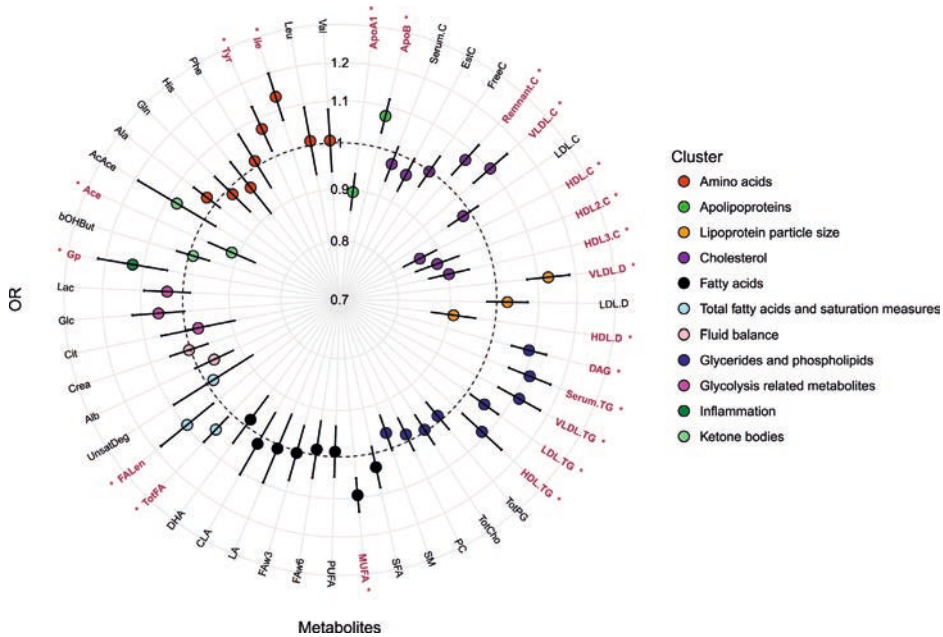

Figure 1. Polar plot illustrating pooled odds ratio and 95% confidence intervals for the association of the 51 lipids, fatty acids and various low-molecular-weight metabolites with depression

*Significant at false discovery rate q < 0.05. Dotted circle indicates an OR of 1. Density: high-density lipoprotein (HDL) subfraction 2 (HDL_2), 1.063–1.125 g/mL; HDL_3, 1.125–1.210 g/mL. AcAce, acetoacetate; Ace, acetate; Ala, alanine; Alb, albumin; ApoA1, apolipoprotein A-I; ApoB, apolipoprotein B; bOHBut, 3-hydroxybutyrate; C, cholesterol; Cit, citrate; CLA, conjugated linoleic acids; Crea, creatinine; D, mean diameter; DAG, diglycerides; DHA, docosahexaenoic acid; Est, esterified; FA, fatty acids; FALen, estimated fatty acids chain length; FAw3, ω-3 fatty acids; FAw6, ω-6 fatty acids; Glc, glucose; Gln, glutamine; Gp, glycoprotein acetyls, mainly α1-acid glycoprotein; His, histidine; IDL, intermediate-density lipoprotein; Ile, isoleucine; LA, linoleic acid (18:2); Lac, lactate; Leu, leucine; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acids (16:1, 18:1); PC, phosphatidylcholine and other cholines; Phe, phenylalanine; PUFA, polyunsaturated fatty acids; Remnant, non-HDL, non-LDL cholesterol; SFA, saturated fatty acids; SM, sphingomyelins; TG, triglycerides; TotCho, total cholines; TotFA, total fatty acids; TotPG, total phosphoglycerides; Tyr, tyrosine; UnsatDeg, estimated degree of unsaturation; Val, valine; VLDL, very-low-density lipoprotein.

Heterogeneity was small (I^2<25% for 15 out of 21 metabolites) and statistically non-significant in almost all (19 out of 21) analyses. As shown in the related forest plots (Figure S1 in Supplement 1) association estimates were quite consistent across the different datasets, including those enriched for cardiometabolic risk. To confirm this, we reran the analyses after removing two datasets (CODAM subgroup with type 2 diabetes mellitus and TMS subgroup with type 2 diabetes mellitus) containing approximately 900 participants with established diabetes and elevated cardiovascular risk factors. Association estimates were highly concordant with those of the original analyses (r=0.99); all the 21 metabolites detected in the

original analyses were associated at nominal level with depression (17 at FDR q<0.05; Table S3 in Supplement 1).

Additional adjustment for BMI partially reduce the strength of the association of these 21 metabolites with depression (regression slope of the 21 beta's before versus after BMI-adjustment=0.65, whereas a beta value of 1 would indicate similar average association sizes; correlation r=0.98): of the 21 metabolites associated with depression, 16 remained significantly related to depression at p<0.05 and 13 at FDR q<0.05 (Table 2). Table S2 in Supplement 2 shows the pooled ORs and heterogeneity findings for all metabolites.

Associations of 98 detailed lipid composition and particle concentration measures of lipoprotein subclasses with depression

Figure 2 shows the ORs of the meta-analyses for the 98 lipid measures of the 14 lipoprotein subclasses, ordered from large to small particle size. Generally, there appeared to be a shift in association with depression by lipoprotein classes: VLDL lipoprotein levels were positively related to depression, intermediate-density lipoprotein (IDL) and LDL lipid levels were not consistently associated, whereas HDL lipoprotein measures were inversely related to depression. Furthermore, depression was related to higher TG levels.

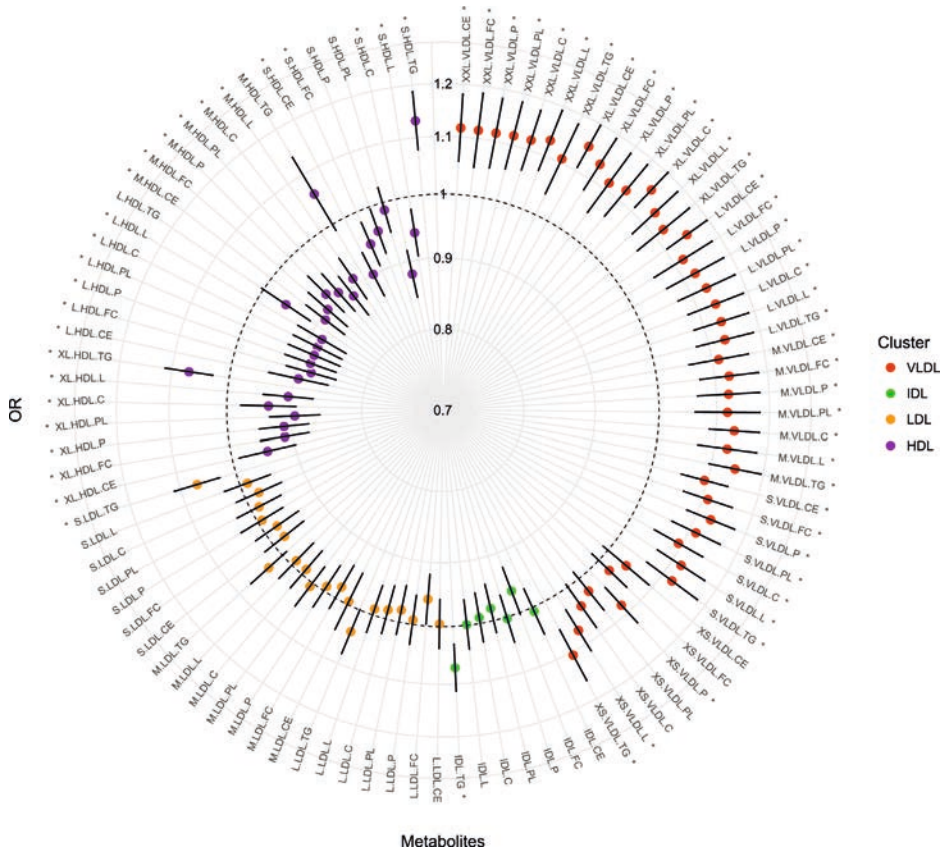

Figure 2. Pooled odds ratios (OR) and 95% confidence intervals for the association of the 98 lipid measures of lipoprotein subclasses with depression.
*Significant at false discovery rate q < 0.05. Dotted circle indicates an OR of 1. Particle sizes: extremely large (XXL) very-low-density lipoprotein (VLDL), >75 nm; very large (XL) VLDL, 64 nm; large (L) VLDL, 53.6 nm; medium (M) VLDL, 44.5 nm; small (S) VLDL, 36.8 nm; very small (XS) VLDL, 31.3 nm; intermediate-density lipoprotein (IDL), 28.6 nm; L low-density lipoprotein (LDL), 25.5 nm; M LDL, 23.0 nm; S LDL, 18.7 nm; XL high-density lipoprotein (HDL), 14.3 nm; L HDL, 12.1 nm; M HDL, 10.9 nm; S HDL, 8.7 nm. C, total cholesterol; CE, cholesterol ester; FC, free cholesterol; L, total lipids; P, particle concentration; PL, phospholipids; TC, triglycerides

Associations of 81 metabolite ratios with depression

Figure S2 in Supplement 1 shows the ORs of the meta-analyses for the 81 metabolite ratios, of which 27 were significant at FDR q<0.05. In general, TG to total lipid ratios were significantly related to an increased odds of depression. Some of the VLDL, IDL, LDL, and HDL lipid measures as percentage of total lipids were positively related to depression, whereas others were inversely related. In general, associations of the metabolite ratios with depression were less pronounced compared to those with absolute metabolite values.

Sensitivity analyses

Current depression

The original 5,283 depression cases included subjects with any lifetime history of depression. In 62% of the cases (3,265 subjects) depression was present between one month before and one month after blood draw. We repeated analyses with only these 3,265 current cases with depression (vs. 10,145 controls). Of the 51 lipids, fatty acids and low-molecular weight metabolites, 22 were associated with current depression at FDR q<0.05 (Figure S3 in Supplement 1). Notably, the strength of the associations with the 51 metabolites tended to be greater for current depression than for the original definition (regression slope of beta's for current versus broadly defined depression=1.22, r=0.95) (Table S2 in Supplement 2). Table S2 in Supplements 2 and Figure S4 and S5 in Supplement 1 show associations of the 98 lipid measures of lipoprotein subclasses, and the 81 metabolite ratios with current depression, which were largely in line with those of original analyses.

Antidepressant medication

To study whether associations were independent of concurrent antidepressant medication use, we removed 1,597 subjects across cohorts who reported use of antidepressants. The majority were depression cases (N=1,305), which was expected given that depression is the main indication for receiving antidepressant treatment. Additionally, one study (LLD) was removed because of model convergence issues. In the remaining 3,966 cases and 8,887 controls - representing a 21% decrease in effective sample size compared with the original analyses, associations with the 51 lipids, fatty acids and low-molecular-weight metabolites were generally in the same direction, but the strength of the associations was attenuated (regression slope of betas before and after exclusion of antidepressant users=0.60, r=0.88) (Figure S6 in Supplement 1). Among the 21 significantly associated metabolites in the overall sample, 8 were still associated at p<0.05, of which 2 (HDL_3- cholesterol, and acetate) at FDR q<0.05 in the antidepressant-free subsample.

Subgroups

Exploration of consistency of associations across subgroups showed that there were no significant differences (Wald-test, FDR q>0.05) in the strength of the association between metabolites and depression across subgroups with depression diagnoses vs. self-reported depression (r=0.75, Figure S7 in Supplement 1), across men vs. women (r=0.64, Figure S8 in Supplement 1), across age <50 years vs. >=50 years (r=0.84, Figure S9 in Supplement 1), and across BMI groups (normal vs. overweight r=0.68, normal vs. obese r=0.55, overweight vs. obese r=0.71, Figures S10-12 in Supplement 1).

DISCUSSION

This meta-analysis of metabolomics and depression, is to our knowledge the largest of its kind. We analyzed data of more than 15,000 subjects from nine Dutch clinical and population-based studies in the Netherlands to identify metabolites associated with depression. Our findings showed that depression is associated with a metabolic signature towards less HDL and more VLDL and triglycerides particles. More specifically, 21 plasma lipids, fatty acids and low-molecular-weight metabolites were significantly related to depression: higher levels of apolipoprotein B, VLDL cholesterol, triglycerides, diglycerides, total and mono-unsaturated fatty acids, fatty acid chain length, glycoprotein acetyls, tyrosine, and isoleucine, and lower levels of HDL cholesterol, acetate, and apolipoprotein A1. Associations were generally consistent across sex, age and body mass index strata, and across cohorts using depression diagnoses vs. depressive symptoms. These metabolic alterations in depression could potentially explain part of the increased risk of cardiometabolic disease in individuals with depression.

Our findings that depression is related to higher VLDL, higher TG and lower VLDL are in line with previous research [3, 11, 45]. In the present study, we predominantly found differences in absolute lipid measures of the VLDL subfractions, whereas findings with lipid measures to lipid ratios in VLDL were less consistently associated with depression. This suggests that the total amount of lipids, rather than the type of lipids, is the main contributor to associations of depression with VLDL. For other metabolites, previous studies indicated more mixed findings. We did not find associations for LDL cholesterol measures, which contrasts with a previous meta-analysis that showed associations between depression and increased LDL cholesterol [11]. For measures of fatty acids, we observed that higher mono unsaturated fatty acids, total fatty acids and estimated fatty acids chain length were associated with an increased odds of depression. Most evidence for links with fatty acids in depression stems from research on omega-3 fatty acids [12], for which we did not observe a consistent, significant association with depression in the present study. The finding of proinflammatory glycoprotein acetyls being positively associated with depression is in line with the large body of evidence linking inflammation to depression [46]. The short chain fatty acid and ketone body acetate was lower in depression. It was hypothesized that a Western-style diet alters gut microbiome composition, resulting in lower acetate levels, which could subsequently induce depression [4]. Furthermore, a smaller study found lower isoleucine levels in depression [47], which contrasts our findings. Finally, a review concluded that there was no association between tyrosine and depression [48], whereas we observed higher tyrosine in depression. Discrepancies could be explained by differences in study characteristics or

variation in analytic approaches, such as selection of potentially confounding factors.

We additionally evaluated the impact of the time frame of depression assessment on the results. In secondary analyses including cases with current depression only, associations tended to become enhanced, suggesting that metabolomics alterations represent state markers reflecting current depression. Nevertheless, a similar profile of associations was found when analyzing depression cases defined in a broader timeframe. The metabolic signature identified may therefore also represent a persisting biological scar after remission of depression, or a pre-existing underlying vulnerability factor for development of depression.

The impact of antidepressant medication use on the results was explored in secondary analyses, although this observational study precludes definitive conclusions, as depression severity most likely represents the clinical indication for antidepressant treatment (confounded by indication) [49]. We reanalyzed data after excluding antidepressant users, and found that the strength of associations was attenuated. Furthermore, the reduction in effective sample size substantially impacted the power to find significant associations. Nevertheless, directions of associations were highly consistent with those obtained in the full sample. Furthermore, the literature shows that potential detrimental effects of antidepressants on dyslipidemia is evident mainly for tricyclic antidepressants (TCA) [50, 51]. Data from the NESDA cohort [51], including patients from mental health care institutions and with the highest prevalence of antidepressant users (27%, Table 1), showed that TCA antidepressant were prescribed only in 3% of the participants. As the overall prevalence of antidepressant use in other cohorts included in the present meta-analysis was lower than approximately 9%, it could be assumed that the number of TCA users may be limited. This observation, combined with the results of our sensitivity analyses, suggests that antidepressant use is unlikely to be the major driver of the associations between metabolites and depression.

Secondary analyses also indicated that results were generally attenuated when BMI was taken into account, suggesting that part of the differential metabolite levels in depression could be explained by BMI. However, interrelationships between BMI, metabolite, depression and antidepressants are particularly complex. A significant genetic correlation has been found between depression and BMI [52], indicating that they may emerge from partially shared etiological mechanisms; at the same time BMI has been shown to influence metabolite concentrations [42]. The ability to disentangle different independent effects of this complex network in observational data is limited. Nevertheless, the majority of metabolites were

associated with depression after taking into account BMI, indicating that this factor explains only a limited portion of the depression-metabolites link.

The present findings may be explained by three, non-mutually exclusive, scenarios. First, alterations of lipids may be a consequence of depression. Depressed persons are more likely to engage in unhealthy behaviors such as sedentariness, excessive alcohol use and poor nutrition (e.g., saturated fats), which may lead to dyslipidemia [16]. Second, lipid dysregulations may be part of the pathophysiological pathways implicated in depression, such as chronic HPA-axis and inflammatory activity, resulting in lipolysis, release of fatty acids, synthesis of VLDL, hypertriglyceridemia and reduction in HDL cholesterol. Third, metabolomic alterations in depression may represent epiphenomena stemming from the same root, such as a common genetic factor. A recent genome-wide association study (GWAS) of major depression involving >450,000 participants, reported a significant genetic correlation ($rg=0.14$, $p=7.8x10^{-7}$) with high TG levels, but not with LDL or HDL [53]. Furthermore, no genetic correlations emerged with metabolites of the same panel that we found to be associated with depression, although the relatively smaller sample size (~25,000) of the metabolomics GWAS may substantially limit the ability to detect correlation; the only exception was a nominally significant correlation with glycoprotein acetyls ($rg=0.15$, $p=0.03$), with the same direction of the phenotypic association we identified. Further experimental studies and genetically informed designs such as Mendelian randomization may disentangle whether depression and lipid dysregulations emerge from shared etiology, and whether depression causally determines lipid alterations or vice versa.

The present study has some limitations. Owing to limited availability or differences in assessment across datasets we cannot rule out confounding by other health-related or lifestyle factors, such as chronic cardiometabolic conditions, alcohol use or specific food intake before sample collection. Nevertheless, the associations between depression and metabolites were consistent across datasets, including those enriched for traits such as diabetes, cardiovascular risk factors and migraine. Furthermore, alcohol use may represent a mediating mechanism rather than a confounder in the metabolites-depression association, as recent evidence [54] showed that alcohol dependence is to quite some extent caused by depression. Analyses were adjusted for fasting status (>94% of subjects were fasting, Table 1), but both fasting and non-fasting samples can be reliably analyzed by the metabolomics platform used [26, 36]. We could not examine whether the associations with metabolites detected vary as a function of specific depression clinical characteristics. Strengths of the study (large samples, metabolites data generated for all studies with the same platform) have enabled the identification of the most reliable metabolic signals associated with depression. These are worth further examination in relation to clinically relevant phenotypes (e.g., age of onset,

recurrence, duration, symptom profiles) in future studies based on psychiatrically well-characterized samples.

This large-scale meta-analysis including more than 15,000 participants identified a metabolomics signature associated with depression. This biological signature is partially shared with other conditions such as diabetes, obesity and cardiovascular diseases [3, 5-7] that commonly co-occur with depression, heavily burdening public health resources. Alterations in the lipid spectrum identified in the present study may represent a substrate linking depression to cardiometabolic diseases and, therefore, a potential target for prevention and treatment of depression and its detrimental somatic sequelae.

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SUPPLEMENTARY MATERIAL

Full version of supplementary materials can be found through the following link:
https://ars.els-cdn.com/content/image/1-s2.0-S0006322319316282-mmc1.pdf
https://ars.els-cdn.com/content/image/1-s2.0-S0006322319316282-mmc2.xlsx
https://ars.els-cdn.com/content/image/1-s2.0-S0006322319316282-mmc3.xlsx

Information about BBMRI-NL consortium can be found through the following link:
https://www.bbmri.nl/bbmri-metabolomics-consortium

Classification of depressed cases and controls

Controls were those with a negative diagnostic interview for lifetime depression, or had a score on the depression questionnaires below established cut-off scores (i.e., CES-D<16, HADS-D<8 and/or IDS-SR30<14). If multiple self-reports of depressive symptoms before blood sampling were available, controls needed to score below the established cut-offs during all these assessments. When diagnostic data on other psychiatric disorders were available (e.g., anxiety disorders), persons with other psychiatric disorders were excluded from the controls.

Metabolomics assessment

A total of 230 metabolites or metabolite ratios were reliably quantified from Ethylenediaminetetraacetic acid (EDTA) plasma samples using targeted high-throughput proton Nuclear Magnetic Resonance (^{1}H-NMR) metabolomics (Nightingale Health Ltd, Helsinki, Finland) [19]. This platform provides simultaneous quantification of routine lipids, lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition, and various low-molecular-weight metabolites including amino acids, ketone bodies and gluconeogenesis-related metabolites in molar concentration units. This metabolomics platform has been extensively used and described in numerous studies (see https://nightingalehealth.com/publications for an overview), including large-scaled epidemiological studies in the field of type 2 diabetes [20], cardiovascular disease [21], mortality [22], and lifestyle factors such as alcohol intake [23]. Details of the experimentation and applications of the ^{1}H-NMR metabolomics platform have been extensively described previously [19, 24, 25].

The entire process from sample handling to data processing is highly standardized and fully automated. Samples were prepared irrespective of depression status, because depression cases and controls entered each study at random order (i.e. unrelated to depression status), and the laboratory analyzing the samples was unaware of depression cases vs. control status when preparing the samples. Automated liquid handlers mixed 260 µL buffer (75 mM Na_2HPO_4 in 80%/20% H_2O/D_2O, pH 7.4; 4.64 mM sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4, and

6.15 mM sodium azide) with the plasma in 1:1 ratio and moved the prepared samples to 96-format racks of NMR tubes, which were subsequently moved to the robotic sample changer, cooled to refrigerator temperature. Each rack contained 2 quality control samples: 1 serum mimic and a mixture of 2 low-molecular-weight metabolites. For the native plasma samples, the lipoprotein (80k data points after 4 dummy scans using 8 transients, 90° pulse) and low-molecular-weight metabolites (64k data points, using 24 (or 16) transients acquired after 4 steady state scans, T2-relaxation-filtered pulse sequence) data were automatically collected at 310.1K either with the 500 MHz or the 600 MHz Bruker AVANCE IIIHD NMR spectrometer, with a relaxation delay of 3.0 seconds [19, 25].

The NMR spectra are converted to absolute concentrations via Bayesian modeling performed via advanced proprietary software and integrates quality control checks. Several of the metabolic biomarkers have already been 'validated' with other techniques (i.e. routine clinical chemistry assays, gas chromatography, an enzymatic method, and/or mass spectrometry) [21, 24, 26-28]. Furthermore, genetic studies [29-31] performed on the same metabolomics platform showed that the labels applied to the metabolites are coherent and linked with biologically relevant and plausible genes.

The 14 lipoprotein subclass sizes were defined as follows: extremely large VLDL with particle diameters from 75 nm upwards and a possible contribution of chylomicrons, five VLDL subclasses, IDL, three LDL subclasses and four HDL subclasses. The following components of the lipoprotein subclasses were quantified: phospholipids (PL), TG, cholesterol (C), free cholesterol (FC), and cholesteryl esters (CE). The mean size for VLDL, LDL and HDL particles was calculated by weighting the corresponding subclass diameters with their particle concentrations.

NMR spectroscopy provides highly consistent biomarker quantification. This is due to the inherently reproducible nature of the technology; the samples never come into contact with the radiofrequency detector in the NMR spectrometer. Biomarker quantification directly from plasma, without any sample extraction procedures, further contributes to the high reproducibility [24]. Representative coefficients of variations (CVs) for the metabolic biomarkers are published as Supplementary Data 3 in Kettunen et al. [30] with the CVs determined for 9,600 samples. Values ranged between 0.3 and 19.5 (mean 4.5%), and most values are comparable to routinely used assays in clinical chemistry.

Covariates

To be largely in line with previous metabolomics meta-analytic studies, [23], we adjusted analyses for the following potentially confounding variables: age (in

years), sex, fasting status (yes/no), use of lipid modifying medication (yes/no), and current smoking (yes/no). The lipid modifying drugs were defined according to the related Anatomical Therapeutic Chemical Classification System (ATC) code C10 (Lipid modifying agents) in order to capture all the medications falling under this category, including the use of single agents (C10A - Lipid modifying agents, plain: C10AA HMG CoA reductase inhibitors; C10AB Fibrates; C10AC Bile acid sequestrants; C10AD Nicotinic acid and derivatives; C10AX Other lipid modifying agents) and all their potential combinations (C10B - Lipid modifying agents, combination: C10BA HMG CoA reductase inhibitors in combination with other lipid modifying agents; C10BX HMG CoA reductase inhibitors, other combinations). The antidepressant medications selected for the sensitivity analyses included all classes listed under the ATC code N06A (N06AA Non-selective monoamine reuptake inhibitors, N06AB Selective serotonin reuptake inhibitors, N06AF Monoamine oxidase inhibitors, non-selective, N06AG Monoamine oxidase A inhibitors, N06AX Other antidepressants). Given the bidirectional relationship between depression and obesity and their shared biological processes (including genes, endocrine and immuno-inflammatory mechanisms) [32], the role of obesity was explored in greater detail in sensitivity analysis (see Statistical analyses). Body mass index (BMI) was calculated as measured weight (kg)/length $(m)^2$, and divided into normal weight (BMI=18.50-24.99), overweight (BMI=25.00-29.99) and obesity (BMI≥30).

Assessment of potential bias due to metabolites data transformation

According to the standardized protocol of data processing applied in the present study a constant of 1 was added to the metabolite values before log-transformation. This common practice, adopted also in several other studies also from the same BBMRI-NL Metabolomics Consortium [33], aims to achieve normalization of the distribution also for metabolites with initial values equaling zero. Nevertheless, it is important to acknowledge that this transformation may have had introduced some bias due to the high variability in the normal range of different metabolite. In the present analyses we aimed to estimate the potential degree of bias introduced by comparing the results of the metabolites-depression associations obtained applying three different transformation before log-transformation: A) adding a constant of 1; B) adding the value of the 10^{th} percentile of the distribution (excluding 0 values) of each metabolite, a value therefore within the normal range of the original metabolite; C) excluding all 0 values, a more conservative approach.

Analyses were performed in the NESDA sample (N=2,509), the most representative dataset for the trait under study, which involves subjects well phenotyped in psychiatric terms including healthy controls and depressed patients from various settings and developmental stages of psychopathology. Furthermore, analyses

focused on the 51 metabolites classified in the cluster of "lipids, fatty acids and various low-molecular-weight metabolites".

Ridge plots in Figure S13 shows the distribution (per SD increase) of the (log) values of the metabolites after the three different transformation. The three sets of values were used in logistic regression analyses estimating the association between metabolites and lifetime depression, adjusting for sex, age, smoking, lipid modifying drugs and fasting status. Results were highly similar across the three transformations. In Figure S14 the estimates obtained used the original transformation A were plotted against estimates obtained with transformation B (panel 1), and against those obtained with transformation C (panel 2). In both instances the correlation between association effect sizes equaled 1 as the estimates were substantially identical across transformation (coefficient from regressing estimates of transformation A on those from transformation B = 1.02, se=0.01; coefficient from regressing estimates of transformation A on those from transformation C = 1.00, se=0.02). Overall, these results suggests that the degree of bias potentially introduced by the transformation applied in original analyses is minimal and negligible.

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4

Circulating metabolites modulated by diet are causally associated with depression

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ABSTRACT

Metabolome reflects the interplay of genome and exposome at molecular level and thus can provide deep insights into the pathogenesis of a complex disease like major depression. To identify metabolites associated with depression we performed a metabolome-wide association analysis in 13,596 participants from five European population-based cohorts characterized for depression, and circulating metabolites using ultra high-performance liquid chromatography/tandem accurate mass spectrometry (UHPLC/MS/MS) based Metabolon platform. We tested 806 metabolites covering a wide range of biochemical processes including those involved in lipid, amino-acid, energy, carbohydrate, xenobiotic and vitamin metabolism for their association with depression. In a conservative model adjusting for life style factors and cardiovascular and antidepressant medication use we identified 8 metabolites, including 6 novel, significantly associated with depression. In individuals with depression, increased levels of retinol (vitamin A), 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1) (lecithin) and mannitol/sorbitol and lower levels of hippurate, 4-hydroxycoumarin, 2-aminooctanoate (alpha-aminocaprylic acid), 10-undecenoate (11:1n1) (undecylenic acid), 1-linoleoyl-GPA (18:2) (lysophosphatidic acid; LPA 18:2) are observed. These metabolites are either directly food derived or are products of host and gut microbial metabolism of food-derived products. Hippurate and mannitol/sorbitol have previously been consistently associated with depression. Our Mendelian randomization analysis suggests that low hippurate levels are causally related to depression. Further analysis of dietary sources of the metabolites in the UK Biobank reveals that increased vitamin A intake may also have causal implications for major depression. Our findings highlight putative actionable targets for depression prevention that are easily modifiable through diet interventions.

INTRODUCTION

Depression is the most common psychiatric disorder with an average lifetime prevalence of 11-15% [1]. A sharp increase in the prevalence of depression worldwide (33.7%; confidence interval 27.5–40.6) has been observed during the recent COVID-19 pandemic [2] and is predicted to increase as the effects of the pandemic unfold further [3]. The molecular mechanisms underlying depression remain elusive. The heritability is estimated to be around 40% [4] and 87 genetic variants have been identified to be associated with depression [5]. There is also a range of environmental risk factors for morbidity including low education, diet and smoking [6]. There is increasing evidence that diet influences mood [7]. Depression also often co-occurs not-only with other neuro-psychiatric pathologies [8, 9], but also clusters strongly with systemic disorders such as cardiometabolic disease, diabetes and arthritis [10-13]. Treatment success for depression is poor and mortality is high [12, 14, 15]. While depression is primarily considered as a disorder of the brain [16], it is associated with metabolic changes in the blood circulation that may be explained by weight loss/gain, changes in diet and altered gut metabolism [17]. There is increasing interest in metabolomic studies of depression that capture the downstream effects of genes, lifestyle factors, pathology and medication [18-20]. A novel hypothesis why circulating metabolites may be involved in depression is that these metabolites are involved in the gut-brain axis, i.e., the bi-directional signalling between the gut, its microbiome and the brain [21, 22]. Metabolomic studies on depression have been small and findings have not always been consistent [23]. Yet, consensus is building that depression is associated with increased levels of glutamate, lactate, alanine, isobutyrate and sorbitol and with decreased levels of kynurenine, gamma aminobutyric acid (GABA), phenylalanine, tyrosine, creatinine, hypoxanthine, leucine, tryptophan, N-methylnicotinamide, β-aminoisobutyric acid, hippurate, amino-ethanol and malonate [24]. Our study of 5,283 patients with depression and 10,145 controls from nine Dutch cohorts [25] using a proton Nuclear Magnetic Resonance (NMR) metabolomics platform (Nightingale Health Ltd., Helsinki, Finland) identified 21 cardiometabolic metabolites that are significantly related to depression. These include an unfavorable spectrum of metabolites associated to cardiometabolic morbidity and mortality [26-28] including apolipoprotein A1 and B, very-low-density and high-density lipoprotein cholesterol, di- and triglycerides, (mono-) unsaturated fatty acids, fatty acid chain length, acetate, glycoprotein acetyls, tyrosine, and isoleucine [29].

