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Mendelian randomization study of the relation between adiponectin and heart function, unravelling the paradox

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ABSTRACT

High adiponectin concentrations are generally regarded as beneficial with regard to cardiometabolic health, but have been paradoxically associated with increased cardiovascular disease risk, specifically heart failure, in individuals at high cardiovascular risk. We aimed to investigate the association between adiponectin and heart function parameters, and inversely, we estimated the effect of genetically-determined heart function and NT-proBNP as the main marker of heart failure on adiponectin using Mendelian randomisation.

Observational analyses between adiponectin and measures of heart function, i.e. E/A ratio, left, and right ventricular ejection fraction, were performed in participants of the Netherlands Epidemiology of Obesity (NEO) study, assessed by MRI of the heart (n = 1,138). Two-sample Mendelian randomisation analyses were conducted to estimate the effect of NT-proBNP and heart function on adiponectin concentrations using publicly-available summary statistics (ADIPOGen; the PLATO trial).

The mean (standard deviation) age was 56 (6) years and mean body mass index was 26 (4) kg/m^2. Per five μg/mL higher adiponectin, the E/A ratio was −0.05 (95 % CI: −0.10, −0.01) lower, left ventricle ejection fraction was −0.5 % (95 % CI: −1.1, 0.1) lower, and right ventricle ejection fraction was 0.5 % (95 % CI: −0.1, 1.2) higher. Genetically-determined NT-proBNP was causally related to adiponectin concentrations in ADIPOGen: per doubling of genetically-determined NT-proBNP, adiponectin concentrations were 11.4 % (95 % CI: 1.7, 21.6) higher.

With causal MR methods we showed that NT-proBNP affects adiponectin concentrations, while adiponectin is not associated with heart function parameters. Therefore, reverse causation may explain the adiponectin paradox observed in previous studies.

1. Introduction

Adiponectin is a protein that is produced by adipocytes, and is negatively associated with ectopic fat depots [1]. Serum adiponectin concentrations are decreased in individuals with overweight or obesity, and in particular in individuals with increased visceral adipose tissue (VAT) depots [1–3]. Several studies suggest that adiponectin has beneficial effects on lipid metabolism, endothelial function and obesity-related low-grade inflammation [4–8]. Also, adiponectin is suggested to have insulin sensitizing properties and to be protective against type 2 diabetes mellitus [4,5,9–12]. However, recent Mendelian randomization studies indicated the absence of a causal relation between adiponectin and type 2 diabetes, and coronary heart disease [13,14]. This contradicts the findings that high adiponectin levels are associated with an increased risk of heart failure in large observational cohort studies of 3,263 and 5,574 individuals [15,16].

Several explanations for this so-called ‘adiponectin paradox’ have been explored in previous studies. One potential explanation is that

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previous studies investigated the association between adiponectin and cardiovascular disease in a population that was selected for a high risk of cardiovascular disease, which could lead to selection bias [17]. But more likely, previous results may be explained by reverse causation, i.e. subclinical cardiovascular disease, specifically heart failure, may have affected adiponectin concentrations. In this explanation, the strong heart failure marker N-terminal-pro-brain natriuretic peptide (NT-proBNP) may cause an increase in adiponectin production and serum concentrations [18–21].

Mendelian randomization (MR) is an effective method to investigate the causality and causal direction of an observed association, because instead of an observed exposure, the genetic predisposition for an exposure is analyzed. This genetic predisposition is set at conception, which excludes the possibility that the outcome or a confounder affects the genetic exposure [22]. Therefore, MR may be a useful tool to investigate the adiponectin paradox.

In this study, a two-sample MR strategy was employed to unravel the directionality and mechanisms of the relations between adiponectin and cardiac function. We used subclinical measures of heart function in this study to reduce the possibility of an 'index event' on which previous studies have selected, which would limit the possibility of collider bias [23]. First, we aimed to investigate the effect of adiponectin on heart function determined by magnetic resonance imaging in a general population using observational analyses. Our second aim was to investigate the reverse causal pathway by determining the effect of genetically determined heart function on adiponectin concentrations using a two-sample MR strategy in two large publicly-available datasets. Thirdly, we aimed to investigate a specific mechanism in the reverse pathway by determining the effect of genetically-determined NT-proBNP concentrations on adiponectin concentrations using two-sample MR in large publicly-available datasets.

2. Methods

2.1. Study design and study population

The first part of this study is a cross-sectional analysis of the Netherlands Epidemiology of Obesity (NEO) study, a population-based, prospective cohort study of 6,671 men and women aged between 45 and 65 years. The study design and population are described in detail elsewhere [24]. 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 blood sampling. In a random subgroup of participants without contraindications (body circumference ≥170 cm, implanted metallic devices, or claustrophobia) magnetic resonance imaging (MRI) of abdominal fat was performed. Research nurses recorded current medication use by means of a medication inventory.

