

Depression And Cardiovascular Risk – Association Among Beck Depression Inventory, PCSK9 Levels And Insulin Resistance

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Original investigation

Keywords: Beck Depression Inventory, cardiovascular risk, depression, Framingham Risk Score, obesity, Proprotein Convertease Subtilisin/Kexin type 9

Posted Date: September 1st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-56070/v1>

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Version of Record: A version of this preprint was published on November 3rd, 2020. See the published version at <https://doi.org/10.1186/s12933-020-01158-6>.

Abstract

Background. Depression and cardiovascular disease (CVD) are among the most common causes of disability in high-income countries, depression being associated with a 30% increased risk of future CV events. The association of depression with CV outcomes is likely *via* behavioral mechanisms, *e.g.* sedentary lifestyle and obesity, this last being more frequently linked to the occurrence of depressive symptoms. Proprotein convertase subtilisin/kexin type 9 (PCSK9) has been related to a large number of CV risk factors, *e.g.* insulin resistance. Aim of this study was to investigate whether in a population of obese subjects, depression could affect PCSK9 levels and how these changes may mediate potentially associated pre-diabetic risk.

Results. In 389 obese individuals, mainly women, the Beck Depression Inventory (BDI-II) was significantly associated with PCSK9 levels, *i.e.* for every unit increment in BDI-II score, PCSK9 rose by 1.85 ng/mL and with the homeostatic model assessment index of insulin resistance (HOMA-IR). Mediation analysis suggested that PCSK9 levels partially mediates by 11% the effect of BDI-II on HOMA-IR.

Conclusions. This study proposes a possible mechanism linking depression and insulin resistance, a well-known CV risk factor, providing evidence on a role for PCSK9.

1. Background

The extensive presence of cardiovascular diseases (CVDs) as a leading cause of mortality and morbidity worldwide [1] has repeatedly indicated that monitoring and correction of modifiable major CV risk factors (hypertension, smoking, dyslipidemia, diabetes and others) alone may not be sufficient to reduce the risk [2]. Lifestyle improvement has an important role in CV prevention and in particular excess body weight associates with a shorter lifespan and a significant increased risk of CV morbidity and mortality [3]. However, this condition may be a confounder for the less frequently evaluated clinical conditions linked to the CV risk, *i.e.* anxiety and depression [4]. Indeed, a reciprocal link exists between depression and obesity with depressive mood increasing the odds for developing obesity by roughly 50% [5]. In addition, all-cause mortality and CVD mortality are markedly raised among depressive individuals with prediabetes [6]. As estimated by the WHO, anxiety and depression are major CV risk factors and will become the second leading cause of disability in high income countries by 2030, carrying social and very high healthcare-related costs [7]. A number of meta-analyses have provided evidence of an association between clinical depression (or depressive symptoms) and CVD risk [8], the latter being raised by 30% in this condition [9].

Since the prognosis of patients with CVD and depression associates with a 2- to 4-fold higher risk of subsequent events, an effect directly proportional to the depression severity [10], the screening and the management of depression are strongly recommended for patients with CVD [11]. Up to 15–20% of patients with CVD suffer from depression and two thirds of patients with myocardial infarction (MI) develop depression either concomitant with the event or during follow-up [12].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) has a pivotal role on low-density lipoprotein cholesterol (LDL-C) levels by regulating the degradation of the LDL receptor (LDLR), although direct effects on additional atheroma components have been reported [13]. The ATHEROREMO-IVUS study showed that higher serum PCSK9 levels are linearly associated with a higher necrotic core fraction in coronary atheroma, regardless of serum LDL-C [14], confirming findings in PCSK9 KO mice, partially protected from neointimal formation [15]. In the context of CV prediction, PCSK9 concentrations, which are increased by insulin-resistance [16], are also associated with a raised susceptibility for MI, as assessed by genome association studies [17], although data from the literature are discordant and not conclusive [13]. Relative to the association between mood disorders and PCSK9 levels, a recent study on subjects affected by the alcohol use disorder has shown that PCSK9 cerebrospinal fluid levels are associated with severity of behavioral disturbances [18].

The growing number of subjects affected by major depressive disorders worldwide and the possible association between depression and major adverse CV events [19] make it imperative to find markers predicting an enhanced CV risk. Depression and/or anxiety disorders have been associated recently with a subclinical marker of atherosclerosis, i.e. the thickness of carotid intima-media [20], a tool for the assessment of CV risk [21]. Since, among lipid biomarkers associated to CV prevention, LDL-C seems to be the only one related to depression and its severity [22], this study was aimed to verify, in a population of obese subjects, the association between depression and PCSK9 levels and how these changes may play a role in linking depression with insulin resistance, a well-established CV risk factor [23].

2. Subjects And Methods

2.1 Study design and participants. The baseline study population has been previously described [24]. We selected 389 obese subjects among participants of the cross-sectional SPHERE (Susceptibility to Particle Health Effects, miRNAs and Exosomes) study whose primary endpoint was to evaluate how air pollution exposure acted in a highly susceptible population (such as in obesity). Subjects were recruited from the Center for Obesity and Work-Activity (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan, Lombardia, Italy). The eligibility criteria of the SPHERE study were: 1) older than 18 years at enrollment; 2) overweight/obese according to the following definitions: overweight, BMI between 25 and 30 kg/m²; obese: BMI of 30 kg/m² or more; 3) resident in the Lombardy Region at the time of recruitment. Each participant provided written informed consent approved by the Ethics Committee of Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico (approval number 1425). On the day of recruitment, each subject underwent physical and anthropometric evaluations as well as cardiovascular and pulmonary function tests. The study was carried out over the period September 2010–April 2014.

2.2. The Beck Depression Inventory. All participants were evaluated according to the Beck Depression Inventory II (BDI-II), considered as an appropriate tool to evaluate depressive symptoms in subjects with medical comorbidities such as obesity [25]. The following scores correspond to the different severity of depressive symptoms [25]: < 14-no depression, 14-19-mild depression, 20-29-moderate depression, 30-63-severe depression.

2.3 Clinical and laboratory measurements. Body weight and height were determined on a standard scale, waist circumference (WC) was measured at the umbilical level with subjects standing and breathing normally by the same physician at the end of the physical examination; BMI and weight to height ratio were also calculated. Systolic and diastolic blood pressures (SBP and DBP, respectively) were taken on the left arm using a mercury sphygmomanometer (mean of two measurements taken after 5 minutes of rest). Plasma lipids/lipoproteins and glucose were determined by established methodologies. C-reactive protein (CRP) and liver function tests (ALT, AST and GGT), as well as the full hematological profile (red blood cells, hematocrit and leukocyte formula) were determined. HbA1c was measured by ion-exchange high performance liquid chromatography on a VARIANT II Turbo Instrument (Glyco Hb Control, Menarini Diagnostics, Firenze, Italy); insulin by electrochemiluminescence immunoassay (ECLIA) on the Modular P automated analyser (Roche, Basel, Switzerland). HOMA-IR (homeostasis model assessment-insulin resistance) was assessed by means of fasting plasma glucose (mg/dL) times fasting plasma insulin (mU/L) divided by 405. Quantitative insulin sensitivity check index (QUICKI) is given by $1/[\text{Log}(\text{Fasting Insulin}, \mu\text{U/mL}) + \text{Log}(\text{Fasting Glucose}, \text{mg/dL})]$.