A problem hampering the translation of findings of metabolomics studies into preventive and therapeutic interventions is that metabolites in the blood circulation are strongly influenced by medication and comorbidity [22]. Although their effects are well recognized, the potential bias is not controlled for in most

studies conducted to date. Another problem to be tackled is to disentangle metabolic changes that occur as a cause from those that occur because of depression progression. To control for confounding, we conducted a comprehensive analysis of the relation between the blood metabolome and depression in five large scale epidemiologic cohorts including a total of 13,596 participants. This setting allows us to control for confounding effects of medication and co-morbidity. The metabolome in the circulation was characterized by mass spectrometry (MS) using Metabolon. To identify the origin of metabolites (gut and/or human) we integrate our findings with those of the Virtual Metabolic Human (VMH) and Assembly of Gut Organisms through Reconstruction and Analysis (AGORA2) databases. To separate potential causal effects from the consequences of the disease, we integrate genomic and metabolomic data using the NIHR BioResource (NBR). We then examine the impact of anti-depressive therapy on the metabolites in the Predictors of Remission in Depression to Individual and Combined Treatments (PReDICT) study. Finally, we study the association of the diet-based sources of these metabolites with depression and brain pathology in the UK Biobank.

METHODS

Study populations

The association analysis of metabolite levels with depression was performed in 13,596 participants separately recruited in five different cohort studies. The following cohort studies were included: the Rotterdam Study (RS), the Study of Health in Pomerania (SHIP-TREND), the Cooperative Health Research in the Region of Augsburg (KORA) study, the European Prospective Investigation into Cancer (EPIC)-Norfolk Study, and the Netherlands Epidemiology of Obesity (NEO) study. Detailed information on these cohorts is provided in the Supplementary Materials. All participants provided written informed consent, studies were approved by their local ethics committees and conformed to the principles of the declaration of Helsinki. Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Association of depression with the dietary sources of the depression-associated metabolites was performed in the UK Biobank study. UK Biobank is a prospective cohort study including ~ 500,000 participants aged 40-69 years at baseline recruited between 2006 and 2010. The aim of the study is to investigate the effects of genetic and environmental factors on the risk of common multifactorial diseases. Participants have provided a detailed information on lifestyle, medical history and nutritional habits; basic variables such as weight, height, blood pressure etc. were measured; and blood and urine samples were taken. Detailed information about the cohort is provided in the Supplementary Materials.

To ascertain the effects of various depression treatments including cognitive behavioural therapy (CBT) and antidepressants SSRI (escitalopram) and SNRI (duloxetine) on the depression-associated metabolites we performed a lookup in the PReDICT study. The design of PReDICT study has been published previously. [28] Details on the study and the metabolomics assessments are provided in the Supplementary Materials.

To select instruments/proxies for metabolites for Mendelian Randomization we used the results of the genome-wide association study (GWAS) performed using the NIHR BioResource (NBR). NIHR BioResource (NBR) – Rare Disease Study is a multi-center whole-exome and whole-genome sequencing study including up to 13,600 patients (http://bioresource.nihr.ac.uk/rare-diseases/rare-diseases/). The NBR–Rare Diseases study was approved by the East of England Cambridge South national research ethics committee (REC) under reference number: 13/EE/0325. The inclusion and exclusion criteria, as well as other steps of quality control, adjustment and transformations followed the same analytical steps as described before [30].

Depression assessment

In the RS, depressive symptoms were assessed with the 20-item version of the Centre for Epidemiologic Studies Depression (CES-D) scale, a self-report measure of depressive symptoms experienced during the prior week [30]. The total score ranges from 0 – 60, where a higher score indicates more depressive symptoms. In the SHIP-trend and KORA cohorts, depressive symptoms were assessed with the Patient Health Questionnaire 9 (PHQ-9) [31], where each of the nine DSM-IV criteria for depression are scored from 0 – 3. The total score ranges from 0 – 27 where higher score indicates a greater depression severity. In KORA a brief interview version of PHQ-9 called Patient Health Questionnaire Depression (PHQ-D) module was used to measure depression [31, 32]. In the EPIC-Norfolk study depression was assessed using the following question: "Has the doctor ever told you that you have any of the following: depression requiring treatment?" with answers "yes" or "no". In the NEO cohort, depressive symptoms were assessed using the Inventory Depressive Symptomatology Self Report questionnaire (IDS-SR30) [33], which assesses specific depressive symptoms (via a 4-level response system) during the last week and their severity. The total score ranges from 0 to 84, with higher scores indicating higher severity. Thus, in all cohorts except EPIC-Norfolk, depression in participants was measured on a quantitative scale and used as such in the analysis.

In the UKB study, we used the derived lifetime probable major depressive disorder measure as described in Smith et al. 2013 [34]. We further defined current depressive symptoms by summing the responses to four questions related to mood

in the past two weeks. These include, (1) Over the past two weeks, how often have you felt down, depressed or hopeless?, (2) Over the past two weeks, how often have you had little interest or pleasure in doing things?, (3) Over the past two weeks, how often have you felt tense, fidgety or restless? and (4) Over the past two weeks, how often have you felt tired or had little energy? Answers could be given on a four-point scale ranging from 0-3 (0 = not at all, 1 = several days, 2 = more than half of the days and 3 = nearly every day). The total score ranged from 0-12 where higher score indicating more severe depression.

In the PReDICT study, participants were treatment-naive adults defined as having never previously received a minimally adequate course of treatment with an antidepressant medication or evidence-based psychotherapy for a mood disorder, aged 18 to 65 years with moderate-to-severe, non-psychotic MDD depression as assessed by the Structured Clinical Interview for DSM-IV [35] and a psychiatrist's evaluation, and if they scored ≥18 on the HRSD17. Eligible patients were randomized equally to one of three 12-week treatment arms: (1) cognitive behavior therapy (CBT, 16 sessions); (2) duloxetine (30–60 mg/d); or (3) escitalopram (10–20 mg/d).

Metabolomics measurements

In all studies, the metabolome was quantified using the Metabolon platform (Metabolon Inc., Durham, USA). Different versions of the platform have been used and details on the platforms are included in the Supplementary Materials. In all studies, metabolites with ≥ 40% missing values were removed and for the remaining metabolites missing metabolite values were replaced with half of the detection limit for that particular metabolite [36]. Subsequently, a natural logarithm transformation was applied to all metabolites and metabolites were scaled to standard deviation units.

In the PReDICT study, metabolites were quantified using targeted metabolomics platforms including ultra-performance liquid chromatography triple quadrupole mass spectrometry (UPLC-TQMS) (Waters XEVO TQ-S, Milford, USA) and gas chromatography time-of-flight mass spectrometry (GC-TOFMS) (Leco Corporation, St Joseph, USA). Metabolites with >20% missing values were excluded. Then, metabolites were log-transformed, imputed and scaled to mean zero and variance 1. Details are provided in the Supplementary Materials.

Non-targeted metabolite detection and quantification was conducted by the metabolomics provider Metabolon, Inc. (Durham, USA) on fasting plasma samples of 10,654 participants from the UK Bioresource. The metabolomic dataset measured by Metabolon included 1069 compounds of known structural identity belonging to the following broad categories - amino-acids, peptides, carbohydrates,

energy intermediates, lipids, nucleotides, cofactors and vitamins, and xenobiotics. Metabolites data were day-median normalized, and inverse normalized, as the metabolite concentrations were not normally distributed. Metabolic traits with more than 20% missing values were excluded leaving 722 metabolites of known chemical identity for analysis.

Genotyping

For the GWAS of metabolites, genotyping in the UK bioresource was carried out with a high-density array data (Affymetrix UK Biobank Axiom® Array). Genotypes were subsequently imputed using information from the Human Reference Consortium imputation panel (version r1.1, 2016) [37]. Only individuals of full European ancestry (N=8,809) were included in the analyses in the discovery cohort.

Statistical analyses

Metabolites association analysis

All cohorts used linear regression analysis to test the association between the metabolite levels (dependent variable) and depression. Three different models were tested, where the first model (model 1) was adjusted for age and sex only, the second model (model 2) was additionally adjusted for antidepressant medication usage, and the third model was an extension of the second model (model 3) with additional adjustment for lipid-lowering medication (yes/no), antihypertensive medication (yes/no), antidiabetic medication (yes/no), BMI (kg/m^2), and current smoking (yes/no). The summary statistics from all cohorts were combined in a sample size-weighted meta-analysis using METAL software [38]. Sample size weighted meta-analysis was used since the depression measurement scales were different among cohorts. Only metabolites that were present in two or more studies were included. To investigate the robustness of our findings, a sensitivity analysis was performed by including only cohorts that assessed metabolites with the most recent version of the Metabolon platform (HD4).

Association analysis of major depressive disorder with dietary sources of the metabolites in the UK Biobank

We used logistic regression analysis to test the association between major depressive disorder and dietary sources of metabolites (vitamin A supplements, retinol intake estimated from food, fresh fruits intake and vitamin K antagonists). Age, sex and principal components were used as covariates in the analysis. For the association of current depressive symptoms, we used linear regression analysis. We further tested the association of volume of white matter hyperintensities (WMH) with vitamin supplements to ascertain the impact of these supplements on brain pathology. Linear regression analysis was used with the volume of WMH

as the dependent variable, vitamin supplements as the independent variable, and age, sex, BMI, head size and principal components as covariates. All analyses were performed in R.

Metabolite GWAS for Mendelian Randomization (MR) analysis

To test for association between metabolite levels and genotypes, we built linear regression models where the outcome was defined as the transformed level of each metabolite, predicted by the allele dosage at each polymorphic (MAF > 0.01) genotyped or imputed genetic variant. In addition, analyses were adjusted for age, sex and BMI. All analyses were conducted using the PLINK software (https://www.cog-genomics.org/plink/2.0/).

Mendelian Randomization (MR) analysis

To understand the relationship between the identified metabolites and major depression we performed bidirectional two-sample MR analysis. For major depression we used the independent genome-wide significant single nucleotide polymorphisms (SNPs) reported by Howard et al. 2019 [5] as instrumental variables (IVs). Summary statistics for these IVs were extracted from Howard et al. The summary statistics for the metabolites were extracted from the GWAS performed in UK Bioresource. Of the identified metabolites in this study (model 3), GWAS results were available for six metabolites including 2-aminooctanoate, 10-undecenoate (11:1n1), 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1), hippurate, mannitol/sorbitol and retinol (Supplementary Table 1). The IVs for these six metabolites and their summary statistics were extracted from the same GWAS. Because of scarcity in GWAS-grade significance for SNPs associated with these metabolites, we used independent SNPs that showed the strongest association with a p-value < 10^{-06} as instruments (Supplementary Table 2). The summary statistics for depression for these IVs were extracted from the publicly available dataset (2019 PGC UKB Depression Genome-wide; https://www.med.unc.edu/pgc/download-results/mdd/). For the analysis we used the 'mr_allmethods' option of the R (https://cran.r-project.org/) library "MendelianRandomization" [39] that reports the results from the median method (simple, weighted and penalized), Inverse variance weighted and Egger methods (penalized, robust and penalized & robust).

Table 1. Descriptive statistics of the study populations.

	RS	SHIP-trend	KORA	EPIC-Norfolk B2	EPIC-Norfolk B3	NEO
N	484	965	1688	4639	5163	599
Ncases/Ncontrols	-	-	-	638/4001	685/4478	-
Mean age (years) (SD)	73.1 (6.3)	50.1 (13.6)	61 (8.8)	59.9 (8.8)	59.6 (8.9)	55.8 (6.0)
Age range (years)	62-96	20-81	32-77	40-78	40-78	45-66
Females (%)	52.5	56.0	51.4	52.4	52.8	52.6
Mean BMI (kg/m^2) (SD)	26.8 (3.7)	27.4 (4.6)	28.2 (4.8)	26.20 (3.7)	26.2 (3.8)	25.9 (4.0)
Smoking (%)	12.6	22.0	14.5	11.4	10.9	11.9
Medication						
Antidepressants (%)	3.7	4.0	5.6	4.5	3.8	5.3
Lipid-loweringmedication (%)	10.5	7.8	16.7	1.4	1.5	7.7
Antihypertensives (%)	0.6	28.2	37.9	19.5	17.0	19.7
Antidiabetics (%)	5.4	0	7.5	1.9	2.0	2.7

Table 2. Top findings of the association analysis of metabolites with depression (FDR corrected *p-value* < 0.05 in model 1).

			Model 1				Model 2				Model 3			
Chemical ID	**Name**	**Super pathway**	**N**	**Zscore**	**Direction***	**FDR**	**N**	**Zscore**	**Direction***	**FDR**	**N**	**Zscore**	**Direction***	**FDR**
100001197	10-undecenoate (11:1n1)	Lipid	13596	-5.12	+-----	8.02E-05	13556	-3.94	+-----	1.7E-02	13549	-3.79	+-----	2.71E-02
100001740	mannitol/sorbitol	Carbohydrate	12631	5.14	+?-+++	8.02E-05	12592	3.39	+?-+++	5.02E-02	12586	3.58	+?-+++	4.47E-02
1090	bilirubin (Z,Z)	Cofactors and Vitamins	13596	-5.33	------	8.02E-05	13556	-3.60	------	3.5E-02	13549	-2.84	------	1.83E-01
100001950	bilirubin (E,E)*	Cofactors and Vitamins	13596	-5.25	------	8.02E-05	13556	-3.33	------	5.13E-02	13549	-2.73	------	2.08E-01
100002049	4-hydroxycoumarin	Xenobiotics	10885	-4.99	-??---	1.30E-04	10847	-4.48	-??---	4.0E-03	10847	-4.17	-??---	1.12E-02
100008984	1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)*	Lipid	10885	4.84	+??+++	1.94E-04	10847	3.58	+??+++	3.5E-02	10847	3.51	+??+++	4.47E-02
100001951	bilirubin (E,Z or Z,E)*	Cofactors and Vitamins	12631	-4.74	-?----	2.83E-04	12592	-3.50	-?----	3.5E-02	12586	-2.98	-?----	1.37E-01
498	retinol (Vitamin A)	Cofactors and Vitamins	10885	4.65	+??+++	3.58E-04	10847	3.89	+??+++	1.7E-02	10847	4.14	+??+++	1.12E-02
100004227	2-aminooctanoate	Lipid	11850	-4.65	--?---	3.58E-04	11811	-4.00	--?---	1.7E-02	11810	-3.92	--?---	1.87E-02
100002253	cinnamoylglycine	Xenobiotics	10885	-4.40	-??---	9.38E-04	10847	-3.50	-??---	3.5E-02	10847	-3.32	-??---	6.71E-02
100000010	3-phenylpropionate (hydrocinnamate)	Xenobiotics	13596	-4.42	-----	9.38E-04	13556	-3.73	------	2.9E-02	13549	-3.24	--+---	7.89E-02
100001251	decanoylcarnitine (C10)	Lipid	13596	-4.35	+-----	1.02E-03	13556	-3.35	+-----	5.13E-02	13549	-3.15	+-----	8.53E-02
1526	1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	Lipid	10885	4.36	+??+++	1.02E-03	10847	2.64	-??+++	2.12E-01	10847	2.44	-??+++	2.68E-01
250	biliverdin	Cofactors and Vitamins	13596	-4.29	------	1.24E-03	13556	-2.88	------	1.40E-01	13549	-2.41	------	2.75E-01
504	serotonin	Amino acid	12631	-4.24	-?----	1.49E-03	12592	0.18	-?-+++	9.55E-01	12586	-0.02	-?-+++	9.92E-01
192	N-acetylputrescine	Amino acid	10885	4.06	-??+++	3.01E-03	10847	2.09	-??-++	3.52E-01	10847	2.16	-??+++	3.44E-01
100002259	cis-4-decenoylcarnitine (C10:1)	Lipid	13596	-4.02	------	3.27E-03	13556	-2.80	------	1.58E-01	13549	-2.47	------	2.68E-01

Table 2. Continued.

			Model 1				Model 2				Model 3			
Chemical ID	**Name**	**Super pathway**	**N**	**Zscore**	**Direction***	**FDR**	**N**	**Zscore**	**Direction***	**FDR**	**N**	**Zscore**	**Direction***	**FDR**
212	5-methylthioadenosine (MTA)	Amino acid	10885	4.03	+??+++	3.27E-03	10847	2.09	+??+++	3.52E-01	10847	1.78	+??+++	4.91E-01
100000997	3-hydroxydecanoate	Lipid	11850	-3.97	+-?---	3.89E-03	11811	-2.60	+-?---	2.23E-01	11810	-2.39	+-?---	2.75E-01
1539	1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	Lipid	10885	3.85	+??++-	6.01E-03	10847	2.93	+??++-	1.36E-01	10847	2.76	+??++-	2.03E-01
100000014	hippurate	Xenobiotics	13596	-3.80	------	7.10E-03	13556	-4.17	------	1.1E-02	13549	-3.72	------	2.99E-02
100001392	laurylcarnitine (C12)	Lipid	13596	-3.73	+-----	8.97E-03	13556	-2.68	+-----	1.96E-01	13549	-2.61	+-----	2.48E-01
1128	2-aminobutyrate	Amino acid	13596	-3.68	------	9.61E-03	13556	-2.08	---+--	3.54E-01	13549	-1.84	---+--	4.74E-01
561	glutamate	Amino acid	13596	3.70	++++++	9.61E-03	13556	1.92	++++-+	4.13E-01	13549	1.15	++++-+	7.26E-01
98	kynurenate	Amino acid	10885	-3.61	-??---	1.22E-02	10847	-2.79	-??---	1.58E-01	10847	-2.50	-??---	2.68E-01
100001658	taurolithocholate 3-sulfate	Lipid	13596	-3.52	------	1.45E-02	13556	-3.09	------	9.31E-02	13549	-3.09	---+--	1.01E-01
100001511	1-palmitoleoyl-GPC (16:1)*	Lipid	13596	3.54	+++++-	1.45E-02	13556	2.43	+++++-	2.74E-01	13549	2.57	++++++	2.48E-01
100001112	3-hydroxylaurate	Lipid	10885	-3.53	-??---	1.45E-02	10847	-2.54	-??---	2.42E-01	10847	-2.43	-??---	2.69E-01
100008990	1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	Lipid	10885	3.52	-??+++	1.45E-02	10847	2.22	-??+++	3.00E-01	10847	2.23	-??+++	3.27E-01
100000773	3-hydroxyoctanoate	Lipid	11850	-3.54	+-?---	1.45E-02	11811	-2.25	+-?---	3.00E-01	11810	-2.13	+-?---	3.63E-01
100001868	4-allylphenol sulfate	Xenobiotics	10885	-3.48	-??---	1.58E-02	10847	-2.86	-??-+-	1.46E-01	10847	-2.46	-??-+-	2.68E-01
100001247	octanoylcarnitine (C8)	Lipid	13596	-3.47	+-----	1.58E-02	13556	-2.62	+-----	2.14E-01	13549	-2.48	+-----	2.68E-01
100001083	indolepropionate	Amino acid	13596	-3.48	------	1.58E-02	13556	-2.95	------	1.35E-01	13549	-2.12	------	3.66E-01
100001977	beta-cryptoxanthin	Xenobiotics	6246	-3.46	-???--	1.60E-02	6208	-3.25	-???--	5.74E-02	6208	-2.59	-???--	2.48E-01
100006430	arabitol/xylitol	Carbohydrate	12631	3.44	+?++++	1.65E-02	12592	2.48	+?++++	2.51E-01	12586	2.69	+?++++	2.15E-01
100009082	1-linoleoyl-GPA (18:2)*	Lipid	10885	-3.39	-??---	1.91E-02	10847	-3.57	-??---	3.5E-02	10847	-3.53	-??---	4.47E-02
100001870	1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	Lipid	10885	3.39	+??+++	1.91E-02	10847	2.02	+??+++	3.78E-01	10847	2.18	+??+++	3.41E-01

Table 2. Continued.

			Model 1				Model 2				Model 3			
Chemical ID	**Name**	**Super pathway**	**N**	**Zscore**	**Direction***	**FDR**	**N**	**Zscore**	**Direction***	**FDR**	**N**	**Zscore**	**Direction***	**FDR**
100000257	glucuronate	Carbohydrate	10885	3.35	+??+++	2.18E-02	10847	2.09	+??+++	3.52E-01	10847	1.93	+??+++	4.51E-01
391	citrulline	Amino acid	13596	-3.31	------	2.45E-02	13556	-3.53	------	3.5E-02	13549	-3.29	------	7.22E-02
100001121	pyridoxate	Cofactors and Vitamins	13596	3.29	++-++-	2.50E-02	13556	2.71	++-++-	1.83E-01	13549	3.17	++-++-	8.53E-02
100002021	5alpha-androstan-3beta,17alpha-diol disulfate	Lipid	10885	-3.25	-??---	2.79E-02	10847	-2.05	-??---	3.67E-01	10847	-1.63	-??---	5.35E-01
100001287	epiandrosterone sulfate	Lipid	13596	-3.16	-+----	3.59E-02	13556	-1.71	-+----	4.70E-01	13549	-1.73	-+----	5.03E-01
100008914	1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4n6)	Lipid	10885	3.13	-??+++	3.89E-02	10847	2.92	-??+++	1.36E-01	10847	2.94	-??++-	1.52E-01
100001320	erythronate*	Carbohydrate	12631	3.12	+?++++	3.89E-02	12592	1.76	+?++++	4.55E-01	12586	1.77	+?++++	4.91E-01
935	sucrose	Carbohydrate	10885	3.13	+??+++	3.89E-02	10847	1.61	+??+-+	4.99E-01	10847	1.16	+??+-+	7.21E-01
100001567	1-palmitoyl-GPE (16:0)	Lipid	13596	3.10	++-++-	4.01E-02	13556	2.15	++-++-	3.28E-01	13549	2.57	++++++	2.48E-01
100008991	1-palmitoyl-2-docosahexaenoyl-GPE (16.0/22.6)	Lipid	10401	3.06	???+++	4.26E-02	10401	2.43	???+++	2.74E-01	10401	2.71	???+++	2.15E-01
100001657	glycolithocholate sulfate*	Lipid	11850	-3.06	--?--+	4.26E-02	11811	-2.46	--?---	2.60E-01	11810	-2.29	--?---	3.09E-01
100008977	1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	Lipid	10885	3.07	-??+++	4.26E-02	10847	1.94	-??+++	4.07E-01	10847	1.93	-??+++	4.51E-01
823	pyruvate	Carbohydrate	13596	3.06	++++-+	4.26E-02	13556	2.28	++++-+	2.91E-01	13549	1.84	+-++-+	4.74E-01
397	leucine	Amino acid	13596	-3.04	--+--+	4.44E-02	13556	-2.73	--+--+	1.76E-01	13549	-3.33	------	6.71E-02
100002945	15-methylpalmitate	Lipid	13596	-3.04	+-----	4.44E-02	13556	-2.77	+-----	1.63E-01	13549	-2.40	+-----	2.75E-01
100009066	1-palmitoyl-2-oleoyl-GPI (16:0/18:1)*	Lipid	6246	3.03	+???++	4.50E-02	6208	1.96	+???+-	4.01E-01	6208	1.86	+???+-	4.66E-01

* The order of the direction column: RS, SHIP-trend, KORA, EPIC-Norfolk B2, EPIC-Norfolk B3, NEO

Effect of antidepressant therapy on metabolites in PReDICT study
To examine the strength and significance of metabolite concentration changes within each of the three treatment arms, i.e., (1) CBT (16 sessions); (2) duloxetine (30–60 mg/d); or (3) escitalopram (10–20 mg/d), linear mixed effect models (with random intercept) with metabolite levels (in log scale) as the dependent variable, were fitted while correcting for age, sex, BMI, and baseline HRSD17. Then, the R package "emmeans" was used to compute the least squared means of the contrasts of interest (week 12 vs. baseline) and their corresponding p-values.

To detect whether metabolites levels were associated with clinical outcomes, linear regression analyses corrected for age, sex and treatment arm were performed. Dependent variables (Baseline HRSD17, Week 12 HRSD17, and 12 weeks change in HRSD17) were regressed on either of following independent variables: 1) baseline metabolite, 2) week 12 metabolite, 3) 2 weeks change in metabolites and 4) 12 weeks change in metabolites.

Linking metabolites to human and/or gut metabolism
To assess whether the identified metabolites are products of human metabolism, gut microbial metabolism, or both, we integrated our findings with those of the Virtual Metabolic Human (VMH) and Assembly of Gut Organisms through Reconstruction and Analysis (AGORA2) databases. Additional information is provided in the Supplementary Materials.

RESULTS

This study includes 13,596 participants from five independent cohorts including the Rotterdam Study (RS), the Study of Health in Pomerania (SHIP-TREND), the Cooperative Health Research in the Region of Augsburg (KORA) study, the European Prospective Investigation into Cancer (EPIC)-Norfolk Study, and the Netherlands Epidemiology of Obesity (NEO) study. A detailed description of the study participants is provided in Table 1. Depression was measured on a quantitative scale in all cohorts except the EPIC-Norfolk study, where the participants reported depression on a yes/no scale. The mean age ranged from 50.1 years in SHIP-Trend to 73.1 years in the Rotterdam Study. The percentage of female participants (51-56%) and mean body mass index (BMI; between 26-28 kg/m^2) were comparable between studies. There were differences in the percentage of smokers between the cohorts, ranging from 11% in EPIC-Norfolk and to 22% in SHIP-Trend.

When testing for an association with depression adjusting for age and sex, 53 (41 novel) metabolites were significantly associated with depression after adjusting for multiple testing (false discovery rate (FDR) < 0.05; Table 2 & Figure 1). These

include nine metabolites in the amino acid metabolism pathway including five previously associated with depression (leucine, kynurenate, citrulline, glutamate and serotonin) [23, 40, 41] and four novel metabolites (N-acetylputrescine, 5-methylthioadenosine (MTA), 2-aminobutyrate and indolepropionate). In addition, significant association was found for six carbohydrates (one novel), six cofactors and vitamins, all of which were novel, 26 lipids (25 novel), and six xenobiotics (five novel) (Table 2).

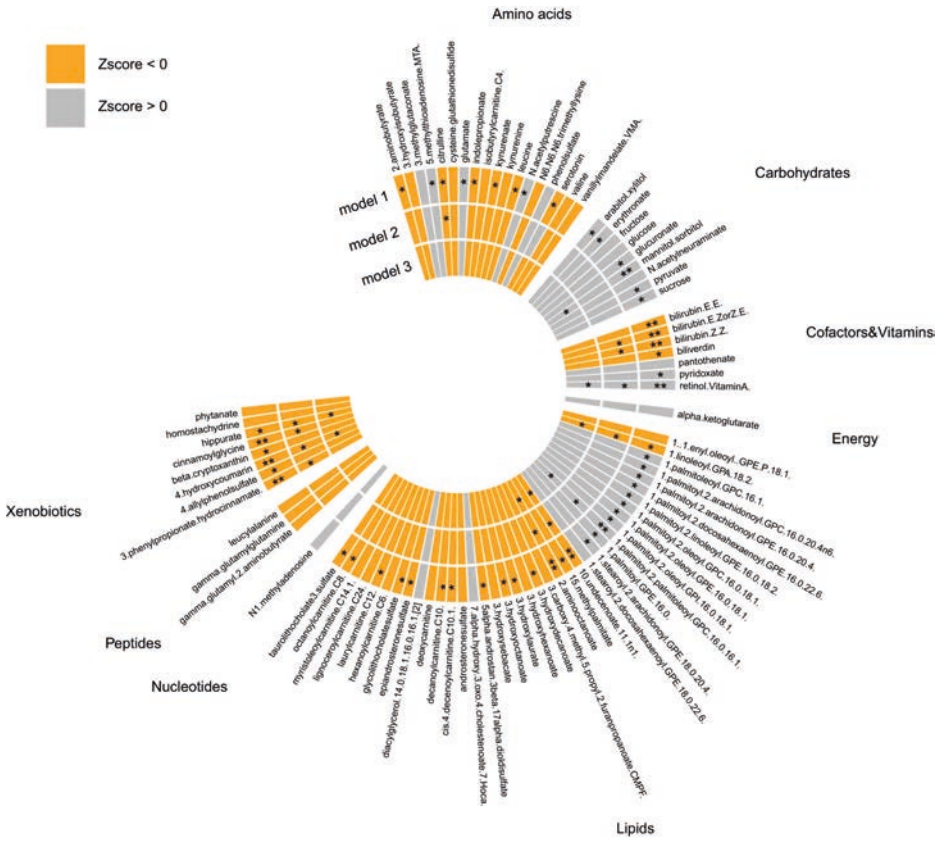

Figure 1. Association plot of metabolites with depression.
This plot shows the top findings of the association analysis of metabolites with depressive symptoms, for all three models tested. Only metabolites with FDR p-value < 0.1 are shown in this Figure. The associations with a negative Z-score are depicted in grey, while the positive associations are depicted in orange. The plot is divided per metabolite subgroup. Significance levels: **: FDR < 0.001, *: FDR < 0.05. Script for Figure modified from Nath et al.(Genome Biol, 2017. **18**(1): p. 146.).