For the present analyses, we included 1,150 randomly selected participants without contra-indications for MRI. For observational analyses, we excluded 12 participants with missing adiponectin concentration, and consequently 1,138 participants were included in the present study. For genetic analyses, we additionally excluded related participants, participants with poor genotyping quality and of non-European descent (n = 132) [25].

The Medical Ethical Committee of the Leiden University Medical Centre (LUMC) approved the protocol. All participants gave their written informed consent.

Further analyses presented in this study used genetic instruments discovered in genome-wide association studies on NT-proBNP concentrations [26,27], and publicly-available summary statistics of genome-wide association studies on measures of heart function [28] and on adiponectin [26], available in GRASP [29].

2.2. Blood sampling

During the visit to the NEO study centre, venous blood samples were obtained from the antecubital vein after a >10 h overnight fast. Fasting serum total cholesterol, HDL-cholesterol, triglycerides, C-reactive protein, and plasma glucose and insulin were determined in the fasting blood samples at the central clinical chemistry laboratory of the LUMC using standard assays. LDL cholesterol concentrations were calculated using the Friedewald equation [30]. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as the product of fasting glucose and insulin concentrations divided by 22.5 [31]. Furthermore, aliquots of plasma and serum were stored after centrifugation at −80 °C. Serum adiponectin concentration was determined in a fasting blood sample that had undergone one previous freeze-thaw cycle using a turbidimetric immunoassay (Cat Nr A0299, Randox Laboratories Limited, United Kingdom) on an automated analyser (Roche Modular P800, Roche, Switzerland). Samples with a concentration above 28 μg/L were diluted 2x with water before re-analysis. Analytical variation was calculated based on 88–94 runs over 35 days and was 2.8 % at a level of 1.9 mg/L and 1.9 % at a level of 11 mg/L using a commercial serum based unassayed control material (Randox Immunoassay Level 1, Randox Laboratories Limited, United Kingdom) and a human pooled serum.

2.3. Heart function

In a random sample of the NEO study population without contraindications for MRI (n = 1,150), the heart was imaged in the short-axis orientation by using electrocardiographically gated breath-hold balanced steady-state free precession imaging by magnetic resonance imaging (MRI, 1.5 T MR imaging, Philips Medical Systems) to assess left ventricle (LV) and right ventricle (RV) dimensions and mass. An electrocardiographically gated gradient-echo sequence was performed with velocity encoding to measure blood flow across the mitral valve to determine diastolic function. We used the ejection fraction (EF) as the systolic parameter. Diastolic parameters included peak filling rates of the early filling phase (E) and atrial contraction (A) and their ratio (E/A ratio). Image postprocessing was performed using in-house–developed software packages (MASS and FLOW; Leiden University Medical Center, Leiden, the Netherlands).

2.4. Genotyping and selection of genetic instruments

As instruments for measures of LV function, we selected 12 genetic variants from a GWAS that was performed in 44,203 primarily Caucasian individuals included in studies in the EchoGen consortium, in which analyses were adjusted for age, sex, height, weight, and study site (when applicable). [28] Instruments for NT-proBNP were selected from a GWAS on NT-proBNP that was performed in 9,232 patients with acute coronary syndrome of whom 99% were of European descent, in which analyses were adjusted for the first four principal components of ancestry [27]. The reported values for explained variance did not exceed 1% for any of the phenotypes of interest. We extracted the effect estimates (betas) and their standard errors of the instruments for heart function and NT-proBNP on adiponectin concentrations from summary statistics of the ADIPOGen consortium (n = 29,347) that were made publicly available via GRASP [26–29]. Therefore we extracted the summary statistics for the variants rs806322, rs6702619, rs17696696, rs7127129, rs17608766, rs2649, rs4765663, rs11207426 (aortic diameter), rs12541595, rs10774625 (left ventricular diastolic internal dimension), rs1454157 (left ventricle mass), and rs9470361 (fractional shortening), and rs13107325, rs198389, and rs4842653 (NT-proBNP)

from the ADIPOGen summary data. For aortic diameter, the variant rs806322 was dropped during harmonisation of the exposure and outcome data due to ambiguous alleles, leading to the use of 7 variants. The variant rs4842653 was used as a proxy (D': 1.0, R^2:1.0) for the original variant rs11105306 from the GWAS on NT-proBNP, as this variant was not available in the ADIPOGen summary data. The overlap between the GWAS for LV function and the ADIPOGgen data was 35 % (of the ADIPOGen data), and 0 % for the GWAS of NT-proBNP.

