Increased CRP: An extended biomarker of microvascular risk in men with type 2 diabetes

Michel P. Hermans [a,*], Sylvie A. Ahn [b], Michel F. Rousseau [b]

[a] Division of Endocrinology & Nutrition, Cliniques universitaires St-Luc and Institut de Recherche Expérimentale et Clinique (IREC), Université catholique de Louvain, Brussels, Belgium
[b] Division of Cardiology, Cliniques universitaires St-Luc and Pôle de Recherche Cardiovasculaire, Institut de Recherche Expérimentale et Clinique (IREC), Université catholique de Louvain, Brussels, Belgium

ARTICLE INFO

ABSTRACT

Background: The usefulness of C-reactive protein (CRP) to predict cardiovascular disease (CVD) in type 2 diabetes (T2DM) remains controversial. As many factors linked to obesity can modulate CRP in T2DM, we comprehensively revisited the cardiometabolic phenotype of patients with normal or raised CRP, taking into account the sexual dimorphism of its serum value.
Methods: 1005 T2DM patients (651 males, 354 females; macroangiopathy 38%; coronary artery disease 26%; microangiopathy 47%) were divided depending on whether CRP level was ≤ or >3 mg/L. Thirty percent of men ($n = 195$) and 39% of women ($n = 137$) had raised CRP. Their cardiometabolic phenotype and presence of micro- and macrovascular complications were compared to those with normal CRP.
Results: In both gender, patients with elevated CRP had higher body mass index, waist circumference, fat mass, visceral fat, insulinemia, HbA1c, and lower muscle mass and insulin sensitivity. They had more atherogenic dyslipidemia, higher non-HDL-C and apolipoprotein B_{100}, and more lipoprotein(a) (+59% in men and +38% in women). In both sexes, there was no difference between patients with normal or high CRP regarding overall macroangiopathy (42% vs. 45% [men]; 27% vs. 28% [women]), coronary and peripheral artery disease, or stroke. Only in men, microangiopathy was more prevalent when CRP was raised (61% vs 44%; $p < 0.0001$).
Conclusions: This study shows major sex-related differences in microangiopathies in T2DM patients with high CRP levels. The latter are unrelated to prevalent CVD despite an unfavorable metabolic phenotype. By contrast, increased CRP may represent an extended biomarker of microvascular risk in men with T2DM.

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1. Introduction

C-reactive protein (CRP) is an acute phase reactant mainly synthesized by the liver. Its chronic elevation is associated, apart from inflammatory or infectious states, with cardiovascular (CV) risk, of which it is considered either as a marker or a factor directly promoting atherosclerosis.[1] The hypothesis of subclinical chronic inflammation as residual risk driver was supported by the CANTOS trial (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study), in which antiinflammatory therapy with canakinumab targeting the interleukin-1β pathway significantly reduced CV events.[2,3] Yet, there is considerable controversy from other cross-sectional or longitudinal studies on the causal role of CRP in the occurrence of cardiovascular diseases (CVD). For some, CRP is a substantial emerging risk factor with potential utility for estimating residual risk, or even targeting its decrease for therapeutic purposes.[2–12] For others, it is only a risk marker for CVD, the direct involvement of which is unsupported.[13–19] A major part of the current debate stems from the many confounding factors contributing to raise CV risk and CRP levels, resulting in entangled epidemiological links. These include extrahepatic sources of CRP (including adipose tissue), ethnic differences, and a marked sexual dimorphism.

Given the disagreement on the role of CRP as true CV risk factor, it is a prerequisite that a candidate risk factor satisfies all Hill's criteria for causative association.[20] One fundamental criterion is to have an occurrence or prevalence of the condition commensurate to exposure to a presumed risk factor when the latter is harmful as a continuous variable, after exclusion of other confounders. Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease that predisposes to early-onset macrovascular complications and late-onset microangiopathies. Compared to the general population, patients with T2DM have higher prevalence of CVD and increased incidence of CV events. Diabetic patients are therefore a choice population to investigate the potential links between CRP and CVD, especially since CRP-interfering items are multiplied in case of diabetes, starting with obesity and its repercussions. Hence the purpose of this work was to revisit the association between serum CRP and the cardiometabolic phenotype

of T2DM patients, focusing on prevalent micro- and macrovascular complications, as well as on emerging CV risk factors or markers.

