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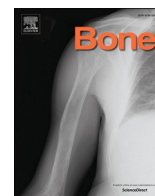
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## Full Length Article

## Circulating lipocalin-2 and features of metabolic syndrome in community-dwelling older women: A cross-sectional study

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## ABSTRACT

Lipocalin-2 (LCN2) is released by several cell types including osteoblasts and adipocytes and has been suggested as a marker of renal dysfunction, metabolic syndrome (MetS) and type 2 diabetes (T2D). Whether LCN2 is linked to these diseases in older women remains unknown. This study investigated whether LCN2 is related to features of MetS and T2D in older women. This cross-sectional study included 705 non-diabetic women (mean age 75.1 ± 2.6 years) for MetS analysis and 76 women (mean age 75.4 ± 2.8 years) with T2D. Total circulating LCN2 levels were analysed using a two-step chemiluminescent microparticle monoclonal immunoassay. MetS was determined by a modified National Cholesterol Education Program Adult Treatment Panel III classification. Multivariable-adjusted logistic regression analysis was used to assess odds ratios between LCN2 quartiles and MetS. Women in the highest LCN2 quartile had approximately 3 times greater risk for MetS compared to women in the lowest quartile (OR 3.05; 95%CI 1.86–5.02). Women with T2D or MetS scores of ≥ 3 had higher LCN2 levels compared to women with a MetS score of 0 ( $p < 0.05$ ). Higher LCN2 correlated with higher body mass index, fat mass, triglycerides and glycated haemoglobin and lower high-density lipoprotein cholesterol and estimated glomerular filtration rate ( $p < 0.05$ ). Higher circulating levels of LCN2 are associated with worsened cardio-metabolic risk factors and increased odds of MetS and T2D in older women. Whether it can be used as a biomarker for identifying those at risk for MetS and T2D should be explored further.

### 1. Introduction

Life expectancy continues to increase globally, with females having a higher life expectancy than males [1]. Ageing, in combination with suboptimal lifestyle habits such as poor diet and low levels of physical activity, has a profound negative effect on human physiology and metabolism leading to increased risk for chronic health conditions, including metabolic syndrome (MetS) and type 2 diabetes (T2D) [2]. MetS is a precursor for T2D and includes a cluster of characteristic criteria including obesity, hypertension, dyslipidemia and elevated

blood glucose levels [3]. Early identification of risk factors can prevent the development of chronic conditions in our ageing population.

Lipocalin-2 (LCN2), also known as neutrophil gelatinase-associated lipocalin (NGAL), is an adipokine released by multiple cell types such as adipocytes, osteoblasts and renal tubular cells [4,5]. LCN2 is also an important protein for satiety and energy regulation [6]. It has been suggested that under “healthy conditions” LCN2 is mainly produced from bone, whereas under “pathological conditions” it is released by adipocytes [6]. Increased levels of LCN2 in the circulation can also be a marker of acute kidney injury [7]. An association between increased

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circulating LCN2 and chronic metabolic conditions has been observed previously in animal studies, diabetic and pre-diabetic humans and cardiac patient populations [8–10]. It is unclear whether higher circulating LCN2 is related to increased number of MetS criteria in community-dwelling older women. Further information is needed to determine whether this relationship is amplified in those with diagnosed T2D compared to those with MetS.

Understanding the relationship between LCN2, MetS characteristics and T2D may provide vital information regarding the use of LCN2 as a biomarker to improve the identification of older women at risk of developing metabolic diseases. As such, the aim of this study was to explore the hypothesis that higher circulating LCN2 levels are associated with the clinical components of MetS and increased odds of having MetS and T2D in a representative population of older women.

## 2. Methods

### 2.1. Study population

Participants were recruited as part of the Calcium Intake Fracture Outcome study (CAIFOS) as described previously [11]. Participants were recruited from the Western Australian general population of women aged over 70 years by mail using registration on the electoral roll, which is a requirement of citizenship. From the 5586 women approached, 1500 women were recruited into the study. Of the 1500 recruited, LCN2 was not measured in 255 women, 412 women were missing at least one variable required to calculate MetS and estimated glomerular filtration rate (eGFR) was not assessed in 52 women. As such, the current study included 705 women for MetS analysis and 76 women with diabetes. The disease burden and medications of the study participants were comparable with the general population of similar age, although the CAIFOS participants were more likely to be from higher socio-economic groups [11]. As this trial commenced and was completed prior to the advent of the clinical trials registry, the trial was retrospectively registered with the Australian New Zealand Clinical Trials Registry ACTRN12615000750583.

