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Adipokines are possible risk markers for aortic stenosis requiring surgery

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ABSTRACT

Objectives: Aortic stenosis (AS) is the most prevalent valvular heart disease among adults. The adipocyte-derived hormones, leptin and adiponectin, have profound metabolic actions. We examined whether these adipokines are independently associated with future aortic valve replacement (AVR).

Design: In this longitudinal case-control study, we identified 336 cases who had undergone AVR due to AS, and who had previously participated in population-based health surveys. Two referents were matched to each case and leptin and adiponectin concentrations were analysed from stored baseline survey samples. Uni- and multivariable logistic regression analyses were used to estimate the risk of future AVR. An additional cohort was identified for validation including 106 cases with AVR and 212 matched referents.

Results: Median age (interquartile range (IQR)) in years at survey was 59.9 (10.4) and at surgery 68.3 (12.7), and 48% were women. An elevated concentration of leptin was not associated with future AVR (odds ratio [95% confidence interval]) (1.10 [0.92–1.32]), although leptin was associated with a higher risk in patients with coronary artery disease (CAD) having more than 5 years between survey and AVR (1.41 [1.08–1.84]). Adiponectin was not associated with higher risk for future AVR (0.95 [0.82–1.11]), although after stratification for age, higher levels were associated with reduced risk for AVR in persons aged \geq 60 years at surgery (0.79 [0.64–0.98]). In the validation study, leptin was associated with future AVR whereas adiponectin was not. None of the associations remained significant after adjustment for body mass index (BMI).

Conclusions: The adipokine leptin may promote the development of AS.

ARTICLE HISTORY

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KEYWORDS

Aortic stenosis; leptin; adiponectin; fat mass; risk markers; prospective study

1. Introduction

Aortic stenosis (AS) is caused by progressive calcification of the aortic valve (AV) leaflets. This leads to decreased leaflet mobility which, in turn, causes restriction of left ventricular (LV) outflow and increases LV pressure. The prevalence of AS increases with age. Degenerative AS is the most common form in developed countries, with a prevalence of around 3% in people 75 years and older [1]. Together with ageing, a congenital bicuspid aortic valve (BAV) is one of the strongest risk factors for developing AS, and nearly half of all aortic valve replacements (AVR) are due to BAVs. These patients tend to develop calcific AS 1-2 decades earlier than individuals with a normal tricuspid aortic valve [2].

As of today, the predominant treatment for AS is surgical replacement of the valve or, in the case of a patient not suitable for this highly invasive method, transcatheter aortic valve replacement (TAVR). AV disease has a long subclinical period, and currently, no treatment exists to halt the progression of AS once it has been detected [3]. Therefore, identification and characterization of mechanisms leading to

AS that could be therapeutic targets to curb the progression of the disease, or manage the disease by lifestyle or pharmacological interventions, are highly sort after.

The pathophysiology behind degenerative AS is not fully understood, however, currently it is described as an active process of lipid deposition, calcification, low-grade inflammation, and ossification. Hence, it is believed that AS shares the same risk factor profile as atherosclerosis, and thus coronary artery disease (CAD) is a frequent finding in patients requiring surgery for AS [4,5]. Other clinical risk factors related to AS include arterial hypertension, smoking, obesity, hypercholesterolemia, and diabetes mellitus [6,7].

Over the years, multiple biomarkers have been investigated with the intention of identifying underlying biochemical mechanisms which, when targeted would halt or reverse AS pathogenesis, and decrease the likelihood of needing AS surgery. B-type natriuretic peptide (BNP) has been the only one successfully identified and validated for this purpose and is currently used in clinical practice [8]. High concentration of lipoprotein (A) and a high ratio of apolipoprotein

B/A1 have been associated with increased risk of future AS surgery, however, only in patients with CAD [9], whereas high-sensitivity (hs) troponin T has been shown to predict AVR irrespective of concomitant CAD [10]. This indicates that there may be different phenotypes of AV disease and that these phenotypes might not share the same risk factors.