2. Patients & methods

This cross-sectional retrospective cohort study analyzed the archived medical records of 1005 T2DM patients (85% white Caucasian) attending the diabetes clinic of St-Luc academic hospital in Brussels. Patients were categorized into 2 groups according to wether their baseline CRP was low to intermediate (i.e. <1 mg/L and 1–3 mg/L, respectively) or high (≥3 mg/L). The cut-off of 3 mg/L corresponds to the level above which the associated CV risk is considered very high as per the Centers for Disease Control/American Heart Association classification of CV risk.[18,21,22] CRP levels being furthermore sexually-dimorphic (higher in females), men and women were analyzed separately to prevent unbalanced gender distribution between groups due to a single cutoff for both sexes.[23]

Exclusion criteria included patients treated with medications that could affect insulin sensitivity (IS) or β-cell function (other than glucose-lowering drugs), including systemic or topical corticosteroids, antiretrovirals, immune-modulators, and anti-psychotics. Were also excluded patients with chronic inflammatory or infectious diseases, ongoing infections and/or other acute conditions, cancer or major organ failure (respiratory, heart, and liver). Each patient gave informed consent, and the study was performed in agreement with the principles of the Declaration of Helsinki and Good Clinical Practice, and the Institutional Review Board.

The following sociodemographic and clinical variables were recorded: age; gender; age at diabetes diagnosis; diabetes duration; education (as proxy for socio-economic status); smoking; ethanol intake; recreational physical activity; and leisure-time spent watching screens (as surrogate for sedentarity); weight; height; body mass index (BMI); blood pressure (BP); fat mass, visceral fat and skeletal muscle mass (Omron BF 500, Omron Healthcare Europe B.V., Hoofddorp, The Netherlands). Neck, waist and hip circumferences were measured, and conicity index was calculated, as surrogate for upper body adiposity (conicity index: waist circumference (m)/0.109√[weight(kg)/height (m)]). First-degree (mother; father; siblings) familial histories of DM and/or premature coronary artery disease (CAD), defined by the occurrence of a 1st CAD event <55 years in men and <65 years in women, were also recorded.

Hypertension was defined as systolic BP ≥140 and/or diastolic BP ≥90 mmHg, as well as ongoing antihypertensive medication(s). Nonalcoholic liver steatosis was considered in case of ultrasonic hyperreflectivity, in the absence of etiological factors associated with fatty liver, including excess ethanol intake. The presence of obstructive sleep apnea syndrome was also noted. Current therapies were identified, including glucose-lowering drugs and cardiovascular drugs (BP-lowering; lipid-modifying drugs (LMDs; statins; fibrates and/or ezetimibe);and aspirin).

Methods used to define the presence of a metabolic syndrome (MetS), atherogenic dyslipidemia, microangiopathies (diabetic retinopathy and peripheral neuropathy), and macrovascular complications (CAD; transient ischemic attack [TIA]/stroke, and peripheral artery disease [PAD]) were previously described.[24,25] β-cell function and insulin sensitivity were assessed with HOMA-2. Values of insulin secretion ([B]; normal 100%) were plotted as a function of insulin sensitivity ([S]; normal 100%), defining a *hyperbolic product* area [B × S] (unit: %^{2}; normal: 100%, corresponding to 10^4 %^{2}), representing residual β-cell function. Secular loss of hyperbolic product ([BxS] loss rate; %.year^{-1}) was obtained by dividing (100%-[BxS]) by patient's age.[26]