The Human Research Ethics Committee of the University of Western Australia approved the study and written informed consents were obtained from all participants. Human ethics approval for the use of linked data for the project was provided by the Human Research Ethics Committee of the Western Australian Department of Health (DOHWA HREC), project number #2009/24.

### 2.2. Biochemistry analyses

Fasting blood samples were collected at the commencement of the CAIFOS study (1998) and stored at  $-80^{\circ}\text{C}$  until assessment. Plasma LCN2 was measured using a two-step chemiluminescent microparticle monoclonal immunoassay on an automated platform (Abbott Diagnostics, Longford, Ireland). The inter-assay coefficient of variation over a 6-week period was 9.3% and 4.6% at concentrations of 19 ng/L and 190 ng/L respectively. Total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglyceride concentrations were determined using a Hitachi 917 auto analyser (Roche diagnostics). Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's method [12]. In 2005, baseline fasting venous serum previously stored at  $-80^{\circ}\text{C}$  were analysed for creatinine using an isotope dilution mass spectrometry (IDMS) traceable Jaffe kinetic assay for creatinine on a Hitachi 917 analyser (Roche Diagnostics GmbH, Mannheim Germany). eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations: 1) for standardised serum creatinine  $\leq 0.7$  mg/dL:  $\text{eGFR} = 144 \times (\text{Scr} / 0.7)^{-0.329} \times (0.993)^{\text{Age}}$  and 2) for standardised serum creatinine  $> 0.7$  mg/dL:  $\text{eGFR} = 144 \times (\text{Scr} / 0.7)^{-1.209} \times (0.993)^{\text{Age}}$  [13]. Stage III chronic kidney disease (CKD) was defined as an eGFR rate  $< 60$  mL/min/1.73m<sup>2</sup>. In 1999, (year 1) glycated haemoglobin (HbA1c) was measured using ion-exchange HPLC using the Variant II (Bio-Rad), the

CV was 2.00% at 5.4 and 1.44% at 13.7.

### 2.3. Assessments

At baseline, participants provided their previous medical history and current medications verified by their General Practitioner. These data were coded using the International Classification of Primary Care – Plus (ICPC-Plus) method [14]. The coding methodology allows aggregation of different terms for similar pathologic entities. Use of anti-diabetic medications (oral hypoglycaemic agents or insulin) were used to determine the presence of pre-existing diabetes (T89001-90009). Linked health records were provided by the Western Australian Data Linkage Branch, Hospital Morbidity Data Collection to identify prevalent atherosclerotic vascular disease (ASVD) for the 18 years prior to baseline clinical assessment, as described previously [15]. Statin and anti-hypertensive medication use was verified by participants' primary care physician when possible. Smoking status was coded as non-smoker or ex-smoker/current smoker if they had consumed  $> 1$  cigarette per day for  $> 3$  months at any time in their life. Weight was assessed using digital scales with participants wearing light clothes and no shoes. Height was assessed using a stadiometer and BMI was calculated in kg/m<sup>2</sup> at baseline. Blood pressure was measured on the right arm with a mercury column manometer using an adult cuff after the participants have been seated in an upright position and had rested for 5min. An average of three blood pressure readings was recorded.

### 2.4. Body composition by dual-energy X-ray absorptiometry

Whole body composition was assessed using a Hologic Acclaim QDR4500A (Hologic Corp, Waltham, MA) dual-energy X-ray absorptiometry machine at baseline or 12 months in a subgroup of women in the current study ( $n = 192$ ) using standard protocols. Of these 192 participants, 15 were diabetic. Anthropometric measurements considered included whole-body fat and lean mass (less head) as well as appendicular fat and lean mass.

### 2.5. Definition of metabolic syndrome (MetS)

Metabolic syndrome was defined using a modified National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) classification scheme [3], identified by three or more of the following; (1) women who had a body mass index  $\geq 30$  kg/m<sup>2</sup>, (2) triglycerides  $\geq 150$  mg/dL (1.69 mmol/L), (3) HDL cholesterol  $< 50$  mg/dL (1.3 mmol/L) or on statins, (4) diastolic blood pressure  $\geq 85$  mmHg and/or systolic blood pressure  $\geq 130$  mmHg or on anti-hypertensives and (5) HbA1c  $\geq 5.7\%$  (impaired fasting glucose or pre-diabetes).