Being overweight or obese is associated with cardiovascular disease (CVD) and premature death, partly by aggravating classical CV risk factors such as hypertension. Other mechanisms connecting fat mass with CVD have been discovered during the last decades, with the adipocyte-derived hormones leptin and adiponectin being identified as possible links, and elevated leptin being found to be associated with myocardial infarction (MI) [11] and stroke [12]. Hyperleptinemia is related to inflammation [13], increased sympathetic nervous activity [14], and type 2 diabetes mellitus (T2DM) [15]. Adiponectin has anti-inflammatory and insulin-sensitizing properties, and low concentration of adiponectin are found in obese subjects and in patients with T2DM [15,16]. Some studies have shown a correlation between elevated leptin concentration and AS [17,18], but not prospectively. Furthermore, hypoadiponectinemia has been linked to faster progression of AS [19]. In this nested case-referent study we hypothesized that elevated leptin levels and reduced adiponectin levels are associated with future risk of AS requiring surgery.

2. Methods

2.1. Study population

The cases were identified from the register of patients having undergone any type of valvular surgery and/or surgery for the ascending aorta at the department of thoracic surgery at Umeå University Hospital, between March 1988 and December 2014. This resulted in a total of 6691 patients. This register has been cross-linked with the Northern Sweden Health and Disease Study (NSHDS) and its three sub-cohorts, the Västerbotten Intervention Program (VIP), MONItoring of Trends and Determinants of CArdiovascular Disease (MONICA) study, and the Mammary Screening Project (MSP) study, with total of more than 150,000 participants. Overall, 873 patients with surgery for valvular disease and/or ascending aorta with previous participation in NSHDS were identified. Among these, 336 patients (237 (70.5%) from VIP, 62 (18.5%) from MONICA, and 37 (11%) from MSP) had been operated with AV replacement due to AS between October 1992 and April 2012. For details, see Figure 1.

For each case, two controls without any valvular surgery were selected from the NSHDS, and were matched for sex, age (±2 years), subtype of survey (VIP, MONICA, or MSP), date of participation in health survey (±4 months) and residency. Altogether 671 controls were identified following these criteria. Following the same criteria as above, additional 106 cases and 212 referents were identified for validation, and the AVR was carried out between May 2012 and April 2014.

The VIP is a continuing health intervention programme for the residents in Västerbotten county, where subjects aged 30 (until 1995), 40, 50 and 60 years were asked to participate in a health survey at their primary healthcare centre. The aim of the project is to prevent cardiovascular disease (CVD) and diabetes in the region [20]. The WHOinitiated MONICA study is carried out in the two northernmost counties in Sweden and was initiated in the mid-eighties. Seven health surveys have been performed between 1986 and 2014, and subjects aged between 25 and 74 years of age were randomly selected and asked to enrol in the health surveys [21]. In the MSP cohort, women who attended routine mammary screening could voluntarily participate in the health survey, during the period between 1995 to 2006. Participants in all three sub-cohorts were asked to complete a questionnaire on various lifestyle factors and donate blood to the Northern Sweden Biobank for future research [22].

Ethical approval has been granted by the Regional Ethical Review Board in Umeå (diary number 2014-348-32 M and 07-174 M), and the study protocol complied with the declaration of Helsinki. Written informed consents was collected and the participants were informed that blood samples were being taken and stored for non-specified research in the future.

2.2. Baseline clinical examinations and biochemical analysis

Clinical examinations and biochemical analyses performed at baseline have been thoroughly described in our previous papers [23–25]. Participants in VIP and MONICA were asked to complete a health questionnaire regarding their lifestyle and CV risk factors. Subjects were categorised as smokers (including current daily smokers and ex-smokers) or never-smokers. An oral glucose tolerance test was performed routinely in the VIP, in 60% of MONICA participants, but not in the MSP participants. Glucose tolerance categories were defined according to WHO guidelines [26]. Anthropometry and blood pressure measurements were performed as previously described [24], and hypertension was defined as a systolic blood pressure \geq 140 mmHg, diastolic blood pressure \geq 90 mmHg, and/or reported use of antihypertensive medication.