2.5. Other variables

Self-reported level of education was classified into low (none, primary school or lower vocational education) or high (other). Participants reported their medical history of diabetes and cardiovascular disease. Pre-existing cardiovascular disease was defined as myocardial infarction, angina pectoris, congestive heart failure, stroke, or peripheral vascular disease. Diabetes was defined as self-reported diabetes, use of glucose-lowering medication, or fasting plasma glucose concentrations of 7.0 mmol/L or higher. Tobacco smoking was reported in three categories: never smoker, former smoker or current smoker. Participants reported their physical activity during leisure time, which was expressed in metabolic equivalent hours per week.

Total body fat (%) was estimated using a bio-impedance device (TBF-310, Tanita International Division, UK). Visceral adipose tissue area (VAT) was quantified by MRI using a turbo spin echo imaging protocol in all participants with a cardiac MRI.

2.6. 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 [32]. This was done by weighting all participants towards the BMI distribution of participants from the Leiderdorp municipality [33], whose BMI distribution was similar to the BMI distribution of the general Dutch population [34]. All results were based on weighted analyses. Consequently, the results apply to a population-based study without oversampling of individuals with a BMI $\geq$ 27 kg/m^2.

Data were summarized by sex as mean (SD; normally distributed data only), median (25th, 75th percentiles; non-normally distributed data only), or as percentage (categorical data).

First, for our observational analyses within the NEO study, we used linear regression to estimate the associations between adiponectin concentration and heart function, and their 95 % confidence intervals. Analyses were performed crude, and adjusted for potential confounding factors age, sex, total body fat, visceral fat, smoking status, HOMA-IR, triglyceride and C-reactive protein concentrations, use of antihypertensive and glucose lowering and lipid lowering medication.

Second, we conducted two-sample Mendelian randomization analysis to quantify the causal effects of NT-proBNP on adiponectin concentrations, and of left ventricle function on adiponectin in publicly-available summary statistics.

The effects of the three SNPs for NT-proBNP on adiponectin in publicly-available data were estimated using weighted linear regression of the SNP-NT-proBNP associations (betas) on the SNP-adiponectin betas in the R package *TwoSampleMR* [35]. The regression was weighted towards the inverse of the standard error of the SNP-adiponectin betas, and the intercept was constrained to zero. Because both NT-proBNP and adiponectin have been log-transformed to the natural logarithm in the original GWASs, we reported coefficients that can be interpreted as the percentage change in adiponectin concentration per doubling of genetically-determined NT-proBNP concentration. Similarly, the effects of the variants for aortic root diameter (n = 7), LV diastolic internal diameter (n = 2), LV mass (n = 1), and factional shortening of the LV (n = 1) on adiponectin concentration were estimated using weighted regression (aortic root diameter, LV diastolic internal diameter) or Wald estimation (LV mass, fractional shortening). The resulting coefficients can be interpreted as the percentage difference in adiponectin concentration per unit of the heart function parameter.

3. Results

3.1. Baseline characteristics

Table 1 shows baseline characteristics of participants (47 % men) with a mean age of 56 years (standard deviation, SD: 6), a BMI of 26 kg/m^2 (SD: 4) and of whom 22 % were using antihypertensive medication. The mean LVEF was 64 % (SD: 6) and the mean E/A ratio was 1.26 (0.46) in men and 1.37 (0.52) in women. Adiponectin concentrations were higher in women (11.2 μg/mL, SD: 5.0) than in men (6.5 μg/mL, SD: 2.8). Baseline characteristics of the subpopulation with cardiac MRI were comparable with those of the total population (data not shown).

3.2. Observational associations between adiponectin and heart function

The associations between adiponectin concentration and E/A ratio and LV systolic function are presented in Table 2. In adjusted analysis, adiponectin was associated with a −0.05 (95 % confidence interval: −0.10, −0.01) lower E/A ratio. We observed no further associations

Table 1
Baseline characteristics of participants of the NEO study that underwent MRI examination of the heart (n = 1,138).