When adjusting for antidepressant use (model 2), 12 metabolites remained significantly associated (FDR <0.05) with depression (Table 2, Figure 1), suggesting that most associations observed with depression were confounded by antidepressant medication use. Of the amino acids, only citrulline remained

significantly associated with depression after adjustment for antidepressant medication (Table 2, Figure 1). Other metabolites that remained significantly associated with depression in the extended model included four xenobiotics (4-hydroxycoumarin, hippurate, 3-phenylpropionate (hydrocinnamate) and cinnamoylglycine), four lipids (2-aminooctanoate, 10-undecenoate (11:1n1), 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1) and 1-linoleoyl-GPA (18:2)), and three cofactors and vitamins (retinol (vitamin A), bilirubin (Z,Z), bilirubin (E,Z or Z,E)). Among these, higher levels of 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1) and retinol (vitamin A) were associated with an increased risk of depression, while the others were associated with a decreased risk (Figure 1).

We subsequently build a more conservative model, further adjusting for other medication use, including lipid-lowering medication, antihypertensive medication, antidiabetic medication, BMI and current smoking (model 3). Seven out of the 12 metabolites remained significantly associated with depression (Table 2). These included retinol (vitamin A), hippurate, 4-hydroxycoumarin, 2-aminooctanoate, 10-undecenoate (11:1n1), 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1), and 1-linoleoyl-GPA (18:2). Additionally, mannitol/sorbitol appeared statistically significant in model 3. Complete results of the meta-analysis are available in Supplementary Table 3.

There was no significant evidence for effect modification by sex (Supplementary Table 4) and the directionality of effects tended to be consistent in men and women. Effect sizes appeared to be stronger in women. Results were consistent across various versions of the Metabolon platform and depression assessing instruments and a sensitivity meta-analysis, which only included results from cohorts that had assessed metabolites on the most recent (HD4) platform, showed that they remained essentially unchanged (Supplementary Table 5).

Association of depression with dietary sources of metabolites in the UK Biobank

To evaluate the association of food sources of the identified metabolites with major depression we conducted a series of analyses in the UK Biobank (UKB). In the UKB information on vitamin supplements including vitamin A, retinol intake from food, consumption of fresh fruits – a major source of hippurate, and medication use including vitamin k antagonist (a proxy for 4-hydroxycoumarin) was available. In a cross-sectional analysis, we found a significant positive association of vitamin A intake from supplements with both measures of depression including current depressive symptoms (beta = 0.23, p-value = 1.25×10^{-25}) and lifetime major depressive disorder (MDD, OR = 1.40, p-value = 9.72×10^{-18}). However, vitamin D supplement intake was also significantly positively associated with both measures of depression (Table 3), suggesting that depressed individuals take more vitamin supplements than non-depressed individuals.

Table 3. Results of association of depression outcomes with dietary sources of metabolites in the UK Biobank.

	Current depressive symptoms				Major Depressive Disorder (MDD)						
	N	Beta	SE	p-value	N	Beta	SE	p-value	OR	Lower 95% CI	Upper 95% CI
Vitamin A supplements	304399	0.23	0.02	1.25E-25	189800	0.34	0.04	9.72E-18	1.40	1.30	1.52
Vitamin D supplement	313100	0.19	0.02	5.07E-32	194681	0.34	0.03	1.22E-34	1.41	1.33	1.48
Retinol intake from food	61363	2.29E-04	4.02E-05	1.26E-08	38758	1.76E-04	5.50E-05	1.37E-03	1.00	1.00	1.00
Fresh fruits intake	434770	-0.06	0.002	1.61E-205	264796	-0.04	0.004	3.27E-22	0.96	0.96	0.97
Vitamin K antagonists use	435867	0.43	0.03	1.04E-46	265648	0.14	0.06	0.016	1.15	1.03	1.28

Since both vitamin A and vitamin D are fat-soluble and can cross the blood-brain barrier, we performed additional association with the measure of brain pathology, i.e., white matter hyperintensity (WMH) volume. Only vitamin A supplement intake was found to be associated with higher volume of WMH (beta = 479.09, p-value = 0.04, Supplementary Table 6), suggesting a possible role of vitamin A in brain diseases. To address the issue of reverse causality, we additionally tested the association of depression with retinol intake estimated from the food consumed in the previous 24 hours. Significant positive association of estimated retinol intake was observed with both measures of depression (current depressive symptoms, p-value = 1.26×10^{-08}; lifetime MDD, p-value = 1.4×10^{-03}). However, the effect estimates were small (Table 3), which may in part be explained by the imprecision of food consumption questionnaires. Fresh fruit intake, a major source of hippurate, was negatively associated with both measures of depression (current depressive symptoms, beta = -0.06, p-value = 1.61×10^{-205}; lifetime MDD, OR = 0.96, p-value = 3.27×10^{-22}) and vitamin K antagonists, a proxy for 4-hydroxycoumarin, was positively associated with both measures of depression (current depressive symptoms, beta = 0.43, p-value = 1.04×10^{-46}; lifetime MDD, OR = 1.15, p-value = 0.016) (Table 3).

Mendelian randomization analysis

Testing the hypothesis that major depression results in changes of circulating metabolites in the Mendelian randomization analysis (MR), nominally significant results were obtained for 2-aminooctanoate and 10-undecenoate (11:1n1), under the MR-Egger method and weighted median method, respectively. However, these findings did not remain significant after adjustment for multiple testing (Supplementary Table 7). MR models in which we tested the hypothesis that levels of circulating metabolites increase the risk of depression provided significant evidence for a causal relation between hippurate and the risk of depression, both in the MR-Egger robust and penalized-robust methods (Supplementary Table 8). The effect estimate was consistent with the inverse relationship observed between hippurate and major depression in this study. However, a significant intercept was also observed suggesting pleiotropy. To exclude a pleiotropic effect, we studied the effect of intervention on the metabolite in the PReDICT trial.

Effect of antidepressant therapy on hippurate

To further evaluate the impact of antidepressant therapy including cognitive behavioral therapy (CBT), duloxetine – a serotonin-norepinephrine reuptake inhibitor (SNRI) and escitalopram – a selective serotonin reuptake inhibitor (SSRI) on hippurate we consulted the PReDICT study. The PReDICT study allows us to test the effect of antidepressant therapy on the metabolite levels in circulation by measuring the metabolite levels before and after the antidepressant therapy. In PReDICT, we found that levels of hippurate in the circulation increased significantly

from baseline to week 12 only after treatment with escitalopram (estimated week 12 vs. baseline difference = 0.45, 95% confidence interval (CI; 0.16,0.74), p-value = 0.002; Supplementary Figure 1), but not in the cognitive behaviour therapy (CBT) and duloxetine treatment arms (CBT: estimated difference = -0.02, 95% CI (-0.39,0.33) and p-value = 0.87; duloxetine: estimated difference = 0.13, 95% CI (-0.17,0.44) and p-value = 0.38). In this study, we could not show a relation between hippurate and depression as the study recruited patients only and lacked healthy controls. In patients receiving pharmacotherapy (escitalopram and duloxetine arms), the association of baseline depression as measured by the 17-item Hamilton Rating Scale for Depression (HRSD17) and baseline hippurate was not statistically significant (beta = 0.04, 95% CI (-0.03,0.11), p-value = 0.27). Further, no significant association was observed between depression in week 12 as measured by the HRSD17 and week 12 hippurate (beta = 0.09, p-value = 0.45) and 12 weeks change in HRSD17 and 12 weeks change in hippurate (beta = 0.02, 95% CI (-0.65, 1.57), p-value = 0.85).

Linking the human circulating metabolome to gut microbiome metabolism

Of the 53 metabolites identified in this study in model 1, 28 metabolites could be matched to a unique VMH metabolite ID. For each metabolite, the presence or absence in the global human reconstruction, Recon3D [42], and a resource of 7,206 reconstructions of human gut microbes, AGORA2 (https://www.biorxiv.org/content/10.1101/2020.11.09.375451v1) was retrieved. In total, 12 metabolites were present in both the human and gut microbial metabolic networks, three were only present in gut microbes, and 13 were only present in human (Supplementary Table 9). To further investigate potential links between the microbiome and metabolites associated with depression, the potential of the 7,206 AGORA2 strains to consume or secrete the 15 microbial metabolites identified in this study was computed. Since hippurate is synthesized in the liver and renal cortex from the microbial metabolite benzoate [43], the uptake and secretion potential for benzoate was also predicted for the 7,206 strains.

A wide range of genera and species were involved in the uptake of mannitol (Supplementary Table 10). Mannitol is largely secreted by several species of the genus Bacteroides followed by Lactobacillus, among others (Supplementary Table 11). Both genera have previously been found to be associated with depression [17]. In total, 3,616 AGORA2 strains mainly of the Gammaproteobacteria and Bacilli classes (Supplementary Table 11) synthesized benzoate as a product of benzamide (VMH reaction ID: BZAMAH). Interestingly, benzamides are a class of antipsychotic medication.

DISCUSSION

In this study, we identified 53 metabolites significantly associated with depression, most of which, including those in the monoamine and neurotransmitter pathways (serotonin, kynurenate and glutamate), were explained by antidepressant use. We identified novel associations with depression for six metabolites, including retinol (vitamin A), 4-hydroxycoumarin, 2-aminooctanoate, 10-undecenoate (11:1n1), 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1), 1-linoleoyl-GPA (18:2) and confirmed the association of hippurate and mannitol/sorbitol. We found that the relation of hippurate and depression may be causal and that hippurate levels can be modified by a specific antidepressant, escitalopram. Analysing the major dietary sources of these metabolites in the UKB study, we found that retinol (vitamin A) intake was significantly higher and fresh fruits intake, a major source of hippurate, significantly lower in depressed individuals compared to those who were not depressed.

One of the most interesting findings of this study is the identification of the association of higher levels of retinol (active form of vitamin A) with depression. There have been several case reports of individuals with vitamin A intoxication with no previous history of depression, who developed symptoms of depression and even psychosis when overdosed with vitamin A [44, 45]. Depressive symptoms resolved upon discontinuation of vitamin A, implying that depression may be a side effect of vitamin A intake [44]. Animal models have suggested elevated monoamine oxidase enzyme activity and depression-related behavior upon vitamin A supplementation [46, 47]. Our study is the first to link higher levels of retinol in blood with depression in the general population. Retinol and its derivatives known as retinoids are lipid soluble and can cross the blood-brain barrier. Vitamin A is required for brain development and functioning [48, 49]. However, excess of vitamin A is neurotoxic and may result in brain shrinkage [49]. Brain areas high in retinoic acid signaling and receptors overlap with areas of relevance to stress and depression [50]. Further, vitamin A is known to increase the synthesis of triglyceride-rich very low-density lipoproteins (VLDLs) and apolipoproteins in the serum [51, 52], which we found associated with depression in our previous study [53]. Since food it the primary source of vitamin A, an important question to answer is whether vitamin A intake is associated to depression. In the UK Biobank we found significant increase in dietary retinol intake in individuals with depression. Thus, our findings ask for intervention studies that evaluate prospectively the effect of vitamin A reduction in depressed patients.

Two of the most strongly associated metabolites with depression were xenobiotics, hippurate and 4-hydroxycoumarin. In line with the findings of our study, decreased levels of hippurate have been previously reported in urine and plasma

of individuals with unipolar and bipolar depression consistently in several studies and it has been suggested as a biomarker for depressive disorders [54]. Our MR analysis suggests that low hippurate levels in circulation are a part of the causal pathway leading to depression. However, as the MR could not exclude a pleiotropic effect, our findings yield a hypothesis that requires further evaluation in a clinical trial. While we could not show an association between hippurate and depression in the PReDICT study, as the study lacked controls, hippurate levels were higher 12 weeks after initiation of selective serotonin reuptake inhibitor (SSRI) therapy (escitalopram) but not for SNRI or CBT, raising the question whether blood levels of hippurate can be used in clinical trials for compliance and efficiency of SSRIs specifically. Hippurate is derived from benzoate and polyphenols and is reported to be a metabolomics marker of gut microbiome diversity [53]. A diet rich in whole grains and fruits has been reported to increase levels of Hippurate [53]. In line with the decreased levels of hippurate in depressed individuals found in our metabolome-wide association analysis, we found significantly decreased fresh fruit intake among individuals with depression in the UKB, which is in line with the previous study that high consumption of fruits, vegetables, nuts, and legumes is associated with a reduced risk of depression [7, 55].

The metabolite 4-hydroxycoumarin is a fungal derivative of coumarin. Coumarins are found naturally in plants and spices [55] and coumarin is converted into 4-hydroxycoumarin by fungi [56]. 4-hydroxycoumarin is then converted into dicoumarol in the presence of formaldehyde [56]. Dicoumarol is an anticoagulant (warfarin) that inhibits the synthesis of vitamin K, also called vitamin K antagonist, and is commonly used to treat thromboembolic diseases [57]. In the UKB, we found significant positive association of anticoagulant use (vitamin K antagonists) with major depression. A history of depression is a risk factor for thromboembolism [58-60]. Antidepressants are also known to interact with warfarin [61] and are also associated with increased risk of thromboembolism [62]. Taking all findings together, we hypothesize that depression/antidepressant use depletes 4-hydroxycoumarin in circulation leading to thromboembolism. Vitamin K has been shown to act in the nervous system as it is involved in sphingolipid synthesis [63]. Sphingolipids are present in high concentrations in cell membranes of neuronal and glial cells [64]. Sphingolipids are essential for important cellular events, including proliferation, differentiation, senescence, cell-cell interactions, and transformation [65] and they have been linked to aging, Alzheimer's disease, and Parkinson's disease [66-68]. Further, sphingolipids were found to play a crucial role in the development of depression- and anxiety-related behaviours in mice [69, 70] and depression is seen often in patients with sphingolipid storage diseases [71-75]. Treatment with escitalopram /citalopram is also associated with changes in sphingolipids [76]. In our study, we did not find an association of depression with circulating sphingolipids present on the Metabolon platform. However, we cannot

exclude that 4-hydroxycoumarin in the blood affects sphingolipid metabolism in the brain specifically.

Other metabolites that were found to be significant in our study include mannitol/sorbitol, of which increased levels were associated with depression. Higher levels of sorbitol in plasma and urine have previously been consistently reported in patients with unipolar and bipolar depression and, like hippurate, it has been suggested as a diagnostic biomarker of depression [23]. Mannitol/sorbitol are sugar alcohols found in food such as fruits and berries and often used in diet/sugar free foods as sweeteners [77]. Fructose reduced diets have been shown to improve gastrointestinal disorders, depression and mood disorders [78]. Our AGORA2 analysis suggests that mannitol is mainly secreted by several species of Bacteroides, Lactobacillus, Fructobacillus, Alistipes and Bifidobacterium. Interestingly, all genera, except for Fructobacillus have previously been associated with depression [17], asking for further studies on the role of the microbiome, circulating levels of mannitol and depression.

Finally, there were four lipids identified in our study (2-aminooctanoate, 10-undecenoate (11:1n1), 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1) and 1-linoleoyl-GPA (18:2)) significantly associated with depression. 1-Palmitoyl-2-palmitoleoyl-GPC (16:0/16:1) also known as phosphatidylcholine (16:0/16:1) or lecithin (HMDB0007969) is commonly found in foods like eggs, soyabean, liver, nuts and seeds and is a precursor of choline. Lecithin is believed to cause depression by increasing the production of acetylcholine in the brain [79]. When fed to animals and humans, lecithin significantly increases the levels of choline in blood and brain and of acetylcholine in brain [80-82]. Our study is the first to show higher circulating levels of lecithin in the depressed individuals from the general population. The other three lipids 2-aminooctanoate, 10-undecenoate (11:1n1) and 1-linoleoyl-GPA (18:2) were negatively associated with depression. 2-Aminooctanoate (alpha-aminocaprylic acid) and 10-undecenoate (11:1n1) (undecylenic acid) are neutral hydrophobic molecules for which there is not much known in the literature. Lower levels of 10-undecenoate (11:1n1) have been found in individuals with non-alcoholic fatty liver disease [83]. 1-linoleoyl-GPA (18:2) is a lysophosphatidic acid (LPA 18:2). LPA is a bioactive membrane lipid that acts on at least six distinct G protein-coupled receptors (LPA1–6) and plays a role in pain sensitivity and emotional regulation [84]. LPA knock out mice exhibit anxiety-related behaviour [84, 85].

We found that decreased plasma levels of serotonin, kynurenate, leucine and citrulline and higher levels of glutamate were associated with depression. Lower plasma/serum levels of serotonin, kynurenate, citrulline and leucine and higher levels of glutamate have been reported in relationship to depression in earlier

studies [40, 41, 86, 87], which also appears consistent with our findings of model 1. However, we and others have shown that antidepressants affect plasma/serum levels of serotonin, glutamate, leucine and kynurenine [87-91]. An important finding of our study is that only citrulline remained significantly associated with depression after adjusting for antidepressant medication use. Lower levels of citrulline and its precursor arginine were previously associated to depression in unmedicated individuals [41, 92]. Interestingly, treatment with SSRIs significantly increase the levels of plasma citrulline [93]. Further, levels of plasma citrulline were found to be significantly increased two hours post treatment with ketamine, suggesting a possible mechanism of action of the rapid acting drug [92]. Citrulline is an intermediate in the urea cycle and linked to nitric-oxide synthesis [93]. It is absorbed by the gut and has useful therapeutic effects against cardiovascular diseases [94]. In our study the association of citrulline with depression lost its significance, albeit not completely, after adjusting for cardiovascular medication use and BMI.

Our study is the first large-scale effort combining metabolites measured on assorted, untargeted metabolomics platforms (Metabolon) studied in relationship to depression. In addition to confirming several previously identified metabolites in smaller studies, we successfully identified novel metabolites that are associated with depression. Our findings are robust across different versions of the Metabolon platform or the criteria assessing presence of clinical or subclinical depression. A possible limitation of our study is that differences in metabolomics platforms and technologies that were used by different cohorts to assess depression may have resulted in a reduction of statistical power. Older versions of the Metabolon platform reported significantly fewer known metabolites compared to the more recent implementations. Another possible limitation of our study is the presence of residual confounding. After adjusting for medication use and the lifestyle factors smoking and BMI, confounding may still be present and may influence the results [95]. Also, our MR analysis was most likely underpowered lacking strong instrumental variables for both depression and the associated metabolites.

Analysing circulating levels of 806 metabolites from untargeted metabolomics platforms in 13,596 individuals, we identified six new associations of metabolites with depression including retinol (vitamin A), 4-hydroxycoumarin and four lipids, 2-aminooctanoate, 10-undecenoate (11:1n1), 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1) and 1-linoleoyl-GPA (18:2), while confirming known associations of hippurate and mannitol/sorbitol. We further show that previously identified associations of depression with metabolites belonging to the amino-acid pathways including serotonin, kynurenate, leucine and glutamate are likely explained by antidepressant medication. Our findings point to effective preventive targets, as most of these metabolites are food derived and thus can be altered in patients by modifying diet.

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SUPPLEMENTARY MATERIAL

Full version of supplementary materials can be found through the following link:
https://assets.researchsquare.com/files/rs-1815755/v1/b5558213de1f6214d9a49648.docx
https://assets.researchsquare.com/files/rs-1815755/v1/69dea74aa784af1fbd638dbb.docx
https://assets.researchsquare.com/files/rs-1815755/v1/8614958ffbce5f372aba9ce7.pdf

5

Metabolomics dissection of depression heterogeneity and related cardiometabolic risk

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ABSTRACT

Background

A recent hypothesis postulates the existence of an "immune-metabolic depression" (IMD) dimension characterized by metabolic dysregulations. Combining data on metabolomics and depressive symptoms, we aimed to identify depressions associated with an increased risk of adverse metabolic alterations.

Method

Clustering data were from 1094 individuals with major depressive disorder in the last 6 months and measures of 149 metabolites from a ^{1}H-NMR platform and 30 depressive symptoms (IDS-SR30). Canonical correlation analyses (CCA) were used to identify main independent metabolite-symptom axes of variance. Then, for the replication, we examined the association of the identified dimensions with metabolites from the same platform and cardiometabolic diseases in an independent population-based cohort (n=6572).

Results

CCA identified an overall depression dimension and a dimension resembling IMD, in which symptoms such as sleeping too much, increased appetite, and low energy level had higher relative loading. In the independent sample, the overall depression dimension was associated with lower cardiometabolic risk, such as (i.e., per SD) HOMA-1B -0.06 (95% CI:-0.09;-0.04), and visceral adipose tissue -0.10 cm^2 (95% CI:-0.14;-0.07). In contrast, the IMD dimension was associated with well-known cardiometabolic diseases such as higher visceral adipose tissue 0.08 cm^2 (95% CI:0.04;0.12), HOMA-1B 0.06 (95% CI:0.04;0.09), and lower HDL-cholesterol levels -0.03 mmol/L (95% CI:-0.05;-0.01).

Conclusions

Combining metabolomics and clinical symptoms we identified a replicable depression dimension associated with adverse metabolic alterations, in line with the IMD hypothesis. Patients with IMD may be at higher cardiometabolic risk and may benefit from specific treatment targeting underlying metabolic dysregulations.

INTRODUCTION

Cardiovascular disease (CVD) together with major depressive disorder (MDD) are leading causes of mortality and disease burden worldwide [1, 2]. Each of these conditions may predispose for the other, and the presence of one condition worsens the prognosis of the other [3]. Although the mechanism of this comorbidity is still not fully understood, adverse metabolic alterations may serve as the element that connects the two conditions [1, 3, 4]. A recent large scale epidemiological study in >15,000 individuals analyzing the association between depression and more than 200 lipid related metabolites [5] found that depression is associated with a metabolic signature that is also found in CVD patients [6]. This metabolic signature was characterized by a shift in the lipids levels encompassing less HDL-cholesterol and more very low density lipoproteins (VLDL) and triglycerides, in line with a higher metabolic syndrome profile in depression [5]. This metabolic signature may represent a substrate linking depression to cardiometabolic diseases. Another large population-based study in >350,000 individuals [4] concluded that the risk factors of CVD (i.e., inflammatory markers (CRP, IL-6) and biomarker (triglycerides)) are likely causal for the development of depression.

MDD is a highly heterogeneous disorder: patients with the same MDD diagnoses according to DSM-V (Diagnostic and Statistical Manual of Mental Disorders) [7] may experience very different symptom profiles [8]. These different clinical expressions may be, in turn, differentially related to underlying biological dysregulations. Recent evidence suggests that adverse metabolic alterations and inflammatory dysregulation map more consistently onto "atypical, energy-related depressive symptoms", such as excessive sleepiness, hyperphagia, weight gain, and fatigue [9]. This set of symptoms is partially shared with other constructs, such as sickness behavior [10] and nosological categories, such as atypical depression, seasonal affective disorder, and bipolar disorder [7]. The clustering of atypical, energy-related depressive symptoms with inflammatory and metabolic alterations indexes an underlying quantitative dimension, labelled "immuno-metabolic depression" (IMD), with transdiagnostic value and potentially present in psychiatric (depression, bipolar or psychotic disorders) and somatic (obesity, diabetes, cardiovascular) disorders characterized by overlapping symptomatology or biological dysregulations [9]. Nonetheless, further empirical evidence is needed to fully characterize the clustering between specific symptom profiles and immuno-metabolic biological dysregulations. The identification of depression dimensions characterized by this clustering of clinical and biological features could give us a better understanding of the shared biological mechanisms between depression and cardiometabolic diseases and potential opening for interventions aimed at avoiding their reciprocal influence [11-13]. Furthermore, the identification of individuals with this specific form of depression may create

awareness amongst healthcare providers and the need to perform more rigorous cardiometabolic health checks and interventions.

The main aim of the present study was to identify depression dimensions associated with increased risk of adverse metabolic profile by combining data on metabolomics and depressive symptoms. First, we applied a data-driven method to identify patterns of correlations between depressive symptoms and metabolites from a lipid-focused metabolomic platform in >1,000 MDD patients. Previous studies aimed at parsing depression heterogeneity through data-driven methods followed two conceptually distinct approach (Supplemental figure 1 adapted from [14]). In one approach (top-down), studies [15, 16] performed symptom-based clustering as a first step and subsequently evaluated the clustering results via association with biomarker levels. In the opposite approach (bottom-up), studies [17, 18] performed biomarker-based clustering as a first step and subsequently evaluated the clustering results via association with clinical features. The novelty of the present study is that we merged the two approaches and performed clustering based on both symptoms and biomarkers, leveraging their co-variance structure. Then, for the replication, we examined the association between the identified dimensions and 51 metabolites from the same panel, and clinical cardiometabolic diseases such as levels of fasting glucose, insulin resistance, total and abdominal adiposity in an independent population-based cohort (n=6572).

METHOD

Study design

The current analysis consists of two parts: the metabolite-symptom clustering and the replication (**Figure 1**). In the first part, we used a data-driven approach to dissect the heterogeneity of depression and to identify main independent metabolite-symptom dimension of variance in 1094 individuals with depression in the last 6 months from the Netherlands Study of Depression and Anxiety cohort (NESDA). Then, in the replication, we examined the association between the dimensions identified and the cardiometabolic metabolites (51 lipids, fatty acids, and low-molecular-weight metabolites) and diseases in an independent dataset of 6572 participants from the general population enrolled in the Netherlands Epidemiology of Obesity (NEO) study. The research protocol of NESDA was approved by the medical ethical committees of the following participating universities: Leiden University Medical Center (LUMC), Vrije University Medical Center (VUMC), and University Medical Center Groningen (UMCG). The NEO study was approved by medical ethics committee of Leiden University Medical Center (LUMC). All participants gave written informed consent.

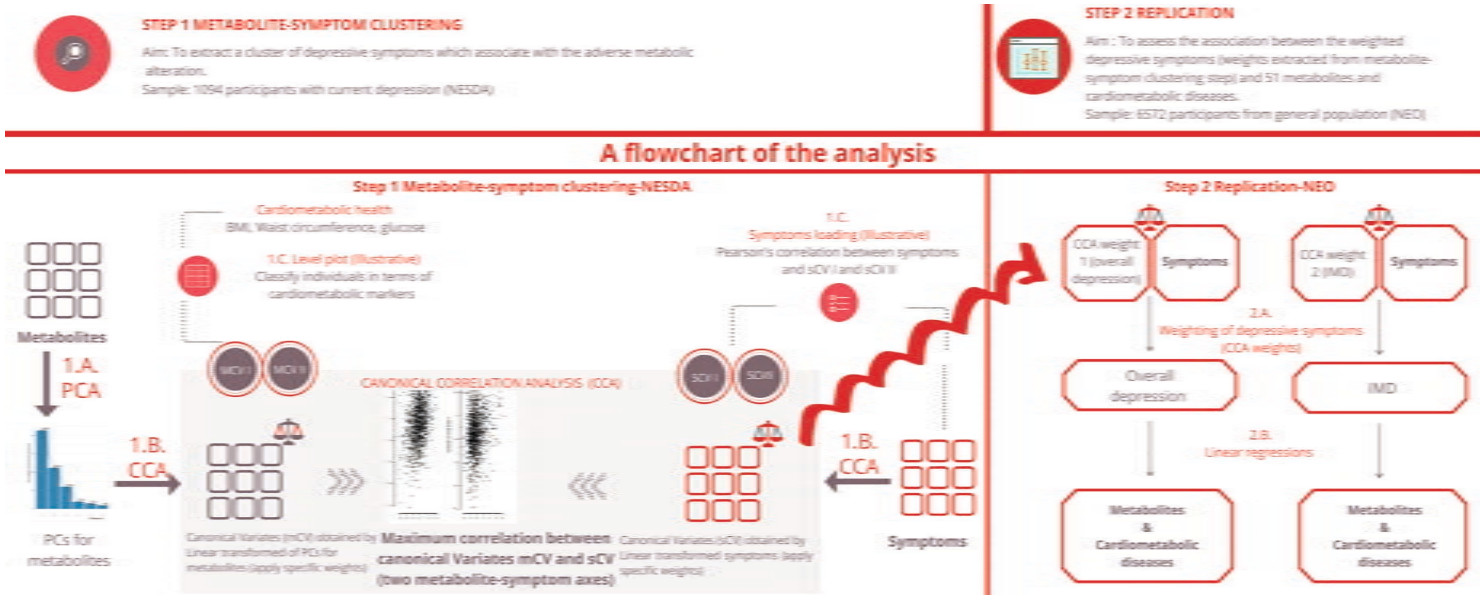

Figure 1. An illustration of the method

Part 1: Metabolite-symptom clustering

We performed this analysis on 1094 participants diagnosed with MDD in the last 6 months via the structured Composite Interview Diagnostic Instrument (CIDI, version 2.1) [19] from NESDA [20]. After an overnight fast, EDTA plasma was collected and stored in aliquots at -80°C until further analysis by ^{1}H-NMR (Nightingale Health Ltd, Helsinki, Finland) [21] metabolomics platform. This metabolomics platform consists of 230 metabolites or metabolite ratios and can be classified into 3 clusters [22] as follow: 1) lipids, fatty acids, and low-molecular-weight metabolites (n= 51); 2) lipid composition and particle concentration measures of lipoprotein subclasses (n= 98); and 3) metabolite ratios (n= 81). In this analysis, we focused on the first two classes (n=149). Metabolite ratios were not used due to redundancy. We processed the metabolomic data based on the protocol described in Appendix 1 that was suggested by the manufacturer of the platform and has been consistently applied in several large-scale epidemiological studies [5, 23]. Blood samples were analyzed in two batches (April 2014 and December 2014) by ^{1}H-NMR (Nightingale Health Ltd, Helsinki, Finland) [21]. We regressed the metabolites on age and batch effect in order to remove their confounding effect.

During the baseline assessment, the presence of major depressive disorder was determined with the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)-based Composite Interview Diagnostic Instrument (CIDI, version 2.1, World Health Organization, 1997) by specially trained research staff. Additionally, participants were asked to complete the Inventory of Depressive Symptomatology (IDS-SR30), which assesses (via a 4-level response system) the presence of 30 depressive symptoms during the last week and their severity [24]. Additional measures of body mass index (BMI), waist circumference and fasting glucose level are described in details in Appendix 2.

Statistical analysis for metabolite-symptom clustering

Our goal was to identify independent dimensions emerging from patterns of correlations between depressive symptoms and metabolites. For that, we used canonical correlation analysis (CCA, [25]).

