Protein-Bound Plasma N-epsilon-(Carboxymethyl) lysine Is Inversely Associated With Central Obesity and Inflammation and Significantly Explain a Part of the Central Obesity-Related Increase in Inflammation The Hoorn and CODAM Studies

Protein-Bound Plasma N^{ε}-(Carboxymethyl)lysine Is Inversely Associated With Central Obesity and Inflammation and Significantly Explain a Part of the Central Obesity–Related Increase in Inflammation

The Hoorn and CODAM Studies

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Objective—Adipose tissue inflammation contributes to the development of complications, such as insulin resistance and type 2 diabetes mellitus. We previously reported that plasma levels of N^{ε}-(carboxymethyl)lysine (CML) were decreased in obese subjects resulting from CML accumulation in adipose tissue and that this CML accumulation plays an important role in adipose tissue inflammation. The objective of this study is to investigate associations between obesity (body mass index, waist circumference, and trunk fat mass), plasma CML (as an inversely correlated marker of CML accumulation in adipose tissue), and low-grade inflammation (LGI) in a large sample of individuals whose weight status ranged from normal to morbid obesity.

Approach and Results—We studied 1270 individuals of the Cohort on Diabetes and Atherosclerosis Maastricht Study and Hoorn Study, in whom protein-bound CML levels were measured by UPLC-Tandem MS (ultra performance liquid chromatography-tandem mass spectrometry), and 6 inflammatory markers were measured with multiarrays. These inflammatory markers were compiled into an LGI score. Multiple linear regression, adjusted for covariates, showed that (1) waist circumference was inversely associated with protein-bound CML plasma levels (standardized regression coefficient [β]=−0.357 [95% confidence interval: −0.414; −0.301]); (2) protein-bound CML was inversely associated with LGI score (β=−0.073 [−0.130;-0.015]); and (3) the association between waist circumference and LGI (β=0.262 [0.203;0.321]) was attenuated after adjustment for protein-bound CML plasma levels and other potential mediators (to β=0.202 [0.138;0.266]), with CML explaining the greatest portion of the attenuation (≈12%). Further analysis with dual-energy X-ray absorptiometry measures of body composition confirmed a strong inverse association of fat mass preferentially accumulated in the trunk with protein-bound CML plasma levels, significantly explaining ≈21% of the trunk fat–LGI association.

Conclusions—Obesity, in particular central obesity, is characterized by greater levels of LGI but by lower levels of circulating CML; the latter significantly explaining a portion of the positive association between central obesity and inflammation. **(*Arterioscler Thromb Vasc Biol*. 2015;35:2707-2713. DOI: 10.1161/ATVBAHA.115.306106.)**

Key Words: advanced glycation endproducts ■ biomarkers ■ central obesity ■ epidemiology ■ inflammation

Obesity, in particular central obesity, is characterized by a state of chronic low-grade inflammation (LGI) that has been linked to the increased risk of insulin resistance and type 2 diabetes mellitus (T2DM).[1–3] Increasing evidence indicates that the adipose tissue is an active endocrine organ that produces several biological active mediators of LGI, which contributes to a proinflammatory milieu.[4] These factors include tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, serum

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Nonstandard Abbreviations and Acronyms	
CML	N^{ε}-(carboxymethyl)lysine
DXA	dual-energy X-ray absorptiometry
IL	interleukin
LGI	low-grade inflammation
RAGE	receptor for advanced glycation endproducts
T2DM	type 2 diabetes mellitus

amyloid A (SAA), C-reactive protein (CRP), and others.[5] In addition, adipocytes release numerous vasoactive factors contributing to endothelial and vascular dysfunction, characterized by increased expression of intracellular cell adhesion molecules, such as intercellular adhesion molecule (ICAM)-1, which attract circulating immune cells into the vascular wall, thereby contributing to a local inflammatory response.[6,7]