	Total population	Men (47 %)	Women (53 %)
Demographic/anthropometric			
Age (y)	56 (6)	56 (6)	56 (6)
Total body fat (%)	31 (8)	25 (6)	37 (6)
BMI (kg/m^2)	26 (4)	27 (3)	26 (4)
Visceral adipose tissue (cm^2)	92 (56)	117 (58)	71 (44)
Tobacco smoking (% never)	42	41	44
Alcohol intake (g/d)	10 (2–22)	17 (4–29)	8 (1–15)
Total energy intake (MJ/d)	9.5 (3.1)	10.8 (3.2)	8.3 (2.5)
Physical activity (METhours/week)	32 (17–53)	34 (17–54)	30 (16–52)
Education level [a] (% high)	47	50	44
Biomarkers			
Total cholesterol (mmol/L)	5.7 (1.1)	5.6 (1.0)	5.9 (1.1)
Triglycerides (mmol/L)	1.3 (0.8)	1.5 (0.9)	1.1 (0.6)
Fasting glucose (mmol/L)	5.5 (1.0)	5.7 (1.2)	5.3 (0.9)
Fasting CRP (mg/L)	1.2 (0.6–2.3)	1.1 (0.5–1.9)	1.3 (0.7–2.8)
Adiponectin (μg/mL)	9.0 (4.7)	6.5 (2.8)	11.2 (5.0)
Comorbidity and medication			
Diabetes [b] (%)	4	5	3
Glucose lowering medication (%)	3	4	2
Lipid lowering medication (%)	10	14	6
Antihypertensive medication (%)	22	22	23
Measures of heart function			
LV Ejection fraction (%)	64 (6)	63 (7)	64 (5)
RV Ejection fraction (%)	55 (6)	53 (6)	57 (6)
E/A ratio	1.32 (0.49)	1.26 (0.46)	1.37 (0.52)

BMI, body mass index; CRP, C-reactive protein; E/A ratio, early/atrial filling ratio; LV, left ventricle; MET, metabolic equivalents of task; MJ, megajoule; MRI, magnetic resonance imaging; NEO, Netherlands Epidemiology of Obesity.
Results are based on analyses weighted towards the BMI distribution of the general population (n = 1,138). Data are shown as mean (SD), median (IQR) or percentage.
[a] Low education: none, primary school or lower vocational education as highest level of education.
[b] Self-reported diabetes or use of glucose-lowering medication or insulin.

Table 2
Results of linear regression analyses to estimate the difference in MRI measures of heart function (ratio or percentage, 95 % confidence interval) per 5 μg/mL difference in adiponectin concentration in men and women participating in the Netherlands Epidemiology of Obesity study (n = 1,138).

	Per 5 μg/mL adiponectin concentration
E/A ratio	
Crude	−0.00 (−0.04, 0.04)
Multivariate	−0.05 (−0.10, −0.01)
LVEF (%)	
Crude	0.1 (−0.3, 0.5)
Multivariate	−0.5 (−1.1, 0.1)
RVEF (%)	
Crude	1.1 (0.5, 1.6)
Multivariate	0.5 (−0.1, 1.2)

Results are based on analyses weighted towards the BMI distribution of the general population (n = 1,138) and presented as difference in MRI measure of heart function per 5 μg/mL of adiponectin concentration. Multivariate: age, sex, total body fat, visceral fat, smoking status, type II diabetes, fasting glucose, triglyceride and C-reactive protein concentrations, use of antihypertensive and glucose lowering and lipid lowering medication.
E/A ratio, early/atrial filling phase ratio; LVEF, left ventricular ejection fraction; MRI, magnetic resonance imaging; NEO, Netherlands Epidemiology of Obesity study; RVEF, right ventricular ejection fraction; SD, standard deviation.

between adiponectin and LVEF and RVEF, nor that the associations differed between women and men (data not shown).

3.3. Associations between genetically-determined measures of left ventricular function, NT-proBNP and adiponectin

We observed wide confidence intervals around the associations between genetically-determined measures of left ventricular function and adiponectin concentrations in the publicly-available summary statistics of genetic variants on adiponectin concentrations (Table 3). However, we observed that a doubling of genetically-determined NT-proBNP was associated with 11.4 (95 % CI: 1.7, 21.6) increased adiponectin concentrations.

4. Discussion

In this study, we combined observational analyses with two-sample Mendelian randomisation analyses in order to unravel the previously described adiponectin paradox. We observed a weak association between higher adiponectin concentrations and lower E/A ratio in our observational analyses, but no associations with left and right ventricular function. Genetic determinants of left ventricular function did not affect adiponectin concentrations directly. Notably, our results indicated a causal relation between NT-proBNP and serum adiponectin concentrations.

Table 3
Two-sample Mendelian randomization analysis using inverse variance weighted regression or Wald estimation in summary statistics of adiponectin in ADIPOGen (n = 24,044–29,347).