2.4 PCSK9 evaluation (ELISA - Enzyme-Linked Immunosorbent Assay). All patients underwent fasting blood sampling around 9 a.m, thus minimizing the possible confounding effect of circadian variations observed in PCSK9 levels. Plasma PCSK9 concentrations were measured by a commercial ELISA kit (R&D Systems, MN). Samples were diluted 1:20 and incubated onto a microplate pre-coated with a monoclonal human-PCSK9-specific antibody. Sample concentrations were obtained by a four-parameter logistic curve-fit, with a minimum detectable PCSK9 concentration of 0.219 ng/mL. Intra- and inter-assay CVs were 3.2% and 5.1%, respectively.

2.5 Statistical analysis. Data were evaluated with standard descriptive statistics. Categorical variables were presented as absolute numbers and percentages. Continuous data were expressed as the mean \pm SD or as the median and interquartile range (Q1-Q3), as appropriate. Normality and linearity assumptions were verified by graphical inspection. Univariate and multivariable linear regression models were used to test the relation between circulating PCSK9 levels and BDI-II score as the continuous predictor. Multiple analysis to test the association between PCSK9 concentrations and BDI-II was adjusted for *a priori* covariates (age, gender, BMI, smoking habit, antidepressant treatment) and for variables that were significantly related with PCSK9 in univariate analysis (P-value < 0.05). Given the existence of multicollinearity among predictor variables (*i.e.* Total cholesterol and HDL cholesterol) the variance inflation factor (VIF) statistic was calculated. To determine the best performing model for evaluating the P-value, the VIF statistic as well as goodness of fit (R^2) of several model equations, including one or more significant explanatory variables were used to predict PCSK9 levels. Finally, the best model selected to predict the association between circulating PCSK9 levels and BDI-II score was adjusted for: use of antidepressant treatment, age, gender, BMI, smoking habit, use of statin medications, non-HDL cholesterol, use of antihypertensive medications and triglycerides. We subsequently evaluated the association between depression score and the HOMA-IR, as adjusted for the above-mentioned selected covariates. The dependent variable, HOMA-IR, was log-transformed in order to achieve normality of

models' residuals. We performed causal mediation analysis to verify whether a third intermediate variable, *i.e.* PCSK9, is related to the observed exposure-outcome relationship. Linear regression coefficients were estimated by three equations:

- $\text{HOMA-IR} = \text{intercept} + \mathbf{C} \cdot \text{BDI-II} + e$, estimating the total effect of depression severity score on HOMA-IR.
- $\text{PCSK9} = \text{intercept} + \mathbf{a} \cdot \text{BDI-II} + e$, estimating part of the indirect effect of depression severity score.
- $\text{HOMA-IR} = \text{intercept} + \mathbf{b} \cdot \text{PCSK9} + \mathbf{c}' \cdot \text{BDI-II} + e$, estimating part of the indirect effect and the direct effect of BDI-II on HOMA-IR.

Total effect **C** is the sum of direct effect (**c'**) and indirect effect (**a*b**). The use of antidepressant treatment, age, gender, BMI, smoking habit, use of statin medications, non-HDL cholesterol, use of antihypertensive medications and triglycerides were considered as confounders.

All statistical analyses were performed with SAS software (version 9.4; SAS Institute Inc., Cary, NC). The mediation analysis was carried out while utilizing the PROCESS program (model 4) provided by Hayes [Hayes, 2013 #103]. The Bootstrap confidence intervals (CIs) are provided with the number of bootstrap samples equal to 10,000.

3. Results

3.1. Study population. As shown in Table 1, the majority of the 389 subjects were females (68.9% vs 31.1%), with a mean BMI 33 ± 5.2 kg/m² and large WC (mean \pm SD: 100.7 ± 12.7 cm). Blood pressure was in the normal range, 29.1% of participants being on hypertensive medications. The case was similar for cholesterolemia, *i.e.* mean values of TC, LDL-C and non-HDL-C being in the upper range of normal (TC = 214.4 ± 41.1 mg/dL, LDL-C = 133.6 ± 36.2 mg/dL and non-HDL-C = 155.1 ± 41.0 mg/dL). Only 7.7% of participants were on statin medications. HDL-C and TG levels were also in the normal range: 59.0 ± 15.3 mg/dL and 117.2 ± 74.5 mg/dL, respectively. Levels of thyroid stimulating hormone (TSH) were normal (1.8 ± 1.1 U/mL) and no participant was on thyroid substitution/suppression therapies. Among other CV risk factors, 15.7% were current smokers and C-reactive protein (CRP) median level was 0.25 mg/L. There were no significant abnormalities in the standard laboratory tests including liver enzymes (AST and ALT), complete blood count (CBC) and white blood cell formula.

Table 1
Demographic and clinical characteristics of the studied population (n = 389).

Characteristics	Value
Age, years	50 ± 13
Gender,	
<i>Males</i>	121 (31.1%)
<i>Females</i>	268 (68.9%)
WC, cm	100.7 ± 12.7
BMI, kg/m ²	33.2 ± 5.4
Blood pressure,	
<i>Systolic</i>	123.6 ± 15.5
<i>Diastolic</i>	77.2 ± 10.2
Total cholesterol, mg/dl	214.4 ± 41.1
HDL-C, mg/dl	59.0 ± 15.3
LDL-C, mg/dl	133.6 ± 36.2
non-HDL-C, mg/dl	155.1 ± 41.0
Triglyceride, mg/dl	117.2 ± 74.5
C-reactive protein, mg/l	0.25 (0.12–0.52)
Glucose, mg/dl	93.1 ± 14.1
Glycated haemoglobin, mmol/mol	39.1 ± 5.7
Insulin level, U/ml	14.3 ± 8.4
AST, U/l	21.7 ± 9.0
ALT, U/l	26.6 ± 18.5
Gamma-glutamyltransferase, U/l	24.6 ± 17.8
TSH, U/ml	1.8 ± 1.1
Neutrophils, %	58 ± 7.8
Eosinophils, %	2.4 ± 1.5

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TSH: thyroid-stimulating hormone; WC: waist circumference. Continuous variables were expressed as mean ± standard deviation (SD) or as median [first quartile-third quartile], if not normally distributed.

Characteristics	Value
Lymphocytes, %	31.5 ± 7.1
Monocytes, %	7.6 ± 2.4
Basophils, %	0.5 ± 0.3
Granulocytes, %	60.9 ± 7.3
Smoking status,	
<i>Never smoker</i>	182 (47.5%)
<i>Former smoker</i>	141 (36.8%)
<i>Current smoker</i>	60 (15.7%)
Occupation,	
<i>Employee</i>	240 (63.7%)
<i>Unemployed</i>	28 (7.4%)
<i>Pensioner</i>	83 (22.0%)
<i>Housewife</i>	26 (6.9%)
ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TSH: thyroid-stimulating hormone; WC: waist circumference. Continuous variables were expressed as mean ± standard deviation (SD) or as median [first quartile-third quartile], if not normally distributed.	