Serum CRP was determined by high-sensitivity immunoturbidimetric assay (Roche Diagnostics GmbH; Mannheim, Germany), with 0.15–20.0 mg/L measurement limits. Specific insulin was measured by an electrochemiluminescence immunoassay (Cobas e analyzer). Fibrinogen; cystatin-C; uric acid; blood count; ferritin; sex-hormone binding globulin (SHBG); total and free testosterone; HbA$_{1c}$ were determined by routine methods, as were fasting lipids and lipoproteins: total cholesterol (C), HDL-C, triglycerides (TG); LDL-C (Friedewald's formula), non-HDL-C, lipoprotein(*a*), and apolipoprotein B$_{100}$. Lipoprotein(a) [Lp(a)] was determined by turbidimetric analysis, with threshold value for increased CV risk >75 nmol/L. Albuminuria was considered in patients whose urine albumin/creatinine ratio (ACR) >30 mg/g creatinine.

Results are shown as means (±1 standard deviation; SD) or proportions (%). The significance of differences between means was assessed using Student's *t*-test, or Welch's test for data sets with significant differences in SDs, and using Fisher's Exact test for differences in proportions. Statistical analyses were performed using IBM® SPSS® 25 software. Results were considered statistically significant or non-significant (NS) for $p < 0.05$ or $p ≥ 0.05$, respectively.

3. Results

The characteristics of the entire cohort ($n = 1005$; 651 men; 65% and 354 women; 35%) are described in the first column of Tables 1 to 4. Patients were divided in two groups according to whether their CRP levels were normal or high. In the entire cohort, mean CRP was 3.7 (5.2) mg/L, with 33% having elevated CRP. CRP levels were significantly different between men and women, with means of 0.34 (0.51) and 0.42 (0.54) mg/L, respectively, female patients having a 24% higher average CRP (p 0.0202). Based on a common cutoff of 3 mg/L, 30% among men ($n = 195$) had elevated CRP, vs. 39% among women ($n = 137$; p 0.0061). (Table 3). Because of this sexual dimorphism bearing on the average CRP level and the prevalence of abnormal CRP, data for men and women are shown separately in Tables 1 to 4.

For both men and women, there were no differences between CRP-based groups regarding age, family history of diabetes and/or premature CAD, duration of diabetes, presence of hypertension, glomerular filtration rate, use of oral glucose-lowering medications and all-class LMD (s). In both sexes, patients with elevated CRP were significantly more sedentary, spent more time in front of screens, and received more insulin (+26% men; +36% in women). Men with raised CRP were more frequently smokers or ex-smokers, had a higher heart rate (+3 beats/min), were more often treated with fibrates (+35%; *not shown*), and less often with statins (−11%). Women with raised CRP had higher systolic BP (+6 mmHg), were more often treated with insulin (+46%), and received more diuretics (+31%; Table 1).

Men and women with increased CRP had a more unfavorable phenotype, including increased BMI; abdominal, cervical and hip circumferences; body and visceral fat; conicity; and decreased muscle mass (Table 2). In terms of body weight, men with normal CRP averaged 88 (17) kg, vs. 95 (22) kg for those with raised CRP, whereas women with normal CRP weighed a mean 73 (17) kg, vs. 86 (17) kg in those with high CRP (both $p < 0.0001$). Both gender had increased prevalence of MetS (+12% in men; +19% in women), higher fasting insulin, and lower insulin sensitivity (−16% in men; −28% in women), and their metabolic control was worse in terms of HbA$_{1c}$. Men with raised CRP had more sleep apnoea syndrome (+47%), and less often achieved the HbA$_{1c}$ target of <7.0%. Women with raised CRP had more fatty liver (+19%), and a more severe MetS, scoring 4.2 vs. 3.7 ($p < 0.0001$). Their residual B-cell function was lower (−24%), and B-cell function loss rate was higher (+16%), respectively (Table 2).

With respect to non-lipid biological variables in both men and women, those with raised CRP had higher fibrinogen, cystatin-C, white blood cells, and neutrophiles. In addition, men with elevated CRP had higher total testosterone, whereas women with high CRP had higher uric acid, ferritin, SHBG, and free testosterone (Table 3).