1.A. Principal component analysis (PCA)

Metabolites are correlated to each other; to avoid overfitting and unstable results of CCA, data reduction [26] of metabolomics was performed applying PCA to age- and batch-adjusted metabolites residuals. PCA is described in more details in Appendix 3. We selected principal components explaining the highest proportion of variance (components that explained more than 10% of variance) in metabolites. Therefore, the next analysis was performed on principal components explaining the highest proportion of metabolites variance and 30 depressive symptoms.

1.B. Canonical correlation analysis (CCA)

CCA [25] is a method that given two sets of variables X and Y (in this case, metabolites and depressive symptoms), find a linear combination of X that is maximally correlated with a linear combination of Y (i.e., a weighted sum of each variable). Detailed definition and description of CCA method explained in Appendix 4. In our analysis we chose to proceed with the first two canonical pairs that provided more information about the two sets of variables. The relationship between the created canonical variables of depressive symptoms and metabolites from the same panel and cardiometabolic diseases was validated in an independent sample (see replication section).

1.C. Illustrative analyses

In order to better explain the results of CCA and the meaning of its output we proposed two additional analyses (point 1.C. In Figure 1). To explore how the first two metabolic canonical variates (mCVI and mCVII) classify individuals in terms of cardiometabolic diseases (i.e., BMI, waist circumference, and fasting glucose) we plotted the predicted level of the cardiometabolic diseases as a function of the two metabolic canonical variates (i.e., smoothing function was used for the prediction). Furthermore, to evaluate the symptoms contribution to the two canonical correlation, for each symptom we calculated the symptoms loadings, expressed in Pearson's correlation coefficient, with the first two symptoms canonical variates (sCVI and sCVII).

Part 2: Replication

To replicate the results of previous step, we investigated the association between the dimensions identified in the previous step via CCA and metabolomics and cardiometabolic diseases in the Netherlands Epidemiology of Obesity (NEO) study [27]. The depressive symptoms in NEO study were assessed by IDS-SR30 [24], the same instrument used in the NESDA study. For the purpose of replication, we included only the first class from the ^{1}H-NMR platform (i.e., 51 lipids, fatty acids, and low-molecular-weight metabolites) in the main results. For completeness of data, we showed the result of the entire metabolomic platform in the supplementary results since they have large overlap with the standard clinical lipid profile. We used the same protocol for processing this metabolomic data in the clustering step. The cardiometabolic diseases are described in detail elsewhere [27]. From fasting glucose and insulin concentrations, we calculated the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) and HOMA of beta-cell function (HOMA-1B) as markers of hepatic insulin resistance and steady-state insulin secretion [28]. HOMA-IR was calculated as fasting insulin (μU/mL) x fasting glucose (mmol/L)/22.5 and HOMA-1B% as 20 x fasting glucose (mmol/l)-3.5 [28, 29].

Statistical analysis for replication

2.A. Weighting of depressive symptoms
To index the two dimensions identified in the clustering step, we created two weighted depressive symptom scores. We weighted each individual item of the IDS-SR30 based on extracted CCA weights from the previous step. Then, we summed the weighted depressive symptoms to create two weighted IDS scores. We standardized weighted IDS scores to a mean of zero and a standard deviation of one to allow comparison across the scores.

2.B. Linear regressions
We used linear regression to examine the relationship between the two weighted IDS scores as the independent variable and 51 ^{1}H-NMR metabolites and cardiometabolic diseases (BMI, total body fat, waist circumference, visceral adipose tissue, HbA1c, fasting glucose, HOMA-IR, HOMA-1B, total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides) as dependent variables. We fitted four linear regression models, the crude model, model 1, model 2 and, model 3. Model 1 was adjusted for age, sex, and educational level. Model 2 was adjusted for age, sex, educational level, smoking, alcohol consumption, physical activity, and ethnicity. Model 3 was model 2 with additional adjustment for lipid-lowering drugs, and antidepressants. The false discovery rate (FDR) method was applied to correct for the multiple testing. As the NEO study is a population-based study with oversampling of individuals with a BMI > 27 kg/m^2, all results are based on BMI-weighted analysis. The weighting factor is based on BMI distribution in the general Dutch population to make our results generalizable to the Dutch population.

RESULTS

Part 1:Metabolite-Symptom clustering

Table 1 shows the main demographic, health- and depression-related characteristic, in the NESDA sample of individuals with MDD in the last 6 months.

1.A. Principal component analysis
Data reduction of metabolomics was performed using PCA, identifying three principal components that explained more than 10% of the variance in metabolites (together explained 75% of the variance) (Scree plot in Supplemental figure 2).

1.B. Canonical correlation analysis
The resulting 3 principal components were used in the CCA analysis and were correlated to the 30 depressive symptoms, to identify the main independent metabolite-symptom dimensions of variance based on their correlation. The correlation between the linear transformation (weights) of metabolites principal

components (metabolic canonical variate I, mCVI) and depressive symptoms (symptom canonical variate I, sCVI) was 0.30 explaining 54 % of the metabolite-symptom covariance, for the second pair of canonical variates the correlation between mCVII and sCVII was 0.24 explaining 33% of the metabolite-symptom covariance (Supplemental figure 3).

Table 1. Characteristics of the study population for the metabolite-symptom clustering step (NESDA)

	Metabolite-symptom clustering (NESDA n=1094)
N	1094
Women, n (%)	741 (67.73)
Age (years) (mean, sd)	40.88 (12.11)
High educational level (high) n (%)	306 (27.97)
Use of lipid-modifying medications, yes n (%)	78 (7.13)
BMI (kg/m^2) (mean, sd)	25.90 (5.51)
Waist circumference (cm)	89.58 (14.56)
Glucose (mmol/L)	5.20 (1.15)
Use of antidepressant Yes n (%)	477 (43.60)
Total IDS-score (0-84) median (25th ,75th percentiles)	32.50 (24.0,41.0)

Normally distributed data shown as mean and standard deviation (SD), skewed distributed data shown as median (25th, 75th percentiles), and categorical data are shown as percentage. High education level: university or college education, while other education level: none, primary school, or lower vocational education. IDS-SR30: Inventory of Depressive Symptomatology (self-report). BMI: body mass index. NESDA: Netherlands study for depression and anxiety.

1.C. Illustrative analyses

To explore how the first two metabolic canonical variates (mCVI and mCVII) classify individuals in terms of cardiometabolic diseases (i.e., measures of BMI, waist circumference, and fasting glucose) we plotted the predicted level of the diseases as a function of the two metabolic canonical variates. Level plots depicted in Figure 2 show that high values in BMI, waist circumference, and fasting glucose tended to cluster at high level of mCVII and low levels for mCVI. Figure 3 shows the loading, expressed as Pearson's correlation coefficient, of IDS-SR item on the two symptoms canonical variates (sCVI and sCVII). In the first variate, correlation coefficients were substantially consistent across the entire spectrum of items, including mood, cognitive and somatic symptoms. In the second variate, the loading of specific items such as difficulty falling asleep, sleeping too much, increase weight and appetite, low energy level and gastrointestinal problems were relatively higher as compared to the other symptoms.

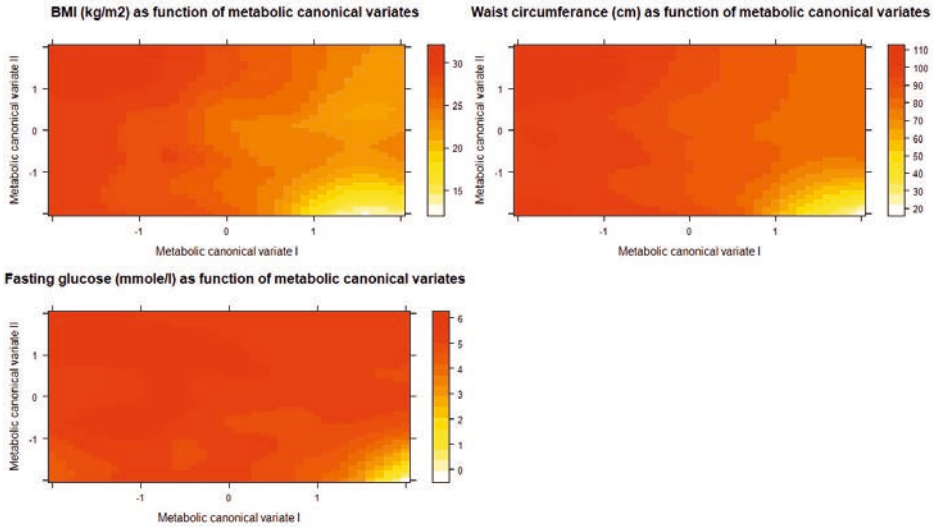

Figure 2. Level plot of the predicted cardiometabolic health conventional biomarker as functions of the first and second metabolic canonical variates.

We interpreted the first canonical variate CVI, explaining a larger proportion of symptom-metabolite covariance (54%), as an overall depression dimension characterized by a wide array of symptoms (sCVI, Figure 3) and lower levels of cardiometabolic diseases (mCVI, Figure 2). The second variate, explaining 33% of the symptom-metabolite covariance, partially resembled the postulated IMD construct [9], with relevance for energy-related behavioral symptoms and higher cardiometabolic diseases. Thus, for interpretability we labelled the two canonical variates, respectively, "overall depression" and "IMD".

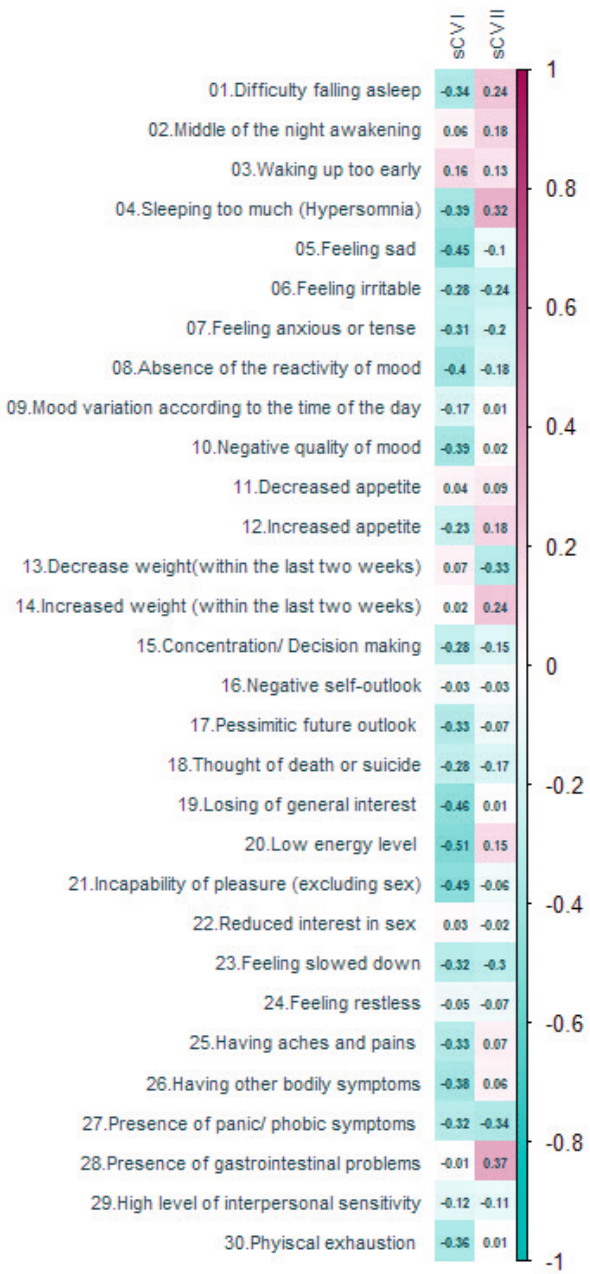

Figure 3. Canonical loading of depressive symptoms on the symptoms canonical variates sCV I: First symptoms canonical variates I. sCV II: Second symptoms canonical variates.

Part 2: Replication

The baseline characteristics for all 6572 participants of the NEO cohort included in the replication step are shown in Supplemental table 1. The mean age in the NEO population was 55.7 years (standard deviation (SD)): 6 years, and the median of the IDS-SR30 questionnaire was 8.0 points (4, 13).

2.A. Weighting of depressive symptoms

We created two weighted depressive symptoms scores labelled "overall depression" and "IMD" with the weights derived in CCA for, respectively, the first and second canonical variate.

2.B. Linear regression

We examined the association of these weighted scores with 51 metabolites and cardiometabolic diseases (levels of BMI, total body fat, waist circumference, visceral adipose tissue, HbA1c, fasting glucose, HOMA-IR, HOMA-1B, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides). Figures 4A and 4B depict the linear regression effect estimates and 95% confidence intervals for the association between the weighted symptom sum score and the 51 lipids, fatty acids, and low-molecular-weight metabolites, and cardiometabolic diseases adjusted for age, sex, and educational level (model 1). The results of all crude and adjusted models can be found in Supplemental table 2 and 3. In general the two weighted symptoms scores showed divergent pattern of results: IMD showed metabolic alterations linked to increased cardiometabolic risk, while overall depression score showed opposite associations. IMD was associated with (per standard deviation (SD)) higher glycoprotein acetylase 0.08 mmol/L (95% CI: 0.06;0.11), apolipoprotein B 0.06 g/L (95% CI:0.03;0.08), triglyceride levels 0.09 mmol/L (95% CI: 0.06;0.11), total body fat 0.06% (95% CI:0.05;0.08), visceral adipose tissue 0.08 cm^2 (95% CI:0.04;0.12), HOMA-1B 0.06 (95% CI: 0.04;0.09), and lower HDL-cholesterol levels -0.03 mmol/L (95% CI: -0.05;-0.01). In contrast, the overall depression was associated with (per SD) glycoprotein acetylase -0.11 mmol/L (95% CI: -0.14;-0.09), apolipoprotein B -0.04 g/L (95% CI: -0.06;-0.01), triglyceride levels -0.08 mmol/L (95% CI: -0.11;-0.06), total body fat -0.07% (95% CI:-0.09;-0.06), visceral adipose tissue -0.10 cm^2 (95% CI:-0.14;-0.07), HOMA-1B -0.06 (95% -0.09;-0.04), and HDL-cholesterol levels 0.07 mmol/L (95% CI: 0.05;0.09) (Figure 4A, 4B). We repeated the analysis of the linear regression with additional adjustment for lipid-lowering drugs (model 3) and results did not notably change (Supplement table 2,3).

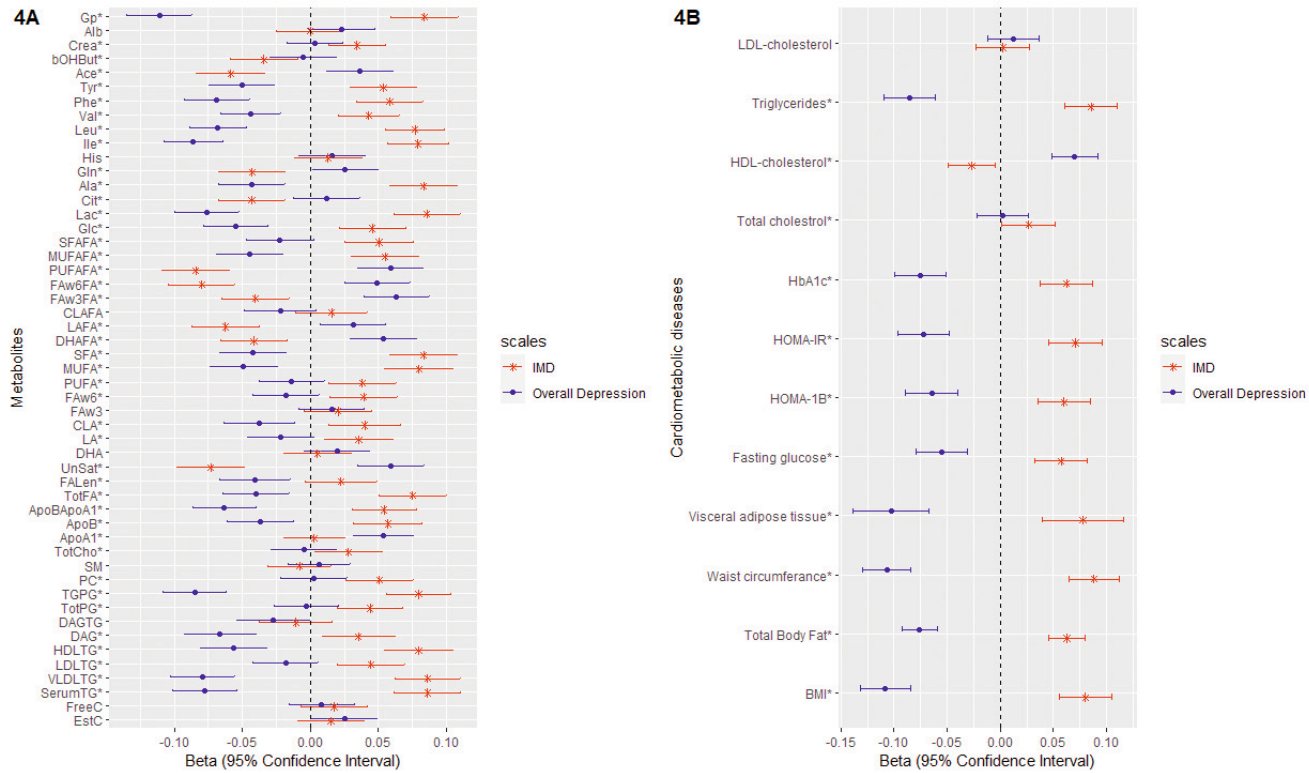

Figure 4. The linear regression analysis of the association between the weighted depressive symptoms score and the cardiometabolic endpoints and metabolites in individuals from NEO study.
The weights extracted from the metabolite-symptom clustering step. Number of individuals with data for BMI: 6572, Total body fat: 6541, Waist circumference: 6566, Visceral adipose tissue: 1869, Fasting glucose: 6554, HOMA-1B: 6541, HOMA-IR: 6545, HbA1c: 6543, Total cholesterol: 6562, HDL-cholesterol: 6561, Triglycerides: 6561, LDL-cholesterol: 6560.

DISCUSSION

Using a data-driven method, we combined metabolomics and clinical symptoms data to dissect depression heterogeneity and identify independent underlying dimensions in participants diagnosed with MDD in the last 6 months from NESDA cohort (n=1094). Then, we replicated our results by examining the association between the identified dimensions and 51 metabolites from the same lipidomic panel, and cardiometabolic diseases in an independent dataset of 6572 participants from the general population enrolled in the NEO study.

We used the NESDA sample including subjects with a recent MDD diagnosis to obtain a sharper picture, leveraging the higher intensity of depressive symptoms of clinical relevance, of the covariance between symptoms and metabolites commonly associated with cardiometabolic risk. We identified a major dimension reflecting overall depression explaining a large proportion (54%) of symptom-metabolite covariance, and innovatively characterized by a wide array of symptoms and reduced levels of cardiometabolic diseases. A second dimension explaining 33% of symptom-metabolite covariance emerged as characterized by higher cardiometabolic diseases and higher relative relevance for symptoms like difficulty falling asleep, sleeping too much, increase weight and appetite, low energy level and gastrointestinal problems. This second dimension partially resemble the recently pustulated [9] construct of IMD, defined by the clustering of inflammatory and metabolic dysregulations with behavioral energy-related symptoms. We labelled therefore the first and second dimensions "overall depression " and "IMD". In the replication step, we found that the IMD dimension was associated with a metabolic profile similar to the metabolic profile reported in individuals with cardiometabolic diseases such as higher triglyceride levels, visceral adipose tissue content, branched chain amino acids, glycoprotein acetylase, insulin resistance and lower HDL-cholesterol levels. In contrast, the associations between these metabolites and the overall depression dimension were in the opposite direction, indicating a lower cardiometabolic risk.

The present finding confirm the presence of partially divergent correlation structures between specific depressive symptom profiles and metabolic dysregulations. The weights estimated in NESDA certainly reduced or magnified the relevance of certain symptoms in relation to metabolic alteration**.** However, results obtained after weighting of the different symptoms are consistent with those obtained using unweighted depressive symptoms in previous studies. In a previous work [30], we investigated the association between individual depressive symptoms measured with IDS-SR30 and overall and abdominal adiposity (known proxy for adverse metabolic alteration) indexes such as total body fat, and visceral adipose tissue in NEO study. Overall, adiposity indexes were

associated with a wide variety of depressive symptoms, but were more strongly associated with energy-related symptoms (i.e., hyperphagia, low energy level, and increased physical exhaustion) found to contribute relatively more strongly to the IMD-like dimension identified in the present study. Moreover, this is in line with the previous research in this field that confirmed that the presence of homeostatic shift toward increase energy (increased appetite) intake and decrease energy expenditure (sleeping too much, difficulty falling asleep [31] and low energy level) were more strongly associated with inflammatory and metabolic biomarkers considered as risk factors for CVD. In earlier work based on NESDA data, among participants with active depression episode, increased a neuroendocrine energy homeostasis marker (leptin) [32] was associated (independently from BMI) with a depressive symptoms profile defined by increase the intake (increase appetite/weight) and decrease the expenditure (fatigue, low energy) [33]. Likewise, in the same population, another study confirmed the relationship between cardiometabolic diseases, such as increased abdominal adiposity, inflammation markers, and metabolic syndrome, and increased appetite during the active depressive episode [13]. In agreement with above-mentioned well characterized clinical cohort studies, similar results were obtained from a large population-based studies [34] that confirmed the association between this cluster of symptoms and higher CRP. Our findings are also consistent with previous literature showing a correlation between mood-related syndrome characterized by the presence of similar atypical energy-related symptom profile and metabolic dysregulation. For example, bipolar disorder has been linked to impairment of glucose metabolism [35], seasonal affective disorder with dysregulations of major metabolic regulator (i.e., adiponectin) [36], and sickness behaviour with immuno-metabolic alterations [37]. Also, in a small study that combined neuroimaging and biochemical approaches, hyperphagia during depression was strongly associated with endocrine dysregulation and inflammation [38]. Interestingly, earlier [39] and recent [40] large-scale genomic studies found that the genetic overlap between BMI, CRP and leptin with depression is symptom specific; this overlap was only found in depressed patients with increased hypersomnia [40], weight and appetite [39, 40]. In addition, a cross-disorder systematic review identified a set of genes - coding for energy balance, metabolism, circadian rhythm, inflammation and HPA-axis activity – as potential shared genetic basis for cardiometabolic diseases, depression and bipolar disorder [41]. Another study [42] that used neuroticism as genetic specifier to stratify depression patients showed that the portion of the common genetic liability between depression and neuroticism was also share with other psychiatric disorders; interestingly, the genetic liability not shared with neuroticism was positively correlated with metabolic phenotypes and CVD. These results confirm the existence of different dimension within the construct of depression rooted in underlying biological and genetic mechanisms. Based on evidence along this line of research, the existence of an "immuno-metabolic

depression (IMD)" dimension of depression was hypothesized [9]. This dimension is characterized by the clustering of immuno-metabolic biological alterations and behavioral symptom related to homeostasis dysregulation, which in turn can be the link between depression and CVD [9].

Many plausible mechanisms can directly or indirectly lead to or result from this homeostatic shift as maintaining energy homeostasis is governed by biological, behavioral and environmental factors [43]. For example, low-grade inflammation which associated with adiposity and depression [44], favor -as proposed previously [45]- the fast aerobic glycolysis in the immune cells over other efficient but yet slower energy production pathways (e.g., lipid oxidation). This appropriation of the available cellular fuel done by immune cells results in low energy available to any other activities. When the body has low energy level, the circadian rhythm and sleep cycle disturb as well (i.e., feeling tired and sleeping during the day which affect sleeping time and quality during the night) [45]. Moreover, dysregulation of neuroendocrinological signaling (e.g., leptin, and insulin which have crucial metabolic roles) may diminish their function as satiety inducers hormones which lead to the development of increased appetite and decreased energy level symptoms [43]. These biological processes interact with behavioral/environmental factors that contribute in regulating of the energy homeostasis. Obesogenic environment (e.g., low physical activity demand, and availability of palatable food) could shift the energy balance toward energy accumulation which in turn can result in low grade inflammation and neuroendocrinal dysregulation [46, 47]. Putting it together, the IMD symptoms profile may reflects a prolonged homeostatic failure that closely interconnected with neuroendocrinal and metabolic dysregulation that also reported in patients with CVD [48].

Fully characterizing the IMD dimension identified in the present study, in terms of its clinical manifestation and underlying biological mechanisms is the first step in the path to a personalized approach for patients with depression [49]. This full characterization may help in guiding the choice of the most suitable intervention to alleviate the symptoms burden or to prevent its adverse prognosis. Moreover, understanding the clinical, and biological characteristics of this depression dimension will increase the precision of the genetic studies that aim to comprehend depression genetic architecture [50]. Future research is needed to help us understand to what extent treating underlying metabolic dysregulation will contribute to mitigate this symptoms profile adversity. Nonetheless, we also need to know to what degree will behavioral intervention that target this symptoms profile such as exercising, dieting and sleep hygiene can improve the cardiometabolic health profile. Moreover, future genetics studies using techniques such as Mendelian Randomization are needed to test the causal direction between metabolic dysregulation and specific depressive symptom profile [51].

To the best of our knowledge, this study is the largest study that exploits jointly metabolomic and clinical symptom data to dissect depression dimensionality in a large, well-defined clinical (i.e., subjects with a psychiatric diagnosis) cohort (NESDA). Moreover, we replicate our findings from the clustering set in a population based large cohort (NEO). Furthermore, while previous studies [16, 52] investigating the biological correlates of depression subtypes commonly examined a very limited number of biomarkers, we used an extensive lipid focused metabolomics platform (149 metabolites) and 12 cardiometabolic diseases, including four extensive adiposity measures, glucose, insulin and lipoprotein measures. While we confirmed the link between an IMD-like depression dimension and cardiometabolic risk [9], a novel aspect of the present findings is that we also provided evidence of an independent dimension associated with lower cardiometabolic risk, potentially eluding to protective factors and resilience. However, some methodological issues should be considered. First, we performed the metabolite-symptom clustering and replication in two different samples. On the other hand, the samples' differences may also be considered a strength: the connection between metabolites indexing cardiometabolic risk and IMD-like depressive symptoms could be already detected in the general population, where symptom severity does not cross the clinical threshold. This may be relevant in terms of potential preventive interventions. Second, we should acknowledge the limitation of the NMR metabolite platform, which mainly is a lipidomic metabolomic platform. Accordingly, the term metabolic dysregulation should be interpreted based on the used metabolomic platform. Third, based on the cross-sectional study design, we are unable to infer the directionality of the relationship between depressive symptoms and adverse metabolic alterations.

In the present study, using a data-driven method we identified two independent depression dimensions differentially related with cardiometabolic diseases, such as higher triglycerides, higher visceral fat content, lower HDL-cholesterol levels and insulin resistance in the replication step. Our findings confirm that depression is associated with metabolic alterations that could represent the mechanism linking depression with CVD. However, these metabolic alteration are not present in all forms of depression. Depressed patients with IMD may be at higher cardiometabolic risk and may require specific additional treatment targeting underlying metabolic dysregulations.

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SUPPLEMENTARY MATERIAL

Full version of supplementary materials can be found through the following link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9874986/bin/S0033291721001471sup001.docx

Appendix 1. Processing the metabolomics data

Values of metabolites that could not be quantified were set as missing for all individuals. Furthermore, metabolite values with outlying concentrations (± 5 SD) were additionally set as missing. A value of 1 was added to all metabolite values, which were subsequently natural log-transformed to approximate normality. The obtained values were scaled to standard deviation units to enable comparison. This protocol for processing the metabolomic data was suggested by the manufacturer of the platform and has been consistently applied in several large-scale epidemiological studies [1, 2]. Blood samples were analyzed in two batches (April 2014 and December 2014) by ^{1}H-NMR (Nightingale Health Ltd, Helsinki, Finland) [3]. We regressed the metabolites on age and batch effect in order to remove their confounding effect.

Appendix 2. Additional measures of body mass index (BMI), waist circumference and fasting glucose level

Body mass index (BMI), waist circumference and fasting glucose level were used in the analysis to examine the relationship between CCA output and cardiometabolic diseases. Height and weight were measured to calculate BMI in kg/m^2 as an index of general adiposity. Waist circumference (cm), defined as the minimal abdominal circumference between the lower edge of the rib cage and the iliac crests, was measured by trained clinical staff according to a standardized procedure as index of abdominal adiposity. Glucose was measured from fasting plasma samples by using standard laboratory technique.

Appendix 3. Principal component analysis (PCA)

PCA is an orthogonal linear transformation, that scalarly projected the data to a new coordinate system in which the maximum variation in the data projected on the first coordinate (i.e. first principal component), the second maximum variation projected on the second coordinate, and so on [4].

Appendix 4. Canonical correlation analysis (CCA)

CCA [5] is a method that, given two sets of variables X and Y (in this case, metabolites and depressive symptoms), finds a linear combination of X that is maximally correlated with a linear combination of Y (i.e., a weighted sum of each variable). The linear transformation weights were chosen such that the correlation

between resulting linear combinations is maximized. These linear combinations are called canonical variates (i.e., mCV (metabolites canonical variates), sCV (symptoms canonical variates)). Together mCV and sCV are called a canonical pair and the correlation between this canonical pair is called the canonical correlation. In a specific dataset, it is possible to find multiple canonical pairs such that canonical pairs are uncorrelated to each other and equal to the number of variables in the smallest dataset. In our analysis we chose to proceed with the first two canonical pairs that provided more information about the two sets of variables. The relationship between the created canonical variables of depressive symptoms and metabolites from the same panel and cardiometabolic diseases was validated in an independent sample (see replication section).

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6

The association between adiposity and atypical energy-related symptoms of depression: a role for metabolic dysregulations

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ABSTRACT

Background
Adiposity has been shown to be linked with atypical energy-related symptoms (AES) of depression. We used genomics to separate the effect of adiposity from that of metabolic dysregulations to examine whether the link between obesity and AES is dependent on the presence of metabolic dysregulations.