PCSK9 levels followed a Gaussian distribution (Fig. 1A) with mean levels of 282±116 ng/mL. These values did not appear to be markedly different from those observed in prior studies by our group in the general population [26]. Glycemia and glycated hemoglobin were in the high normal range 93.1 ± 14.1 mg/dL and 39.1 ± 5.7 mmol/mol, respectively, as was the HOMA-IR. This last had a skewed distribution, with a median level of 2.8 (Q1-Q3: 1.9–4.1; Fig. 1B).

3.2. Depression scores. As shown in Table 2, the mean Beck's total score was 11.8 + 8.6. BDI-II mean values were higher in women compared to men, *i.e.* 14 ± 8.7 and 6.9 ± 8.7, respectively ($p < 0.0001$). Less than 10% of the total sample received an antidepressant treatment, *i.e.* selective serotonin or norepinephrine/serotonin reuptake inhibitors (Supplemental Table 1). Antidepressant treatment was frequently prescribed to subjects with more severe depression, the therapeutic approach being quite homogeneously distributed among the different clusters of depression severity.

3.3. PCSK9 levels positively associate with BDI-II score and HOMA-IR. In the univariate analysis the severity of depression (BDI-II score) positively associated with PCSK9 circulating levels with a rise of 0.44% ($\beta = 2.75$, Table 3) for every ten-unit increase of BDI-II ($\Delta\% = 0.44$, 95%CI 0.23–0.65, $p < 0.0001$). The strength of this association was confirmed in multivariable analysis, *i.e.* for every ten-unit increment in

BDI-II score, PCSK9 rose by 0.3% ($\beta = 1.85$, $\Delta\% = 0.30$, 95%CI 0.08–0.51, $p = 0.0074$; Fig. 2A), after correction for variables that in univariate analysis were associated with PCSK9 and were not collinear among them (Supplemental Table 2). Relative to the association between PCSK9 and HOMA-IR, in this cohort, a statistical 6.39% rise of the HOMA-IR for every 100 ng/mL increment in PCSK9 levels ($\beta = 0.00619$, $\Delta\% = 6.39$ 95%CI: 0.83–12.23, P-value 0.0237) was observed (Fig. 2B).

Table 2
Descriptive analysis of depressive symptoms (N = 389).

BDI-II scores	Value
Depression total score, <i>Mean ± SD</i>	11.8 ± 8.6
Depression total score by gender*, <i>Mean ± SD</i>	
<i>Male</i>	6.9 ± 6.0
<i>Female</i>	14 ± 8.7
Depression severity category,	
<i>Minimal (0–13)</i>	240 (61.7%)
<i>Mild (14–19)</i>	78 (20.1%)
<i>Moderate (20–28)</i>	58 (14.9%)
<i>Severe (≥ 29)</i>	13 (3.3%)
Use of antidepressive drugs,	
<i>Yes</i>	37 (9.5%)
<i>No</i>	352 (90.5%)
BDI-II, Beck Depression Inventory II	
*P-value from t-test < 0.0001	

Table 3

Linear regression models to evaluate the association of proprotein convertase subtilisin/kexin type 9 [PCSK9 (ng/mL)] levels with severity of depression (BDI-II score).

	β	SE	P-value
Univariate model			
Intercept	249.98	9.85	< .0001
BDI-II Score (for unit increase)	2.75	0.68	< .0001
Adjusted model			
Intercept	188.43	53.14	0.0004
BDI-II Score (for unit increase)	1.85	0.69	0.0074
Age, years	-0.07	0.48	0.8824
Gender			0.0004
<i>Male</i>	-46.10	12.86	
<i>Female</i>	Ref		
BMI	-0.29	1.00	0.7673
Smoking status			0.0157
<i>Current smoker</i>	21.29	15.67	0.1752
<i>Former smoker</i>	34.13	11.88	0.0043
<i>Never smoker</i>	Ref		
non-HDL, mg/dl	0.49	0.15	0.0015
Statin medications			
<i>Yes</i>	63.35	21.22	0.0022
<i>No</i>	Ref		
Triglyceride, mg/dl	0.07	0.08	0.3730
Antihypertensive medications			
<i>Yes</i>	13.07	13.9	0.3479
<i>No</i>	Ref		
Antidepressive medications			

BDI-II, Beck Depression Inventory II score; BMI: body mass index; HDL: high-density lipoprotein; PCSK9: pro protein convertase subtilisin/kexin type 9.

	β	SE	P-value
Yes	0.34	18.02	0.9848
No	Ref		

BDI-II, Beck Depression Inventory II score; BMI: body mass index; HDL: high-density lipoprotein; PCSK9: pro protein convertase subtilisin/kexin type 9.

3.4. Causal Mediation. The results of mediation analysis suggested that the association between HOMA-IR and BDI-II score is partially mediated by PCSK9 levels. The analysis showed that the BDI-II score has a significant overall effect on HOMA-IR (total effect C, $\Delta\%$ =1.04, 95%CI: 0.34–1.75, $p = 0.0038$; Fig. 2C and Fig. 3). The direct effect of the BDI-II on HOMA-IR was significant (direct effect c' , $\Delta\%$ =0.93, 95% CI: 0.22–1.65, $p = 0.0105$, Fig. 3). Another part of such effect may operate through PCSK9 (indirect effect C- c' , $\Delta\%$ =0.11 bootstrapped 95%CI: 0.02–0.31, Fig. 3), Consequently, of the total effect of BDI-II on HOMA-IR, 11% appears to act via PCSK9.

4. Discussion

The present study carried out on 389 obese individuals, mainly females, [24] supports the following conclusions: 1) PCSK9 levels appear to be raised by depressive symptoms as assessed by BDI-II score, 2) depression symptoms directly associate with HOMA-IR, a pre-diabetes related CV risk variable [23] and 3) 11% of such an effect operates via an indirect path, through PCSK9. Reliability of these conclusions relies on the following three pillar criteria : a) statistically significant association between depression and outcome (HOMA-IR); b) exposure (BDI-II score) must have an effect on the mediator (PCSK9), c) the mediator has to be associated with the outcome (HOMA-IR) when exposure is controlled, *i.e.*, after adjusting for PCSK9 levels.

In order to identify individuals depressed or at high risk of depression with a potential CV risk, a number of screening tests are now available [27]. These can be applied successfully also to individuals who may be at CV risk. Aside from the widely reported questionnaires, commonly used for the screening of depression, such as the PHQ-2 [28] of some value in order to evaluate large numbers of individuals, the more classical and sensitive tests are of better value to obtain a baseline assessment of symptom severity and to monitor subsequent improvement on antidepressant treatments. In the present report the classical BDI was selected, as widely applied also in earlier studies in cardiac patients [29, 30]. More specifically the BDI-II as per the 1996 improved version [31] can be successfully administered both to adolescents and adults, as a tool of medical assistance but also for research. The BDI lists 21 symptoms, with item responses scoring from 0 to 3 and total scores ranging from 0 to 63. Moreover, considering that depression is approximately twice as prevalent in women [32], in our cohort BDI-II mean values were higher in women compared to men, *i.e.* 14 and 6.9, respectively [33].