As regards lipid and lipoproteins, men and women with raised CRP had higher non-HDL-C, apolipoprotein B$_{100}$, and triglycerides levels, more atherogenic dyslipidemia (about one quarter), as well as lower HDL-C. Lipoprotein(a) levels were markedly higher in patients with raised CRP (+59% in men and +38% in women). In addition, men with high CRP had higher total cholesterol and increased frequency of

Table 1
Patients' characteristics.

		All patients	Males CRP ≤ 3 mg/L	CRP > 3 mg/L	P	Females CRP ≤ 3 mg/L	CRP > 3 mg/L	P
n		1005	456	195		217	137	
Age	Years	70 (12)	69 (12)	70 (12)	NS	71 (12)	69 (11)	NS
Family history of diabetes	%	51	47	54	NS	50	60	NS
Family history of premature CAD	%	12	10	13	NS	13	15	NS
Diabetes duration	Years	18 (9)	17 (9)	18 (9)	NS	18 (9)	18 (10)	NS
Smoking*	%	48–52	38–62	27–73	0.0071	73–27	67–33	NS
Exercise**	%	70–30	59–41	74–26	0.0005	76–24	88–12	0.0053
Screen watching	h.day^{-1}	2.9 (1.7)	2.8 (1.6)	3.3 (2.0)	0.0013	2.6 (1.5)	3.4 (1.7)	0.0001
Hypertension	%	89	90	91	NS	85	88	NS
Systolic BP	mmHg	138 (20)	140 (19)	139 (21)	NS	133 (18)	139 (22)	0.0079
Heart rate	b.p.m.	76 (12)	74 (13)	77 (13)	0.0072	77 (12)	78 (12)	NS
eGFR	mL.min^{-1}·1.73 m^2	75 (27)	77 (24)	73 (31)	NS	75 (27)	71 (28)	NS
Metformin - BCS - IBT - TZD	%	64 - 43 - 16 - 4	65 - 44 - 17 - 3	63 - 43 - 12 - 5	NS	68 - 41 - 19 - 3	59 - 41 - 13 - 4	NS
Insulin	%	45	42	49	NS	39	57	0.0015
Insulin	IU.day^{-1}	66 (56)	62 (50)	78 (70)	0.0484	55 (44)	75 (60)	0.0173
RAB - CCB - BB - diuretics	%	65 - 24 - 38 - 36	64 - 25 - 37 - 31	60 - 26 - 44 - 39	NS	59 - 22 - 34 - 36	65 - 23 - 42 - 47	0.0460[a]
Lipid-modifying drug(s)	%	78	79	80	NS	76	77	NS
Statin	%	70	74	66	0.0295	67	70	NS
Fibrate	%	20	20	27	0.0379	18	14	NS
Ezetimibe	%	10	12	8	NS	12	7	NS

Results are expressed as means (1 SD) or proportions (%). *: never vs. former or current smoking; **: absence vs. presence of leisure time physical activity; BB: beta-blocker; BCS: beta-cell stimulant; BP: blood pressure; CAD: coronary artery disease; CCB: calcium-channel blocker; CRP: high-sensitivity C-reactive protein; eGFR: estimated glomerular filtration rate; IBT: incretin-based therapies; RAB: renin-angiotensin blocker; TZD: thiazolidinedione; a: P < 0.05 for diuretic use; NS: not significant.

lipoprotein(a) >75 nmol/L, whereas women with raised CRP had higher LDL-C levels (Table 3).

In all patients, the prevalence of microangiopathy from any cause was 47%, and that of all-cause macroangiopathy 38%, with a 26% overall CAD prevalence. There was no significant difference between patients with normal or elevated CRP for the presence of all-cause macroangiopathy, CAD, cerebrovascular disease, and peripheral artery disease, and this for both sexes. Regarding microangiopathies, men with elevated CRP had increased frequency of all-cause microangiopathy, polyneuropathy, abnormal ACR and erectile dysfunction. Women with elevated CRP had higher ACR, without increased prevalence of microangiopathies (Table 4). A multivariate logistic regression analysis, was performed with the following univariate variables selected as potential confounders for micro- or macrovascular complications: age,

