

## Effect of Traditional Cardiovascular Risk Factors on the Independent Relationship of Leptin with Atherosclerosis in Rheumatoid Arthritis

To the Editor:

Leptin is an adipokine that regulates appetite and energy expenditure<sup>1</sup>. Both high and low leptin production can further increase cardiovascular (CV) risk<sup>1</sup>. Leptin is also produced in inflamed joints and implicated in the pathophysiology of rheumatoid arthritis (RA)<sup>2</sup>.

Whether leptin increases CV risk in RA is currently uncertain. Two studies reported a lack of association between leptin concentrations and carotid artery intima-media thickness (cIMT) in RA<sup>3,4</sup>. Leptin concentrations were also found to be unrelated to coronary artery classification scores in RA<sup>5</sup>. However, we recently reported an independent relationship between leptin concentrations and surrogate markers of early atherosclerosis in young patients with RA<sup>2</sup>. Importantly, in the present context, carotid artery plaque is a more reliable indicator of atherosclerosis than cIMT<sup>6</sup>.

In our present study, we examined the independent relationships of leptin concentrations with cIMT and plaque in 217 (112 black and 105 white) patients with RA. Because the production and effects of adipokines on CV risk depend on pathophysiological context<sup>1,2,7</sup>, we also determined whether the presence of conventional and nonconventional CV risk factors modified leptin concentrations and their associations with atherosclerosis.

All patients were receiving disease-modifying agents for rheumatic disease that included tumor necrosis factor- $\alpha$  blockade and rituximab in 3.7% and 1.2% of them, respectively. The Human Research Ethics Committee (Medical) from The University of the Witwatersrand in Johannesburg, South Africa, approved the protocol (approval number: M06-07-33) and each participant gave informed written consent.

Carotid ultrasound measurement methods and its reproducibility in our setting were previously reported<sup>7</sup>. Carotid artery plaque was defined as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding intima-media thickness value, or demonstrates a thickness of > 1.5 mm as measured from the media-adventitia interface to the intima-lumen interface<sup>8</sup>.

Leptin concentrations were measured using solid-phase sandwich ELISA (QuantikineHS, R&D Systems Inc.). The lower detection limit was 7.8 pg/ml, and the interassay and intraassay coefficients of variation were 4.4% and 3.2%, respectively.

These characteristics were previously reported overall and in the different age quartiles in the present cohort<sup>2</sup>: demographic features, lifestyle factors, anthropometric measures, conventional metabolic risk factors, C-reactive protein concentrations, glomerular filtration rate (GFR), CV agents, and leptin concentrations. The median (interquartile range: IQR) erythrocyte sedimentation rate was 12 (5–28) mm/h.

The mean (SD) cIMT and plaque prevalence were 0.709 (0.109) mm and 40.6%, respectively. Leptin concentrations in all patients and relevant subgroups<sup>7</sup> are given in Table 1. In univariate analysis, leptin concentrations were larger in black compared to white participants, and in patients with compared to those without major conventional CV risk factors and overall and abdominal obesity. Each of these differences was no longer significant in confounder and/or mediator-adjusted analysis<sup>2</sup>.

Leptin concentrations were unrelated to cIMT in all patients and in those with and without conventional or nonconventional risk factors (data not shown). Table 1 also shows the relationships of leptin concentrations with carotid artery plaque. In univariate analysis, leptin concentrations were consistently unassociated with plaque. The number of major conventional CV risk factors affected the leptin concentrations-plaque prevalence association (interaction  $p = 0.02$ ). In adjusted analysis, a 1-SD increment in leptin concentrations increased the OR for plaque 2.75-fold in patients with major conventional CV risk factors; the respective OR (95% CI) was 2.64 (1.18–5.92,  $p = 0.01$ ) in patients with 2 ( $n = 40$ ) or  $\geq 3$  ( $n = 10$ ) major risk factors. Patients with major CV risk factors had a larger body mass index (BMI) and lower GFR than those without these risk factors [median (IQR) = 27.7 (24.0–32.8) vs 24.0 (21.7–28.5) kg/m<sup>2</sup>,  $p = 0.0001$ , and 94

(81–111) and 102 (89–123) ml/min,  $p = 0.05$ , respectively]. As shown in Table 2, the leptin concentrations-plaque prevalence relation was driven by the GFR and BMI. This suggests an effect of leptin on carotid plaque that is mediated by increasing adiposity and renal impairment. Alternatively, our results may conceptually represent a compensatory increase in leptin production that is targeted at reducing atherosclerosis<sup>5</sup>.