Among the reported mechanistic links between depression and CVD [8], conditions characterized by insulin resistance have been pointed out as sharing pathogenic mechanisms with depression and

diabetes [34]. Evaluation of the HOMA-IR provides a sensitive biomarker of CVD risk in the forecasting of coronary atherosclerosis, independent of established risk factors, including hsCRP [23], as also confirmed in a large series of Italian type 2 diabetics [35]. The liaison between PCSK9 and insulin resistance has been confirmed in obese individuals undergoing gastric bypass. Roux-en-Y gastric bypass promotes a significant reduction in plasma PCSK9 levels, changes that appeared to be more associated with glucose improvement rather than with lipid homeostasis [36]. Very recently, in severely obese patients undergoing bariatric surgery and euglycemic-hyperinsulinemic clamp, our group confirmed the pivotal role played by adipose tissue and insulin resistance on PCSK9 homeostasis [37].

Concerning the relationship between PCSK9 and CV risk, although some studies on PCSK9 levels did not report any association with CV events [38, 39], other population studies, meta-analyses and genome-wide association studies have supported the predictivity of PCSK9 levels in CV events [40–42]. A genetic reduction of PCSK9 levels by 50% is associated with a similar percent reduction of coronary heart disease (CHD) risk [43] and a genetic large-scale study on 337,536 individuals of British ancestry reported that the rs11591147 loss-of-function *PCSK9* mutation had a protective effect not only on hyperlipidemia but also on the risk of CHD (-27%) and ischemic stroke (-39%) [44]. A further hypothesis linking PCSK9 to atherosclerosis is the direct role that this protein can play on atherogenesis. PCSK9 is expressed in vascular smooth muscle cells and in human atherosclerotic plaques [45] as well as in epicardial fat tissue [46].

Besides raised PCSK9 levels, an enhanced CV risk, in our cohort, may be consequent to the increased incidence of insulin resistance and diabetes mellitus [47]. In a targeted study on depressive symptom incidence and insulin resistance, the HOMA-IR was found to be associated with depression markers in women, not in men [48]. The rise of this major insulin resistance biomarker, also linked to an increased CV risk, points out to a novel pathophysiological background for the development of depressive symptoms, thus, confirming the mechanistic association between PCSK9 and impaired insulin secretion [49].

Finally, it should be recalled that although PCSK9 was discovered in the brain as the human equivalent of NARC-1 (Neural Apoptosis Regulated Convertase-1) implicated in the differentiation of cortical neurons [50], to our knowledge, no data have been reported relative to its relationship with depressive mood. While there is little evidence that PCSK9 may regulate the CNS or the autonomic nervous system [51], a genetic approach showed a significantly increased risk of depression in the case of SNPs related to inhibition of HMG-CoA reductase (OR 1.15) and PCSK9 (OR 1.19) [52].

Since earlier studies indicated that depression and obesity often come hand in hand, the relationship between the two is difficult to tease apart. Data from a Mendelian randomization analysis provided evidence that a higher BMI is likely to have a causal role in determining the likelihood of an individual developing depression [53]. In our cohort, a trend toward a positive association between BMI and depressive symptoms ($\beta = 0.121$, $p = 0.13$) was found, after correcting for age and gender. The complex biological profile of depression does not appear to be clearly linked to definite genetic variants. A more informative approach may take into account life habits and psychological risk factors, eventually leading

to altered hypothalamic responses to metabolic signaling molecules [54]. Findings from the present report, advocating the potential clinical significance of the association between PCSK9 and the HOMA-IR in determining depression, may indicate that plain evaluation of the body weight variable may not be adequate for a full explanation of the PCSK9 elevation.

The present study, conducted on a large number of subjects, has limitations. It is a retrospective analysis in obese patients with no diagnosis of prediabetes or diabetes but with BDI-II scores available at recruitment. Thus, in order to limit possible comorbidities linked to obesity, *a priori* we selected the healthiest participants and further corrected for the possible confounders. Moreover, only a small proportion of those classified with depression were taking psychotropic medications, an aspect similar to prior observations in EUROASPIRE cardiac patients [55]. This may partly be due to our assessment of current, but not previous, symptoms and of the use of psychotropic medications; further, a large proportion of those with depression were likely to have subclinical or mild symptomatology.

5. Conclusions

Among the effects of common modifiable risk factors on CVD and mortality, symptoms of depression are commonly listed. In view of the common occurrence of diabetes linked life-habits with depression [56], PCSK9 may thus offer a pathophysiological link, being associated with an insulin resistance marker, predictor of a raised CV risk. Since depression is highly prevalent in patients with CVD and portends adverse CV outcomes and increased healthcare costs, the identification of a biomarker linked to depression which correlates with insulin resistance may identify people who could benefit most by targeted interventions [57].

List Of Abbreviations

BDI-II, Beck Depression Inventory

BMI, Body Mass Index

CI, Confidence interval

CRP, C-reactive protein

CVD, Cardiovascular disease

HDL, High-density lipoprotein

HOMA, Homeostasis model assessment-insulin resistance

ELISA, Enzyme-linked immunosorbent assay

FRS, Framingham Risk Score

LDL, low-density lipoprotein

NARC-1, Neural Apoptosis Regulated Convertase-1

PCSK9, Proprotein convertase subtilisin/kexin type 9

QUICKI, Quantitative insulin sensitivity check index

SD, Standard deviation

TC, Total cholesterol

TG, Triglycerides

WC, Waist circumference

Declarations

Ethics approval and consent to participate

Ethics Committee of Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico (approval number 1425)

Consent for publication

All the participants gave their written consent

Availability of data and materials

The data will be available on request due to privacy/ethical restriction. The data that support the findings of this study are available on request from the corresponding author.

Competing interests

C Macchi, M Ruscica, A Ceresa, DM Conti, N Ferri, C Favero, L Vigna, AC Pesatori, G Racagni, CR Sirtori, V Bollati, M Buoli declare that there is no conflict of interest. A Corsini received honoraria from AstraZeneca, AMGEN, Sanofi, Recordati, Novartis, MSD, Mediolanum, DOC, Mylan and Pfizer.