sex, diabetes duration, HbA$_{1c}$, smoking, BMI, systolic blood pressure, LDL-C, and log(TG)/HDL-C. It confirmed an association between the presence of high CRP (>3 mg/L) and prevalent microangiopathy only in men. No associations were found in multivariate analyses between high CRP and microangiopathic complications in women, or between high CRP and prevalent macroangiopathies of any cause or organ specific in either sex. Using a step-by-step method, the selected model retained, as significant variables, age, diabetes duration, HbA$_{1c}$, smoking, systolic blood pressure, atherogenic dyslipidemia, and CRP >3 mg/dL. For the latter, the odd ratio of the association with microangiopathy was high and significant: 2.20 [95% confidence interval 1.49–3.25] (p < 0.0001). Using a step-by-step model to assess risk of diabetic nephropathy based on chronic kidney disease (CKD) stage ≥3 (GFR < 60 mL/min/1.73 m^2; present in 28% of men), the selected model retained,

Table 2
Cardiometabolic phenotype.

		All patients	Males CRP ≤ 3 mg/L	CRP > 3 mg/L	P	Females CRP ≤ 3 mg/L	CRP > 3 mg/L	P
n		1005	456	195		217	137	
BMI	kg.m^{-2}	29.4 (5.9)	28.3 (4.8)	31.1 (6.7)	<0.0001	28.1 (6.1)	32.5 (6.2)	<0.0001
Neck circumference	cm	40 (4)	41 (3)	43 (3)	<0.0001	37 (3)	39 (3)	<0.0001
Waist circumference	cm	104 (14)	104 (12)	111 (16)	<0.0001	97 (15)	107 (13)	<0.0001
Hip circumference	cm	106 (11)	103 (8)	106 (10)	<0.0001	106 (12)	115 (13)	<0.0001
Fat mass	%	31 (9)	26 (6)	29 (6)	<0.0001	40 (7)	43 (6)	<0.0001
Visceral fat	0–30	13 (5)	13 (5)	15 (5)	<0.0001	10 (3)	12 (3)	<0.0001
Conicity index	m^2.kg^{-1}	1.35 (0.09)	1.35 (0.09)	1.39 (0.08)	<0.0001	1.32 (0.10)	1.36 (0.09)	0.0002
Skeletal muscle mass	%	31 (5)	33 (3)	32 (3)	<0.0001	26 (3)	24 (2)	<0.0001
Hepatic steatosis	%	72	72	80	NS	64	76	0.0100
Sleep apnoea syndrome	%	17	17	25	0.0399	11	16	NS
Metabolic syndrome	%	83	78	87	0.0066	81	96	<0.0001
Metabolic syndrome	0–5	3.7 (1.1)	3.6 (1.2)	3.9 (1.1)	NS	3.7 (1.2)	4.2 (0.9)	<0.0001
Fasting insulinaemia	pmol.L^{-1}	113 (78)	108 (77)	121 (75)	0.0472	107 (77)	133 (82)	0.0027
Insulin sensitivity	%	54 (38)	57 (39)	48 (34)	0.0033	60 (42)	43 (28)	<0.0001
Hyperbolic product [B x S]	%	28.6 (19.1)	29.4 (19.8)	28.1 (19.5)	NS	30.4 (17.9)	23.1 (17.2)	0.0002
[B x S] loss rate	%.yr^{-1}	1.27 (0.44)	1.27 (0.45)	1.29 (0.47)	NS	1.18 (0.39)	1.37 (0.44)	0.0002
HbA$_{1c}$	%	7.5 (1.4)	7.4 (1.4)	7.8 (1.5)	<0.0001	7.4 (1.3)	7.7 (1.5)	0.0102
HbA$_{1c}$	mmol.mol^{-1}	59 (11)	57 (11)	61 (12)		58 (10)	61 (11)	
HbA$_{1c}$ < 7.0%	%	39	43	32	0.0111	41	36	NS

Results are expressed as means (1 SD) or proportions (%). BMI: body mass index; [B x S]: hyperbolic product between insulin sensitivity and B-cell function; CRP: high-sensitivity C-reactive protein; HbA1c: glycated hemoglobin A1c; NS: not significant.