Method
Data were from NEO (*n*=5734 individuals) and NESDA (*n*=2238 individuals) cohorts, in which the Inventory of Depressive Symptomatology (IDS-SR30) was assessed. AES profile was based on four symptoms: increased appetite, increased weight, low energy level, and leaden paralysis. We estimated associations between AES and two genetic risk scores (GRS) indexing increasing total body fat with (metabolically unhealthy adiposity, GRS-MUA) and without (metabolically healthy adiposity, GRS-MHA) metabolic dysregulations.

Results
We validated that both GRS-MUA and GRS-MHA were associated with higher total body fat in NEO study, but divergently associated with biomarkers of metabolic health (e.g., fasting glucose and HDL-cholesterol) in both cohorts. In the pooled results, per standard deviation, GRS-MUA was specifically associated with a higher AES score (β=0.03, 95%CI: 0.01; 0.05), while there was no association between GRS-MHA and AES (β=-0.01, 95%CI: -0.03; 0.01).

Conclusion
These results suggest that the established link between adiposity and AES profile emerges in the presence of metabolic dysregulations, which may represent the connecting substrate between the two conditions.

INTRODUCTION

The bidirectional relationship between obesity and depression has been well-established [1]: the presence of one of these conditions increases the risk of developing the other [2-5]. There is some evidence for a causal role of obesity in developing depression, though much still has to be elucidated [6, 7]; not every individual with depression is obese, and not every obese individual is depressed. The association between obesity and depression is complicated by heterogeneity on both sides.

Obesity is a metabolically complex and heterogenous condition. One type of obesity, known as "metabolically unhealthy", is interwoven with cardiometabolic diseases, endocrinological alteration, and inflammation [8]. However, about 30 % of obese individuals are "metabolically healthy" [9], and excess total body fat is disconnected from these metabolic alterations [8]. A previous study by Ji et al., which combined data from genome-wide association studies on total body fat percentage and biomarkers of metabolic health, identified 14 single nucleotide polymorphisms (SNPs) associated with increased total body fat and a favourable metabolic profile characterised by higher circulating levels of HDL-cholesterol, and lower levels of triglycerides [10].

Similar to obesity, depression is a heterogeneous disorder. Individuals with a diagnosis of depression may express different symptom profiles that, in turn, are linked to different metabolic adversities. Emerging evidence [1, 11] indicates that the overlap between obesity and depression is stronger in individuals expressing atypical depressive symptoms related to altered energy intake/output balance, such as increased sleepiness, increased appetite, increased weight, low energy level and leaden paralysis. Consistently, in our earlier work [12], the four most strongly associated symptoms with increased total body fat were atypical energy-related symptoms (AES), namely increased appetite, leaden paralysis, low energy level, and increased weight. This connection is also supported by large-scale genomics studies showing genetic covariance between metabolic traits and these AES [13, 14].

The mechanism underlying the relationship between obesity and specific depressive symptoms known as atypical energy-related symptoms (AES) profile is unknown. We expect that metabolic dysregulations may represent the shared link connecting obesity with the AES profile. Studies have shown that the atypical energy-related symptom profile is associated with an adverse immuno-metabolic profile, such as BMI and fasting glucose [15, 16], and biomarkers of neurotoxicity (kynurenine and quinolinic acid) related to low grade inflammation [17]. In the present study, we used genomics to separate the effect of adiposity from that of

metabolic dysregulations to examine whether the link between obesity and AES is dependent on metabolic dysregulations. We used the same genetic instruments applied by Tyrrell et al. [7] to inspect the causal role of adiposity in the development of depression in the UK Biobank. They used two genetic risk scores (GRS, reflecting an individual's genetic liability for a given trait) with a similar effect on total body fat but an opposing relationship with metabolic dysregulations (one predicting high total body fat without metabolic dysregulations and the other predicting high total body fat with metabolic dysregulations). The authors could not observe different patterns of associations between the two GRS and overall depression [7] but were unable to analyse specific depression symptom profiles. We expect that the association may differ when focusing on specific depressive symptom profiles.

For the current study, we used two large datasets from The Netherlands Epidemiology of Obesity study (NEO study, a population-based cohort including >6600 participants with oversampling of overweight and obese individuals) and from the Netherlands Study of Depression and Anxiety (NESDA, a prospective cohort enriched with ~3000 participants with depressive disorders). In these studies, we derived two GRS: 1) a GRS of metabolically healthy adiposity (GRS-MHA), consisting of the SNPs associated with higher total body fat but a favourable metabolic profile identified by Ji et al. [10]; (2) a GRS of metabolically unhealthy adiposity (GRS-MUA), linked to higher adiposity and unfavourable metabolic profile based on a GWAS of BMI (See method section and appendix 1) [7, 10, 18]. We hypothesised that two GRS scores, built to index consistent association with total body fat but opposite direction associations with biomarkers of metabolic health (e.g., HDL-cholesterol and fasting glucose), and AES (i.e., increased appetite, increased weight, low energy level, and leaden paralysis). In particular, we expected that AES profile to be specifically linked with GRS-MUA reflecting increased adiposity accompanied by metabolic dysregulations.

METHOD

Study cohorts

The Netherlands Epidemiology of Obesity (NEO) study

NEO study is a population-based cohort study including 6671 men and women aged 45 to 65 years [19]. All inhabitants with a self-reported body mass index (BMI) of 27 kg/m^2 or higher and living in the greater area of Leiden, the Netherlands, were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one adjacent municipality (Leiderdorp, the Netherlands) were invited to participate irrespective of their BMI, allowing for a reference distribution of BMI. Prior to the study visit, participants completed questionnaires at home with respect to demographic, lifestyle, and clinical

information. Participants visited the NEO study centre after an overnight fast for an extensive physical examination, including anthropometry. This analysis included 5734 unrelated participants of European ancestry with available genetic and phenotypic information.

Netherlands Study of Depression and Anxiety (NESDA)
NESDA is an ongoing longitudinal cohort study that aims to describe the long-term course and consequences of depression and to examine its interaction with biological and psychosocial factors [20]. At baseline, 2981 individuals aged 18 through 65 years with depressive and/or anxiety disorders (confirmed by the Composite International Diagnostic Interview (CIDI, version 2.1.)) and healthy controls were included from the community, primary care, and secondary care settings between 2004 and 2007. The assessment included a diagnostic interview to assess the presence of depressive and anxiety disorders, a medical exam, and several questionnaires on symptom severity, other clinical characteristics and lifestyle. Participants were followed-up during four biannual assessments. For the current study, we used data from unrelated individuals of European ancestry with genetic information at the baseline data (n=2238) and 4 subsequent follow-up waves in which IDS-SR30 symptoms were assessed (total observations=11152). The research protocol of NESDA was approved by the medical ethical committees of the following participating universities: Leiden University Medical Centre, Vrije University Medical Centre, and University Medical Centre Groningen.

Genetic risk scores

Genotyping, quality control, and imputation of GWAS data for both cohorts were previously described in detail [21, 22] (Appendix 2). In each cohort, we created two genetic risk scores (GRS) following the procedure previously proposed by Tyrrell et al. [7] (Appendix 1): the first one is metabolically healthy adiposity (GRS-MHA) included the 14 SNPs that were identified by Ji et al. and associated with higher total body fat but with a favourable metabolic profile indexed by the following biomarkers: HDL-cholesterol, sex hormone binding globulin, triglycerides, fasting insulin, adiponectin, and alanine transaminase (Appendix 1) [10]. The second GRS (the metabolically unhealthy adiposity (GRS-MUA)) included 76 SNPs that were linked to higher adiposity and unfavourable metabolic profile GRS index an individual's lifetime genetic liability for a certain trait and are built as weighted sums of genetic variants associated with that trait. For each individual, the number of trait-increasing alleles carried at each SNP (0,1 or 2) is weighted for the effect size of that SNP in a GWAS of the trait of interest and then summed. In each cohort, the two GRS were standardized to a mean of zero and a standard deviation of one.

Atypical energy-related depressive symptoms (AES)

As described in a previous study [15], the AES profile was based on the sum score of items extracted from the Inventory of Depressive Symptomatology (IDS-SR30)). The IDS-SR30 assesses (via a 4-points likert scale) the presence of 30 depressive symptoms during the last week and their severity [23]. The symptoms used in the AES included the first four top-ranking symptoms associated with total body fat in a previous analysis in the NEO study [12], namely increased appetite, leaden paralysis, low energy level, and increased weight. Increased sleepiness, previously included among atypical energy-related symptoms [15], was not among the top-ranking body-fat related symptoms and was not considered in primary analyses. In NESDA, we used baseline and four follow-up waves. AES scores at each wave were averaged in order to index the participant's long-term exposure to depressive symptoms. In each cohort, the AES score was standardized to a mean of zero and a standard deviation of one.

Total body fat and biomarkers of metabolic health

To benchmark the relationship between the two GRS and the total body fat and blood biomarkers of metabolic health, we used measurements of total body fat (i.e., total body fat was only available in the NEO study) and biomarkers of the same – or very closely related - traits used in the training of GRS-MHA, including triglyceride, LDL-cholesterol, HDL-cholesterol (i.e., lipid profile), and fasting glucose (i.e., glucose profile). Additionally, we tested the association with the inflammatory biomarkers, C-reactive protein (CRP) in both cohorts and interleukin-6 (IL-6) in NESDA, previously shown [15] to be associated with atypical energy-related symptoms. Measurements details about biomarkers of metabolic health are provided in Appendix 3.

Statistical analysis

A schematic representation of the main elements of the study structure and the two analytical steps is depicted in Figure 1.

A. Benchmarking of GRS-MUA, GRS-MHA and AES against total body fat and biomarkers of metabolic health

This step consists of two parts (A.1 and A.2) (Figure 1). In the first part of step one (A.1), the associations of GRS-MUA, GRS-MHA and AES with total body fat were investigated in the NEO study. This step aimed to validate that the increase in all three instruments were associated with higher total body fat as the benchmark measure for adiposity. In the second part of step one (A.2), we estimated the association of GRS-MUA and GRS-MHA with the following biomarkers of metabolic health: triglyceride, LDL-cholesterol, HDL-cholesterol, fasting glucose, and CRP both in NEO and NESDA cohorts. The aim was to validate the different directions associations with biomarkers of metabolic health of the two GRS (GRS-MUA and

GRS-MHA). Associations were estimated with linear regression models adjusted for age, sex and genetic ancestry-informative principal components. A.1 analyses were run only in NEO (due to availability of total body fat measure); A.2 analyses were run in parallel in NEO and NESDA and study-specific estimates were pooled using a fixed-effect meta-analysis.

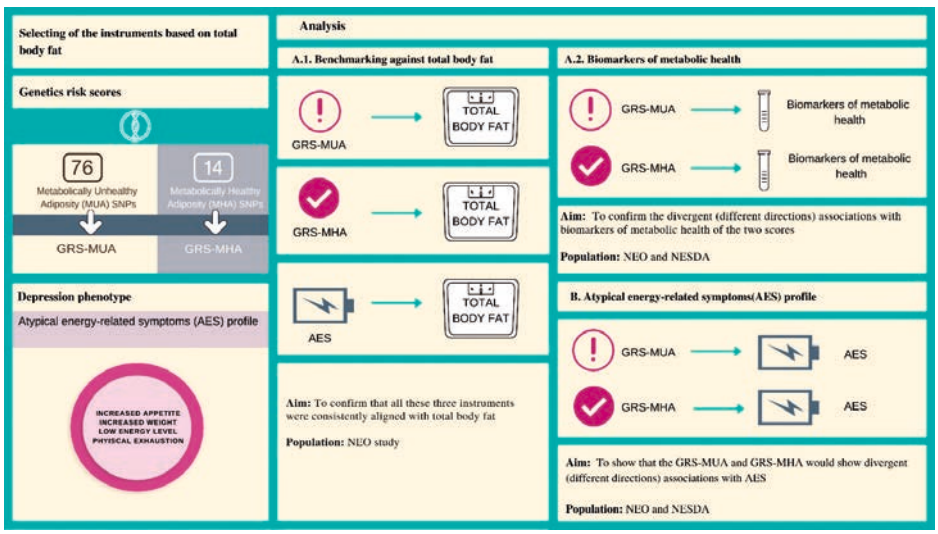

Figure 1. A schematic representation of the main elements of the study structure and the two analytical steps
GRS-MUA: genetic risk score-metabolically unhealthy adiposity. GRS-MHA: genetic risk score: metabolically healthy adiposity. AES: atypical energy-related depressive symptoms. NEO study: The Netherlands epidemiology of obesity study. NESDA: The Netherlands study of depression and anxiety.

B. Association between GRS-MUA, GRS-MHA and Atypical energy-related symptom profile (AES)

In this main step, we estimated the association of GRS-MUA and GRS-MHA with AES. The aim of these analyses was to show divergent associations, consistently with the associations with metabolic biomarkers in A.2. GRS-MUA would be expected to show a positive association with AES, and GRS-MHA would be expected to show a negative association with AES. As in A.2, we used linear regression models adjusted for age, sex and genetic ancestry-informative principal components, and we pooled estimates obtained in NEO and NESDA using fixed-effect meta-analysis. To illustrate the findings of this step, we also used logistic regression models adjusted for age, sex and genetic ancestry-informative principal components for the associations between GRS-MUA, GRS-MHA and individual atypical energy-related symptoms (dichotomized as low vs high). The

dichotomization was applied differently in NEO (low =0 vs high=1-3) and NESDA (low=0-1 vs high= 2-3) cohorts based on the different level of average symptom endorsement – lower in the population-based NEO and higher in the clinically-enriched NESDA cohort - as previously prescribed [12, 24]. In NESDA, individual atypical energy-related symptoms in the baseline and the four follow-up waves were averaged before the dichotomization. Furthermore, we added two sensitivity analyses in the linear regression model in which we first investigated the impact of the inclusion of increased sleepiness symptom among atypical energy-related symptom profile (i.e., by adding it as an extra symptom to the score) on the results. Second, to further confirm the specificity of the associations detected for AES, we derived similarly to previous work [15-17] a melancholic symptom profile score (0-24 range) including the following melancholic features [25]: diurnal variation (mood worse in the morning), early morning awakening, distinct quality of mood, excessive guilt, decreased appetite, decreased weight, psychomotor agitation and psychomotor retardation. All analyses were done using R version 4.0.2, and for the meta-analysis step, package (rmeta) was used.

RESULTS

The baseline characteristics for 5734 participants of the NEO study and 2238 participants of the NESDA included in this study are shown in Supplemental Table 1. The median of the AES in the NEO population was 1 point (25th-75th percentiles: 0-3), while the median of AES in the NESDA population was 2 points (25th-75th percentiles: 1-3.6). The correlation between metabolic and inflammatory biomarkers are depicted in Supplemental Figure 1.

A. Benchmarking of GRS-MUA, GRS-MHA and AES against total body fat and biomarkers of metabolic health

The analyses in the first part (A.1) were done only in the NEO study. All three instruments (GRS-MUA, GRS-MHA, and AES) were associated with increased total body fat in the same direction. Effect estimate (β) in percentage total body fat per standard deviation (SD) increase of 1) GRS-MUA equal to: 0.23% (95% CI: 0.08; 0.39), 2) GRS-MHA 0.31% (95% CI: 0.15; 0.46), and 3) AES 1.43% (95% CI: 1.28; 1.59). The association between total body fat and AES was substantially similar when increased weight symptom was removed from the AES score 1.49, 95%CI (1.34;1.65). Supplemental Table 2 shows the results of the linear regression analysis of the associations between the three instruments (GRS-MUA, GRS-MHA, and AES) and total body fat in the NEO study. Figure 2 depicts the predicted values of total body fat as a function of above mentioned three instruments. These results confirmed that the two GRS and the AES profile were consistently aligned to body fat. Then, the second part of this step (A.2) confirmed that the GRS-MUA and GRS-MHA were differently associated with the biomarkers of metabolic health in NEO

and NESDA cohorts (Supplemental Table 2 for cohort specific association). Figure 3 depicts the pooled (and supplemental table 3 shows cohort-specific) effect estimates and 95% confidence intervals for the association of the two genetic risk scores and the biomarkers of metabolic health. GRS-MUA was associated with an adverse metabolic profile such as (per SD) higher fasting glucose 0.03 mmol/L (95% CI: 0.01; 0.05) and lower HDL-cholesterol -0.02 mmol/L (-0.04; 0.00). The GRS-MHA was linked to a favourable metabolic profile, such as (per SD) lower fasting glucose -0.03 mmol/L (-0.05; 0.00) and higher HDL-cholesterol 0.07 mmol/L (0.05; 0.09). GRS-MUA and GRS-MHA were not associated with the inflammatory biomarker C-reactive protein (CRP) in both cohorts and IL-6 in NESDA (Supplemental Table 3).

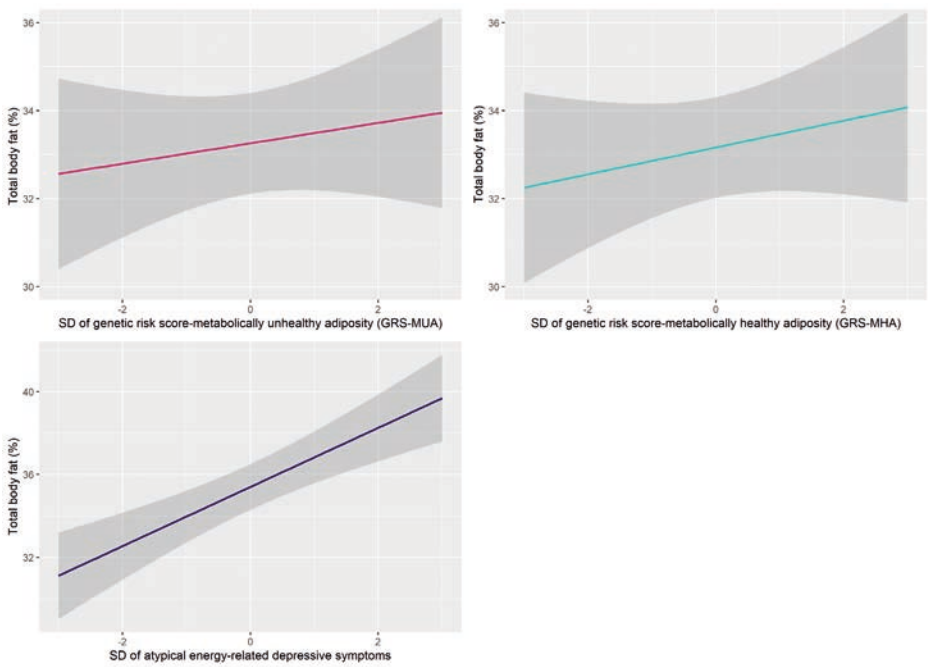

Figure 2. Predicted values of total body fat in the NEO study as function of the GRS-MUA, GRS-MHA, and AES
SD: standard deviation. AES: Atypical energy-related symptom profile: a sum score of the four depressive symptoms, increased appetite, increased weight, low energy level, and leaden paralysis. The grey area represents 95% confidence interval.

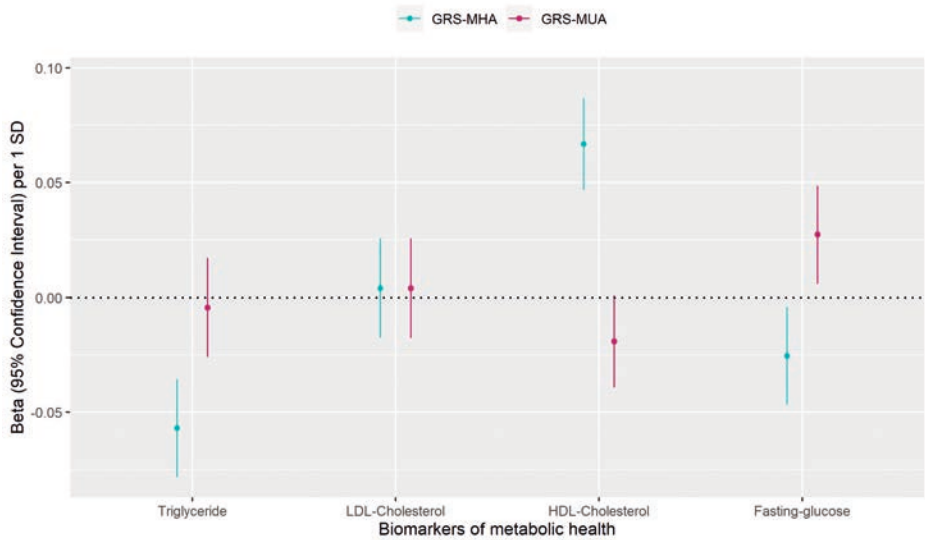

Figure 3. Pooled results of effect estimates of the linear regression between the genetic instruments (GRS-MUA, GRS-MHA) and biomarkers of metabolic health, model adjusted for age, sex , and genetic ancestry-informative principal components
GRS-MUA: Genetic risk score metabolically unhealthy adiposity, GRS-MHA: Genetic risk score metabolically healthy adiposity. SD: standard deviation

B. Association between GRS-MUA, GRS-MHA and atypical energy-related symptom profile

Finally, we examined the association between the two genetic risk scores (GRS-MUA, GRS-MHA) and the AES profile. Figure 4 shows pooled estimates and 95% CIs, and supplemental table 4a shows cohort-specific effect estimates and 95% CIs of the associations with AES from linear regression models adjusted for age, sex, and genetic ancestry-informative principal components. GRS-MUA was specifically associated with higher AES (per SD) 0.03 (95% CI: 0.01;0.05); in contrast, GRS-MHA was not associated with AES -0.01 (-0.03;0.01). Supplemental Table 6 shows the results of the association between GRS-MUA, GRS-MHA and individual atypical energy-related symptoms that showed profiles of associations similar to the overall score of AES. This may suggest that the selected symptoms may have converging biology and that the overall AES association is not driven by a particular individual symptom. Adding increased sleepiness to the AES yielded similar results indicating that a substantial proportion of genetic co-variance between GRS-MUA and AES was already captured by the four symptoms of increased appetite, increased weight, low energy level, and leaden paralysis. Figure 4 and Supplemental Table 5a show that neither GRS-MUA nor GRS-MHA were associated with melancholic symptom profile. This finding suggests that the detected link between GRS-MUA

and AES is specific for this symptom profile. Finally, we repeated this step (B) using BMI-weighted analyses in the NEO study. Since NEO is a population-based study with oversampling of individuals with a BMI > 27 kg/m^2, a weighted analyses were performed as sensitivity analyses. The weighting factor is based on BMI distribution in the general Dutch population to make our results generalizable to the Dutch population. This procedure did not substantially change the results (Supplemental Table 4b and 5b).

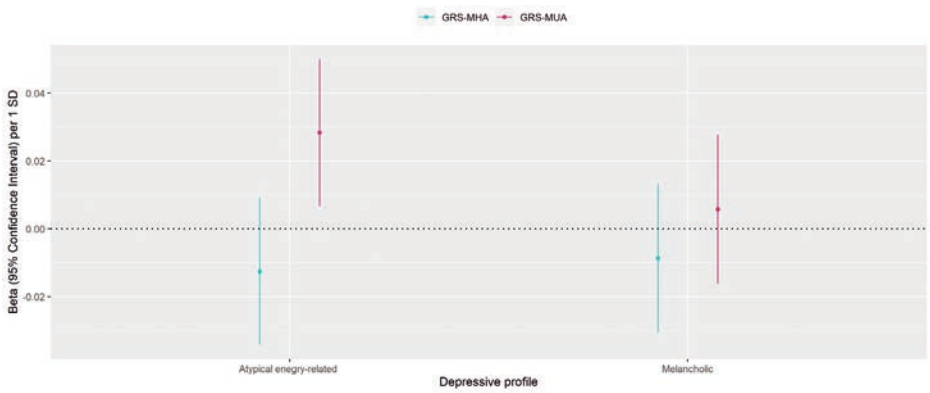

Figure 4. Pooled results of effect estimate of the linear regressions between the genetic instruments (GRS-MUA, GRS-MHA) and atypical energy related symptoms and melancholic symptoms profile, model adjusted for age, sex, and genetic ancestry-informative principal components.
GRS-MUA: Genetic risk score metabolically unhealthy adiposity, GRS-MHA: Genetic risk score metabolically healthy adiposity. SD: standard deviation. Atypical energy-related symptom profile: a sum score of the four depressive symptoms, increased appetite, increased weight, low energy level, and leaden paralysis. Melancholic depressive symptoms profile: a sum score of the symptoms, decreased appetite, decreased weight, early morning awakening, mood variation in relation to the time of the day, distinct quality of mood, excessive guilt, psychomotor agitation, and psychomotor retardation

DISCUSSION

This study investigated whether the established link between adiposity and AES of depression is rooted in underlying metabolic dysregulations. For that, we uncoupled the effect of adiposity from that of metabolic dysregulations. We studied the relationships between two adiposity increasing genetic risk scores (i.e., GRS-MUA and GRS-MHA) and AES. Both genetic instruments used in this study increased the predisposition to high adiposity. The discrepancy between them is that GRS-MUA also increases the predisposition to metabolic dysregulations, and GRS-MHA associates with a favourable metabolic profile. We firstly validated the two GRS by estimating their associations with the traits they were trained to capture: GRS-MUA and GRS-MHA both predicted a high total

body fat level and were divergently associated with metabolic dysregulations. In a subsequent step we tested our main hypothesis by showing that AES was specifically associated with GRS-MUA indexing the liability for increased total body fat accompanied by metabolic dysregulations. GRS-MUA and GRS-MHA were divergently associated with metabolic dysregulations and AES. In particular, GRS-MUA was specifically associated with higher AES scores. Overall, these results suggest that the established link between adiposity and atypical energy-related depressive symptoms emerges in the presence of metabolic dysregulations, which may represent the connecting substrate between the two conditions.

The mechanisms underlying this relationship between adiposity and this specific depression profile are unknown. The recently introduced transdiagnostic model of immuno-metabolic depression (IMD) [26] suggests that metabolic dysregulations and inflammation act as a shared substrate influencing the development of specific behavioural symptoms common to depression and obesity. For instance, alterations in central signalling of leptin and insulin may associate with shifting body energy balance from expenditure to accumulation, favouring the development of hyperphagia, present in both obesity and atypical form of depression. Finally, these metabolic dysregulations have been hypothesised to be the link between depression and cardiovascular diseases. For example, immuno-metabolic dysregulations commonly linked to CVD, such as triglyceride, IL-6, and CRP, were causally related to depression [27]. It was recently reported that adiposity-related inflammation can be dissociated from metabolic dysregulation and that it represents the main predictor of depressive symptoms independently of metabolic dysregulation [28]. Interestingly, a recent study showed that higher inflammation measured by IL-6 activity is a potential causal for a specific symptom profile of depression, such as sleep problems or fatigue [29]. In the present study, using genetic instruments related to metabolic health, we identified a potential role for metabolic dysregulation in the link between obesity and atypical energy-related symptoms profile. This role may be independent and complementary as compare to that of inflammatory alterations. The two GRS were not consistently associated with inflammatory biomarkers commonly linked to AES. This may suggest that inflammatory biomarkers levels may depend on underlying pathways independent from those of metabolic dysregulations tagged by our specific GRS, although both convergent on atypical, energy-related depressive symptoms [15]. Alternatively, the lack of association may be due to the limited power of GRS composed of a reduced set of SNPs to capture different traits with limited genetic covariance with those on which they were trained.

Other mechanisms related to body fat but not associated with immuno-metabolic biological alterations (e.g., weight shame [30], body image dissatisfaction [31]) may play a role in developing and experiencing depression. However, considering

that in our results, GRS-MHA was not related to higher AES, these alternative mechanisms seem less likely. A previous individual-participants meta-analysis study [32] pooled data from 8 studies (n>30000) to test the relationship between metabolically healthy adiposity and depression. They divided individuals into four groups, non-obese metabolically healthy (reference), non-obese metabolically unhealthy, obese metabolically healthy, and obese metabolically unhealthy. They found an increased risk of depression in all three categories in comparison to the reference [32]. This might mean that the body image dissatisfaction explanation may be still valid for the other types of depression.

The present findings highlight the importance of resolving depression heterogeneity when examining its biology. Tyrrell et al. [7] and Marten et al. [33] inspected the causal role of adiposity (via two instrumental variables, metabolically unhealthy adiposity GRS and metabolically healthy adiposity GRS) in the development of depression in the UK Biobank. For example, Tyrrell et al. [7] hypothesised that the GRS-MUA would be associated with depression due to the underlying metabolic dysregulation and GRS-MHA would not be associated with depression for the link with the favourable metabolic profile. Instead, they found that both GRS-MUA and GRS-MHA were associated with depression. The results of [7] and [33] exemplify how depression heterogeneity hinders efforts to identify its biological underpinnings. In this work, we found a positive association between GRS-MHU and AES and a negative association between GRS-MHA and AES, which was in the direction initially hypothesised by Tyrrell et al. by focusing on a specific depressive symptom profile. The present findings are consistent with previous genetic studies that showed the AES was associated with the genetic risk scores that related to a higher risk of adiposity and its related immuno-metabolic dysregulations such as GRS of BMI [34]. Moreover, two large scale genetics studies in > 30000 individuals from the UK Biobank [35] and >26000 individuals from Psychiatric Genomics Consortium [13] found a genetics overlap between adiposity related traits such as BMI, and leptin levels and AES (e.g., increased weight). Overall, evidence from those previous studies and the present one support the hypothesis that the link between adiposity and AES is driven by immune-metabolic dysregulation [26].

The strengths of the present study are, first, we used a large sample size (n> 7000) by combining participants from two cohorts. Second, both the NEO study (i.e., a population-based study that focuses on obesity) and the NESDA cohort (i.e., a clinical cohort study that focuses on depression) have similar genetics and symptoms instruments and detailed biomarkers of metabolic health. However, some limitations need to be addressed. First, based on the different sample sizes between NEO and NESDA, the meta-analysed results of the pooled analyses are driven by the largest study. Nonetheless, the results in both studies were similar.