Factors and mechanisms responsible for the inflammatory response in (central) obesity are not fully clear. In this context, we recently reported that obese subjects were characterized by an accumulation of the advanced glycation/lipoxidation endproduct, N^{ε}-(Carboxymethyl)lysine (CML), in 2 key metabolic tissues, where it activates inflammatory signaling pathways contributing to obesity-related insulin resistance.[8,9] In addition, we demonstrated that plasma CML concentrations were strongly reduced in obese subjects because of trapping of CML in adipose tissue.[8] As a result of CML trapping, adipose tissue of obese subjects is characterized by increased accumulation of CML.[8,10] This accumulation of CML in obesity was also recently established by Schmidt et al, which demonstrated that high-fat diet affects the concentration of CML in perigonadal adipose tissue and liver compared with low-fat diet–fed mice.[10] CML is a proinflammatory ligand for the receptor for advanced glycation endproducts (RAGE), and we demonstrated that RAGE is required for the accumulation of CML in obese adipose tissue.[8] In addition, CML-RAGE binding can activate cell-signaling pathways, thereby modulating the expression of downstream genes and regulating metabolic and inflammatory pathways.[10,11] In obese, RAGE-deficient mice and in human adipocyte cell cultures, we and others demonstrated that RAGE and activation of the CML-RAGE axis were associated with increased inflammation.[8,10] Furthermore, we showed that CML-RAGE–mediated inflammation plays a role in the induction of insulin resistance.[8] These observations were also confirmed by Schmidt et al, who demonstrate a major role of RAGE in adipose tissue inflammation and insulin resistance.[10]

Investigating the mechanisms described above, that is, the associations between (central) obesity, CML accumulation in adipose tissue, and LGI, at the population level is hindered by the impossibility of assessing the levels of CML accumulation in adipose tissue in large cohort studies. However, levels of CML can be easily measured in plasma, and considering that plasma CML levels have been shown to be inversely related to individuals' levels of (central) adiposity[12–14] and in view of the mechanisms described above, plasma CML may serve as an inversely correlated marker of CML accumulation in the adipose tissue and related inflammation. In view of these considerations, we investigated the associations between (central) obesity, CML, and inflammation in a large sample of individuals whose weight status cover the whole range from normal to severe/morbid obesity. We hypothesized that levels of CML in plasma would be inversely associated with overall obesity (as measured by body mass index) and even more so with central obesity (reflected by waist circumference); therefore, we also hypothesized that plasma CML would be inversely associated with levels of LGI and would explain, at least in part, the well-known association between (central) obesity and LGI. These hypotheses were also tested with the use of more direct measures of body composition (ie, central and peripheral fat and lean mass) in a subsample of individuals in whom body composition was assessed by means of dual-energy X-ray absorptiometry (DXA).

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

General characteristics of the study populations (Cohort on Diabetes and Atherosclerosis Maastricht [CODAM] study, Hoorn study, and clinical sample) are shown in Table 1. Levels of CML across categories of weight status (BMI) or waist circumference (quartiles) are shown in Figure 1A and 1B, respectively. Plasma CML decreased significantly with increasing categories of both BMI and waist circumference (P for linear trends <0.001 for both). The clinical cohort, which was characterized by the highest levels of BMI, showed the lowest values of plasma CML.

Analyses in the CODAM and Hoorn Studies Combined (n=1270)

Associations of BMI or Waist Circumference With Plasma Levels of CML

BMI and more strongly waist circumference were inversely associated with plasma CML (standardized regression coefficients β=−0.279 [95% CI, −0.332;−0.226] and β=−0.357 [−0.414;−0.301], P<0.001 for both, in analyses adjusted for age, sex, cohort, serum creatinine, T2DM, and smoking status and also body height in analyses with waist circumference as independent variable; [see Figure I, path a in the online-only Data Supplement]).

Association of Plasma Levels of CML With LGI

Plasma levels of CML, in turn, were inversely associated with the LGI score (Table 2, model 1) even after adjustments for other risk factors that may mediate the associations of BMI or waist circumference with LGI (Table 2, model 2; see Figure I, path b in the online-only Data Supplement). After further adjustments for BMI or waist circumference, these associations were attenuated but remained statistically significant: respectively, β=−0.088 (−0.144;−0.031), P=0.002 and β=−0.073 (−0.130;−0.015), P=0.013 (Table 2, models 3a or 3b).