Table 3
Laboratory values.

		All patients	Males			Females		
			CRP ≤ 3 mg/L	CRP > 3 mg/L	P	CRP ≤ 3 mg/L	CRP > 3 mg/L	P
n		*1005*	*456*	*195*		*217*	*137*	
CRP	mg.L^{-1}	**3.7** (5.2)	**1.3** (0.8)	**8.2** (7.2)	~	**1.6** (0.8)	**8.4** (6.7)	~
Fibrinogen	mg.dL^{-1}	**344** (79)	**325** (73)	**363** (85)	<0.0001	**342** (74)	**387** (74)	<0.0001
Cystatin C	mg.L^{-1}	**0.95** (0.49)	**0.90** (0.47)	**1.03** (0.51)	0.0017	**0.89** (0.44)	**1.09** (0.54)	0.0003
Uric acid	mg.dL^{-1}	**5.7** (1.8)	**5.8** (1.7)	**6.0** (2.1)	NS	**5.1** (1.6)	**5.6** (1.7)	0.0055
White blood cells	10^3.mm^{-3}	**7.5** (1.9)	**7.3** (1.8)	**7.9** (1.8)	0.0001	**7.4** (1.8)	**8.0** (2.1)	0.0032
Neutrophiles	%	**59** (9)	**58** (9)	**61** (9)	0.0001	**57** (9)	**61** (8)	<0.0001
Lymphocytes	%	**29** (8)	**29** (8)	**27** (9)	0.0077	**32** (8)	**29** (8)	0.0007
Ferritin	µg.L^{-1}	**159** (177)	**187** (206)	**181** (176)	NS	**101** (100)	**135** (143)	0.0158
SHBG	nmol.L^{-1}	**42** (29)	**39** (24)	**38** (26)	NS	**53** (35)	**42** (31)	0.0022
Testosterone	nmol.L^{-1}	**9.0** (5.5)	**11.6** (4.2)	**10.0** (3.5)	<0.0001	**0.80** (0.55)	**0.90** (0.54)	NS
Free testosterone	nmol.L^{-1}	**0.15** (0.09)	**0.19** (0.07)	**0.18** (0.06)	NS	**0.012** (0.008)	**0.018** (0.015)	<0.0001
Cholesterol	mg.dL^{-1}	**164** (39)	**157** (37)	**166** (39)	0.0053	**167** (36)	**176** (46)	NS
Non-HDL-C	mg.dL^{-1}	**116** (38)	**111** (37)	**123** (39)	0.0020	**112** (35)	**125** (45)	0.0044
Apolipoprotein B$_{100}$	mg.dL^{-1}	**88** (25)	**85** (23)	**92** (25)	0.0006	**87** (22)	**94** (31)	0.0223
LDL-C	mg.dL^{-1}	**84** (33)	**81** (32)	**86** (32)	NS	**83** (31)	**91** (40)	0.0474
Lipoprotein(a)	nmol.L^{-1}	**54** (75)	**44** (62)	**70** (92)	0.0004	**53** (66)	**73** (96)	0.0129
Lipoprotein(a) > 75 nmol/L^{-1}	%	**24**	**18**	**29**	0.0282	**25**	**34**	NS
HDL-C	mg.dL^{-1}	**48** (16)	**46** (15)	**43** (12)	0.0071	**55** (18)	**51** (15)	0.0245
Triglycerides	mg.dL^{-1}	**164** (109)	**156** (104)	**195** (136)	0.0004	**150** (102)	**169** (85)	0.0041
Atherogenic dyslipidemia	%	**46**	**41**	**51**	0.0254	**44**	**55**	0.0380

Results are expressed as means (1 SD) or proportions (%). C: cholesterol; CRP: high-sensitivity C-reactive protein; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SHBG: sex hormone-binding globulin; NS: not significant.

as significant variables, age, diabetes duration, ACR, and CRP >3 mg/dL. For the latter, the odd ratio of the association with CKD stage ≥3 was 1.77 [95% confidence interval 1.16–2.69] (p 0.008).