### 2.6. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Version 21 (2012, Armonk, NY: IBM Corp), STATA (version 13 StataCorp LP, College Station, TX) and SAS (Version 9.4, SAS Institute Inc., Chicago, IL). Baseline data are presented as mean  $\pm$  SD for normally distributed continuous variables, median and IQR for non-normally distributed continuous variables, and number and (%) for categorical variables. Participants were grouped into quartiles based on circulating total LCN2 levels for data presentation purposes; quartile 1  $< 62.8$  mg/L, quartile 2 62.9 to  $< 76.3$  mg/L; quartile 3 76.3 to  $< 94.2$  mg/L, and quartile 4  $\geq 94.2$  mg/L. Bonferroni tests, chi-squared and Kruskal-Wallis tests were performed to assess significant differences between sub-groups (LCN2 quartiles and diabetes) for participant characteristics. The relationship between LCN2 and components of metabolic syndrome, age and eGFR was assessed using an unadjusted Spearman's rank correlation. *P*-values of  $< 0.05$  in two tailed testing were considered statistically significant. Odds ratios (ORs) (as part of restricted cubic splines) were calculated relative to the median of the first LCN2 quartile as a reference value and

plotted against the exposure variable, MetS, with 95% confidence bands provided. Wald tests were used to determine *P*-values for ORs. We tested for non-linearity using a likelihood ratio test to compare nested models with and without the nonlinear terms for the exposure. Three models of adjustment were considered; Model 1: age-adjusted, Model 2: Model 1 + physical activity and smoking status and Model 3: Model 2 + estimated glomerular filtration rate. Statistical significance was set at  $p < 0.05$  for all tests.

## 2.7. Additional analysis

Linear regression was used to examine the relationship between log-transformed LCN2 and DXA-derived whole-body fat and lean mass (less head), as well as appendicular fat and lean mass in the subset of women without diabetes ( $n = 177$ ).

## 3. Results

Characteristics of the 705 women without T2D (mean age  $75.1 \pm 2.6$  years) for MetS analysis and 76 women (mean age  $75.4 \pm 2.8$  years) with T2D are presented in Table 1 according to LCN2 Quartiles.

Women without diabetes in LCN2 Q4 (highest levels) had a higher BMI and lower eGFR compared to Q1-Q3 and higher triglycerides compared to Q1 and Q2 (all  $p < 0.05$ ). LCN2 was significantly lower in patients with diabetes compared to Q4 in those without diabetes ( $p < 0.001$ ). The proportion per category differed significantly between quartiles and diabetes for anti-hypertensive use ( $\chi^2(4) = 25.52, p < 0.001$ ) and statin use ( $\chi^2(4) = 14.81, p = 0.005$ ). The distribution of physical activity was different across quartiles and diabetes sub-groups ( $p = 0.001$ ).

Compared to women without MetS (median LCN2 = 73.70 (27.20)), those with MetS (median LCN2 = 87.85 (43.97)) and those with T2D (median LCN2 = 85.15 (46.38)) had higher LCN2 ( $p = 0.001$  and  $p < 0.001$  respectively). The proportion of women with diabetes within LCN2 quartile 1–4, with quartiles determined by the sample without diabetes, is displayed in Supplementary Fig. 1.

Higher circulating LCN2 levels were significantly correlated with four of the five components of MetS including increased BMI ( $r = 0.26, p < 0.001$ ), HbA1c ( $r = 0.11, p = 0.004$ ) and triglycerides ( $r = 0.23, p <$

0.001), and lower HDLC ( $r = -0.18, p < 0.001$ ) in the entire cohort excluding those with T2D. Higher circulating LCN2 was also associated with age ( $r = 0.12, p = 0.001$ ) and eGFR ( $r = -0.35, p < 0.001$ ). In addition, when considering the subset of women with available DXA scans without diabetes ( $n = 177$ ), higher LCN2 levels were significantly correlated with whole body, and appendicular fat mass, but not with lean mass (Fig. 1).

Women with MetS scores of 3 ( $p < 0.001$ ), 4 ( $p < 0.02$ ) and 5 ( $p < 0.05$ ) and T2D ( $p < 0.02$ ) had higher LCN2 levels compared to individuals with a MetS score of 0 (Fig. 2).