Plasma samples were obtained after fasting for a minimum of 4 h (extended to 8 h, after 1992). The samples were stored in a blood bank at $-80\,^{\circ}$ C until analysis. Total serum cholesterol was measured in the VIP participants until September 2009 using a bench-top analyzer (ReflotronR, Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany). All other cholesterol measurements were done at a central laboratory with an enzymatic method (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany). Cholesterol values acquired from the two different methods were adjusted to each other. High sensitivity C-reactive protein (hsCRP) was analysed as previously described [10], and was available only in the discovery cohort.

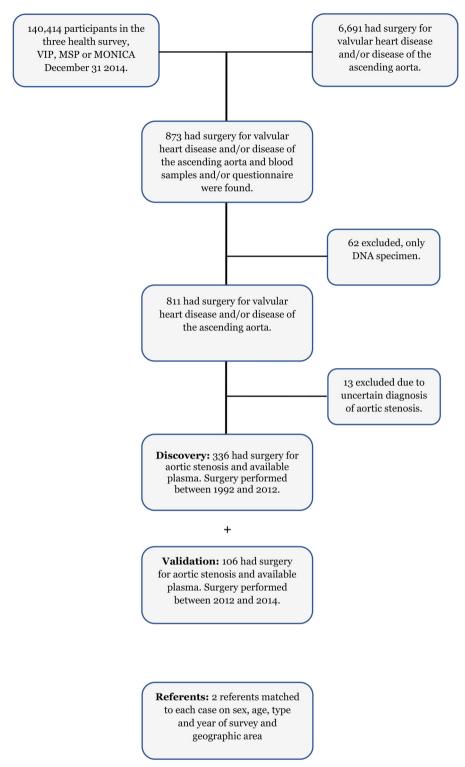


Figure 1. Flowchart showing identification of cases and referents.

Leptin and adiponectin were measured using a Sandwich enzyme-linked immunosorbent assay (ELISA) in 2019 (EMD Millipore Corp., Billerica, MA, USA). The lowest detectible leptin level was 0.2 ng/mL. Interassay-intraassay coefficients of variation (CV) were 4.9-8.6% and 1.9-1.3% at concentrations of 0.9 and 14.1 ng/mL, respectively. Lowest detection concentration of adiponectin was 0.2 ng/mL. Interassay-intrassay CVs were 7.4-8.4% and 1.8-6.2% at levels of 17.7 and 39.1 ng/mL, respectively. Intraindividual stability of adipokine levels and robustness of biochemical analyses have previously been shown by us and others [27-29].

2.3. Perioperative characteristics

Preoperative and perioperative data was collected from hospital medical records, including medical history, echocardiography, and surgical information regarding the aortic valve when available. Any signs of atherosclerosis were regarded as CAD. Valve pathology was determined from the preoperative echocardiography report and from the perioperative surgical report. A BAV was reported if any of the examinations indicated bicuspid morphology.

2.4. Statistical analysis

Normal distribution of continuous variables was tested with test of skewness (Kolmogorov-Smirnov) and by visual inspection of histograms, and when needed (ln) transformation of variables was performed. The (ln) z-scores were calculated separately for males and females.

Data is presented as medians with interquartile range (IQR) or as (geometric) means and 95% confidence intervals (CI), when appropriate. Differences in means between cases and referents were tested with Student's *t*-test. Quartiles for continuous variables were calculated according to the distribution of referent values, for males and females respectively. Distribution of cases and referents in the quartiles was tested for linear association with a chi-square test. Bivariate Pearson's correlations were tested and analysed separately for sex and for case status.