Funding

This study was partially supported by Cariplo Foundation (2015 – 0552 and 2018 – 0511 to MR), University of Milan Intramural Grant PSR 2018 to MR and EU Programme ‘Ideas’, European Research Council (ERC-2011-StG 282,413 to VB). Grants from MIUR Progetto Eccellenza

Authors' contributions

CM performed the ELISA assays and wrote the manuscript; MB, MR and VB conceived the study and wrote the manuscript; GR and NF critically revised the manuscript; CF performed all the statistical analyses; ACP, CRS and AC critically revised the manuscript; LV and DMC visited patients. AC and MB visited the patients and assigned the BDI-II score. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Acknowledgements

Not applicable

References

1. Roth GA, Forouzanfar MH, Moran AE, Barber R, Nguyen G, Feigin VL, Naghavi M, Mensah GA, Murray CJ. Demographic and epidemiologic drivers of global cardiovascular mortality. *N Engl J Med*. 2015;372(14):1333–41.
2. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, Chapman MJ, De Backer GG, Delgado V, Ference BA, et al: **2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk**. *Eur Heart J* 2019.
3. Khan SS, Ning H, Wilkins JT, Allen N, Carnethon M, Berry JD, Sweis RN, Lloyd-Jones DM. Association of Body Mass Index With Lifetime Risk of Cardiovascular Disease and Compression of Morbidity. *JAMA Cardiol*. 2018;3(4):280–7.
4. Hare DL, Toukhsati SR, Johansson P, Jaarsma T. Depression and cardiovascular disease: a clinical review. *Eur Heart J*. 2014;35(21):1365–72.
5. Luppino FS, de Wit LM, Bouvy PF, Stijnen T, Cuijpers P, Penninx BW, Zitman FG. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. *Arch Gen Psychiatry*. 2010;67(3):220–9.
6. Liwo ANN, Howard VJ, Zhu S, Martin MY, Safford MM, Richman JS, Cummings DM, Carson AP. **Elevated depressive symptoms and risk of all-cause and cardiovascular mortality among adults with and without diabetes: The REasons for Geographic And Racial Differences in Stroke (REGARDS) study**. *J Diabetes Complications* 2020:107672.
7. organization WH. **The global burden of disease: 2004 update**. Geneva. In..
8. Vaccarino V, Badimon L, Bremner JD, Cenko E, Cubedo J, Dorobantu M, Duncker DJ, Koller A, Manfrini O, Milicic D, et al: **Depression and coronary heart disease: 2018 ESC position paper of the working group of coronary pathophysiology and microcirculation developed under the auspices of the ESC Committee for Practice Guidelines**. *Eur Heart J* 2019.
9. Gan Y, Gong Y, Tong X, Sun H, Cong Y, Dong X, Wang Y, Xu X, Yin X, Deng J, et al. Depression and the risk of coronary heart disease: a meta-analysis of prospective cohort studies. *BMC Psychiatry*.

- 2014;14:371.
10. Nicholson A, Kuper H, Hemingway H. Depression as an aetiologic and prognostic factor in coronary heart disease: a meta-analysis of 6362 events among 146 538 participants in 54 observational studies. *Eur Heart J*. 2006;27(23):2763–74.
 11. Jha MK, Qamar A, Vaduganathan M, Charney DS, Murrough JW. Screening and Management of Depression in Patients With Cardiovascular Disease: JACC State-of-the-Art Review. *J Am Coll Cardiol*. 2019;73(14):1827–45.
 12. Kim JM, Stewart R, Lee YS, Lee HJ, Kim MC, Kim JW, Kang HJ, Bae KY, Kim SW, Shin IS, et al. Effect of Escitalopram vs Placebo Treatment for Depression on Long-term Cardiac Outcomes in Patients With Acute Coronary Syndrome: A Randomized Clinical Trial. *JAMA*. 2018;320(4):350–8.
 13. Macchi C, Banach M, Corsini A, Sirtori CR, Ferri N, Ruscica M. Changes in circulating pro-protein convertase subtilisin/kexin type 9 levels - experimental and clinical approaches with lipid-lowering agents. *Eur J Prev Cardiol*. 2019;26(9):930–49.
 14. Cheng JM, Oemrawsingh RM, Garcia-Garcia HM, Boersma E, van Geuns RJ, Serruys PW, Kardys I, Akkerhuis KM. PCSK9 in relation to coronary plaque inflammation: Results of the ATHEROREMO-IVUS study. *Atherosclerosis*. 2016;248:117–22.
 15. Ferri N, Marchiano S, Tibolla G, Baetta R, Dhyani A, Ruscica M, Uboldi P, Catapano AL, Corsini A. PCSK9 knock-out mice are protected from neointimal formation in response to perivascular carotid collar placement. *Atherosclerosis*. 2016;253:214–24.
 16. Costet P, Cariou B, Lambert G, Lallanne F, Lardeux B, Jarnoux AL, Grefhorst A, Staels B, Krempf M: **Hepatic PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory element-binding protein 1c.** *J Biol Chem* 2006, **281**(10):6211–6218.
 17. Myocardial Infarction Genetics Consortium. Kathiresan S, Voight BF, Purcell S, Musunuru K, Ardissino D, Mannucci PM, Anand S, Engert JC, Samani NJ, et al: Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. *Nat Genet* 2009, **41**(3):334–41.
 18. Lee JS, Rosoff D, Luo A, Longley M, Phillips M, Charlet K, Muench C, Jung J, Lohoff FW. PCSK9 is Increased in Cerebrospinal Fluid of Individuals With Alcohol Use Disorder. *Alcohol Clin Exp Res*. 2019;43(6):1163–9.
 19. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet*. 2004;364(9438):937–52.
 20. Ellins EA, Shipley MJ, Rees DA, Kemp A, Deanfield JE, Brunner EJ, Halcox JP. **Associations of depression-anxiety and dyslipidaemia with subclinical carotid arterial disease: Findings from the Whitehall II Study.** *Eur J Prev Cardiol* 2019:2047487319876230.
 21. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med*. 1999;340(1):14–22.