Whereas in our previous study, leptin concentrations were associated with endothelial activation among patients aged < 50 years<sup>2</sup>, a relationship of leptin with plaque was not found in the respective group in our present investigation. This indicates that whereas leptin may be involved in early atherogenesis, this adipokine may not contribute to advanced atherosclerosis in young patients with RA. Interestingly, the leptin-endothelial activation relationship was also obesity-driven in RA<sup>2</sup>.

Several recorded characteristics were non-normally distributed in our present investigation. When we repeated the analyses using log-transformed variables in the mixed regression models, the results were materially unaltered (data not shown).

We found an effect of leptin on carotid artery plaque that is dependent on the number of major conventional risk factors, which were identified in 70% of participants. Indeed, Rho, *et al* also reported that the effect of leptin on coronary atherosclerosis may be mediated through interactions with other CV risk factors in RA<sup>5</sup>. Plaque represents advanced atherosclerosis<sup>6</sup> that is strongly associated to coronary heart disease risk factors and incident coronary heart disease in both non-RA and RA subjects<sup>9,10</sup>. Leptin may contribute to the reported link between conventional risk factors and enhanced CV risk in RA. Consideration of leptin concentrations may improve CV risk stratification in patients with RA. These findings merit further investigation in future longitudinal studies.

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PATRICK H. DESSEIN, MC, FCP(SA), FRCP(UK), PhD; LINDA TSANG; ANGELA J. WOODIWISS, PhD, Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand; AHMED SOLOMON, MBBCh, FCP(SA), Department of Rheumatology, Charlotte Maxeke Johannesburg Academic Hospital, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. Address correspondence to Prof. P.H. Dessein, P.O. Box 1012, Melville 2109, Johannesburg, South Africa. E-mail: dessein@telkomsa.net

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Table 1. Leptin concentrations (ng/ml) and the relationships with carotid artery plaque (per 1 SD increment) in all RA patients and subgroups. Univariate comparisons of leptin concentrations between subgroups were made using the Mann-Whitney U test and univariate associations of leptin concentrations with plaque in logistic regression models. Major risk factors comprised hypertension, dyslipidemia, smoking, and diabetes<sup>2</sup>.