In this nested, matched case-referent study, the cases and referents had the same follow-up time within strata. Thus, to estimate odds ratios (ORs) and 95% CIs, we used logistic regression analysis (rather than Cox regression). Univariable and multivariable linear regression models were used to test the influence of leptin and adiponectin on the risk for future AS surgery. Additionally, the ratio of leptin and adiponectin was tested against the risk for future AS surgery.

Missing values were treated as a separate category for categorical variables (not included in the tables) and missing continuous values were replaced with the median for the control group to enable use of the entire dataset. The analysis was stratified for sex, time between surgery and survey (<5 years or ≥ 5 years), age at surgery (<60 years or ≥ 60 years), and for the presence of any visible CAD on the preoperative angiogram. These groups were pre-specified and used in our previous analysis [9,10]. For the multivariable analysis, we adjusted for hsCRP and body mass index (BMI) in separate analyses. he same methods and models were used in the validation cohort; however, no replacements were done as there was no missing data. All calculations were performed in the statistical program SPSS version 27 (IBM Corp.).

3. Results

Altogether 336 patients (48% women) received an AVR due to AS. In 84% of patients, the main reason for surgery was AS; the remaining 16% received AV surgery combined with another primary intervention, such as coronary artery bypass surgery (10%) or surgery for ascending aortic disease (5%). The median age (IQR) at survey was 59.9 (10.4) years and at surgery 68.3 (12.7) years. The median time between survey and surgery was 10.9 (9.3) years.

In the validation cohort, 106 patients (45% women) received an AVR, and in 88% of cases the primary indication was AS, whereas the primary indication was coronary artery bypass surgery in 5%, surgery for ascending aortic disease in 5%, and surgery for combined aortic stenosis and insufficiency in 2%. The median age at survey was 56.1 (10.6) years, at surgery 71.5 (13.6) years, and the median time between survey and surgery was 15.4 (7.6) years. Out of 106 patients, only 6 had surgery within 5 years from the health survey, and only 12 were younger than 60 years at surgery.

All cases except one (99.7%) underwent coronary angiography, and CAD was found in 203/336 (60.4%) of patients. Similarly, all except one patient had a preoperative coronary angiogram in the validation cohort, of these patients 67/105 (63.8%) had CAD. The patients with CAD were older and had more often a history of previous myocardial infarction (MI) and higher systolic blood pressure. According to the preoperative echocardiography carried out in nearly all cases, the aortic valves of the CAD group had slightly larger valve areas and lower transvalvular gradients. Patients without CAD more often presented with a BAV than patients with CAD. Similarly, patients younger than 60 years at surgery often presented with BAV compared to those aged 60 years or more (55% vs. 26% (p < 0.001), and 83% vs. 21% (p < 0.001) in the discovery and validation cohorts). The preoperative characteristics in the validation cohort were similar to those in the validation cohort, except patients with or without CAD were older at surgery compared to the discovery cohort. Preoperative characteristics are summarized in Supplementary Table S1.

At the baseline health survey, those with future surgery for AS, had a higher prevalence of glucose intolerance and hypertension, as well as a higher BMI (Table 1). Cases had higher serum cholesterol compared to referents. Leptin and adiponectin levels did not differ between cases and referents. In the validation cohort, cases had higher BMI and they were more often present or ex-smoker compared to referents (Supplementary Table S2). Leptin levels were higher in cases, and this difference remained in males, and in cases with CAD, after stratification. Adiponectin levels did not differ between cases and referents.

Body weight increased from the baseline survey to preoperative examination, in the discovery cohort (median [IQR]) 1.0 [8] kg in men and 0 [6.5] kg in women, and in the validation cohort 0.2 [11] kg in men and 0.9 [10.5] kg in women. Measurements were available for 94% of the cases in the discovery cohort and for 93% in the validation cohort.

A correlation was observed between leptin and BMI, both in males (r = 0.62, p < 0.001) and in females (r = 0.63, p < 0.001). These associations remained after stratification for case status (data not shown). Adiponectin correlated inversely to BMI, both in men (r = -0.16, p < 0.001) and in women (r = -0.24, p < 0.001). This association remained in both cases and referents after stratification (data not shown).