Second, considering the observational design of the study, causality questions about the association between the two genetic risk scores and AES cannot be answered in this study. Third, GRS were derived using summary genotype data and GWAS summary statistics obtained from subjects of European ancestry GWASs, which make our results not fully generalizable to other ethnicities.

This study showed that the established link between adiposity and atypical energy-related depressive symptoms emerges in the presence of metabolic dysregulation. This supports the hypothesis that metabolic dysregulation represents a key connecting mechanism between adiposity and a specific form of depression. Albeit health care providers shift from assessing adiposity based on BMI solely by incorporating waist circumference and lipid profile to diagnose the overall health profile, less has been done regarding the depression heterogeneity. Monitoring the metabolic health of patients who express atypical energy-related symptomatology could be beneficial to prevent the development of cardiometabolic disorders.

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SUPPLEMENTARY MATERIAL

Full version of supplementary materials can be found through the following link:
https://ars.els-cdn.com/content/image/1-s2.0-S0889159122004627-mmc1.doc
https://ars.els-cdn.com/content/image/1-s2.0-S0889159122004627-mmc2.xlsx

Appendix 1. Genetic risk scores

In each cohort (i.e., NESDA and NEO), we created two genetic risk scores (GRS): the first one is metabolically healthy adiposity (GRS-MHA) included the 14 SNPs [1] that associated with higher total body fat but a favourable metabolic profile. Ji et al [1] identified these 14 SNPs in three steps analyses. First, SNPs related to increase total body fat were identified based on a GWAS of total body fat in more than 442,000 individuals in the UK Biobank. Second, multivariate GWAS of metabolic biomarkers performed based on the summary statistics of the GWASs of the following metabolic biomarkers: total body fat (n=120000) [2], HDL-cholesterol (n=99900) [3], adiponectin (n = 29,400) [4], sex hormone-binding globulin (n=21800) [5], triglyceride (n=96600) [3], fasting insulin (n=51800) [6] and alanine transaminase (n=55500) [7]. Third, genetic variants associated with step 1 and step 2 were selected (SNPs related to metabolically healthy adiposity). The second GRS was linked to higher adiposity and unfavourable metabolic profile (metabolically unhealthy adiposity (GRS-MUA)) based on a GWAS of BMI in 339,224 individuals [8, 9], where 76 SNPs associated with metabolically unhealthy adiposity were identified. Following the procedure previously proposed by Tyrrell et al [9], we calculated GRS-MUA based on 76 SNPs (i.e., 75 SNPs were available in NEO and 72 SNPs in NESDA) [8, 9]. GRS were calculated as follows: each individual variants were recoded as 0, 1 and 2, according to the number of adiposity increasing alleles. Each variant was weighted by its effect size (β-coefficient) obtained from the primary GWAS [8], then a sum of the weighted variants was derived as previously done by Ji et al and Tyrrell et al [1, 9]. In each cohort, the two GRS were standardized to a mean of zero and a standard deviation of one, allowing interpretability.

Appendix 2. Genetic data technical report (genotyping and imputation)

Genotyping, quality control, and imputation of GWAS data for NEO and NESDA cohorts were previously described in detail [10, 11].

Genotyping and Imputation in NEO study

DNA was extracted from venous blood samples obtained from the antecubital vein. Genotyping was performed in Centre National de Génotypage (Evry Cedex, France), using the Illumina HumanCoreExome-24 BeadChip (Illumina, San Diego, CA). The detailed quality-control process has previously been described [10]. Genotypes were further imputed to the 1000 Genome Project reference panel (version 3,

2011) [12] using IMPUTE (version 2.2) software [13]. No genetic variants with an imputation quality <0.4 or a minor allele frequency (MAF) <0.01 were considered for the analyses in the current study (Supplemental Table 7).

Genotyping and Imputation in NESDA

Methods for biological sample collection and DNA extraction have been described previously [14]. Quality control and imputation pipelines were also previously described [11]. Briefly, 95% of the samples were genotyped on the Affymetrix 6.0 Human SNP array and the remaining on the Perlegen-Affymetrix 5.0 array. After platform-specific QC the missing SNP genotypes between each platform were imputed using the GONL (Genome of the Netherlands) [15-17] reference panel and then merged, followed by additional more stringent QC. This cross-platform GONL imputed dataset was used to identify ancestry outliers, defined based on Principal Components Analysis (PCA) by projecting 10 PCs from 1000G reference set populations on the cross-platform imputed data using the SMARTPCA program as described earlier [18, 19]. Individuals with PC values located outside of the range of European and/or British populations were defined as outliers. Upon exclusion of outliers, 10 PCs were recomputed for cross-platform imputed data to capture the variation within the Netherlands. The SNPs from the cross-platform GONL imputed dataset (~1.3M) were used for a second round of imputations to the Haplotype Reference Consortium [20] reference panel using the Michigan Imputation Server [21]. The cross-platform imputed dataset was used to build a relationship matrix measuring genetic similarity using GCTA [22], which was pruned at 0.05 threshold in order to retain unrelated participants. After application of additional post-imputation QC (MAF > 0.01, HWE-*p* > 1e-6) 87 SNPs were extracted for the present analyses (Supplemental Table 7). All the selected SNPs had high imputation quality (<0.6).

Appendix 3. Total body fat and biomarkers of metabolic health

To confirm the relationship between the two GRS and the total body fat and blood biomarkers of metabolic health, we used measurements of total body fat (i.e., total body fat was only available in NEO study), and triglyceride, LDL-cholesterol, HDL-cholesterol (i.e., lipid profile), and fasting glucose (i.e., glucose profile). We additionally used HOMA of beta-cell function (HOMA-1B), Homeostasis Model Assessment for Insulin Resistance (HOMA-IR), and HbA1c (%) that were only available in the NEO study. Finally, we used two inflammatory biomarkers (C-reactive protein (CRP) and Interleukin-6 (IL-6). IL-6 was only available in NESDA. Total body fat was measured by Tanita bioelectrical impedance balance (TBF-310, Tanita International Division, UK) [23]. Lipid and glucose profile were measured from fasting plasma samples by using standard clinical laboratory techniques [24, 25]. From fasting glucose and insulin concentrations, we calculated the HOMA-IR and HOMA-1B as markers of hepatic insulin resistance and steady-

state insulin secretion [26]. HOMA-IR was calculated as fasting insulin (μU/mL) x fasting glucose (mmol/L)/22.5 and HOMA-1B% as 20 x fasting glucose (mmol/l)-3.5 [26, 27]. Concentrations of C-reactive protein (CRP) were determined using a high sensitivity CRP assay (TINA-Quant CRP HS system, Roche, Germany and Modular P800, Roche, Germany) in NEO study [28]. In NESDA, plasma levels of CRP were measured by an in-house high-sensitivity enzyme-linked immunosorbent assay (ELISA) based on purified protein and polyclonal anti-CRP antibodies (Dako, Glostrup, Denmark) [29]. IL-6 in NESDA was measured by a high-sensitivity solid-phase ELISA (Human IL-6 Quantikine HS kit, R&D Systems, Minneapolis, MN, USA) [29]. In each cohort, all biomarkers of metabolic health were standardized to a mean of zero and a standard deviation for each variable of interest of one, allowing interpretability. Additionally, CRP and Il-6 were *loge* transformed before standardization.

Supplemental Table 4a. Results of the linear regression analysis of the association between the genetic instruments (GRS-MUA, GRS-MHA) and atypical energy-related depressive symptoms.

		GRS-MUA		GRS-MHA	
		β(95% CI)	**p-value**	**β(95% CI)**	**p-value**
AES	**NEO**	0.02 (0.00;0.05)	1.11×10^{-01}	-0.02 (-0.05;0.01)	1.17×10^{-01}
	NESDA	0.05 (0.01;0.09)	2.27×10^{-02}	0.01 (-0.03;0.05)	7.02×10^{-01}
	Pooled	0.03 (0.01;0.05)	1.06×10^{-02}	-0.01 (-0.03;0.01)	2.56×10^{-01}
AES (sensitivity)	**NEO**	0.02 (-0.01;0.04)	2.39×10^{-01}	-0.02 (-0.04;0.01)	2.35×10^{-01}
	NESDA	0.04 (0.00;0.09)	3.71×10^{-02}	0.00 (-0.04;0.04)	9.61×10^{-01}
	Pooled	0.02 (0.00;0.05)	3.59×10^{-02}	-0.01 (-0.03;0.01)	3.25×10^{-01}

AES: Atypical energy-related symptom profile: a sum score of the four symptoms, increased appetite, increased weight, low energy level, leaden paralysis. AES (sensitivity): a sum score of the five symptoms, increased sleepiness, increased appetite, increased weight, low energy level, leaden paralysis.

Supplemental Table 5a. Results of the linear regression analysis of the association between the genetic instruments (GRS-MUA, GRS-MHA) and melancholic depressive symptoms.

		GRS-MUA		GRS-MHA	
		β(95% CI)	**p-value**	**β(95% CI)**	**p-value**
Melancholic symptoms profile (sensitivity)	**NEO**	0.00 (-0.02;0.03)	9.40×10^{-01}	-0.02 (-0.04;0.01)	1.71×10^{-01}
	NESDA	0.02 (-0.02;0.06)	3.95×10^{-01}	0.02 (-0.03;0.06)	4.71×10^{-01}
	Pooled	0.01 (-0.02;0.03)	6.07×10^{-01}	-0.01 (-0.03;0.01)	4.34×10^{-01}

Melancholic symptoms profile (sensitivity): a sum score of the symptoms, decreased appetite, decreased weight, early morning awakening, mood variation in relation to the time of the day, distinct quality of mood, excessive guilt, psychomotor agitation, psychomotor retardation.

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Symptomatology of depression and onset of cardiometabolic diseases - A 7-year follow-up study

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ABSTRACT

Background
Depression is associated with an increased risk of developing cardiometabolic diseases (i.e., a composite of type 2 diabetes and cardiovascular disease). This association may vary for different depressive symptom profiles and individual cardiometabolic diseases. We examined the association between depression and specific depressive symptom profiles with individual and composite cardiometabolic diseases.

Method
In 6561 participants from the Netherlands Epidemiology of Obesity (NEO) study, depressive symptoms were measured with the Inventory of Depressive Symptomatology (IDS-SR30) and two dimensional profiles were created: atypical energy-related symptom (AES) and melancholic symptom profiles. Participants were followed for 41 896 person-years, and incidents of type 2 diabetes and cardiovascular disease were extracted from medical records at general practitioners. The Cox proportional-hazard model was used to examine the relationships of overall, atypical energy-related symptoms and melancholic depression scores with overall cardiometabolic diseases and individual components of type 2 diabetes, and cardiovascular disease.

Results
The median follow-up time for type 2 diabetes and cardiovascular disease was seven years (8% developed a cardiometabolic disease, 5% type 2 diabetes, 5% cardiovascular disease). A one SD increase of IDS-SR30 at baseline was associated with an increased risk of cardiometabolic diseases (HR:1.20 CI 95% (1.10-1.31)). For the specific symptom profiles, atypical energy-related symptoms profile was associated with an increased risk of type 2 diabetes (HR 1.26 (95 % CI (1.14-1.42)), while melancholic symptom profile was associated with an increased risk of cardiovascular disease (HR 1.15 CI 95% (1.03-1.28)).

Conclusion
Depressive symptoms were associated with the onset of type 2 diabetes and cardiovascular disease (median follow-up of seven years). This association varied for different depressive symptom profiles and cardiometabolic diseases. Considering a more personalized approach that takes into account differential depression symptomatology may be beneficial to prevent or delay the development of cardiometabolic diseases.

INTRODUCTION

The relation between depression and cardiometabolic diseases (i.e., type 2 diabetes and cardiovascular disease) is complex, multifactorial, and not fully understood. The two conditions negatively impact individual health and well-being and burden the healthcare system. Large meta-analyses of longitudinal studies [1-3] indicate that depression is associated with a 30-60% increased risk of cardiometabolic diseases (i.e., heart disease, myocardial infarction, type 2 diabetes, and stroke). Interestingly, for all these cardiometabolic outcomes, bidirectional associations with depression have also been suggested showing that heart disease [4], diabetes [1] and stroke [5] are associated with an increased risk of developing depression.

Depression's heterogeneity likely contributes to variability in its link with cardiometabolic diseases. Patients with depression report different symptom profiles that, in turn, may represent the expression of different underlying pathophysiological processes. It is, therefore, likely that the association with cardiometabolic diseases may be stronger in individuals with specific symptom profiles. Emerging evidence suggests that inflammatory and metabolic dysregulation, commonly accompanying cardiometabolic diseases, tend to cluster with "atypical" depressive symptoms characterized by altered energy intake and expenditure balance [6]. For instance, recent studies showed that an atypical energy-related symptom (AES) profile characterized by increased sleepiness, increased appetite, increased weight, low energy level and leaden paralysis was associated with altered inflammatory and metabolic markers (i.e., fasting glucose, HDL-cholesterol, triglycerides, blood pressure, waist circumference, CRP, and IL-6) and inflammation-related tryptophan catabolites (i.e., kynurenine and quinolinic acid) [7, 8]. In contrast, these markers were not associated with a melancholic symptom profile characterized by early morning awakening, worse mood in the morning, distinct quality of mood, decreased appetite, weight loss, negative self-outlook, psychomotor retardation, and psychomotor agitation [7]. Based on this evidence, it is hypothesized that individuals expressing atypical energy-related depressive symptoms have a higher risk of cardiometabolic diseases than those mainly reporting melancholic symptoms.

This hypothesis is partially in line with results from two recent follow-up studies. In the first one [9], among 2522 individuals with at least one cardiovascular risk factor, 506 had relevant depressive symptoms based on Beck's Depression Inventory (BDI) questionnaire then melancholic and non-melancholic depressive symptoms groups were created [10]. The participant is classified into the melancholic group if the score of adding the following symptoms: the feeling of sadness, failure, anhedonia, guilt, being punished, irritability, loss of interest, and changes in sleeping and appetite is equal or higher than the score of the rest of BDI

symptoms (if the score is lower than the score above then participant is classified into the non-melancholic group) [9]. In both groups, the incidence of cardiovascular disease extracted from national registers over 8 years of follow-up was higher than in controls, with the largest effect size for the non-melancholic group. In the second study [11], among 28,726 individuals from the general population, 4711 had a lifetime diagnosis of major depressive disorder and were classified as either atypical or non-atypical based only on the presence or absence of hyperphagia and hypersomnia symptoms extracted from Alcohol Use Disorder and Associated Disabilities Interview Schedule-IV (AUDADIS-IV). Again, as compared with individuals without depression, both these groups had a higher risk of incident cardiovascular disorders over 3 years, with the largest effect size for the atypical subgroup.

In the present study, we further expanded the examination of the association between depressive symptoms and incident cardiometabolic diseases, including both type 2 diabetes and cardiovascular disease recorded in general practitioner registries followed up for seven years. Furthermore, we refined the examination of different clinical manifestations of depression by using dimensional profilers for AES and melancholic symptoms rather than binary subtypes, as in previous studies [9, 11]. As a result, we were better able to capture the variability of a wider array of depressive symptomatology. We hypothesize that overall depressive symptoms are associated with cardiometabolic diseases. Furthermore, we expect this association to be driven by the AES profile, previously associated with markers of cardiometabolic risk.

METHODS

Study design and population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based cohort study including 6671 men and women aged 45 to 65 years [12]. All inhabitants aged between 45 and 65 years with a self-reported body mass index (BMI) of 27 kg/m^2 or higher and living in the greater area of Leiden, the Netherlands, were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one adjacent municipality (Leiderdorp, the Netherlands) were invited to participate irrespective of their BMI. Prior to the study visit (2008-2012), participants completed questionnaires at home with respect to demographic, lifestyle, and clinical information. Participants visited the NEO study center after an overnight fast for an extensive physical examination.

Participants were followed over time (median = 6.7 years) for the occurrence of type 2 diabetes and cardiovascular disease via their electronic medical records at the general practitioners (see outcome and censoring). The present study is a

prospective analysis of the relationship between depressive symptoms (overall depression) and depressive symptom profiles measured by the Inventory of Depressive Symptomatology (self-report) IDS-SR30 at the baseline and 1) cardiometabolic diseases (i.e., merged type 2 diabetes and cardiovascular disease), 2) type 2 diabetes, and 3) cardiovascular disease. We excluded participants without IDS-SR30 total score data (n=16) or follow-up information (n=94), leaving 6561 participants for the main analyses. The NEO study was approved by the medical ethics committee of Leiden University Medical Center (LUMC) and all participants gave written informed consent.

Assessment of depressive symptoms and profiles

At baseline, we asked all participants to complete the Dutch version of the IDS-SR30 questionnaire [13], which assesses specific depressive symptoms during the past week and their severity. The IDS-SR30 rates (via a 4-level response system) the presence of a wide array of depressive symptoms, including core symptoms of major depressive episodes, melancholic (e.g., anhedonia, nonreactive mood, psychomotor retardation/agitation, appetite or weight decrease, early morning awakening, and self-outlook) and atypical energy-related (e.g., hypersomnia, increased appetite, weight gain, low energy level, and leaden paralysis (physical exhaustion)) features, and commonly associated symptoms (e.g., irritability, anxiety, somatic complaints). We used the total score ranges from 0 to 84, with higher scores indicating higher severity as a continuous variable. Furthermore, we categorized the total score in the secondary analyses. For that, we grouped the participant according to the clinically predefined severity cut-offs as follow: score ≤ 13 as "no depressive mood" status (n = 4625, reference), 14–25 as "mild depressive mood" (n = 1413), 26–84 as "severe depressive mood" (n = 523) [13].

We derived depressive profiles in line with previous studies [7, 14] using items from IDS-SR30. The AES profile was based on the sum score of the following items: increased sleepiness, increased appetite, weight gain, low energy level, and leaden paralysis. Then, we also used a melancholic depressive profile as another clinically established symptom profile for comparison with AES, as it also reflects severity [7, 15]. This symptom profile was created by summing the score of the following items: early morning awakening, mood worse in the morning, distinct quality of mood, decreased appetite, weight loss, self-outlook, psychomotor retardation, and psychomotor agitation. Additionally, in order to better illustrate the shape of the association between symptom profiles and cardiometabolic diseases, for each symptom profile, we grouped the participant into four severity score groups: no symptoms: 0 (reference), mild symptoms:1-2, moderate symptoms: 3-4 and severe symptoms: ≥5.

Ascertainment and definition of outcomes

Diagnoses of type 2 diabetes and cardiovascular disease incidence were extracted from electronic medical records of general practitioners (GPs). This record covers all medical information of the patients regarding prescriptions, GP consultations, and reports from laboratories and specialist visits available at the GP office. Data extraction was performed based on three criteria: (1) the diagnostic coding by the GPs to indicate the health problems or type of care, based on the International Classification of Primary Care (ICPC) [16], (2) finding of predefined type 2 diabetes and cardiovascular disease related keywords in the descriptions of the GP database, and (3) prescription of specific medication, registered according to the Anatomical Therapeutic Chemical (ATC) codes or by screening medication names [17]. The date of diagnosis was defined as the first date of an ICPC-coded diagnosis, a strong indication for the diagnosis based on keywords in the medical records, or prescription of relevant medication. In case only a keyword was found without a confirmed ICPC code, we confirmed the diagnoses using the laboratory values and reading the free text in the medical records. If it remained unclear whether a particular participant was diagnosed with type 2 diabetes or cardiovascular disease, the general practitioner was contacted. A participant was considered as having an incidence of type 2 diabetes or cardiovascular disease when the date of diagnosis occurred after the baseline visit date.

In the present analysis, we used the preliminary follow-up data, as the extraction of information from the GP medical records is still ongoing. Our analyses were focused on the development of three outcomes: (1) cardiometabolic diseases (i.e., having either type 2 diabetes or/and cardiovascular disease), (2) type 2 diabetes, and (3) cardiovascular disease. For each outcome of interest, we excluded participants who had the prevalent condition of interest at baseline based on information extracted from the GP medical records (Figure 1). For this reason, the sample sizes for our analyses differ based on the studied outcome of interest (i.e., type 2 diabetes, cardiovascular disease, both type 2 diabetes and cardiovascular disease). Participants were coded as having type 2 diabetes when the extracted data from GP registration in 2018 indicated 1) the diagnosis of type 2 diabetes (i.e., ICPC codes T90 or T90.02). In addition, the medication list of participants was checked for the use of insulin, metformin and sulfonylurea derivative, and participants using these medications were considered to have type 2 diabetes (n of participants who developed the outcome=276). Similarly, participants were coded as having cardiovascular disease if the extracted data from GP registration in 2018 indicated any of the following diagnoses of 1) myocardial infarction (ICPC Code: K75 or K76.02), 2) transient ischemic attack (K89), or 3) stroke/cerebrovascular accident (K90 or its subtypes: K90.01, subarachnoid haemorrhage; K90.02, intracerebellar haemorrhage; or K90.03, cerebral infarction.

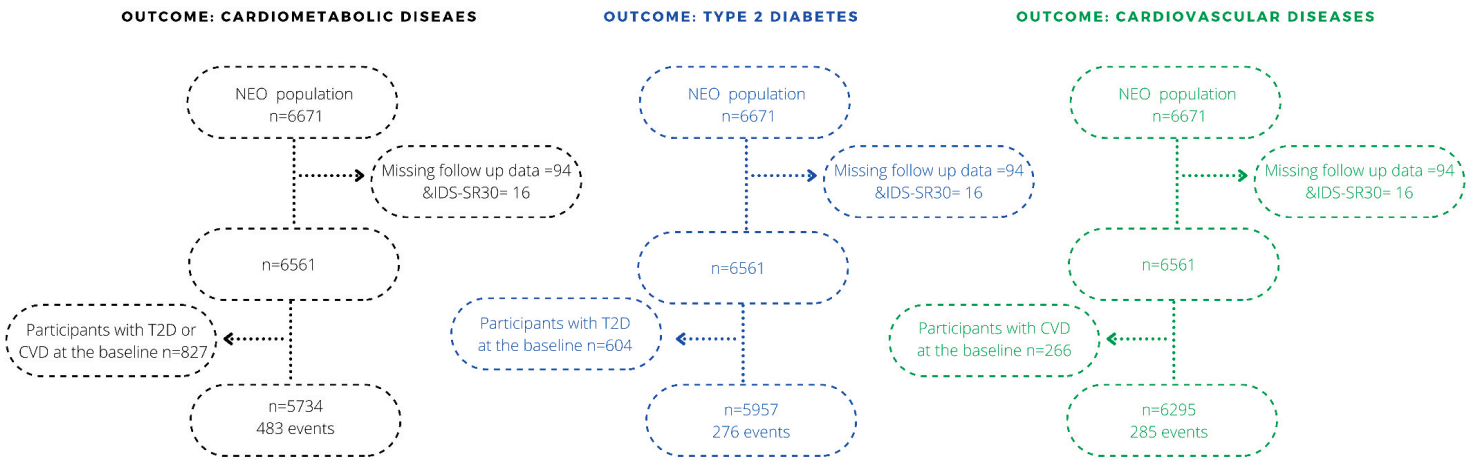


Figure 1. Study population

Keywords included synonyms of myocardial infarction, chest pain, cardiovascular surgery procedures such as coronary artery bypass grafting (CABG) or angioplasty, and synonyms of cerebrovascular accident or haemorrhage. The medication list of participants was checked for the use of specific anticoagulants. In this preliminary data, other types of cardiovascular disease were not yet included (n of participants who developed the outcome=285). We merged the two outcomes (i.e., type 2 diabetes and cardiovascular disease) into a new outcome called cardiometabolic diseases if the participants had either or both diseases (n of participants who developed the outcome=483).

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 (move to another GP or outside of the Netherlands), or the end of the follow-up (extraction date at the GP in 2018), whichever comes first. However, not all participants were followed from start to finish.

Statistical analysis

Characteristics of the study population were expressed as a mean with standard deviation (SD), a median (25th, 75th percentiles) or percentages (%). The incidence rates per 1000 person-years for each outcome were estimated as: (new cases of outcome/ person-years of the population at risk) x 1000.

Cox regression analyses

We performed Cox proportional-hazard models to investigate the relationship between the depressive symptoms at the baseline and the outcomes using 3 steps. In step 1, we performed adjusted Cox proportional-hazard models to investigate the relationships between depressive symptoms and cardiometabolic outcome. In step 2, we explored the relationship between the baseline depressive symptoms and (1) type 2 diabetes and (2) cardiovascular disease as individual outcomes. In step 3, to take the heterogeneity of depressive symptomatology into account, we conducted adjusted Cox proportional-hazard models to investigate the relationships between two depressive symptom profiles (atypical energy-related and melancholic) with type 2 diabetes and cardiovascular disease.

Analyses of the three steps were adjusted for age, sex (model 1) and further BMI adjustment (model 2). Model 2 is important because BMI is a strong risk factor for type 2 diabetes and is related to depression. Finally, in model 3 we further adjusted for type 2 diabetes at baseline when applicable (i.e., in analyses with cardiovascular disease as outcome). All analyses were done using R version 4.0.5, and for the Cox proportional-hazard model analysis "survival" package was used.

RESULTS

For cardiometabolic diseases as the outcome, some participants were lost to follow-up (n=45), died (n= 58), or only had data from an intermediate data extraction in 2012-2013 (n=306). For type 2 diabetes and cardiovascular disease as the outcomes of interest, 46 and 50 were lost to follow-up, 60 and 75 participants died, and 321 and 342 participants only had data from intermediate extraction in 2012-2013, respectively. For participants who did not develop the outcome of interest, data were censored at the known follow-up time or date of death or the last known follow-up time before death.

Table 1 shows the characteristics of the NEO population (mean age 56.0), men and women (52.0% women). For the cardiometabolic diseases as the outcome, the population at risk was 5734, the median (25th, 75th percentiles) follow-up time was 6.7 years (5.9, 7.9), and the incidence rate (IR) was 13/1000 person-years. For type 2 diabetes as the outcome, the population at risk was 5957, and the median (25th, 75th percentiles) follow-up time was 6.8 (6.0, 7.9). 5% developed the outcome, IR 7/1000 person-years. For cardiovascular disease, the population at risk was 6295, the median (25th, 75th percentiles) follow-up time was 6.7 (5.9,7.8). 5% developed the outcome, IR 7/1000 person-years. The Pearson's correlation between the two symptom profiles was 0.4, indicating that they are capturing partially different dimensions of depressive symptomatology.

Table 1. Baseline characteristics for 6561 men and women aged 45 to 65 years included in the analysis from Netherlands Epidemiology of Obesity study

Characteristic	N=6561
Age (years) Mean (sd)	56.0 (6.0)
Sex (women) (n(%))	3443 (52.0)
BMI Mean (sd)	30.1 (4.8)
Ethnicity (White) (n(%))	6227 (95.0)
Education (High) (n(%))	2452 (38.0)
Smoking (n(%))	
No	2274 (35.0)
Former	3217 (49.0)
Current	1067 (16.0)
Alcohol consumption (g/day) Median (25th, 75th percentiles).	9.0 (2.0, 22.0)
Type 2 diabetes incidence (outcome) (n(%))	276 (4.2%)
Type 2 diabetes prevalence (baseline) (n(%))	604 (9.2%)
Cardiovascular diseases incidence (outcome) (n(%))	285 (4.3%)

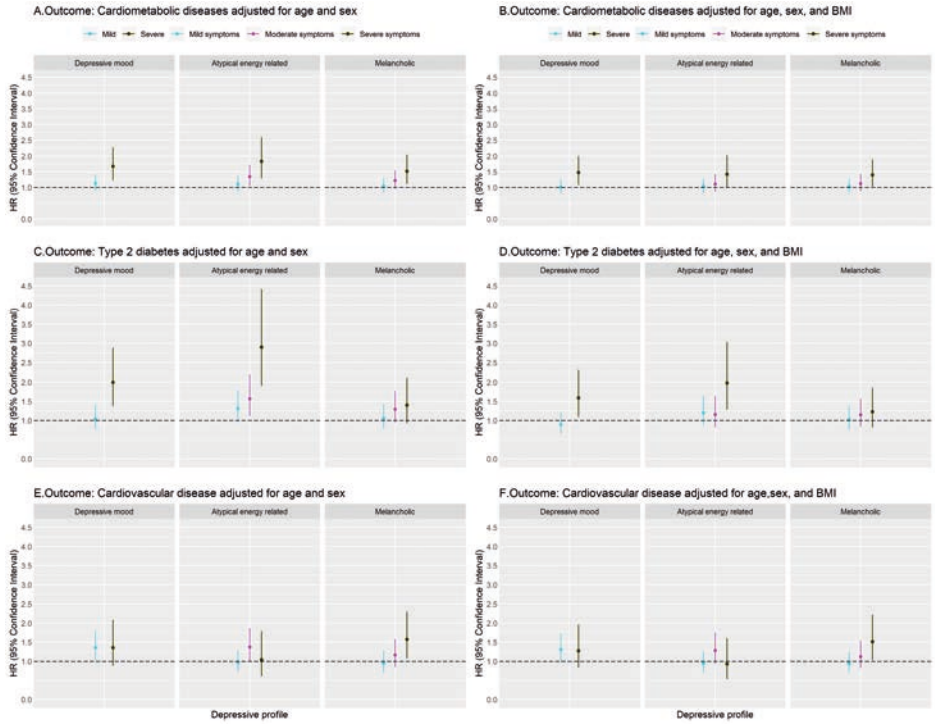
Table 1. Continued.