Per exposure	% difference in adiponectin (95 % CI)	n (SNPs)	Method
NT-proBNP (doubling)	11.4 (1.7, 21.6)	3	IVW
Aortic root diameter (cm)	9.8 (−10.1, 34.1)	7	IVW
LV diastolic internal dimension (cm)	−26.0 (−54.9, 21.3)	2	IVW
LV mass (g)	0.2 (−0.5, 0.9)	1	Wald
Fractional shortening (%)	5.2 (−1.0, 11.9)	1	Wald

Results are presented as percentage difference in adiponectin concentration per exposure.
CI, confidence interval; IVW, inverse variance weighting; LV, left ventricle; NT-proBNP, N-terminal-pro-brain natriuretic peptide; SNP, single nucleotide polymorphism.

The results of our observational analyses of the associations between subclinical measures of heart function and adiponectin in a general population of the NEO study are discordant with previous studies that found an observational association between high adiponectin concentrations and a higher risk of developing heart failure in populations at high-cardiovascular risk [5,9,16]. An explanation for this discordance

We observed an association between genetically-determined NT-proBNP and adiponectin concentrations. This confirmed the hypothesis that was generated by previous studies, and may well explain the paradoxical findings in previous observational studies [16,36,37]. NT-proBNP strongly affected adiponectin concentrations, as for a doubling of NT-proBNP, adiponectin concentrations increased by 11.4 %. A doubling of NT-proBNP is clinically relevant as NT-proBNP concentrations may range by 5-fold between the median and the 97.5th percentile in middle-aged men [38]. This finding gives additional insight in the intricate neurohumoral regulation of heart function and its feedback mechanisms. It is known that increased BNP exerts a protective effect on cardiovascular disease in healthy subjects, which may be confusing regarding the adiponectin paradox [39]. However, in the studies with results indicating this paradox, (NT-pro)BNP is likely increased in a compensatory mechanism with regard to underlying heart disease. In high-risk subjects, NT-proBNP is regarded as a prognostic factor in the development of cardiovascular disease. Adiponectin may serve as a downstream effector in the BNP-mediated response of the heart to increased cardiac burden by increasing vascular compliance. This effect of adiponectin is supported by studies that show a reduction in vascular tone and anticontractile effects as a consequence of increased adiponectin. Further studies into the effects of adiponectin on vascular function may be of large importance [40].

The main strength of our study is that we investigated the associations of interest in an observational study with participants who were not at high cardiometabolic risk. Thereby we aimed to mitigate the effects of reverse causation. Furthermore, previous studies that have selected a population at high risk of the outcome may have introduced a selection bias: collider stratification bias. Collider stratification bias is the phenomenon that the association between two causes of one disease is biased in the diseased population, or in a population at high risk of the disease [18,23]. In this case, as a marker of visceral fat, adiponectin may seem a risk factor in a selected population already at high risk of cardiovascular disease, because due to the selection it may become inversely associated with other strong risk factors for heart failure, such as smoking or hyperlipidaemia. As a consequence, the high adiponectin concentrations accompanying low visceral fat may be paradoxically associated with high risk of mortality in individuals selected on their high risk of mortality. In our study, we aimed to decrease the risk of collider stratification bias by including individuals from the general population and using subclinical variation in measures of heart function. A third strength of the present study is the focus on subclinical measures of heart function instead of clinical cardiovascular disease. In general, our observational results suggest that adiponectin is not associated with heart function except for a marginal association with E/A ratio. However, this association may still be explained by residual confounding. While previous studies suggested that adiponectin has modest effects on heart function through beneficial effects on insulin sensitivity and endothelial function, our results do not support this hypothesis [4,8,10,41,42].

This study also has limitations. First, publicly-available summary statistics did not include sex-specific results. Large differences between women and men exist with regard to prevalence of risk factors for cardiovascular disease, in particular visceral fat and adiponectin, and also in markers like NT-proBNP [3]. Therefore, the possibility remains that effects of adiponectin on heart function or vice versa are different between women and men. Second, due to the limited number of genetic variants, it was not possible to perform sensitivity analyses such as MR-Egger and median- or modal-estimator methods [43].

In conclusion, we observed no associations between adiponectin concentrations and measures of heart function, and no reverse causal relation, but we showed that a potential explanation for the adiponectin paradox could be that NT-proBNP raises circulating adiponectin concentrations.

CRediT authorship contribution statement

Tim Christen: Conceptualization, Methodology, Software, Formal analysis, Writing – original draft. **Renée de Mutsert:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Hildo J. Lamb:** Resources, Writing – review & editing. **Ko Willems van Dijk:** Resources, Writing – review & editing. **Saskia le Cessie:** Methodology, Writing – review & editing. **Frits R. Rosendaal:** Conceptualization, Methodology, Writing – review & editing, Supervision. **J. Wouter Jukema:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Stella Trompet:** Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors report no declarations of interest.

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