There were 190 (27%) women with MetS. The proportion of women with MetS in LCN2 quartiles Q4 (43.2%) and Q3 (27.1%) was significantly higher than Q1 (17.7%) (Table 2). After adjustment, women with the third and fourth highest quartiles of LCN2 had approximately 1.8 and 3.6 times greater odds of having MetS compared to women in the lowest quartile, and that remained significant after adjusting for eGFR (Table 2). The inclusion of statin medication use and prevalent ASVD in the multivariable-adjusted model did not change the interpretation of the results (Supplementary Fig. 3).

A diagrammatic representation of the relationship between increasing LCN2 levels and the odds for presenting with MetS is presented in Fig. 3 ( $p$  for non-linearity = 0.09).

## 3.1. Additional analysis

In a backwards stepwise linear regression model with HbA1c as the dependent variable and LCN2 (log-transformed) adjusting for all other variables included in MetS criteria for the 705 women without diabetes, covariates included BMI, triglycerides, HDL, statin use, mean SBP and DBP and antihypertensive medication use. The most parsimonious model included triglycerides ( $p = 0.005$ ), LCN2 ( $p = 0.040$ ), BMI ( $=0.045$ ), mean DBP ( $p = 0.058$ ).

## 4. Discussion

We report here that women with higher circulating levels of LCN2 (Q4) had approximately 3 times greater odds for MetS than women with the lowest LCN2 levels (Q1). Furthermore, higher circulating LCN2 was related to increases in each individual component of MetS. It appears

**Table 1**  
Characteristics of study participants according to quartiles of lipocalin-2 levels.

	All non-diabetic participants	Quartiles of total LCN2				Diabetes
		Quartile 1 < 62.8 mg/L	Quartile 2 62.9 to < 76.3 mg/L	Quartile 3 76.3 to < 94.2 mg/L	Quartile 4 ≥ 94.2 mg/L	
Number (%)	705 (100)	175 (24.8)	177 (25.1)	177 (25.1)	176 (25)	76
LCN2 (mg/L)	76.3 (62.8–94.1)	55.4 (48.7–59.7)	69.8 (67.0–73.0)*	85.2 (80.0–136.3)*#	115.8 (103.1–136.3)*#	85.2 (63.0–109.4)*#
Age (years)	75.1 ± 2.6	74.7 ± 2.5	75.01 ± 2.5	75.1 ± 2.7	75.7 ± 2.8*	75.4 ± 2.8
BMI (kg/m <sup>2</sup> )	26.9 ± 4.3	25.8 ± 4.0	26.1 ± 4.0	26.8 ± 3.9	28.9 ± 4.8*#	29.8 ± 5.4*#
Smoked ever, yes (n, %)	249 (35.4)	60 (34.3)	49 (27.8)	68 (38.4)	72 (41.1)	30 (39.5)
Physical activity (kcal/day)	114 (44–202)	126 (62–184)	107 (47–183)	115 (0–240)	94 (0–213)	109 (0–193)
SBP (mmHg)	135.2 ± 16.9	134.3 ± 16.1	135.5 ± 16.2	135.2 ± 18.2	135.6 ± 17.2	141.1 ± 18.6 <sup>§</sup> *
DBP (mmHg)	71.9 ± 10.1	71.9 ± 9.98	72.4 ± 9.4	72.1 ± 10.6	71.2 ± 10.3	71.3 ± 11.2
Antihypertensives use, yes (n, %)	295 (41.8)	65 (37.1)	64 (36.2)	69 (39)	97 (55.1)	45 (59.2)
HbA1c (%)	5.2 ± 0.5	5.0 ± 0.5	5.3 ± 0.6*	5.2 ± 0.4	5.2 ± 0.6	6.9 ± 1.5*# <sup>§</sup>
HDLC (mg/dL)	57.1 ± 14.7	60.4 ± 15.7	57.6 ± 14.9	56.2 ± 12.4*	54.2 ± 14.7*	48.8 ± 11.3 <sup>§</sup> *#
LDLC (mg/dL)	142.5 ± 38.9	141.3 ± 42.8	142.4 ± 37.8	146.7 ± 37.8	139.4 ± 36.8	120.9 ± 37.7 <sup>§</sup> *#
Triglycerides (mg/dL)	133.2 ± 54.7	120.6 ± 52.2	125.1 ± 49.6	138.5 ± 55.8	148.6 ± 57.1*#	179.3 ± 83.1 <sup>§</sup> *#
Total cholesterol (mg/dL)	226.5 ± 42.5	226.1 ± 46.3	225.3 ± 43.3	230.9 ± 40.8	223.5 ± 39.5	205.8 ± 45.7 <sup>§</sup> *#
Statin use, yes (n, %)	124 (17.6)	32 (18.3)	29 (16.4)	34 (19.2)	29 (16.5)	27 (35.5)
eGFR (mL/min/1.73m <sup>2</sup> )	66.3 ± 12.9	71.9 ± 10.3	68.1 ± 11.2	65.3 ± 12.6*	59.7 ± 14.0*#	62.3 ± 15.7*#