22. Wagner CJ, Musenbichler C, Bohm L, Farber K, Fischer AI, von Nippold F, Winkelmann M, Richter-Schmidinger T, Muhle C, Kornhuber J, et al. LDL cholesterol relates to depression, its severity, and the prospective course. *Prog Neuropsychopharmacol Biol Psychiatry*. 2019;92:405–11.
23. Reilly MP, Wolfe ML, Rhodes T, Girman C, Mehta N, Rader DJ. Measures of insulin resistance add incremental value to the clinical diagnosis of metabolic syndrome in association with coronary atherosclerosis. *Circulation*. 2004;110(7):803–9.
24. Bollati V, Iodice S, Favero C, Angelici L, Albeti B, Cacace R, Cantone L, Carugno M, Cavalleri T, De Giorgio B, et al. Susceptibility to particle health effects, miRNA and exosomes: rationale and study protocol of the SPHERE study. *BMC Public Health*. 2014;14:1137.
25. Smarr KL, Keefer AL: **Measures of depression and depressive symptoms: Beck Depression Inventory-II (BDI-II), Center for Epidemiologic Studies Depression Scale (CES-D), Geriatric Depression Scale (GDS), Hospital Anxiety and Depression Scale (HADS), and Patient Health Questionnaire-9 (PHQ-9).** *Arthritis Care Res (Hoboken)* 2011, **63 Suppl 11**:S454-466.
26. Ruscica M, Ferri N, Fogacci F, Rosticci M, Botta M, Marchiano S, Magni P, D'Addato S, Giovannini M, Borghi C, et al: **Circulating Levels of Proprotein Convertase Subtilisin/Kexin Type 9 and Arterial Stiffness in a Large Population Sample: Data From the Brisighella Heart Study.** *J Am Heart Assoc* 2017, **6**(5).
27. Siu AL, Force USPST, Bibbins-Domingo K, Grossman DC, Baumann LC, Davidson KW, Ebell M, Garcia FA, Gillman M, Herzstein J, et al. Screening for Depression in Adults: US Preventive Services Task Force Recommendation Statement. *JAMA*. 2016;315(4):380–7.
28. Spitzer RL, Kroenke K, Williams JB. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. *Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire.* *JAMA*. 1999;282(18):1737–44.
29. Rubin RR, Peyrot M, Gaussoin SA, Espeland MA, Williamson D, Faulconbridge LF, Wadden TA, Ewing L, Safford M, Evans-Hudnall G, et al. Four-year analysis of cardiovascular disease risk factors, depression symptoms, and antidepressant medicine use in the Look AHEAD (Action for Health in Diabetes) clinical trial of weight loss in diabetes. *Diabetes Care*. 2013;36(5):1088–94.
30. Westhoff-Bleck M, Winter L, Aguirre Davila L, Herrmann-Lingen C, Treptau J, Bauersachs J, Bleich S, Kahl KG. **Diagnostic evaluation of the hospital depression scale (HADS) and the Beck depression inventory II (BDI-II) in adults with congenital heart disease using a structured clinical interview: Impact of depression severity.** *Eur J Prev Cardiol* 2019:2047487319865055.
31. Beck AT, Steer RA, Brown GK. **BDI-II, Beck depression inventory: manual;** 1996.
32. Albert PR. Why is depression more prevalent in women? *J Psychiatry Neurosci*. 2015;40(4):219–21.
33. Lichtman JH, Bigger JT Jr, Blumenthal JA, Frasure-Smith N, Kaufmann PG, Lesperance F, Mark DB, Sheps DS, Taylor CB, Froelicher ES, et al. Depression and coronary heart disease: recommendations for screening, referral, and treatment: a science advisory from the American Heart Association Prevention Committee of the Council on Cardiovascular Nursing, Council on Clinical Cardiology, Council on Epidemiology and Prevention, and Interdisciplinary Council on Quality of Care and

- Outcomes Research: endorsed by the American Psychiatric Association. *Circulation*. 2008;118(17):1768–75.
34. Lyra ESNM, Lam MP, Soares CN, Munoz DP, Milev R, De Felice FG. Insulin Resistance as a Shared Pathogenic Mechanism Between Depression and Type 2 Diabetes. *Front Psychiatry*. 2019;10:57.
 35. Bonora E, Formentini G, Calcaterra F, Lombardi S, Marini F, Zenari L, Saggiani F, Poli M, Perbellini S, Raffaelli A, et al. HOMA-estimated insulin resistance is an independent predictor of cardiovascular disease in type 2 diabetic subjects: prospective data from the Verona Diabetes Complications Study. *Diabetes Care*. 2002;25(7):1135–41.
 36. Blanchard C, Ledoux S, Verhaegen A, Wargny M, Letessier E, Stepanian A, Hutten N, Jacobi D, Krempf M, Le Bras M, et al: **Roux-en-Y gastric bypass, but not sleeve gastrectomy, decreases plasma PCSK9 levels in morbidly obese patients.** *Diabetes Metab* 2020.
 37. Zenti M, Lupo M, De Martin S, Altomri A, Galvan S, Aventaggiato M, Maneschi C, Sandri D, Paiola E, Battistoni M, et al: **Impact of bariatric surgery-induced weight loss on circulating PCSK9 levels in obese patients.** *Nutr Metab Cardiovasc Dis* 2020.
 38. Ridker PM, Rifai N, Bradwin G, Rose L. Plasma proprotein convertase subtilisin/kexin type 9 levels and the risk of first cardiovascular events. *Eur Heart J*. 2016;37(6):554–60.
 39. Gencer B, Montecucco F, Nanchen D, Carbone F, Klingenberg R, Vuilleumier N, Aghlmandi S, Heg D, Raber L, Auer R, et al. Prognostic value of PCSK9 levels in patients with acute coronary syndromes. *Eur Heart J*. 2016;37(6):546–53.
 40. Vlachopoulos C, Terentes-Printzios D, Georgiopoulos G, Skoumas I, Koutagiar I, Ioakeimidis N, Stefanadis C, Tousoulis D. Prediction of cardiovascular events with levels of proprotein convertase subtilisin/kexin type 9: A systematic review and meta-analysis. *Atherosclerosis*. 2016;252:50–60.
 41. Macchi C, Ferri N, Favero C, Cantone L, Vigna L, Pesatori AC, Lupo MG, Sirtori CR, Corsini A, Bollati V, et al. Long-term exposure to air pollution raises circulating levels of proprotein convertase subtilisin/kexin type 9 in obese individuals. *Eur J Prev Cardiol*. 2019;26(6):578–88.
 42. Guo W, Gong Y, Li J, Qin P, Lu J, Li X, Zhu W, Xu N, Zhou H, Zhang Q. Association of serum proprotein convertase subtilisin/kexin type 9 with early atherosclerosis in newly diagnosed type 2 diabetes mellitus. *Nutr Metab Cardiovasc Dis*. 2019;29(8):815–21.
 43. Pott J, Schlegel V, Teren A, Horn K, Kirsten H, Bluecher C, Kratzsch J, Loeffler M, Thiery J, Burkhardt R, et al. Genetic Regulation of PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) Plasma Levels and Its Impact on Atherosclerotic Vascular Disease Phenotypes. *Circ Genom Precis Med*. 2018;11(5):e001992.
 44. Rao AS, Lindholm D, Rivas MA, Knowles JW, Montgomery SB, Ingelsson E. Large-Scale Phenome-Wide Association Study of PCSK9 Variants Demonstrates Protection Against Ischemic Stroke. *Circ Genom Precis Med*. 2018;11(7):e002162.
 45. Ferri N, Tibolla G, Pirillo A, Cipollone F, Mezzetti A, Pacia S, Corsini A, Catapano AL. Proprotein convertase subtilisin kexin type 9 (PCSK9) secreted by cultured smooth muscle cells reduces macrophages LDLR levels. *Atherosclerosis*. 2012;220(2):381–6.