Characteristic	N=6561
Cardiovascular diseases prevalence (baseline) (n(%))	266 (4.1%)
Atypical energy-related symptom profile Median (25th, 75th percentiles)	1.0 (0.0, 3.0)
Atypical energy-related symptom profile (Categorized) (n(%))	
None (≤0)	1994 (30.0)
Mild (>0 and <3)	2560 (39.0)
Moderate (≥3 and <5)	1553 (24.0)
Severe (≥5)	454 (6.9)
Melancholic symptom profile Median (25th, 75th percentiles)	1.0 (0.0, 3.0)
Melancholic symptom profile (Categorized) (n(%))	
None (≤0)	2597 (40.0)
Mild (>0 and <3)	1940 (30.0)
Moderate (≥3 and <5)	1394 (21.0)
Severe (≥5)	630 (9.6)
IDS-SR30 total score Median (25th, 75th percentiles)	9 (5, 15)
Depressive mood (Categorized) (n(%))	
None (≤ 13)	4625 (70.0)
Mild (14–25)	1413 (22.0)
Moderate to severe (26–84)	523 (8.0)

Step 1: Overall depressive symptoms and cardiometabolic diseases

Table 2 shows the results of the Cox proportional-hazard model of the continuous and categorized total score of IDS-SR30 and cardiometabolic diseases. We found that a one SD increase of IDS-SR30 in the baseline was associated with an increased risk of cardiometabolic diseases (HR:1.20 CI 95% (1.10-1.31)) for model 1 (adjusted for age and sex). In particular, compared to those without depressive mood, individuals in the severe depressive mood group had the highest risk of cardiometabolic diseases (HR:1.67 CI 95% (1.23-2.27) (Figure 2A). Additional adjustment for BMI (model 2) slightly reduced the strength of the associations; the HR of cardiometabolic diseases in individuals with severe depressive mood, as compared to those without depressive mood, was 1.47 CI (95% 1.08-2.00) (Figure 2B).

Figure 2. Cox proportional-hazard regressions for the depressive mood, atypical energy-related and melancholic symptoms profile and all three outcomes



Depressive mood: we grouped the participant according to the clinically predefined severity cut-offs as follow: score ≤ 13 as "no depressive mood" status (n = 4625, reference), 14–25 as "mild depressive mood" (n = 1413), 26–84 as "severe depressive mood" (n = 523). Atypical energy-related symptom profile (a sum score of the five symptoms, increased sleepiness, increased appetite, increased weight, low energy level, leaden paralysis). Melancholic symptoms profile: a sum score of the symptoms, decreased appetite, decreased weight, early morning awakening, mood variation in relation to the time of the day, distinct quality of mood, excessive guilt, psychomotor agitation, psychomotor retardation. For each symptom profile, we grouped the participant in four severity score groups: no symptoms: 0 (reference), mild symptoms:1-2, moderate symptoms: 3-4 and severe symptoms: ≥5.

Table 2. Cox proportional-hazard regressions for IDS-SR30 total score, atypical energy-related and melancholic symptoms profiles with cardiometabolic diseases.

		Model 1 HR (95% CI)	Model 2 HR (95% CI)
Cardiometabolic diseases (n= 5734, 483 events)	**IDS-SR30 total score (continuous)**	1.20 (1.10-1.31)	1.14 (1.04-1.25)
	Depressive mood (categorical)		
	None	*Reference*	*Reference*
	Mild	1.12 (0.89-1.41)	1.01 (0.81-1.27)
	Severe	1.67 (1.23-2.27)	1.47 (1.08-2.00)
Cardiometabolic diseases (n= 5734, 483 events)	**AES (continuous)**	1.15 (1.06-1.26)	1.07 (0.97-1.17)
	AES (categorical)		
	None	*Reference*	*Reference*
	Mild	1.10 (0.88-1.37)	1.03 (0.82-1.29)
	Moderate	1.34 (1.05-1.71)	1.11 (0.86-1.42)
	Severe	1.83 (1.29-2.59)	1.41 (0.99-2.02)
Cardiometabolic diseases (n= 5734, 483 events)	**Melancholic (continuous)**	1.14 (1.05-1.24)	1.11 (1.02-1.21)
	Melancholic (categorical)		
	None	*Reference*	*Reference*
	Mild	1.04 (0.84-1.30)	1.03 (0.82-1.28)
	Moderate	1.22 (0.96-1.54)	1.12 (0.88-1.43)
	Severe	1.51 (1.11-2.04)	1.39 (1.03-1.89)

Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, and BMI. IDS-SR30: Inventory of depressive symptomatology-self report (standardized). None group was set as the reference group throughout the analyses. AES: Atypical energy-related symptom profile (a sum score of the five symptoms, increased sleepiness, increased appetite, increased weight, low energy level, leaden paralysis) (standardized). Melancholic symptoms profile: a sum score of the symptoms, decreased appetite, decreased weight, early morning awakening, mood variation in relation to the time of the day, distinct quality of mood, excessive guilt, psychomotor agitation, psychomotor retardation (standardized). For each symptom profile, we grouped the participant in four severity score groups: no symptoms: 0 (reference), mild symptoms:1-2, moderate symptoms: 3-4 and severe symptoms: ≥5.

Step 2: Overall depressive symptoms and (1) type 2 diabetes and (2) cardiovascular disease)

Table 3 shows the results of the Cox proportional-hazard models of the continuous and categorized total score of IDS-SR30, atypical energy-related and melancholic symptom profiles and individual cardiometabolic diseases. We found that a one SD increase of IDS-SR30 in the baseline is associated with an increased risk of type 2 diabetes (HR:1.26 CI 95% (1.14-1.41)) for model 1. As compared to individuals without depressive mood, individuals in the severe depressive mood group had

the highest risk of type 2 diabetes (HR: 1.99 CI 95% (1.38-2.89) (Figure 2C), also after adjusting for BMI (HR: 1.59 CI 95% (1.09-2.31) (Figure 2D). Furthermore, a one SD increase of IDS-SR30 in the baseline is associated with an increased risk of developing cardiovascular disease (HR:1.15 CI 95% (1.03-1.29)) in model 1. Individuals in the severe depressive mood group, compared to those without depressive mood, had the highest risk of cardiovascular disease (HR: 1.36 CI 95% (0.88-2.08) for model 1 (Figure 2E). Additionally, adjusting for BMI or type 2 diabetes at baseline did not change the hazard ratios.

Table 3. Cox proportional-hazard regressions for IDS-SR30 total score, atypical energy-related and melancholic symptoms profiles with type 2 diabetes and cardiovascular disease.

		Model 1 HR (95% CI)	Model 2 HR (95% CI)
Type 2 diabetes (n= 5957, 276 events)	**IDS-SR30 total score (continuous)**	1.26 (1.14-1.41)	1.16 (1.04-1.30)
	Depressive mood (categorical)		
	Mild	1.04 (0.76-1.41)	0.89 (0.66-1.21)
	Severe	1.99 (1.38-2.89)	1.59 (1.09-2.31)
Type 2 diabetes (n= 5957, 276 events)	**AES (continuous)**	1.27 (1.14-1.42)	1.14 (1.02-1.27)
	AES (categorical)		
	Mild	1.31 (0.96-1.78)	1.20 (0.88-1.64)
	Moderate	1.57 (1.12-2.20)	1.16 (0.82-1.64)
	Severe	2.90 (1.90-4.41)	1.98 (1.29-3.04)
Type 2 diabetes (n= 5957, 276 events)	**Melancholic (continuous)**	1.13 (1.01-1.26)	1.07 (0.95-1.20)
	Melancholic (categorical)		
	Mild	1.06 (0.79-1.42)	1.02 (0.76-1.37)
	Moderate	1.29 (0.95-1.77)	1.15 (0.84-1.57)
	Severe	1.40 (0.93-2.11)	1.23 (0.82-1.86)
Cardiovascular disease (n=6295, 285 events)	**IDS-SR30 total score (continuous)**	1.15 (1.03-1.29)	1.13 (1.00-1.26)
	Depressive mood		
	Mild	1.35 (1.02-1.80)	1.30 (0.98-1.73)
	Severe	1.36 (0.88-2.08)	1.27 (0.83-1.96)
Cardiovascular disease (n=6295, 285 events)	**AES (continuous)**	1.08 (0.96-1.22)	1.05 (0.93-1.18)
	AES (categorical)		
	Mild	0.97 (0.73-1.29)	0.94 (0.71-1.25)
	Moderate	1.37 (1.01-1.86)	1.28 (0.93-1.75)
	Severe	1.04 (0.61-1.79)	0.93 (0.54-1.60)

Table 3. Continued.

		Model 1 HR (95% CI)	Model 2 HR (95% CI)
Cardiovascular disease (n=6295, 285 events)	**Melancholic (continuous)**	1.15 (1.03-1.28)	1.13 (1.01-1.26)
	Melancholic (categorical)		
	Mild	0.95 (0.71-1.27)	0.94 (0.70-1.26)
	Moderate	1.16 (0.85-1.58)	1.13 (0.82-1.54)
	Severe	1.57 (1.08-2.30)	1.51 (1.03-2.21)

Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, and BMI. IDS-SR30: Inventory of depressive symptomatology-self report (standardized). None group was set as the reference group throughout the analyses. AES: Atypical energy-related symptom profile (a sum score of the five symptoms, increased sleepiness, increased appetite, increased weight, low energy level, leaden paralysis) (standardized). Melancholic symptoms profile: a sum score of the symptoms, decreased appetite, decreased weight, early morning awakening, mood variation in relation to the time of the day, distinct quality of mood, excessive guilt, psychomotor agitation, psychomotor retardation (standardized). For each symptom profile, we grouped the participant in four severity score groups: no symptoms: 0 (reference), mild symptoms:1-2, moderate symptoms: 3-4 and severe symptoms: ≥5.

Step 3: Depressive profiles and type 2 diabetes and cardiovascular disease

Table 3 shows Cox proportional-hazard model results for the continuous and categorized depressive profiles (atypical energy-related and melancholic symptom profiles) and type 2 diabetes, and cardiovascular disease. We found that the atypical energy-related symptom profile and melancholic symptom profile had a different pattern of association with type 2 diabetes and cardiovascular disease. One SD increase in the atypical energy-related symptom profile was associated with an increased risk of type 2 diabetes HR 1.27 (95 % CI (1.14-1.42)) in model 1. As compared to those without AES, results showed an increased risk of type 2 diabetes for individuals with moderate ((HR: 1.57 CI 95% (1.12-2.20)) and severe depressive AES (HR: 2.90 CI 95% (1.90-4.41) (Figure 2C). In Model 2, further adjustment for BMI decreased the strength of the association: the HR of individuals with severe AES, when compared with those without AES, was 1.98 (CI 95% 1.29-3.04) (Figure 2D). The same symptom profile was not associated with cardiovascular disease in any of the adjusted Cox proportional-hazard models.

For melancholic symptom profile, one SD increase in the score was associated with an increased risk of type 2 diabetes (HR 1.13 CI 95% (1.01-1.26)) for model 1. Nevertheless, adding BMI to the model substantially decreased the hazard ratio (HR 1.07 CI 95% (0.95-1.20)). For cardiovascular disease, one SD increase in the melancholic symptom profile was associated with an increased risk of the outcome (HR 1.15 CI 95% (1.03-1.29)) for the model adjusted for age and sex. As compared to participants without melancholic symptoms, participants with the severe

melancholic symptoms have an increased risk of cardiovascular disease (HR: 1.57 CI 95% (1.08-2.30)) (Figure 2E). All further adjustments for type 2 diabetes at the baseline and BMI did not change the hazard ratio.

DISCUSSION

This study explored the association between depressive symptoms and the risk of developing cardiometabolic diseases in large population-based cohort with a median follow-up of seven years. We were able to disentangle the heterogeneity of the exposure (i.e., depressive symptoms) and the outcome (i.e., cardiometabolic diseases) by examining the association of two specific depressive symptom profiles, atypical energy-related symptom and melancholic profiles, with type 2 diabetes and cardiovascular disease. We found that having higher overall depressive symptoms at the baseline is associated with an increased risk of developing cardiometabolic diseases over time. When zooming in the atypical energy-related symptom profile, we found that it was specifically associated with a higher risk of developing type 2 diabetes, while the melancholic was associated with a higher risk of developing cardiovascular disease.

The incidence rate of type 2 diabetes was 2.5 times higher in cohort of this study compared to the general Dutch population [18]. This was expected because of the oversampling of obese and overweight individuals (i.e., higher BMI individuals are at higher risk of developing type 2 diabetes) in the NEO study. However, the incidence rate of cardiovascular disease was similar to the general Dutch population [19]. Moreover, our finding that depressive symptoms increased the risk of developing cardiometabolic diseases and its component (i.e., type 2 diabetes and cardiovascular disease) are in line with the previous knowledge. Meta-analyses of longitudinal studies showed that depression (both clinical depression and depressive symptoms) increased the risk of developing type 2 diabetes (relative risk= 1.37 -1.67) [1, 20-22]. Similarly, another recent meta-analysis that included twenty-one follow-up studies reported that depression (i.e., combined depressive scales and depression diagnosis) increased the risk of type 2 diabetes (risk ratio 1.18) [23]. Additionally, depression was also reported as a risk factor for developing cardiovascular disease (i.e., myocardial infarction (MI), stroke, or coronary death) in meta-analyses of longitudinal studies (hazard ratio= 1.31-2.6) [2, 3, 24, 25]. The direction of this association is in agreement with a Mendelian Randomization (MR) study that suggested that genetic predisposition to depression is associated with increased risk of cardiovascular disease (i.e., coronary artery disease (14%) and myocardial infarction (21%)) [26]. Additionally, data from another MR study [27] suggest that obesity, type 2 diabetes, smoking, and high lipid level mediate this causal relationship.

Many mechanisms were studied earlier and described as potential links between depressive symptoms with cardiometabolic diseases. These mechanisms are behavioral (i.e., physical inactivity, unbalanced diet, smoking, alcohol abuse, and low level of medical/lifestyle adherence), biological (i.e., HPA, immuno-metabolic, autonomic dysregulations), and iatrogenic (i.e., the pharmacological impact of depression medication on cardiometabolic diseases) [28]. Furthermore, possible common causes for the independent expression of both depression and cardiometabolic diseases include childhood trauma, personality, and genetic pleiotropy [28]. A recent study [29] identified 24 pleiotropic genes likely to be shared between depression and cardiometabolic diseases (i.e., defined in this study as type 2 diabetes, cardiovascular disease, and their risk factors such as obesity, hypertension, HDL and LDL cholesterol, triglycerides, and fasting glucose and insulin). Four of these genes were shared between depression with type 2 diabetes or cardiovascular disease and regulate neurogenesis, appetite, neurotransmitters, and melatonin receptor [29].

To deepen our understanding, we investigated the association between specific depressive symptom profiles and individual cardiometabolic diseases. Our study suggests that atypical energy-related symptom profile was the main driver for the association between depression and increased risk of type 2 diabetes. This noted link could be explained by interconnected behavior factors and biological mechanisms such as surplus calorie intake and immuno-metabolic dysregulation (i.e., low-grade inflammation and adipokines over secretion), which may later manifest as type 2 diabetes [6, 30]. The hemostatic shift toward positive energy balance, which distinguishes AES, may lead to lipid accumulation in ectopic organs, a known risk factor for insulin resistance and type 2 diabetes [31, 32]. This positive energy balance also creates cellular nutrient stress, especially on the site of protein folding (i.e., endoplasmic reticulum) [33]. This cellular stress triggers the "metaflammation" response. The "metaflammation" describes the situation when the low-grade inflammation alters the function of insulin in metabolic tissues such as the liver and brain [33]. Accumulated white adipose tissue secrete adipokines (e.g., leptin) that play a significant role in inhibition of insulin secretion from pancreatic β cells [34]. This aligns with the previous work that confirmed the increased pro-inflammatory markers and metabolic dysregulation (e.g., CRP and IL-6, high BMI and total body fat, insulin resistance, leptin resistance, dyslipidemia, and hyperglycemia) in individuals with depression reporting AES profile [6, 7, 35-41]. Additionally, pro-inflammatory markers may trigger neuroinflammation associated with decreased tryptophan and increased catabolites associated with the atypical energy-related symptom profile and worse health outcomes such as type 2 diabetes [8, 42]. Furthermore, chronic low-grade inflammation, has been suggested to mediate the relationship between atypical energy-related symptoms and type 2 diabetes [43]. Several genetic studies converged in showing that MDD

patients reporting AES symptoms carried a higher number of genetic risk variants for the following metabolic traits such as increased obesity, CRP, triglycerides and leptin [44-46].

In contrast to that atypical energy-related symptoms, the melancholic symptoms profile was specifically associated with cardiovascular disorders. Different potentially shared risk factors or mechanisms may explain this association. For instance, depressed individuals expressing a melancholic symptom profiles have been shown to be more likely smokers as compared to other patients [44, 45]. Biologically, individuals with depression who reported insomnia, early morning awakening, and decreased appetite were also experiencing HPA and locus ceruleus-norepinephrine LC-NE systems hyperactivation [46]. Hyperactivation of both systems was also linked to an imbalance in the autonomic tone (i.e., sympathetic and parasympathetic nervous systems). Not only activation of the sympathetic system, but the withdrawal of vagal tone (i.e., decreased activity of parasympathetic nervous system) was also associated with the melancholic subtype [47]. Researchers found that decreased heart rate variability (HRV) accompanied by increased resting heart rate were associated with this subtype of MDD compared to control [47]. This hyperactivation of the sympathetic and decreased parasympathetic nervous systems was associated with pro-inflammatory factors and heart rate variability associated with cardiovascular disease [48-50]. It is plausible that the differential association between the two depressive profiles (i.e., AES and melancholic) with the incidence of the two cardiometabolic profiles is rooted in partially distinct complex network of the underlying biological pathways and behavioral lifestyles. In addition to the abovementioned evidence, this explanation is supported by the recent postulation of possible distinct symptoms specific psychopathological pathways that links depression with cardiac risk, one through BMI and inflammation and the other through dysregulation of HPA and the autonomic nervous system [51]. Nonetheless, the exact nature of this specific associations is still unknown and requires further investigation in future research including mechanistic studies.

Several methodological aspects of this study should be addressed. The large sample size, the detailed information of the depressive symptomatology, the follow-up and the detailed information about cardiometabolic outcomes allowed us to investigate the heterogeneity of depression and cardiometabolic diseases. However, there were some limitations. For example, depressive symptoms were evaluated via a self-report questionnaire. Nonetheless, IDS-SR30 is time and cost-efficient for research purposes and showed high concordance with clinical diagnosis of depression [52]. Second, depressive symptomatology data was only available at the baseline, so we were unable to evaluate the depressive symptoms at the time of the occurrence of the cardiometabolic diseases. However, a recent

study [53] showed a remarkable stability of depressive symptoms measured with IDS-SR30 over nine years follow-up in 1941 participants of the NESDA study. Third, we cannot rule out the possibility of reverse causality. We do however consider this highly unlikely, especially due to the fact that we excluded participants with cardiometabolic diseases at the baseline.

In conclusion, we confirmed the previous association between depressive symptoms and increased risk of developing cardiometabolic diseases. Additionally, disaggregating depressive symptoms in different profiles showed a specific trend of associations with cardiometabolic risk. Following up on patients with depression for developing cardiometabolic diseases and measuring depressive symptoms in individuals at risk for cardiometabolic diseases could be beneficial in primary and secondary preventive efforts. Our findings suggest that such preventive efforts may benefit from a more personalized approach taking into account differential symptom manifestations.

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8

Discussion

DISCUSSION

Brief introduction of the main aims and findings

Obesity, depression and cardiometabolic diseases are complex phenotypes [1, 2]. Their heterogeneity complicates studying them individually and hinders efforts to understand the links between them. This thesis aimed to elucidate the relationship between obesity and depression and possible mechanisms linking both conditions together and with cardiometabolic diseases.

Figure 1 in chapter 1 illustrates the outline of this thesis. First, our aim in **chapter two** of this thesis was to examine the relationship between obesity and depression in N=6459 participants. We uniquely dissected both obesity and depression in our analysis. Instead of relying only on body mass index (BMI), we used it together with three other adiposity measures. Two of the four measures reflect the overall adiposity (BMI and total body fat), and the other two reflect the abdominal adiposity (waist circumference and visceral adipose tissue). For the depression side, we assessed 30 depressive symptoms (IDS-SR30). We found that all four measures of adiposity were positively associated with depressive mood and individual symptoms of depression. Furthermore, this link between measures of adiposity (particularly total body fat) and depressive symptoms appeared to be more robust for atypical energy-related depressive symptoms (increased weight, increased appetite, low energy level and leaden paralysis).

Second, to identify plasma metabolites associated with depression, in **chapters three and four**, we performed two studies with two different metabolomics platforms measuring more than 1000 metabolites with a limited cross-platform overlap (N=18 metabolites). The first and the second metabolomics studies used data from, respectively, nine (total N=15 428) and five (total N= 13 596) Dutch and European cohorts from the general population and clinical settings. In **chapter three**, by using a targeted lipid-based metabolomics platform, we found a metabolic signature for depression characterized by twenty-one lipids, fatty acids, and low-molecular-weight metabolites: as compared to non-depressed controls, participants with depressed mood had lower levels of high-density lipoprotein (HDL), short-chain fatty acid and ketone body acetate and higher levels of very low-density lipoprotein (VLDL), triglyceride particles, glycoprotein acetyls, tyrosine and isoleucine. Associations were generally consistent across sex, age, and BMI strata and across cohorts assessing depression diagnoses with psychiatric interview versus those assessing depressive symptoms with self-report instruments. Furthermore, in **chapter four,** leveraging a wide untargeted metabolomic platform, we identified 53 metabolites associated with depression, including those in the monoamine and neurotransmitter pathways (serotonin, kynurenate and glutamate). These associations were partially explained by

antidepressant use (i.e., a possible proxy for depression severity). We also identified novel associations for retinol (vitamin A), 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1) (lecithin), and lower levels of 2-aminooctanoate, 10-undecenoate (11:1n1), 1-linoleoyl-GPA (18:2) with depression. These novel associations were not explained by antidepressant use, cardiovascular medication and lifestyle factors.

Next, in **chapter five**, we extended the use of the same metabolomic platform applied in chapter three to investigate depression heterogeneity. We performed a data-driven clustering analysis based on depressive symptoms and metabolomics in N=1094 participants diagnosed with clinical Major Depressive Disorder (MDD) (i.e., in the last six months) from the Netherlands Study of Depression and Anxiety (NESDA). We aimed to identify depression dimensions associated with an adverse metabolic profile. Clustering analysis identified the following two metabolite-depression dimensions. The first dimension was characterized by a substantially uniform endorsement of mood, cognitive, and somatic depressive symptoms and lower levels of metabolic dysregulations. The second is a dimension with relatively stronger contribution from energy-related behavioral symptoms (such as sleeping too much, increased appetite, and low energy levels) and increased levels of metabolic dysregulations. After the clustering step, we examined the association between these dimensions and the same metabolomics panel and individual components of cardiometabolic diseases (fasting glucose levels, insulin resistance, total body fat, and visceral adipose tissue) in N=6572 participants from the NEO study. The first depression dimension was associated with a lower cardiometabolic risk profile. In contrast, the dimension with relevance for energy-related depressive symptoms was associated with higher visceral adipose tissue, triglyceride levels, branched-chain amino acids, glycoprotein acetylase, insulin resistance and lower HDL-cholesterol levels.

In chapter six, we investigated whether the established link between adiposity and atypical energy-related symptoms of depression is rooted in underlying metabolic dysregulations. In this analysis, we uncoupled the effect of adiposity from that of metabolic dysregulations in relation to atypical energy-related symptoms profile by studying the relationships between two previously defined adiposity increasing genetic risk scores (GRS) and atypical energy-related symptoms profile. Both genetic instruments used in this study were associated with increased body fat. The difference between them was that one genetic risk score was associated with the predisposition to an unfavorable metabolic profile (i.e., metabolic dysregulations), whereas the other was associated with a favorable metabolic profile. We meta-analyzed results from two individual studies; the NEO study (N= 5734) and NESDA (N= 2238). We found that higher atypical energy-related depressive symptoms was positively and specifically associated with GRS that increased the risk of adiposity accompanied by metabolic dysregulations,

but not with the GRS of obesity with a favorable metabolic profile; these findings suggest that metabolic dysregulation represents a connecting mechanism between adiposity and atypical energy related symptoms of depression.

Finally, in **chapter seven**, we explored the association between different depressive symptom profiles and the risk of development of cardiometabolic diseases in N= 6561 individuals from the NEO study, over a median follow-up of seven years. We were able to disentangle the components of the exposure (depressive symptoms categorized in overall depression and atypical energy-related symptoms profile) and the outcome (cardiometabolic diseases categorized as type 2 diabetes and cardiovascular disease). We found that overall depression was associated with an increased risk of cardiometabolic disease. More specifically, the atypical energy-related symptoms profile was significantly associated with an increased risk of type 2 diabetes onset.

Insights based on the main findings

The results of this thesis render two major insights. First, the interrelatedness between obesity and depression goes deeper than distal factors such as social stigma, self-image, or the use of medication and lifestyle, since our analysis reported an overlap between metabolic signatures in depression and obesity that was not fully explained by these factors. We hypothesized that metabolic dysregulation is a potential biological candidate that could (at least partially) explain the comorbidity between obesity and depression (see The potential role of metabolic dysregulation in the link between obesity and depression section). Second, the connection between depression, metabolic dysregulation and obesity varied due to depression heterogeneity and was strongest for a specific depressive symptom profile. We found that metabolic dysregulations correlated more consistently with atypical energy-related symptoms profile. This symptoms profile was positively associated with adiposity only in the presence of metabolic dysregulations. Depression heterogeneity also impacted the link between depression and cardiometabolic diseases with atypical energy-related symptoms profile increasing specifically the risk of type 2 diabetes.

The potential role of metabolic dysregulation in the link between obesity and depression

Many interconnected biological pathways can explain how metabolic dysregulation links obesity and depression and how the two conditions can further lead to cardiometabolic diseases. Firstly, it is possible that obesity causes depression, mediated through inflammation, insulin resistance, and metabolic dysregulation. Previous molecular epidemiological studies (i.e., Mendelian Randomization) suggested a causal role of obesity in developing depression [3]. Similarly, another recent Mendelian randomization suggested a causal role of obesity in

increased C-reactive protein (CRP) levels [4]. Inflammation has been shown to impact on psychopathological processes relevant for depression, alterations in monoaminergic neurotransmission, tryptophan degradation towards neurotoxic end-products, glutamate-related increased excitotoxicity, decreased neurotrophic factors synthesis or hypothalamic-pituitary-adrenal(HPA)-axis activity disruption [5]. Inflammation may also alter the function of two closely connected hormones (leptin and insulin) giving rise to insulin resistance [6] and leptin resistance [7]. Leptin is secreted proportional to the body's adiposity and is known, along with insulin, as the "fed state" hormones [8, 9]. Both hormones have receptors in the hypothalamus, the area of the brain responsible for maintaining the overall body homeostasis, which, if compromised, is linked with depression [10, 11]. Longitudinally, elevated acute phase cytokines and proteins in the baseline increased the risk of developing depressive symptoms [12, 13]. Also, CRP interferes with leptin binding with its receptor leading to leptin resistance [14]. Leptin resistance causes elevated leptin concentrations, which in turn inhibits insulin secretion from pancreatic β cells [15].

Impairment of insulin function is linked to metabolic dysregulation that may lead to depression. A wide range of metabolic dysregulations, such as disrupted lipid and glucose metabolism, has frequently been reported in obesity and depression [10, 16-18]. This is in line with results from our metabolomic-depression analysis (chapters three and four), where we used two large scales metabolomic platforms to investigate the metabolic signature of depression. For example, we reported increased VLDL, triglyceride, and lower HDL cholesterol. These findings show an overlap between metabolic signatures in both obesity [19] and depression.

Secondly, another possibility is the reverse, i.e., that depression causes obesity, mediated by metabolic dysregulation. Adulthood and early life stress cause depression that may intervene with food choices, physical activity, and metabolic homeostasis leading to dyslipidemia, inflammation, and metabolic dysregulation. Alterations in circulating lipid concentrations may be linked to pathophysiological pathways related to depression and obesity, such as chronic activation of the hypothalamic-pituitary-adrenal (HPA) axis or chronic low-grade inflammation [20]. Glucocorticoid-induced hypercortisolemia is known to result in lipolysis, the release of fatty acids and synthesis of very-low density lipoprotein (VLDL) [21]. Similarly, activation of the pro-inflammatory response leads to a reduction in HDL cholesterol and phospholipids, and an increase in triglyceride, caused by the compensatory production and accumulation of phospholipid-rich VLDL [22]. From metabolomic-depression analysis (chapters three and four), we found that lower levels of high-density lipoprotein (HDL), short-chain fatty acid and ketone body acetate and higher levels of very low-density lipoprotein (VLDL), triglyceride particles, glycoprotein acetyls, tyrosine, and vitamin A were associated with

depression. Vitamin A has previously been suggested as a cause of dyslipidemia by increasing the synthesis of triglyceride-rich very-low-density lipoproteins (VLDLs), inhibiting fatty acid degradation, and affecting the synthesis of apolipoproteins in the liver [23, 24].

Lastly, a common cause could influence both depression and obesity. Carrying a genetic disposition to leptin and insulin resistance independently or with a genetic predisposition for inflammation may precede and give rise to metabolic dysregulation, leading to both obesity and depression. Leptin stimulates the appetite-suppressing [25] proopiomelanocortin (POMC) neuron in the nucleus of the hypothalamus that activates the transcription of the melanocortin peptides (i.e., α, β, and γ MSH, and Mc3r and Mc4r) [26]. It has been proposed [27] that alterations of the leptin–melanocortin pathway impair not only its anorexigenic effect, leading to obesity, but also its effect on mood regulation, potentially leading to the development of depression. A recent study [28] identified five shared genetic risks between depression (or its treatment) and obesity. Two of these genes are components of the leptin-melanocortin pathway (i.e., proopiomelanocortin (POMC) and brain-derived neurotrophic factor (BDNF)). The link between obesity and metabolic dysregulation through leptin resistance and further depression may explain our findings from chapter two. We reported a positive association between depression and total body fat. Symptoms of depression related to disturbance of energy homeostasis were associated with total body fat (see below 'Heterogeneity of depression and obesity'). This result is in line with the previous work that examined the relationship between adiposity and depression (by using BMI as a proxy for total body fat) in epidemiological studies [10, 29-31]. Thus, metabolic dysregulation may act in two non-mutually exclusive ways: as a common underlying factor influencing the liability to both depression and obesity or as mediating mechanism in causal relationships between these conditions [10].