Data presented as mean ± SD, median (interquartile range); for non-normally distributed variables) or number *n* and (%). LCN2; lipocalin-2, BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, HbA1c; glycated haemoglobin HDLC; high-density lipoprotein cholesterol, LDLC; low-density lipoprotein cholesterol, eGFR; estimated glomerular filtration rate. <sup>a</sup>  $n = 73$ ; <sup>b</sup>  $n = 49$ . For continuous variables, \* indicates  $p < 0.05$  compared to quartile 1, # indicates  $p < 0.05$  compared to quartile 2, ^ indicates  $p < 0.05$  compared to quartile 3, § indicates  $p < 0.05$  compared to quartile 4.

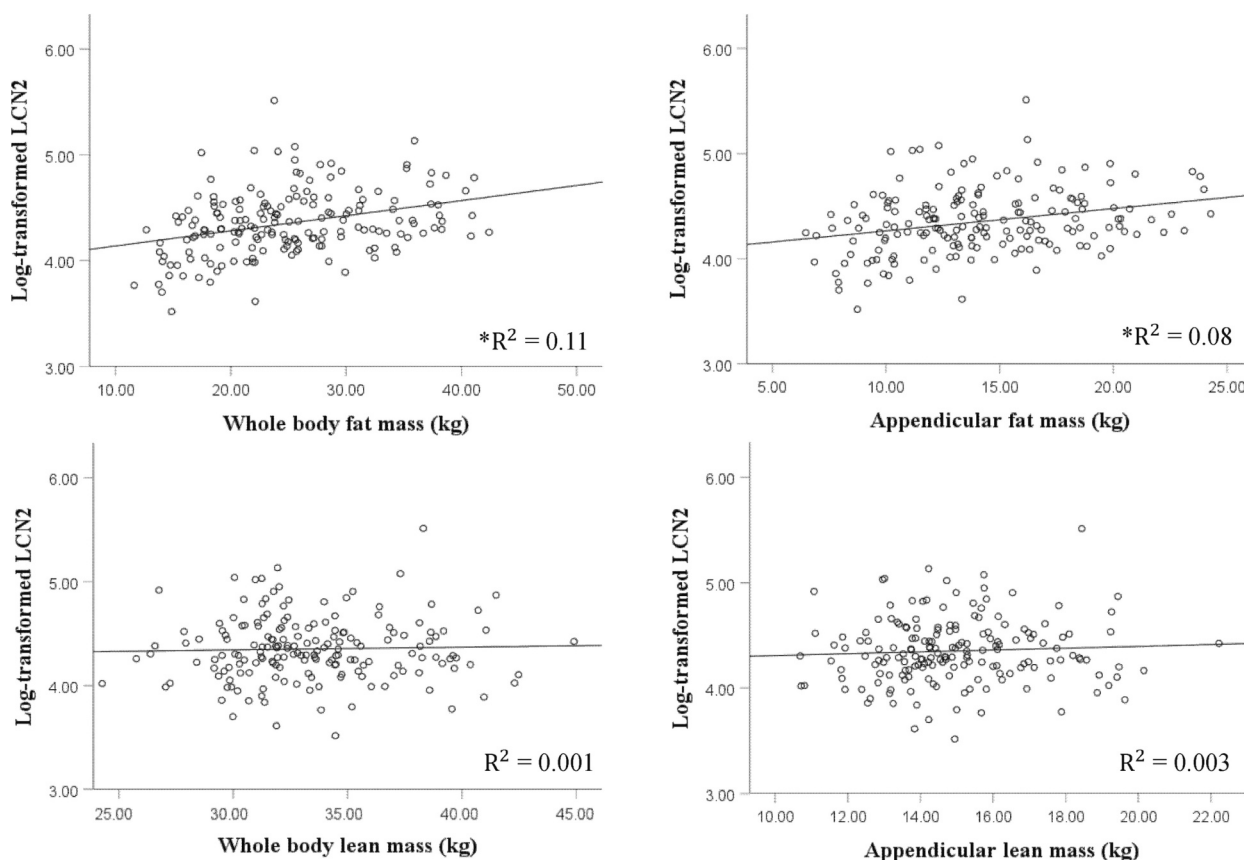


Fig. 1. Relationship between fat and lean mass compartments with log-transformed lipocalin-2 in the non-diabetic population (n = 177). \* indicates  $p < 0.001$ .