46. Dozio E, Ruscica M, Vianello E, Macchi C, Sitzia C, Schmitz G, Tacchini L, Corsi Romanelli MM. PCSK9 Expression in Epicardial Adipose Tissue: Molecular Association with Local Tissue Inflammation. *Mediators Inflamm.* 2020;2020:1348913.
47. Knol MJ, Twisk JW, Beekman AT, Heine RJ, Snoek FJ, Pouwer F. Depression as a risk factor for the onset of type 2 diabetes mellitus. A meta-analysis. *Diabetologia.* 2006;49(5):837–45.
48. Webb M, Davies M, Ashra N, Bodicoat D, Brady E, Webb D, Moulton C, Ismail K, Khunti K. The association between depressive symptoms and insulin resistance, inflammation and adiposity in men and women. *PLoS One.* 2017;12(11):e0187448.
49. Da Dalt L, Ruscica M, Bonacina F, Balzarotti G, Dhyani A, Di Cairano E, Baragetti A, Arnaboldi L, De Metrio S, Pellegatta F, et al. PCSK9 deficiency reduces insulin secretion and promotes glucose intolerance: the role of the low-density lipoprotein receptor. *Eur Heart J.* 2019;40(4):357–68.
50. Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, Basak A, Prat A, Chretien M. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc Natl Acad Sci U S A.* 2003;100(3):928–33.
51. Adorni MP, Ruscica M, Ferri N, Bernini F, Zimetti F. Proprotein Convertase Subtilisin/Kexin Type 9, Brain Cholesterol Homeostasis and Potential Implication for Alzheimer's Disease. *Front Aging Neurosci.* 2019;11:120.
52. Alghamdi J, Matou-Nasri S, Alghamdi F, Alghamdi S, Alfadhel M, Padmanabhan S. Risk of Neuropsychiatric Adverse Effects of Lipid-Lowering Drugs: A Mendelian Randomization Study. *Int J Neuropsychopharmacol.* 2018;21(12):1067–75.
53. Tyrrell J, Mulugeta A, Wood AR, Zhou A, Beaumont RN, Tuke MA, Jones SE, Ruth KS, Yaghootkar H, Sharp S, et al: **Using genetics to understand the causal influence of higher BMI on depression.** *Int J Epidemiol* 2018.
54. Milaneschi Y, Simmons WK, van Rossum EFC, Penninx BW. Depression and obesity: evidence of shared biological mechanisms. *Mol Psychiatry.* 2019;24(1):18–33.
55. Kotseva K, De Backer G, De Bacquer D, Ryden L, Hoes A, Grobbee D, Maggioni A, Marques-Vidal P, Jennings C, Abreu A, et al. Lifestyle and impact on cardiovascular risk factor control in coronary patients across 27 countries: Results from the European Society of Cardiology ESC-EORP EUROASPIRE V registry. *Eur J Prev Cardiol.* 2019;26(8):824–35.
56. Vraney EA, Berntson JM, Khambaty T, Stewart JC. Depressive Symptoms Clusters and Insulin Resistance: Race/Ethnicity as a Moderator in 2005–2010 NHANES Data. *Ann Behav Med.* 2016;50(1):1–11.
57. Essmat N, Soliman E, Mahmoud MF, Mahmoud AAA. Antidepressant activity of anti-hyperglycemic agents in experimental models: A review. *Diabetes Metab Syndr.* 2020;14(5):1179–86.

Figures

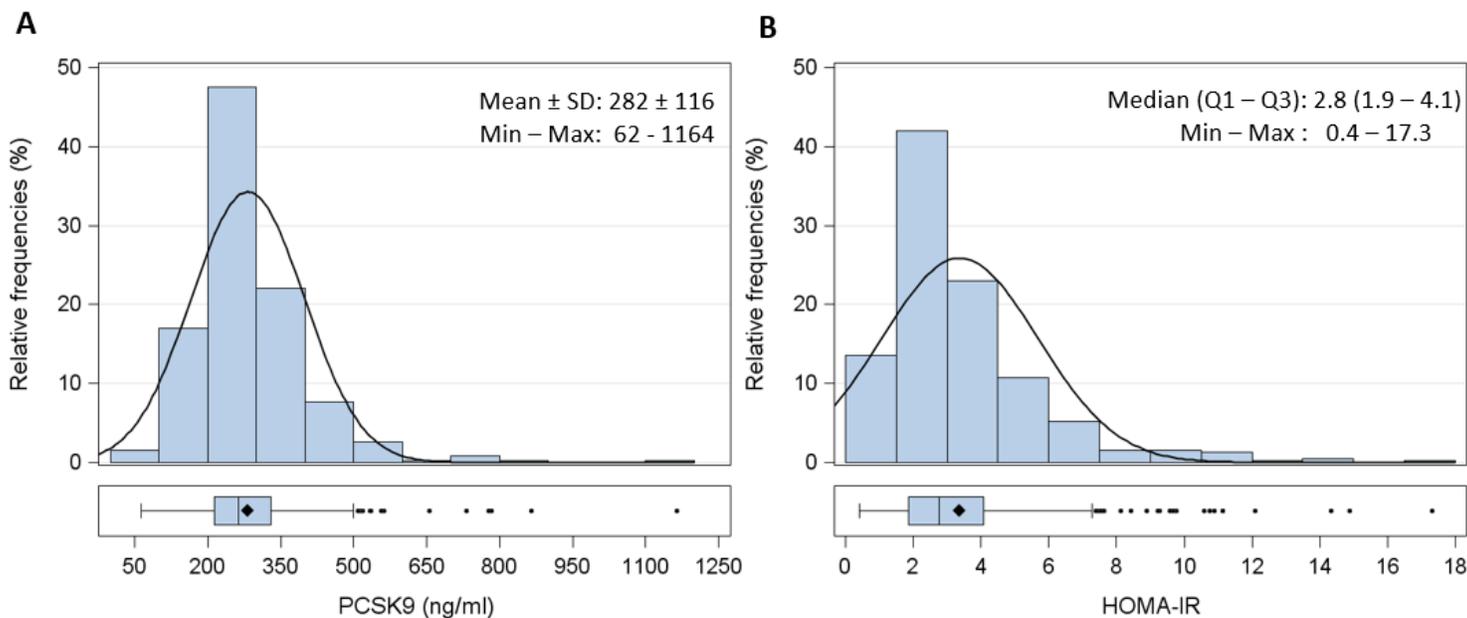


Figure 1

Distribution of fasting plasma concentrations of PCSK9 levels (ng/mL; panel A) and HOMA-IR (panel B) in 389 individuals. Data are presented as histograms and box-plots. PCSK9 is normally distributed, whereas HOMA-IR shows a skewed distribution. PCSK9, pro-protein convertase subtilisin/kexin type 9; HOMA, homeostasis model assessment.