Associations of BMI or Waist Circumference With LGI and Extent Independently Explained by Plasma CML

Higher levels of BMI and more strongly of waist circumference were associated with higher levels of LGI (Table 3, path c;

Table 1. General Characteristics of the Study Populations

	CODAM Study (n=532)	Hoorn Study (n=738)	Clinical Cohort (n=37)
Age, y	59.5 (7.0)	69.6 (7.0)	46.1 (9.5)
Sex (% women)	37	49	68
BMI, kg/m^2	28.4 (4.2)	27.6 (4.2)	48.2 (11.4)
Normal weight/overweight/moderate obesity/severe obesity, %	19/52/21/8	28/48/19/5	0/0/0/100
Waist circumference	99.1 (11.9)	96.0 (12.0)	136.8 (11.4)
Fasting plasma glucose, mmol/L	5.6 (5.2–6.4)	6.0 (5.5–6.9)	6.1 (5.4–7.8)
HbA1C, %	6.0 (0.8)	6.1 (0.7)	6.9 (1.6)
Prevalence of type 2 diabetes mellitus, %	25	36	50
Total-to-HDL cholesterol ratio	4.7 (1.6)	4.4 (1.3)	6.0 (2.4)
Triglycerides, mmol/L	1.4 (1.0–2.0)	1.3 (1.0–1.9)	1.7 (1.2–2.6)
Mean blood pressure, mm Hg	101.4 (11.6)	102.7 (12.5)	n/a
Pulse pressure, mm Hg	58.1 (14.4)	58.6 (16.7)	n/a
Hypertension, %	62	69	54
Creatinine, μmol/L	72.2 (14.5)	95.3 (17.3)	n/a
Smoking status (never/ex/current), %	27/52/21	37/46/17	57/27/16
Glucose-lowering medication, %	12	6	27
Lipid-lowering medication, %	19	17	19
Blood pressure–lowering medication, %	38	38	38
Prior CVD, %	27	54	n/a
Markers of low-grade inflammation*			
CRP, mg/L	2.0 (0.9–3.9)	2.4 (1.2–4.9)	6.7 (3.3–14.8)
SAA, mg/L	1.4 (1.0–2.3)	1.8 (1.1–3.2)	4.1 (1.9–8.5)
IL-6, ng/L	1.6 (1.1–2.3)	1.5 (1.1–2.3)	4.8 (3.1–6.4)
IL-8, ng/L	4.4 (3.6–5.6)	14.4 (11.1–18.8)	5.1 (3.8–6.8)
TNF-α, ng/L	6.2 [5.2–7.5)	8.3 (7.0–10.0)	8.3 (6.9–10.6)
sICAM-1, μg/L	213 (187–244)	252 (221–293)	257 (240–302)
Protein-bound CML, μmol/L	1.77 (0.45)	1.61 (0.38)	1.14 (0.18)

Data are means (standard deviation), median (interquartile range), or percentage. BMI indicates body mass index; CML, N$^{\varepsilon}$-(carboxymethyl) lysine; CODAM, Cohort on Diabetes and Atherosclerosis Maastricht; CRP, C-reactive protein; CVD, cardiovascular disease; HbA1c, glycohemoglobin; HDL, high-density lipoprotein; IL-6, interleukin-6; IL-8, interleukin-8; n/a, not assessed; SAA, serum amyloid A; TNF-α, tumor necrosis factor-α; and sICAM-1, soluble intercellular adhesion molecule-1.

*Inflammatory markers and protein-bound CML levels were measured in different pools (in EDTA-plasma samples in CODAM study and in clinical sample and in serum samples in the Hoorn study), and this may explain some of the differences in absolute concentrations between cohorts.

see Figure I, path c and Figure I, path c′ in the online-only Data Supplement). The association between BMI and the LGI score was attenuated from β=0.197 (0.139;0.254) to β=0.141 (0.080;0.201), and the association between waist circumference and LGI was attenuated from β=0.262 (0.203;0.321) to β=0.202 (0.138;0.266) after further adjustment for potential mediators and the plasma levels of CML (Table 3, paths c′). The magnitude of the attenuations attributable to all risk factors together were statistically significant [c-c′$_{(BMI)}$=0.056 (0.033;0.081) and c-c′$_{(waist\ circumference)}$=0.060 (0.031;0.090)] explaining ≈28% and 23% of the BMI–LGI or the waist circumference–LGI associations, respectively. Plasma CML independently explained the greatest portion (12% and 10%, respectively) of these attenuations.