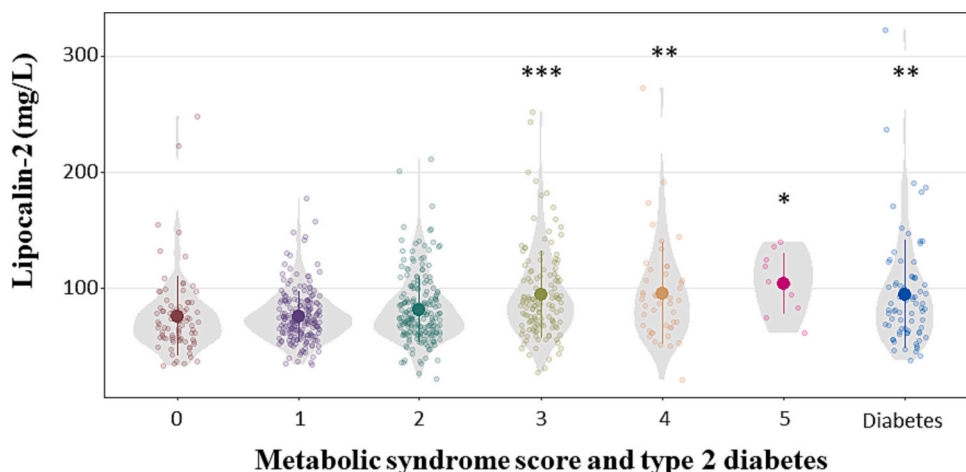


Fig. 2. Median (95 % CI) circulating lipocalin-2 concentrations based on metabolic syndrome score (0–5; n = 705) and type 2 diabetes (n = 76). \* $P < 0.05$ , \*\* $P < 0.02$  and \*\*\* $P < 0.001$  indicates significantly different to individuals with a metabolic syndrome score of 0.

that those with T2D has similar or slightly lower LCN2 (not significant) than those with a MetS score of 5, perhaps due to the use of glucose lowering medications in this group, suggesting that LCN2 may be a modifiable biomarker. In conjunction with previous studies, these data suggest that LCN2 may be a clinically useful biomarker of MetS [10]. Longitudinal studies are needed to better understand the predictive capacity of LCN2 in identifying women who are at risk of MetS and T2D and validate our cross-sectional findings.

LCN2 is a hormone released by multiple cell-types such as adipocytes and osteoblasts and has been implicated in atherosclerosis [16] and fat and bone metabolism [4]. With ageing, and under pathological

metabolic conditions, LCN2 expression is increased in both adipose and liver tissue, potentially contributing to metabolic dysfunction [8]. In recent years, accumulating evidence suggests circulating LCN2 levels are higher in patients with renal and cardio-metabolic diseases [16–18]. Ageing is related to the deterioration of physiological processes including adipose tissue dysfunction that has similar effects as obesity on cardio-metabolic health, such as increased risk for dyslipidaemia, insulin resistance and T2D [19–21]. Chronic inflammation is also known to contribute to metabolic abnormalities leading to chronic disease. It is suggested that the involvement of LCN2 in metabolic disorders, including its relationship to reduced insulin sensitivity, is related to its

**Table 2**

Odds ratios (95% CI) for the presence of metabolic syndrome by quartiles of circulating lipocalin-2 levels.

Estimated odds ratios and 95 % CI from logistic regression analysis comparing the median lipocalin-2 from each quartile with quartile 1 as the reference in 705 women. Median lipocalin 2 for Quartile 1, Quartile 2, Quartile 3 and Quartile 4 was 55.6, 70.1, 85.2 and 115.8 mg/L, respectively. Model 1: age-adjusted; Model 2: Model 1 + physical activity, smoking status; Model 3: Model 2 + estimated glomerular filtration rate. Bolded indicates  $p < 0.05$  compared to quartile 1. LCN2; lipocalin-2.