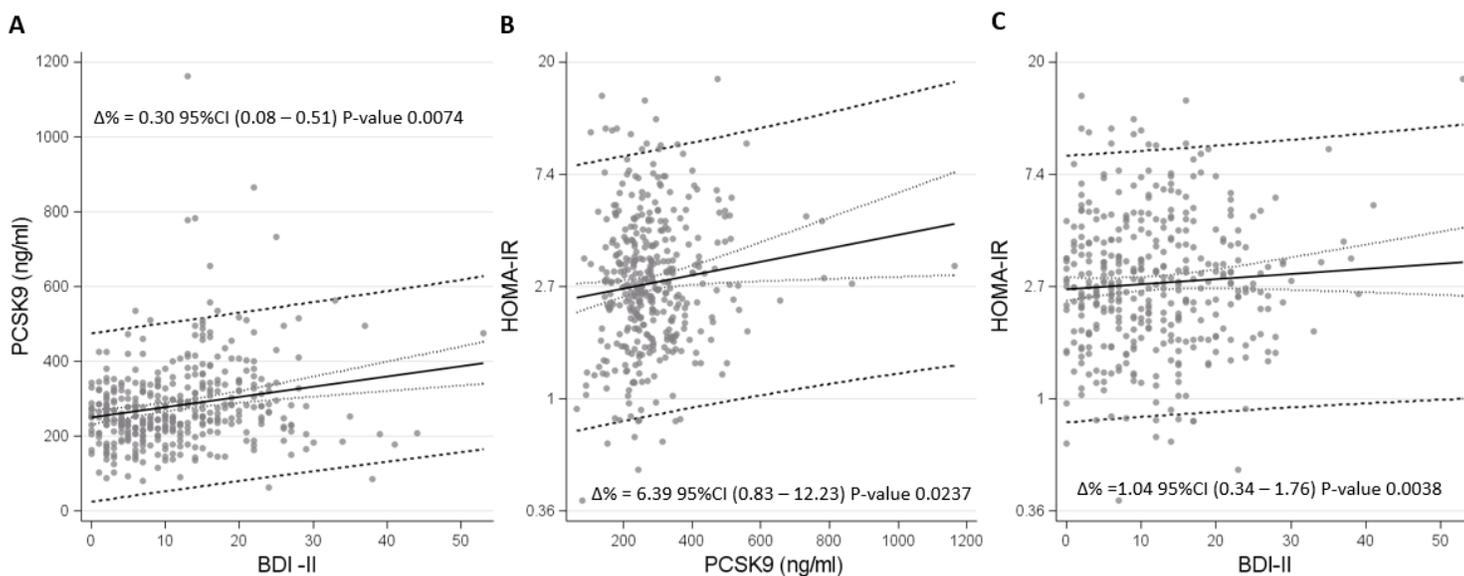


Figure 2

Scatterplots with regression line and confidence interval. Panel A - Association between severity of depression (BDI-II) and PCSK9. $\Delta\%$ represents the percentage increase in PCSK9 for ten-unit increase in BDI-II score. Panel B - Association between PCSK9 and HOMA-IR on natural logarithmic scale, adjusted

also for BDI-II. $\Delta\%$ represents the percentage increase in HOMA-IR for 100 ng/ml increase in PCSK9 concentration. Panel C - Association between severity of depression (BDI-II) and HOMA-IR on natural logarithmic scale. $\Delta\%$ represents the percentage increase in HOMA-IR for ten-unit increase in BDI-II score. BDI-II, Beck Depression Inventory; BMI, body mass index; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; PCSK9, pro-protein convertase subtilisin/kexin type 9. Beta regression coefficients of panel A and B were used to estimate indirect effect of BDI-II on HOMA-IR.

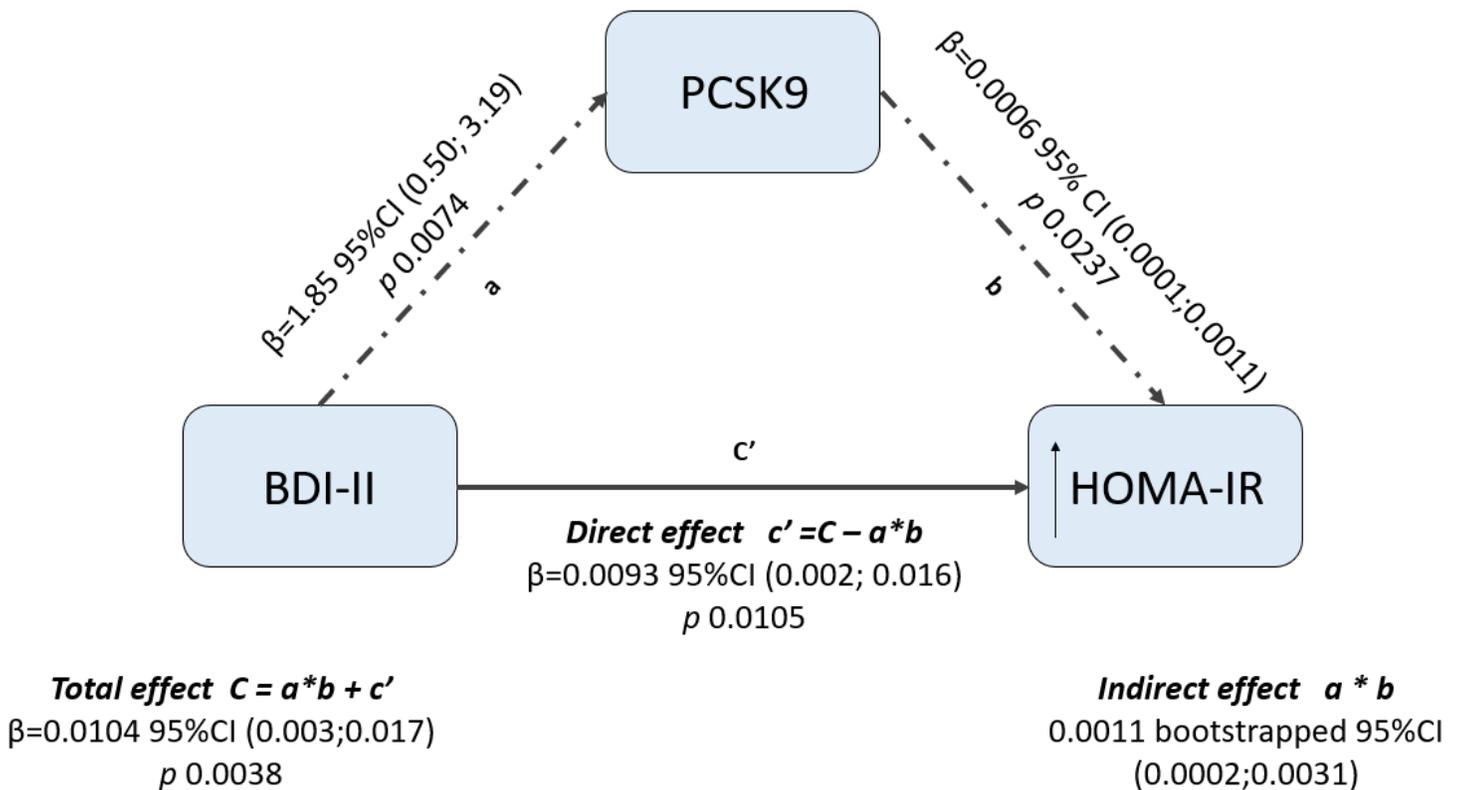


Figure 3

Conceptual diagram of causal mediation analysis, that hypothesized mechanism linking BDI-II and HOMA-IR. The solid black arrow represents the effect of BDI-II on HOMA-IR levels that operates directly or through a pathway different from the mediator analyzed in the current study (PCSK9). The dotted black arrows represent the suggested alternative pathway, where an indirect effect of BDI-II on HOMA-IR is mediated by PCSK9 levels. The black thin arrows indicate increased levels of HOMA-IR. BDI-II, Beck Depression Inventory; HOMA, homeostasis model assessment.

Supplementary Files

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