Heterogeneity of depression and obesity

We confirmed the existence of different dimensions within the construct of depression rooted in partially divergent underlying biological and genetic mechanisms. In this thesis, we observed that the link between obesity and depression was more apparent when considering the heterogeneity of depression and obesity. Similarly, the association of depression with cardiometabolic diseases changed as a function of depression heterogeneity. We found from the results of chapter two that depressive symptoms related to energy homeostasis were relatively more strongly linked to total body fat (i.e., adiposity) as compared to other symptoms. From the results of chapter six, we found that atypical energy-related symptoms profile was positively associated with the genetic variants that increased the predisposition to increase total body fat with metabolic dysregulation but not with the genetic variants that increased the predisposition to

obesity without metabolic dysregulation. This aligns with the recently introduced transdiagnostic model of immuno-metabolic depression (IMD) [32], suggesting that inflammatory and metabolic dysregulations act as a shared substrate influencing the development of specific behavioral symptoms common to depression and obesity. For instance, as mentioned above, alterations in central signaling of leptin and insulin may associate with shifting body energy balance from expenditure to accumulation. This shift favors the development of hyperphagia, present in both obesity and atypical energy-related form of depression. Previous research [33] has shown that among patients with a current diagnosis of depression, higher leptin concentration in the blood is associated with depressive symptoms related to energy metabolism like hyperphagia, fatigability and physical exhaustion, independently from BMI. This agrees with our results from chapter two, where we reported that the effect estimates for symptoms of this cluster were the top-ranked for the associations between individual depressive symptoms with total body fat (i.e., closely linked to leptin concentration). Additionally, our results from chapter five, where we performed a data-driven clustering analysis between metabolites commonly associated with cardiovascular health and depressive symptoms, show the presence of a specific dimension with higher relative relevance for symptoms like difficulty falling asleep, sleeping too much, increased appetite, and low energy level correlates with metabolic dysregulations. These metabolic dysregulations have been hypothesized to link depression and cardiometabolic diseases. For example, immuno-metabolic dysregulations such as marked by elevated plasma concentrations of triglycerides, IL-6, and CRP, were causally related to depression [34]. Interestingly, a recent study has shown that inflammation as measured by IL-6 activity but not CRP is a potential cause for a specific symptoms profile of depression, such as sleep problems or fatigue [35]. Finally, our findings suggest that metabolic dysregulation links obesity and depression with some but not all elements of cardiometabolic diseases. For example, atypical energy-related symptoms profile was specifically related to an increased risk of type 2 diabetes but not cardiovascular disease.

Future work

We suggest three important areas of research in this field for the coming years. Firstly, more longitudinal studies that aim to study the relationship between depression symptoms profile and obesity and cardiometabolic diseases are needed to understand the directionality of the reported associations. Second, experimental mechanistic studies and genetically informed designs such as Mendelian Randomization may identify the presence of causal processes underlying these associations. Finally, future randomized control trials aiming to target the underlying immuno-metabolic dysregulations via pharmacological or behavioral interventions (such as exercising, dieting and sleep hygiene) in patients with depression expressing atypical energy-related symptoms are needed to help

us understand to what extent treating underlying metabolic dysregulation will contribute to mitigate this symptoms profile adversity.

Methodological considerations

Several methodological aspects of this thesis should be considered. The main strength of the analysis of this thesis is using data from two large and deeply phenotyped cohorts. The NEO study has detailed information about obesity phenotype with additional information about depression, and the NESDA has detailed depression phenotype with additional information about obesity. Both cohorts have the same depressive symptoms instruments, lipid-related metabolomic data, and obesity-related genetics that allowed us to perform discovery-replication and pooled analysis in the two cohorts. However, some methodological limitations should be acknowledged. First, the observational nature of the analyses in this thesis does not allow us to completely rule out the possibility of residual confounding. However, due to the design of the cohorts, we could adjust for a broad set of relevant confounding factors related to the studies' associations, including age, sex, educational level, smoking, alcohol consumption, physical activity, antidepressants, lipid-lowering drugs, and ethnicity. Second, most of the studies of this thesis were performed in a cross-sectional design which does not allow us to infer causality in the detected associations. Third, we cannot rule out the possibility of reverse causality due to the nature of observational studies in chapter seven, where we performed a longitudinal analysis between baseline depressive symptoms profiles and developing type 2 diabetes and cardiovascular disease. However, we consider this highly unlikely, mainly because we removed participants with cardiometabolic diseases at the baseline. Fourth, in the NEO study, the depressive symptoms were assessed only via the self-report IDS-SR30 without a clinical diagnosis of depression. Nonetheless, IDS-SR30 is time and cost-efficient for research purposes and showed high concordance with the clinical diagnosis of depression [36].

The implication of this work

This thesis adds to the existing knowledge that encourages the consideration of a more refined classification for depression based on depressive symptoms profiles and their possible biological underpinnings. Albeit healthcare providers are shifting from assessing adiposity solely based on BMI by incorporating waist circumference and lipid profile to diagnose the overall health profile, less has been done so far regarding depression heterogeneity. It is essential to increase awareness about the different manifestations of depression symptomatology, which may arise from potentially divergent pathophysiological pathways. Two individuals with the same DSM-5 scores could have completely different symptoms profiles, biological vulnerabilities and disease trajectory or prognosis. Thus, it is important that healthcare providers become aware of the link between depressive

symptom profiles and their associations with biological biomarkers related to other health problems such as obesity, insulin resistance, type 2 diabetes and cardiovascular disease. Target screening of specific symptom profiles can provide better healthcare for patients with depression. This screening can also be used to protect from, or delay, the manifestation of metabolic dysregulations to cardiometabolic diseases (i.e., type 2 diabetes and cardiovascular disease). When patients with depression are expressing atypical energy-related symptoms profile, it may be useful to monitor their metabolic health biomarkers to prevent the development of cardiometabolic diseases. Our results highlight the importance of considering the instruments to assess depressive symptoms in research and clinical practice. In most studies, psychometric instruments are used to ask about changes in neurovegetative symptoms such as appetite and sleep, but not about the direction of that change. The overwhelming majority of questionnaires assessing depressive symptoms conflate opposite changes in neurogenerative symptoms (example: one question conflating decreased and increased appetite: "Poor appetite or overeating" from UK Biobank mental health questionnaire (MHQ) [37, 38] and another question from the UK Biobank computerized touchscreen interface questionnaire [39] evaluating the presence of a change in the weight but not the direction of that change, such as loss or gain weight: "Compared with one year ago, has your weight changed?" with the following multiple choices No - weigh about the same, Yes - gained weight, Yes - lost weight, Do not know, Prefer not to answer). However, based on the results of this thesis, the connection between changes in appetite and metabolic dysregulation seems stronger for one specific direction of the changes (i.e., increased appetite and weight gain). Adding to that, refining the depression phenotype will increase the precision of the genetic studies that aim to comprehend depression genetic architecture [40]. In the clinical setting, we also should increase awareness about the correlation between depressive symptoms profiles with distinct biological and clinical manifestations when treating patients with depression. It is crucial to take a close look at the symptoms expressed in each patient. Based on the results of this thesis, we demonstrated that participants with depression expressing atypical energy-related depressive symptoms might carry genetic and clinical vulnerability to insulin-resistance related illness (i.e., adiposity, metabolic dysregulations, and type 2 diabetes). Similarly, diseases that are usually put under the label of cardiometabolic diseases should be studied separately as research has shown that each may have a partially distinct pathophysiology. The original definition of cardiometabolic diseases was used to describe the elements of metabolic syndrome and the diseases that they predict (i.e., stroke, heart disease, and type 2 diabetes). However, the definition of cardiometabolic diseases has expanded recently to include cardiovascular diseases, insulin resistance-related diseases, and renal function related diseases (example [28, 41]). Although all these conditions are closely related, it may be beneficial to distinguish groups of diseases that share similar underlying pathophysiology.

In chapter seven, we found that atypical energy-related depressive symptoms were associated with an increased risk of type 2 diabetes but not cardiovascular disease (i.e., both labelled as cardiometabolic diseases). The 2016 guidelines on cardiovascular disease prevention from The European Society of Cardiology's (ESC) [42] recommend active screening for increased cardiometabolic risk factors such as obesity, type 2 diabetes and depression starting from age 40 for men and age 50 for women at least once every five years. We argue that following up on patients with depression for cardiometabolic diseases and measuring specific depressive symptoms in individuals at risk for cardiometabolic diseases could be beneficial in primary and secondary preventive efforts. Additionally, preventive and treatment efforts may benefit from a more personalized approach taking into account differential depressive symptoms manifestations. Very recently, clinical trials [43-45] have started testing the efficacy of targeting immuno-metabolic pathways in the treatment of specific subgroups of depressed patients selected based on their bio-clinical profile. Among these clinical studies, the INFLAMED trial [45] is currently testing the efficacy of an anti-inflammatory add-on to standard antidepressants in the treatment of MDD patients expressing atypical energy-related symptoms and with sign of low-grade inflammation.

Conclusion

Our findings highlight the importance of considering the heterogeneity of adiposity, depression, and cardiometabolic diseases. The complex nature of the relationship between the three conditions makes it challenging to draw a one-size-fits-all conclusion. Our results suggest that metabolic dysregulation is a potential biological mechanism that links specific forms of depression with obesity. This proposed mechanism could lead to the development of cardiometabolic diseases. In this thesis, we found that the atypical energy-related symptoms profile - characterized by behavioral symptoms reflecting altered energy intake and expenditure (i.e., increased appetite, increased sleepiness, low energy level, leaden paralysis, increased weight) - is the main driver of the relationship between depression, adiposity, immune-metabolic dysregulation and their later manifestation (type 2 diabetes). It is important to raise awareness about the depression heterogeneity and how distinct symptoms profile such as atypical energy-related symptoms profile could further correlate with clinical manifestation of metabolic dysregulation and increase the risk of debilitating diseases such as type 2 diabetes. Future detailed genetics and experimental studies that aim to answer the causation question are needed in order to move forward to better precise and personalize diagnosis and treatment for all patients with depression, obesity and cardiometabolic diseases.

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A

NEDERLANDSE SAMENVATTING

De relatie tussen obesitas en depressie blijkt complex te zijn en wordt niet volledig begrepen. Obesitas en depressie zijn in twee richtingen met elkaar verbonden: enerzijds verhoogt depressie het risico op obesitas, terwijl obesitas op zijn beurt het risico op depressie verhoogt. Echter, niet elk persoon met depressie heeft obesitas en niet elk persoon met obesitas is depressief. Zowel obesitas als depressie zijn geassocieerd met een verhoogd risico op cardiometabole ziekten. Hieronder vallen hart- en vaatziekten en diabetes mellitus type 2. Het verrichten van onderzoek naar de associatie tussen obesitas, depressie en cardiometabole ziekten wordt bemoeilijkt door hun complexiteit en heterogeniteit. Bovendien is aangetoond dat deze associatie slechts gedeeltelijk wordt verklaard door leefstijl, medicatie en de aanwezigheid van comorbiditeiten. De hypothese luidt derhalve dat er mogelijk sprake is van biologische verbindingen tussen de drie ziektebeelden.

Obesitas wordt gekenmerkt door een verschuiving van de energiebalans naar overmatige vetopslag, dat over het algemeen plaatsvindt in het gehele lichaam en voornamelijk in de buikholte. Dit teveel aan vet is geassocieerd met verstoringen van het immuunsysteem als gevolg van laaggradige inflammatie. Daarnaast is er sprake van metabole ontregeling die verstoringen veroorzaakt in het transport van essentiële stoffen door het lichaam, die nodig zijn voor de energiebalans (bekend als 'homeostase'). Volgens de World Health Organization (WHO) wordt obesitas gedefinieerd als een body mass index (BMI) groter dan of gelijk aan 30 kg/m^2, waarbij BMI wordt berekend als gewicht gedeeld door lengte in het kwadraat. Alhoewel BMI een hoge correlatie heeft met de hoeveelheid vet die in het lichaam is opgeslagen als vetweefsel, wordt hierbij geen onderscheid gemaakt met hoge vetvrije massa, oftewel spiermassa. Bovendien kan het gebruik van BMI problematisch zijn wanneer etniciteiten met elkaar worden vergeleken, aangezien daarbij sprake is van verschillende lichaamsstructuren en -samenstellingen. Dit kan tot onjuiste conclusies leiden als BMI-afkappunten zonder correctie voor de verschillende etniciteiten worden gebruikt. Daarom meten wij in dit proefschrift zowel het totale lichaamsvet als de vetverdeling in het lichaam. Naast de heterogeniteit van meeteenheden en definities van obesitas, zijn er vele subtypes van obesitas. Twee subtypen zijn tegengesteld aan elkaar en zullen in het kader van dit proefschrift hier beschreven worden: a) 'metabolisch ongezonde obesitas', die geassocieerd wordt met overtollig lichaamsvet en de aanwezigheid van ontsteking en metabole ontregeling; b) 'metabolisch gezonde obesitas' die geassocieerd wordt met overtollig lichaamsvet en een gezond (of gunstig) metabool profiel.

Depressie uit zich in aanhoudende neerslachtigheid en/of het onvermogen om plezier te voelen. Dit treedt op in combinatie met cognitieve symptomen (zoals verminderde concentratie of besluiteloosheid) en somatische symptomen (zoals

vermoeidheid, pijn, toe- of afname van gewicht en eetlust). De diagnose depressie wordt gesteld volgens de criteria van de Diagnostic and Statistical Manual of Mental Disorders (DSM-V). Iemand dient dan gedurende meer dan twee weken aanzienlijke functionele beperkingen hebben met vijf van de negen symptomen, twee daarvan moeten fundamentele symptomen van depressieve stemming en anhedonie zijn. Depressie kan worden vastgesteld middels een gestructureerd klinisch diagnostisch interview, zoals het Composite International Diagnostic Interview (CIDI, versie 2.1), of middels gevalideerde zelfrapportagevragenlijsten, zoals de Inventory of Depressive Symptomatology (IDS-SR30). De IDS-SR30 evalueert op een 4-puntenschaal de aanwezigheid van 30 depressieve symptomen tijdens de laatste week en scoort de ernst van deze symptomen. Doordat op vele verschillende manieren aan de DSM-criteria voor depressie kan worden voldaan, kwam er recentelijk meer aandacht voor depressie heterogeniteit. Verschillende subtypes en dimensies van depressie zijn reeds beschreven. De meest cruciale dimensie van depressie voor dit proefschrift is een cluster van somatische symptomen die gerelateerd zijn aan de beschikbaarheid en het verbruik van energie in het lichaam. Deze symptomen zijn toegenomen slaperigheid, toegenomen eetlust, toegenomen gewicht, laag energieniveau en fysieke uitputting. Dit symptomenprofiel werd consequent in verband gebracht met obesitas, ontsteking, metabole ontregeling en cardiometabole ziekten.

Aanvankelijk werd de term 'cardiometabole ziekten' geïntroduceerd om alle metabole ontregelingen ten gevolge van insulineresistentie te beschrijven; zoals eerder vermeld betreffen dit het metabool syndroom, hart- en vaatziekten en diabetes mellitus type 2. Echter, momenteel heeft de term geen duidelijke definitie. Het wordt gebruikt om diabetes mellitus type 2 en hart- en vaatziekten te beschrijven, maar ook hun risicofactoren zoals insulineresistentie, hypertensie, hyperglykemie, dyslipidemie en soms ook nierziekten. Dit impliceert een heterogeen karakter van de term cardiometabole ziekten, met name door het feit dat factoren die enerzijds diabetes voorspellen, zoals componenten van het metabool syndroom, niet altijd (of maar zwak) hart- en vaatziekten voorspellen.

In dit proefschrift streefden we ernaar om de aard van de relatie tussen obesitas, depressie en cardiometabole ziekten te ontrafelen. We karakteriseerden de associatie tussen verschillende maten van obesitas en metabole dysregulaties (die gewoonlijk gelinkt worden aan obesitas), en depressie. Verder onderzochten we of deze associatie varieerde tussen verschillende depressieve symptoomprofielen. Ook wilden we de rol van metabole ontregeling onderzoeken als mogelijk verbindingsmechanisme tussen obesitas en een depressief profiel, dat gekenmerkt wordt door atypische symptomen die de energiehomeostase weerspiegelen. Ten slotte wilden we het risico van algemene depressie en specifieke depressieve

symptoomprofielen op het ontwikkelen van cardiometabole ziekten nader bestuderen.

Het doel van hoofdstuk 2 van dit proefschrift was om meer kennis te vergaren over de relatie tussen obesitas en depressie. De associatie tussen obesitas en depressie was al eerder bestudeerd, maar wij bekeken de definitie van obesitas op unieke wijze vanuit verschillende invalshoeken. In plaats van ons alleen te baseren op het BMI, die bekend staat als een beperkte maat voor obesitas, gebruikten we het samen met drie andere adipositasmaten. Twee van de vier maten (BMI en totaal lichaamsvet) geven de totale adipositas weer, terwijl de andere twee maten (tailleomtrek en visceraal vetweefsel) de abdominale adipositas weergeven. Het totale lichaamsvet werd geschat middels bio-elektrische impedantieanalyse. Voor het meten van visceraal vetweefsel werd beeldvormend onderzoek verricht middels MRI-scan. Voor het onderzoeken van depressie werd de IDS-SR30 vragenlijst gebruikt. Wij vonden dat alle vier de maten van adipositas (BMI, totaal lichaamsvet, middelomtrek, visceraal vetweefsel) positief samenhingen met depressieve stemming en individuele symptomen van depressie. Bovendien bleek het verband tussen adipositasmaten (met name totaal lichaamsvet) en depressieve symptomen sterker te zijn voor atypische energie-gerelateerde depressieve symptomen; oftewel toegenomen gewicht, toegenomen eetlust, laag energieniveau en loodzware verlamming (fysieke uitputting).

In de hoofdstukken 3 en 4 trachtten we plasmametabolieten te identificeren die geassocieerd zijn met depressie. Metabolieten zijn kleine moleculen die voortkomen uit biochemische processen in het lichaam. Dit werd onderzocht in twee grootschalige analyses met twee verschillende metabolomics-platforms waarbij meer dan 1000 metabolieten werden gemeten met een beperkte overlap tussen de platforms (N=18 metabolieten), in negen Nederlandse en vijf Europese cohorten uit de algemene bevolking en klinische populaties. In de eerste metabolomics studie vonden we een metabole signatuur voor depressie die vergelijkbaar is met dat van cardiometabole ziekten: lagere niveaus van HDL-cholesterol en hogere niveaus van VLDL-cholesterol, triglyceriden en de ontstekingsmarker glycoproteïne acetyls. De associaties werden niet beïnvloed door geslacht, leeftijd en BMI, en waren gelijk voor cohorten met depressie-diagnoses en cohorten met depressieve symptomen. Daarnaast identificeerden we in de tweede metabolomics studie ook nieuwe associaties tussen retinol (vitamine A) en depressie.

In hoofdstuk 5 beoogden we depressiedimensies te identificeren die samenhangen met een verhoogd risico op een ongunstig metabool profiel, door gegevens van metabolomics en depressieve symptomen te combineren. We voerden *data-driven clustering* uit op basis van zowel symptomen als metabolomics bij deelnemers met de diagnose klinische depressie. Om onze bevindingen naar aanleiding van de

clustering te repliceren, onderzochten we in een onafhankelijk bevolkingscohort de associatie van de geïdentificeerde dimensies met hetzelfde metabolomics-panel en individuele cardiometabole ziekten (zoals concentraties van nuchtere glucose, insulineresistentie, totaal lichaamsvet en visceraal vetweefsel). Middels clusteringanalyse werden twee metaboliet-depressiedimensies geïdentificeerd. De eerste dimensie werd gekenmerkt door een vrijwel uniforme bevestiging van een reeks stemmings-, cognitieve en somatische depressieve symptomen en lagere niveaus van metabole disregulaties. De dimensie met vertoonde een relatief sterke bijdrage van energie-gerelateerde symptomen (zoals slaapzucht, verhoogde eetlust en lage energieniveaus) en een verhoogde mate van metabole ontregelingen. Uit de replicatieanalyses bleek dat de dimensie met relevantie voor energie-gerelateerde depressieve symptomen geassocieerd was met meer visceraal vetweefsel, insuline resistentie en hogere concentraties van triglyceriden, vertakte-keten aminozuren, glycoproteïne acetylase en lagere concentraties van HDL-cholesterol dan de dimensie van algemene depressie.

In hoofdstuk 6 gebruikten we genetica (genetics risk score analyse) om het effect van adipositas te onderscheiden van dat van metabole dysregulaties, om na te gaan of het verband tussen obesitas en atypische energie-gerelateerde depressieve symptomen afhankelijk is van de aanwezigheid van metabole dysregulaties. In deze analyse hebben wij het effect van adipositas losgekoppeld van dat van metabole dysregulaties door twee genetische risicoscores (GRS) te creëren die beide geassocieerd waren met adipositas. De ene GRS was ook geassocieerd met de aanleg voor een ongunstig metabool profiel (oftewel metabole dysregulaties), terwijl de andere GRS geassocieerd was met een gunstig metabool profiel. We hebben de resultaten van twee afzonderlijke studies gemeta-analyseerd, namelijk van de NEO-studie en de NESDA. We observeerden dat de GRS dat het risico op adipositas in combinatie met metabole disregulaties verhoogde, geassocieerd was met een verhoogd atypisch energie-gerelateerd depressie profiel. De GRS die gepaard gaat met obesitas met een gunstig metabool profiel was echter niet geassocieerd met een atypisch energie-gerelateerd symptoomprofiel.

Ten slotte onderzochten we in hoofdstuk 7 de associatie van algemene depressie en atypisch energie-gerelateerd symptoomprofiel met het risico op cardiometabole ziekten. We voerden een time-to-event analyse (mediane follow-up periode van 7 jaar) uit om het risico op cardiometabole ziekten en de componenten daarvan (diabetes mellitus type 2 en hart- en vaatziekten) en koppelden deze uitkomsten aan depressie en een atypisch energie-gerelateerd symptoomprofiel van depressie. De uitkomst hiervan was dat algehele depressie samenhing met een verhoogd risico op cardiometabole ziekten. In het bijzonder was het profiel van atypische energie-gerelateerde symptomen geassocieerd met een verhoogd risico op diabetes mellitus type 2.

De uitkomsten beschreven in dit proefschrift dragen bij aan de bestaande overtuiging dat een verfijndere classificatie voor depressie, op basis van symptoomprofielen en hun mogelijke biologische onderbouwing, overwogen dient te worden. Inmiddels wordt adipositas in de dagelijkse praktijk op meer dan alleen het BMI beoordeeld, namelijk ook de tailleomtrek en het lipidenprofiel. Echter, dergelijke aandacht bestaat nog niet voor de heterogeniteit van depressie. Een grotere bewustwording van de verschillende manifestaties van depressie-symptomatologie, die het gevolg kunnen zijn van uiteenlopende pathofysiologische mechanismen, is van essentieel belang. Wanneer een patiënt met depressie een atypisch energie-gerelateerd symptoomprofiel heeft, kan het nuttig zijn om diens metabole biomarkers te controleren om mogelijke ontwikkeling van cardiometabole ziekten te voorkomen. In de klinische praktijk moeten wij ons bij de behandeling van patiënten met depressie ook meer bewust worden van de correlatie tussen symptoomprofielen van depressie en afzonderlijke biologische en klinische manifestaties. Het is cruciaal om goed te kijken naar de symptomen die bij elke patiënt tot uiting komen. De resultaten van dit proefschrift tonen aan dat patiënten met een depressie die atypische energie-gerelateerde depressieve symptomen vertonen, genetisch en klinisch kwetsbaar zijn voor aan insulineresistentie gerelateerde ziekten (namelijk adipositas, metabole ontregelingen en diabetes mellitus type 2). Een gepersonaliseerde aanpak kan behulpzaam zijn in preventie van deze chronische en complexe ziekten. Hierbij dient er rekening gehouden worden met de heterogeniteit van depressie en de associatie tussen atypische energie-gerelateerde symptomen van depressie en deze ziekten.

LIST OF PUBLICATIONS IN THIS THESIS

Alshehri, T., Boone, S., de Mutsert, R., Penninx, B., Rosendaal, F., le Cessie, S., . . . Mook-Kanamori, D. (2019). The association between overall and abdominal adiposity and depressive mood: A cross-sectional analysis in 6459 participants. *Psychoneuroendocrinology, 110*, 104429. doi:10.1016/j.psyneuen.2019.104429

Bot, M., Milaneschi, Y., Al-Shehri, T., Amin, N., Garmaeva, S., Onderwater, G. L. J., . . . Consortium, Bbmri-Nl Metabolomics. (2020). Metabolomics Profile in Depression: A Pooled Analysis of 230 Metabolic Markers in 5283 Cases With Depression and 10,145 Controls. *Biol Psychiatry, 87*(5), 409-418. doi:10.1016/j.biopsych.2019.08.016

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Alshehri, T., Mook-Kanamori, D. O., de Mutsert, R., Penninx, B. W., Rosendaal, F. R., le Cessie, S., & Milaneschi, Y. (2023). The association between adiposity and atypical energy-related symptoms of depression: A role for metabolic dysregulations. *Brain Behav Immun, 108*, 197-203. doi:10.1016/j.bbi.2022.12.005

Alshehri, T., Mook-Kanamori, D. O., Willems van Dijk, K., Dinga, R., Penninx, Bwjh, Rosendaal, F. R., . . . Milaneschi, Y. (2021). Metabolomics dissection of depression heterogeneity and related cardiometabolic risk. *Psychol Med*, 1-10. doi:10.1017/s0033291721001471

Alshehri, T., Milaneschi, Y., de Mutsert, R., Winters-van Eekelen, E., Boone, S., Verkouter, I., ..., Mook-Kanamori, D. Symptomatology of depression and onset of cardiometabolic diseases - A 7-year follow-up study. (In preparation)

PORTFOLIO

Oral and Poster Presentation	Year	Credit
Annual Dutch Diabetes research (NVDO). The Netherlands.	2017	0.25
Netherlands Epidemiological Conference (WEON) Science for society research. The Netherland	2018	1.00
The translational neuroscience network (TN2) conference. Innovation in psychiatry, neurostimulation and inflammation. The Netherlands	2018	0.25
Congresses and Symposia		
The Netherlands association for the study of obesity (NASO) scientific spring meeting	2017	0.75
BBMRI-omics: Introduction and Hands-on Application	2017	0.25
Netherlands Epidemiological Conference (WEON) Epidemiological methods for implementation research. The Netherland	2018	1.00
Courses, Seminars, and Master Classes		
Basic Methods and Reasoning in Biostatistics, Boerhaave Instituut, Leiden, the Netherlands	2017	1.50
Epidemiology "An Introduction" (Based on the book by Kenneth Rothman), Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands	2017	2.00
Regression Analyses (advances course), Boerhaave Instituut, Leiden, the Netherlands	2017	1.50
Survival Analyses, Boerhaave Instituut, Leiden, the Netherlands	2017	1.50
Analysis of Repeated Measurements, Boerhaave Instituut, Leiden, the Netherlands	2017	1.50
Clinical Epidemiology Principals, Methods, and Applications for Clinical Research. Based on the book: "Clinical Epidemiology" by D.E. Grobbee & A.W.Hoes, Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands	2018	3.00
Causal Inference (Hernan), Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands	2018	3.00
Systematic Reviews and Meta-Analyses, Boerhaave Instituut, Leiden, the Netherlands	2018	1.00
International course on clinical Epidemiology Schiermonnikoog, Boerhaave Instituut, Leiden, The Netherlands	2019	2.00

Weekly Research Lunch, Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands	2016-2023	3.00
Weekly Capita Selecta, Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands	2016-2023	3.00
Bi-weekly Journal Club of the NEO study, Department of Clinical Epidemiology, Leiden University Medical Center, the Netherlands	2016-2023	3.00
Weekly (NESDA) lab meeting, Department of Psychiatry, Amsterdam UMC. the Netherlands	2018-2022	3.00
The Netherlands Study of Depression and Anxiety (NESDA)	2018-2022	3.00

Student Monitoring and Teaching	**Year**	**Workload Hours**
Tutoring Working Groups of Courses for Bachelor/ Master Students	2017-2023	40
Supervision of Critical Appraisal of Topic (CAT) Project, Bachelor of Medicine student (Linda Y.S. Jans)	2022	5
Supervision of Master students (Michelle Sinteur and Jeff Kamerman)	2020	20
Supervision of Bachelor students (Sem Dreier Gligoor and Mary-Ann Van Der Linden)	2022	40

Generic and Transferable skills Training by the Graduate School of Leiden University	**Year**	**Workload Hours**
PhD Introductory Meeting	2017	4
Communication skills in science	2017	40

Other activities	**Year**
Reviewing scientific epidemiological publications	2022-2023

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CURRICULUM VITAE

Tahani Alshehri was born on the 23rd of February 1984 in Riyadh, Saudi Arabia. She completed high school in 2001 in Riyadh. She obtained her BSc from the Biochemistry department in 2005 at King Saud University in Riyadh, graduating with First Class Honours. After graduation, she volunteered to work as a technician at the same department, where she was hired as a demonstrator in 2007. In the same year, she joined the Master's program in the biochemistry department. During her Master's study, she gained three years of experience in a specialized molecular biology laboratory in Behavior Genetics (Genetic Department/Research Center/King Faisal Specialist Hospital and Research Center. Riyadh, Saudi Arabia). Her Master's thesis was "Genotype-phenotype correlation in Saudi β-thalassemia patients". In January 2011, she earned her MSc degree of Sciences in Biochemistry from King Saud University. In January 2012, she moved to Iowa City, Iowa, United States, to study English as a second language at Iowa Intensive English Program (IIEP). She was back in Riyadh in January 2014, where she resumed her duties as a demonstrator and, soon after that, was promoted to lecturer in the biochemistry department in 2015. In 2016, she moved to Leiden, the Netherlands, to pursue her PhD in the clinical epidemiology department under the supervision of Prof. dr. F. R. Rosendaal, Dr. D. O. Mook-Kanamori, and Dr. Y. Milaneschi. Tahani's research topic focused on the association between obesity and depression. In her research, she investigated this association from an epidemiological, biochemical (metabolomics), and genetic point of view. In December 2022, she moved back to Riyadh and resumed her work at King Saud University. After earning her PhD, she will be promoted to assistant professor at the same university.