Table 1. Baseline characteristics.

	Referents/cases (n)	Referents	Cases	р
BMI (kg/m ²)	655/322	26.1 (25.8–26.4)	26.9 (26.4–27.4)	0.01
Hypertension (%)	545/269	49.2 (45.0-53.4)	61.0 (55.1–66.8)	0.001
Systolic BP (mmHg)	545/270	135 (134–137)	138 (136–141)	0.05
Diastolic BP (mmHg)	545/269	84 (84–85)	86 (85–87)	0.05
Total cholesterol (mmol/L)	535/265	6.2 (6.1–6.3)	6.4 (6.2–6.5)	0.05
Glucose intolerance (%)	490/242	19.8 (16.3–23.3)	26.4 (20.8-32.0)	0.05
Smoker (%)	531/258	53.7 (49.4–57.9)	59.7 (53.7–65.7)	0.1
Creatinine (µmol/L)	647/310	73.2 (72.1–74.2)	72.3 (70.7–73.8)	0.3
Leptin (ng/mL)*				
All	648/308	7.1 (6.6–7.8)	7.7 (6.8–8.6)	0.4
Men	334/155	3.8 (3.4–4.2)	4.0 (3.5-4.5)	0.6
Women	314/153	14 (12.8–15.4)	14.9 (13.0–17.0)	0.5
Surgery <5 years	145/68	7.5 (6.4–8.8)	7.1 (5.8–8.8)	0.7
Surgery >5 years	503/240	7 (6.4–7.8)	7.8 (6.8–9.0)	0.2
No CAD	257/127	8.7 (7.7–9.8)	8.1 (6.8–9.8)	0.6
CAD	389/180	6.3 (5.7–7.0)	7.4 (6.3–8.6)	0.1
Adiponectin (ng/mL)*				
All	650/309	15.6 (15.0–16.2)	15.3 (14.4–16.3)	0.7
Men	335/155	12.3 (11.7–12.9)	12.2 (11.2–13.2)	0.9
Women	315/154	20.1 (19.1–21.2)	19.3 (17.7–21.0)	0.4
Surgery <5 years	146/69	15.6 (14.3–16.9)	16.3 (14.2–18.7)	0.6
Surgery >5 years	504/240	15.6 (14.9–16.3)	15.0 (14.0–16.2)	0.4
No CAD	258/127	16.2 (15.1–17.3)	16.9 (15.3–18.6)	0.5
CAD	390/181	15.2 (14.5–16.0)	14.2 (13.1–15.5)	0.2

Values shown are numbers, means (*geometric) and proportions with 95% confidence interval. P-values were based on Students t-test. Bold text indicates p-value <0.05. BP: blood pressure, BMI:body mass index, and CAD: coronary artery disease.

Table 2. Univariable analysis in the discovery cohort.