4. Discussion

This cross-sectional study investigated the phenotype of T2DM patients according to whether they had normal or raised CRP. The results show that the elevation of this inflammatory protein was essentially associated with metabolic and anthropometric characteristics related to central obesity. In terms of anthropometry, patients with CRP >3 mg/L were more obese, with more adipose and sarcopenic body composition, and increased visceral fat. Regarding lifestyle, patients with subclinical inflammation had less physical activity and spent more recreational time in front of screens. Patients with high CRP were more resistant to insulin, with higher hyperinsulinemia, more MetS, and poorer glycemic control. Their lipids were characterized, on the one hand, by quantitative and qualitative changes typical of atherogenic dyslipidemia relating to TG-rich lipoproteins as well as abnormal HDLs and, on the other hand, by a marked elevation of Lp(a). Unsurprisingly, other biological markers of inflammation were also higher, including fibrinogen cystatin-C, and white blood cells.[1]

Most of the variables just quoted are considered as CV risk factors for macroangiopathy. Several prospective studies suggested an increased incidence of macrocascular events in diabetic and nondiabetic patients with elevated CRP at baseline.[2–4,6–12] Hence the unexpected finding that there was no difference in macroangiopathy prevalence between T2DM patients with normal or increased CRP. Although the present study was not longitudinal, such complete lack of difference raises many questions. It indirectly suggests that CRP may not be causally involved in the onset of CV events.

One cannot exclude that chronically-raised CRP could reflect unfavorable phenotypes more prone to develop CV events. Obesity is obviously an adverse phenotype to consider in light of our data, since most of the variance between CRP-based groups concerned differences in BMI, body composition, fat mass, visceral fat, and their outcomes on lipoproteins, insulin resistance, and the MetS. This is all the more plausible as a significant fraction of the circulating CRP synthesis takes place in adipose tissues.[27–30] It was indeed remarkable that sorting T2DM subjects on the basis of CRP sorted patients with very different weights, with an average difference of 7 kg for men, and 13 kg for women.

The elevation of Lp(a) in case of raised CRP could represent a rise of this particular LDL driven by subclinical inflammation; a proinflammatory effect of Lp(a) on CRP; or a combination of both. Yet, the higher Lp(a) levels were

Table 4
Microvascular and macrovascular complications.

		All patients	Males			Females		
			CRP ≤ 3 mg/L	CRP > 3 mg/L	P	CRP ≤ 3 mg/L	CRP > 3 mg/L	P
n		*1005*	*456*	*195*	~	*217*	*137*	~
Microangiopathy	%	**47**	**44**	**61**	<0.0001	**43**	**47**	NS
Retinopathy	%	**23**	**19**	**25**	NS	**25**	**30**	NS
Peripheral polyneuropathy	%	**25**	**25**	**33**	0.0349	**18**	**22**	NS
Erectile dysfunction	%	**47**	**43**	**56**	0.0056			
Abnormal ACR	%	**36**	**36**	**49**	0.0029	**28**	**30**	NS
ACR	mg.g creatinine^{-1}	**127** (412)	**124** (407)	**195** (540)	NS	**56** (128)	**155** (491)	0.0222
Macroangiopathy	%	**38**	**42**	**45**	NS	**27**	**28**	NS
Coronary artery disease	%	**26**	**30**	**35**	NS	**18**	**17**	NS
Cerebrovascular disease	%	**10**	**11**	**10**	NS	**10**	**9**	NS
Peripheral artery disease	%	**10**	**11**	**14**	NS	**6**	**8**	NS

Results are expressed as means (1 SD) or proportions (%). ACR: urinary albumin-to-creatinine ratio; CRP: high-sensitivity C-reactive protein; NS: not significant.

not associated with more CVD. One cannot rule out that higher Lp(a) and/or CRP may promote microangiopathy onset, at least in men.[24]

Having found no connection between CRP and macroangiopathy is in line with previous findings of no association between polymorphisms in the CRP gene, which markedly raise CRP levels on a lifelong basis, and ischemic heart or cerebrovascular diseases in the general population.[15] Even if prospective studies show an increased incidence of CV events based on a high baseline CRP, this does not necessarily demonstrate a causal relationship, since a high CRP could represent, in some patients, an integrative marker of other true risk factors, such as central obesity or the presence of unstable atherosclerotic plaques.