	Quartiles of LCN2			
	Quartile 1 < 62.8 mg/L	Quartile 2 62.9 to < 76.3 mg/L	Quartile 3 76.3 to < 94.2 mg/L	Quartile 4 ≥ 94.2 mg/L
Metabolic syndrome With MetS, n (%)	31 (17.7)	35 (19.8)	48 (27.1)	76 (43.2)
Model 1	Ref.	1.08 (0.84–1.39)	<b>1.80</b> <b>(1.29–2.50)</b>	<b>3.68</b> <b>(2.30–5.89)</b>
Model 2	Ref.	1.08 (0.84–1.40)	<b>1.79</b> <b>(1.29–2.49)</b>	<b>3.64</b> <b>(2.27–5.84)</b>
Model 3	Ref.	1.04 (0.81–1.35)	<b>1.62</b> <b>(1.15–2.28)</b>	<b>3.05</b> <b>(1.86–5.02)</b>

pro-inflammatory characteristics [18]. In the current study, we have demonstrated a clear link between increased circulating LCN2 levels and metabolic disease in older women, extending on previous research in young and middle-aged adults [5,8–10,18].

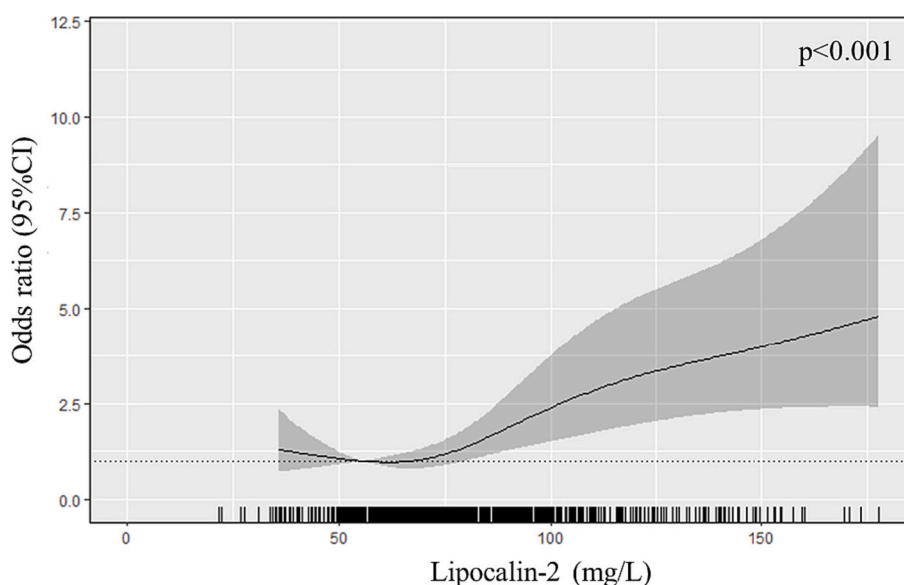
The association between increasing LCN2 and MetS in older women appeared to be of a linear nature. This is consistent with findings that higher serum levels of LCN2 are related to MetS in men on a waitlist for cardio-angiography [10]. We previously reported that lower physical activity is associated with increased circulating LCN2 levels in older women [22] as well as being associated with increased cardiovascular risk [19]. In the current study, we report that higher LCN2 levels were associated with markers of cardio-metabolic risk including higher HbA1c, triglycerides, BMI and fat mass (but not lean mass) and lower HDLC, in older women. Our data supports LCN2 as a potential biomarker to be further investigated as an indicator of overall risk for poor glycaemic control and increased risk of cardio-metabolic diseases. Similar findings in cross-sectional studies have been reported previously, where

increased LCN2 levels were associated with markers of impaired glucose metabolism in males and females aged 33–72 [8] and 50–82 years [23], including hyperglycemia, as well as higher BMI, triglycerides and insulin. It has also been reported that LCN2 deficiency is protective against age-related insulin resistance in animal studies [24]. Yet, in a different population including young men, LCN2 was not an independent predictor of metabolic risk [25]. Further studies will need to examine how different age, sex and disease status may affect the circulating levels of LCN2.

Interestingly, it appears that women with T2D had similar levels of LCN2 to women with 3 risk factors for MetS in the current study. Previous work has shown that diabetes treatment can modify LCN2 levels independent of changes to BMI, suggesting modification of LCN2 levels could potentially influence metabolism beyond the effect of excess adiposity [8]. It also suggests that LCN2 is a modifiable risk factors that may be targeted with both pharmacological and non-pharmacological interventions (such as exercise). This observation is worthwhile investigating in future studies, as ageing presents unique challenges in the treatment of diabetes due to highly diverse health profiles considering comorbidities, function and cognition.