Groups	Number	Concentrations			Relationships with Plaque			
		Median (IQR)	p	p*	Crude OR (95% CI)	p	Independent OR (95% CI)	p†
All Population	217	10.2 (5.5–18.5)	—	—	1.00 (0.99–1.01)	0.4	1.00 (0.99–1.01)	0.3
Black	112	<b>12.4 (6.3–21.4)</b>			1.01 (0.99–1.02)	0.4	1.01 (0.99–1.02)	0.5
White	105	<b>8.4 (4.9–14.3)</b>	<b>0.001</b>	0.2	0.96 (0.65–1.43)	0.8	1.00 (0.67–1.50)	1
Age ≥ 50 yrs								
Yes	169	10.1 (5.5–18.4)			1.00 (0.99–1.01)	0.5	1.00 (0.99–1.01)	0.4
No	48	10.8 (5.2–20.0)	0.9	0.3	1.05 (0.86–1.28)	0.6	1.03 (0.88–1.21)	0.7
Age > 55 yrs								
Yes	127	9.5 (5.5–17.8)			1.00 (0.99–1.01)	0.9	1.00 (0.99–1.01)	1
No	90	11.6 (5.5–21.2)	0.4	0.1	1.01 (0.99–1.03)	0.4	1.01 (0.99–1.02)	0.5
≥ 1 major risk factor								
Yes	151	<b>11.2 (5.7–20.9)</b>			1.53 (0.82–2.84)	0.2	2.75 (1.19–6.37)	<b>0.01</b>
No	58	<b>9.0 (3.8–15.3)</b>	<b>0.03</b>	0.5	1.00 (0.99–1.01)	1	1.00 (0.99–1.01)	0.6
Missing	8							
Obesity								
Yes	64	<b>18.2 (9.5–27.6)</b>			0.99 (0.98–1.01)	0.5	1.00 (0.98–1.01)	0.7
No	147	<b>7.5 (4.6–13.3)</b>	<b>&lt; 0.0001</b>	0.9	1.01 (1.00–1.02)	0.09	1.01 (1.00–1.02)	0.1
Missing	6							
MetS waist								
Yes	100	<b>15.0 (8.2–23.3)</b>			0.99 (0.98–1.01)	0.6	1.00 (0.98–1.01)	0.5
No	114	<b>6.9 (4.3–12.4)</b>	<b>&lt; 0.0001</b>	0.7	1.01 (0.99–1.02)	0.09	1.01 (1.00–1.02)	0.1
Missing	3							
RA duration > 10 yrs								
Yes	116	9.8 (4.9–17.8)			1.01 (0.99–1.02)	0.2	1.01 (1.00–1.02)	0.2
No	100	10.6 (5.9–20.4)	0.3	0.8	1.00 (0.98–1.01)	0.9	1.00 (0.99–1.01)	1
Missing	1							
CDAI > 10								
Yes	92	9.4 (5.4–18.3)			1.00 (0.99–1.02)	0.7	1.00 (0.98–1.02)	1
No	124	10.3 (5.2–21.2)	0.3	0.8	1.00 (0.99–1.02)	0.4	1.01 (1.00–1.02)	0.3
Missing	1							
ESR > 12 mm/h								
Yes	106	10.03 (5.9–19.3)			1.05 (0.96–1.13)	0.3	1.05 (0.96–1.14)	0.3
No	105	9.2 (5.2–18.0)	0.4	0.09	1.00 (0.99–1.01)	0.8	1.00 (1.00–1.01)	0.8
Missing	6							
Deformed joints								
Yes	165	10.0 (5.4–17.9)			1.00 (0.99–1.01)	0.7	1.00 (0.99–1.02)	0.7
No	51	11.9 (5.5–22.4)	0.3	0.06	1.00 (0.99–1.02)	0.6	1.01 (0.99–1.03)	0.4
Missing	1							
RF-positive								
Yes	168	9.5 (5.4–18.5)			1.01 (0.99–1.02)	0.1	1.01 (1.00–1.02)	0.2
No	48	12.7 (6.7–19.4)	0.3	0.9	1.00 (0.98–1.01)	0.5	1.00 (0.98–1.01)	0.8
Missing	1							

\*p value for associations in age at disease onset, sex, race (except for in models by population grouping), body mass index, glomerular filtration rate, and cardiovascular drug use adjusted linear regression models. †p value for associations in Framingham score, race, glomerular filtration rate, body mass index, and C-reactive protein adjusted logistic regression models. Significant associations are shown in bold face. RA: rheumatoid arthritis; IQR: interquartile range; MetS: metabolic syndrome; CDAI: Clinical Disease Activity Index; ESR: erythrocyte sedimentation rate; RF: rheumatoid factor.

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Table 2. Relations of leptin concentrations (1 SD increment) with carotid artery plaque among patients with major conventional cardiovascular risk factors. Associations were determined in logistic regression models.

Adjusted Characteristics	OR (95% CI)	p
None	1.53 (0.82–2.84)	0.2
Framingham score	1.58 (0.83–3.02)	0.2
Framingham score, race	1.78 (0.90–3.52)	0.1
Framingham score, race, CRP	1.80 (0.90–3.58)	0.1
Framingham score, race, CRP, GFR	<b>2.07 (1.00–4.29)</b>	<b>0.04</b>
Framingham score, race, CRP, BMI	<b>2.35 (1.06–5.21)</b>	<b>0.03</b>
Framingham score, race, CRP, GFR, BMI	<b>2.75 (1.19–6.37)</b>	<b>0.01</b>

Significant relations are shown in bold face. CRP: C-reactive protein; GFR: glomerular filtration rate, BMI: body mass index.

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