	R/C (n)	All	R/C (n)	No CAD	R/C (n)	CAD
Leptin (In-Z-score)						
All (missing)	602/308	1.06 (0.87-1.28)				
All	671/336	1.10 (0.92-1.32)	264/132	0.88 (0.65-1.19)	405/203	1.25 (0.99-1.57)
Men	349/175	1.09 (0.85-1.40)	108/54	0.72 (0.46-1.13)	239/120	1.32 (0.97–1.81)
Women	322/161	1.11 (0.85-1.45)	156/78	1.05 (0.68-1.60)	166/83	1.16 (0.82-1.63)
Surgery <5 years	148/74	0.86 (0.57-1.29)	62/31	1.01 (0.52-1.98)	84/42	0.78 (0.46-1.30)
Surgery ≥5 years	523/262	1.17 (0.96-1.44)	202/101	0.85 (0.60-1.19)	321/161	1.41 (1.08-1.84)
Age <60 years	140/70	1.15 (0.74–1.79)	100/50	1.02 (0.59–1.75)	40/20	1.47 (0.67-3.23)
Age ≥60 years	531/266	1.09 (0.89-1.33)	164/82	0.82 (0.56-1.19)	365/183	1.23 (0.96-1.56)
Adiponectin (In-Z-score)						
All (missing)	605/309	0.95 (0.81-1.11)				
All	671/336	0.95 (0.82-1.11)	264/132	1.11 (0.87-1.42)	405/203	0.85 (0.69-1.03)
Men	349/175	1.00 (0.81-1.23)	108/54	1.33 (0.89-1.99)	239/120	0.86 (0.66-1.11)
Women	322/161	0.91 (0.73-1.13)	156/78	0.99 (0.72-1.35)	166/83	0.83 (0.61-1.14)
Surgery <5 years	148/74	1.10 (0.77-1.57)	62/31	1.26 (0.71-2.23)	84/42	0.92 (0.57-1.49)
Surgery ≥5 years	523/262	0.92 (0.78-1.09)	202/101	1.08 (0.82-1.41)	321/161	0.83 (0.67-1.04)
Age <60 years	140/70	1.17 (0.86-1.60)	100/50	1.12 (0.76-1.64)	40/20	1.29 (0.75-2.19)
Age \geq 60 years	531/266	0.89 (0.75-1.06)	164/82	1.10 (0.80-1.51)	365/183	0.79 (0.64-0.98)
Leptin/adiponectin (ratio In-leptin/In-adiponectin)						
All (missing)	600/307	1.25 (0.86-1.83)				
All	671/336	1.32 (0.92-1.90)	264/132	0.82 (0.44-1.52)	405/203	1.73 (1.09-2.75)
Men	349/175	1.21 (0.74-1.98)	108/54	0.45 (0.17-1.18)	239/120	1.81 (0.99-3.29)
Women	322/161	1.46 (0.85-2.52)	156/78	1.29 (0.57-2.92)	166/83	1.62 (0.78-3.36)
Surgery <5 years	148/74	0.85 (0.34-2.12)	62/31	0.98 (0.21-4.47)	84/42	0.81 (0.25-2.57)
Surgery ≥5 years	523/262	1.44 (0.96-2.14)	202/101	0.79 (0.40-1.56)	161/321	2.00 (1.20-3.34)
Age <60 years	140/70	1.36 (0.63-2.94)	100/50	1.07 (0.40-2.82)	40/20	2.12 (0.53-8.50)
Age ≥60 years	531/266	1.31 (0.86-1.98)	164/82	0.68 (0.31-1.53)	365/183	1.68 (1.03-2.75)

Values shown are numbers (cases/referents) and OR with 95% confidence intervals. Bold text indicates p-value <0.05. R/C: Referents/Cases.

Elevated leptin concentration was not associated with future surgery for AS (OR [95% CI]) (1.10 [0.92-1.32]) (Table 2). An association was however seen in patients with CAD and surgery more than 5 years after the health survey (1.41 [1.08-1.84]), specifically in males (1.49 [1.04-2.12]). These associations were slightly attenuated after adjustment for hsCRP (1.32 [1.01-1.74] and 1.42 [0.99-2.03], respectively).

Adiponectin did not associate with future risk for AS surgery (0.95 [0.82-1.11]) (Table 2). Yet, after stratification for age, high concentration of adiponectin associated with lower risk of future AVR in patients with CAD aged 60 years or more at surgery (0.79 [0.64-0.98]). This association was attenuated after adjustment for hsCRP (0.81 [0.65-1.01]). Analysis of leptin and adiponectin categories did not add more information (data not shown).

A high ratio of leptin to adiponectin was associated with AS surgery in patients with CAD, after stratification in patients with CAD and more than 5 years after the health survey, and in patients with CAD aged 60 years or more at

Table 3. Univariable analysis in validation cohort.