In the current study, we have reported some attenuation in odds of MetS for higher LCN2 levels when eGFR is adjusted for, consistent with established research connecting LCN2 to kidney function [26]. The link between metabolic disease such as diabetes and renal disease is established, though the signalling pathways involved are incompletely understood [27]. LCN2 has been established as a biomarker for acute kidney injury as glomeruli in the kidney are involved in filtering circulating LCN2 [28] and it is suggested to play a role in pathways involved in the development and progression of CKD [8,29].

We have previously reported higher levels of physical activity are associated with lower LCN2 levels in this cohort of elderly women [22]. Chronic exercise is suggested to reduce inflammatory markers such as CRP, TNF- $\alpha$ , and IL-6 in ageing populations [31]. LCN2 is an inflammatory mediator with both pro and anti-inflammatory actions reported in the literature [32]. In the current study we report decreased levels of LCN2 are related to reduced odds of MetS in older women. This observation may be linked to reductions in inflammation due to exercise. The effect of activity levels on LCN2 is not clear due to conflicting results [33,34]. It is possible that long-term physical activity, as seen in our previously reported observational data, as opposed to shorter chronic



**Fig. 3.** Restricted cubic spline based on multivariable-adjusted logistic regression models highlighting the relative odds between lipocalin-2 and the presence of metabolic syndrome in 705 women. Shaded represent 95% CI. The reference value is the value associated with the median lipocalin-2 level (55.6 mg/L) for women in the lowest quartile. Model adjusted for age, physical activity, smoking status and estimated glomerular filtration rate. Rug-plot on the x-axis indicates an observation.

training interventions may be necessary to elicit significant change in LCN2 levels though intervention studies are needed. It is recognised that physical activity is influential in the mechanoreceptive role of the LCN2 gene in bone homeostasis [35]. For this reason, we have included adjustments for physical activity levels in our analysis.

The study has some potential limitations. Due to the observational nature of the study, cause-and-effect between LCN2 and metabolic outcomes and the mechanisms involved cannot be determined. However, the relationships observed in the current study suggest LCN2 may be a promising target for future research. The NCEP-ATPIII was modified to include BMI and HbA1c as waist circumference and fasting glucose were not collected in the original study at this time point. The dataset largely included community-dwelling Caucasian women and as such, further explorations are necessary to determine whether these findings are observed in males, diverse ethnicities and younger populations. Due to the relatively low number of participants with type 2 diabetes who completed the DXA, the analysis included the non-diabetic population only. Future studies should explore the link between LCN2 and body composition in patients with type 2 diabetes. It was previously reported that LCN2 can be increased due to other age-related conditions such as cancer and kidney disorders, therefore it is difficult to determine the degree of LCN2 level elevation that can be accounted for with MetS and diabetes. However, the current study included women who were free from conditions likely to limit their survival over the next 5 years and included a large dataset of women with rigorous data collection procedures and comparisons between MetS and T2D.

In conclusion, we report that higher circulating levels of LCN2 are associated with increased risk of MetS and T2D in older women. This association is related to increased metabolic and cardiovascular risk factors. Further explorations are needed to determine whether LCN2 may be used as a biomarker in clinical practice to identify those who are at a high risk of developing MetS and T2D.

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### CRediT authorship contribution statement

**Carlie Bauer:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Marc Sim:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Conceptualization. **Richard L. Prince:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition. **Kun Zhu:** Writing – review & editing, Methodology, Investigation. **Ee M. Lim:** Writing – review & editing. **Elizabeth Byrnes:** Writing – review & editing, Investigation. **Nathan Pavlos:** Writing – review & editing, Conceptualization. **Wai H. Lim:** Writing – review & editing. **Germaine Wong:** Writing – review & editing. **Joshua R. Lewis:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Itamar Levinger:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

### Declaration of competing interest

None.

### Data availability

Some or all datasets generated during and/or analysed during the current study are not publicly available but are available from JRL on reasonable request.

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### Authors' roles

Study design and drafting of manuscript: CB, MS, JRL and IL. Study conduct and data collection: JRL, KZ, EB, RLP. Data analysis: MS, JRL. Data interpretation: CB, MS, JRL and IL. All authors reviewed the manuscript and approve the final version. JRL and MS take responsibility for the integrity of the data analysis.

### Australian New Zealand Clinical Trials Registry

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