The lack of relationship between CRP and macrovascular disease cannot be ascribed to insufficient statistical power, since raised CRP concerned a third of patients and almost 40% of the cohort had ischemic complications. This suggests that the association between CRP and CVD is likely not causal, confining this biomarker to a bystander role. Failure to observe increased CVD as a function of raised CRP de facto transgresses several of Austin Bradford Hill's criteria for causative association, including consistency, temporality, and coherence.[15,20,31]

Even though we did not find a link between macrovascular disease and CRP, there was a marked sexual dimorphism regarding other aspects associated with raised CRP. First and foremost, average CRP was significantly different between sexes. This could result from different hormonal environment, and/or increased body fat in women. Even adjusting the cutoff for this dimorphism, which would equate to lowering it to 2.5 mg/dL (men) and increasing it to 3.5 mg/dL (women), did not affect macrovascular disease prevalence (*not shown*).

In men with elevated CRP, there were increased prevalences of sleep apnea and erectile dysfunction, in line with the proneness of the male gender for these obesity-related comorbidities. Men also had additional stigmas of AD with qualitative HDL abnormalities. They also had more microangiopathic complications, including polyneuropathy, whose epidemiological association with AD is clearly demonstrated. In women, having a raised CRP was associated with non-alcoholic steatosis, presumably related to increased adipose tissue and its metabolic consequences. It was also associated with BCF loss and further reduced insulin secretion. The predicted association between CRP and BCF loss must, however, be requalified in view of negative results of interleukin-1β inhibition with canakinumab, which, in CANTOS, did not prevent new-onset diabetes.[32]

The limits of this study are related to its transversal design which does not allow to assert causal relationships. The lack of difference in prevalent CVD between patients with normal or high CRP could have been favored by inclusion bias related to survival. This seems implausible given the observed characteristics and distribution of comorbidities in both groups. Our data came from a mostly Caucasian population, and our observations may not be applicable to other ethnicities. Finally, as total pentameric CRP was measured in this study, its level might not faithfully represent exposure to the pro-inflammatory monomeric structural isoforms.[1,18]

In conclusion, this study shows major sex-related differences in microvascular disease and residual risk in T2DM patients with high CRP. Raised CRP levels in T2DM are not associated with prevalent CVD, despite a more adverse cardiometabolic phenotype in terms of obesity and dyslipidemia. Increased CRP may represent an extended biomarker of microvascular risk in men with T2DM.

Abbreviations

ACR	albumin-to-creatinine ratio
[BxS]	hyperbolic product between B-cell function and IS
BMI	body mass index
BP	blood pressure
CAD	coronary artery disease
CKD	chronic kidney disease
CRP	C-reactive protein
CV	cardiovascular
CVD	cardiovascular disease
DM	diabetes mellitus
eGFR	estimated glomerular filtration rate
HbA$_{1c}$	glycated hemoglobin
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
HOMA	homeostasis model assessment
IS	insulin sensitivity
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
LMD	lipid-modifying drug
MetS	metabolic syndrome
non-HDL-C	non-high-density lipoprotein cholesterol
NS	non-significant
SD	standard deviation
SHBG	sex hormone-binding globulin
T2DM	type 2 diabetes mellitus
TG	triglycerides (triacylglycerols)

Research involving human participants and/or animal

Statement of human rights

For this type of study formal consent is not required.

Statement on the welfare of animals

Not applicable.

Informed consent

Informed consent was obtained from all individual participants included in the study.

The study protocol was approved by the Commission d'Ethique Biomédicale Hospitalo-facultaire de l'Université catholique de Louvain (Bruxelles) B403-2017-16NOV-521.

Funding

Author's contributions

All authors contributed equally to the manuscript.
All authors read and approved the final version of the manuscript for publication.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgement

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