	R/C (n)	All	R/C (n)	No CAD	R/C (n)	CAD
Leptin (In-Z-score)						
All	211/106	1.78 (1.24-2.54)	77/39	2.01 (1.05-4.02)	134/67	1.67 (1.09-2.55)
Men	116/58	1.79 (1.14-2.82)	36/18	1.79 (0.78-4.11)	80/40	1.79 (1.04-3.08)
Women	95/48	1.75 (0.97-3.16)	41/21	2.51 (0.88-7.14)	54/27	1.47 (0.73-2.97)
Age ≥60 years	187/94	1.84 (1.26-2.69)	59/30	2.19 (1.05-4.58)	128/64	1.72 (1.11-2.67)
Adiponectin (In-Z-score)						
All	211/106	0.81 (0.63-1.06)	77/39	1.03 (0.64-1.66)	134/67	0.73 (0.53-1.01)
Men	116/58	0.75 (0.52-1.08)	36/18	0.77 (0.37-1.61)	80/40	0.74 (0.49-1.13)
Women	95/48	0.90 (0.61-1.32)	41/21	1.27 (0.67-2.42)	54/27	0.73 (0.44-1.19)
Age ≥60 years	187/94	0.79 (0.60-1.05)	59/30	0.92 (0.55-1.53)	128/64	0.74 (0.53-1.04)
Letin/adiponectin ratio (ratio In-leptin/In-adiponectin)						
All	211/106	1.94 (1.20-3.13)	77/39	2.42 (0.90-6.48)	134/67	1.81 (1.05-3.12)
Men	116/58	1.95 (1.05-3.60)	36/18	2.30 (0.66-8.08)	80/40	1.84 (0.91-3.72)
Women	95/48	1.93 (0.90-4.13)	41/21	2.60 (0.54-12.57)	54/27	1.76 (0.75-4.16)
Age \geq 60 years	187/94	2.20 (1.31-3.70)	59/30	3.27 (1.04–10.33)	128/64	1.97 (1.10-3.52)

Values shown are numbers (cases/referents) and OR with 95% confidence intervals. Bold text indicates p-value <0.05. R/C: Referents/Cases.

surgery (Table 2). These results were largely replicated for leptin in the validation cohort (Table 3). High leptin levels were associated with future surgery for AS in all (1.78 [1.24–2.54]), and after stratification in both patients with no CAD (2.01 [1.05-4.02]) and with CAD (1.67 [1.09-2.55]), in males (1.79 [1.14-2.82]), and in those aged 60 years or more at surgery (1.84 [1.26-2.69]). Analysis of those aged less than 60 years at surgery, and of those with less than 5 years between health survey and surgery, was not meaningful due to small numbers. In contrast, adiponectin did not associate with surgery for AS in the validation cohort (0.82 [0.63-1.06]). Analysis of the ratio of leptin to adiponectin replicated the results from the analysis of leptin (Table 3). After adjustment for BMI, no associations remained between high leptin levels and low adiponectin levels on one side, and future AVR on the other.

Discussion

In this longitudinal, nested case-referent study, we showed that elevated leptin was associated with future AS requiring surgery in males, and in patients with concomitant CAD. These findings were largely replicated in our validation cohort. Low concentration of adiponectin associated with AS in a subgroup of older patients, which was not replicated in the validation cohort. However, none of the associations remained after adjustment for BMI. In previous studies, elevated leptin has been associated with AS, however, to our knowledge only in cross-sectional studies [17,18], and low concentration of adiponectin have been related to valvular inflammation and faster disease progression of AS [19].

Several different mechanisms may explain the association between these adipokines and AS, for example both have been associated with the atherosclerotic process [30,31]. High leptin is associated with adverse effects on the fibrinolytic system [32], on platelet aggregation [33], and leptin is also known to promote hypertension through specific receptors in the carotid bodies [34]. Specifically, leptin is found in calcified aortic valves and promotes osteoblast differentiation of human valvular interstitial cells [18], and is produced in atherosclerotic plaques [35]. This is consistent with our findings, where elevated leptin was seen specifically in patients with CAD. Furthermore, leptin replacement therapy

for lipodystrophy has recently been associated with development of severe AS [36].

Adiponectin has shown anti-atherosclerotic effects through inhibition of smooth muscle proliferation [37] and increasing macrophage cholesterol efflux [38]. Opposing results have however been presented regarding the association between adiponectin and aortic stenosis [19, 39]. The fact that adiponectin associated with AVR in patients >60 years might indicate that a connection between adiponectin and AS is more likely in patients with tricuspid stenotic valves, rather than in the younger group (<60 years) where the majority of AS cases are due to BAVs [40]. Accordingly, we found that younger patients without CAD more often presented with a BAV, which may support the theory that adipokines affect the valves differentially depending on the underlying valve pathology. These findings should be interpreted cautiously considering the difficulty to determine the presence of BAV pathology in AS.

In addition, paracrine effects of adipokines produced by the epicardial adipose tissue (EAT) may affect the aortic valve and the coronaries, however the pathways are not fully understood [41]. Both leptin and adiponectin levels relate to the amount of EAT and CAD [42–44], and the thickness of EAT predicts the severity of AS [45].

It is unlikely that inflammation fully explains the association between these adipokines as adjustment for hsCRP only marginally attenuated the associations, and we have previously shown that inflammation expressed as elevated hsCRP did not predict AVR in this cohort [10].

The results related to leptin and AVR differed somewhat between the discovery and validation cohorts, where the age at surgery was higher, and the time between health survey and surgery was longer, in the latter. We speculate that longer exposure time to elevated leptin is responsible for the stronger association in the validation cohort. The difference between sexes, with stronger association in males than in females, is in line with our previous findings where leptin was seen to be associated with stroke and diabetes in males only [11,12]. The association between leptin and future surgery for AS did not remain in any subgroup or cohort after adjustment for BMI. An explanation of this finding could be that adipose tissue produce other adipokines with effect on the valvular tissue and that leptin is only a proxy for fat



mass and other adipocyte-derived factors. However, leptin could still be considered causative as the known pathophysiological effects of leptin described above, and the adjustment for BMI, is not methodologically appropriate considering the strong association between leptin and fat

There are some limitations of this study including the following: Even if the number of cases is relatively large (336 plus 106 cases of AVR), the lack of significant finding may be due to lack of power which may have resulted in us failing to unveil weaker associations. Since the VIP study only included patients of 30, 40, 50 and 60 years of age, younger patients may be under-represented. The cases are exclusively patients accepted for AVR, thus eliminating asymptomatic patients, those with yet unknown AV disease and individuals with contraindications for surgery such as frailty and old age. However, the validation cohort indicates that more elderly patients have had surgery during recent years. Additionally, our patient group explicitly represents a population rooted in Northern Sweden, and thus the generalisability of this study to other populations might be

However, the strengths of this study should also be acknowledged. The study design is truly prospective, although cases are identified retrospectively. The baseline data and blood samples were strictly collected from population-based studies. Moreover, the blood sample analysis of both cases and referents was completed in a standardised manner at a defined period of time, in the same laboratory. As circulating levels of leptin and adiponectin are strongly correlated to body weight, and we rely on only one measurement, it can be argued that these levels do not reflect the life-long exposure. However, we have previously shown that leptin levels are stable over many years [29], and our cases increased modestly in body weight over ten years although with great variation.

This research aimed to identify whether high leptin and low adiponectin levels predict AS requiring surgery. We concluded that elevated leptin associated with increased risk for AS surgery, specifically in males with CAD. Low adiponectin was associated with risk for future AVR in patients over 60 years. However, it is unclear whether these associations are independent of obesity. To our knowledge findings have not been shown previously using a longitudinal study design. Further research should be encouraged to determine possible clinical integration of the adipokines.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Data from the NSHDS surveys are not freely available according to GDPR due to the presence of individual data. However, pseudonymized data can be shared after ethical evaluation and formal request (https://www.umu.se/en/biobankresearch-unit/research/access-to-samples-anddata/access-to-nsdd/).

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