Analyses in the Hoorn Study Subsample With DXA Measures of Body Composition (n=576)

General characteristics of the Hoorn Study subsample with DXA measures of body composition are shown in Table I in the online-only Data Supplement. Trunk fat mass was inversely (β=−0.515 [−0.634;−0.396], *P*<0.001) whereas peripheral fat mass (β=0.205 [0.067;0.396], *P*=0.004) and peripheral lean mass (β=0.117 [−0.039;0.274], *P*=0.144) were both positively associated with plasma CML levels (Figure 2). Only trunk fat mass (β=0.189 [0.066;0.312], *P*=0.003) but not peripheral fat mass (β=0.017 [−0.126;0.160], *P*=0.812) were positively associated with LGI. In contrast, peripheral lean mass was inversely associated with LGI (β=−0.177 [−0.339;−0.016], *P*=0.032; Figure 2). The positive association between trunk fat mass and LGI was attenuated from β=0.189 (0.066;0.312) to β=0.079 (0.066;0.312) after adjustment for CML and the other potential mediators considered (Table 3, paths c and c′, respectively). All together these variables explained 58% of the association between trunk fat mass and LGI, with the greatest portion of the attenuation being attributable to plasma CML (21%).

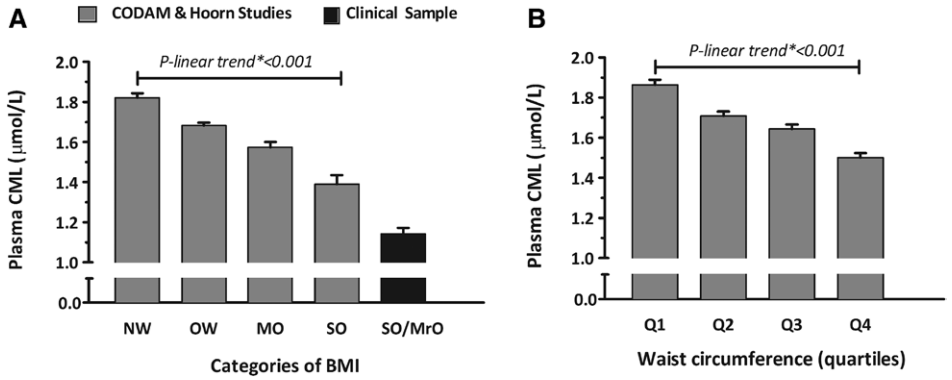

Figure 1. Protein-bound N^{ε}-(carboxymethyl)lysine (CML) plasma levels across categories of weight status (**A**); quartiles (Q) of waist circumference (**B**). Bars are mean levels and whiskers are standard errors; all data are adjusted for age, sex, cohort, serum creatinine, type 2 diabetes mellitus, and smoking status (and in analyses with Q of waist circumference also for height). Median values of body mass index (BMI; in kg/m^2) at each weight status category were 23.6 (normal weight [NW]), 27.3 (overweight [OW]), 31.8 (moderate obesity [MO]), 38.4 (severe obesity [SO]), and 45.5 (severe/morbid obesity [SO/MrO] in the clinical sample). Median values of waist circumference (in cm) at each Q were 83 (Q1), 94 (Q2), 100 (Q3), and 111 (Q4).

Additional Analyses

Additional adjustments for the use of lipid-, blood pressure-, and glucose-lowering medication and prior cardiovascular disease did not materially affect any of the associations reported earlier. We have also investigated whether these associations (ie, all described in paths a, b, or c) differed by cohort, sex, and T2DM status, but found no evidence to support this (*P* values for interaction terms added to the regression models all >0.1).

Discussion

The major findings of the present study are (1) plasma levels of CML decrease with increasing obesity, particularly central obesity, and are inversely related to levels of LGI, and (2) the lower levels of CML associated with (central) obesity explain, in part, the association between (central) obesity and LGI.

The link between (central) obesity and inflammation has been demonstrated extensively, and in agreement with several studies, we found a positive association between (central) obesity and inflammatory markers, such as CRP,[15,16] SAA,[17] IL-6,[18] and soluble ICAM-1.[19] Importantly, production and secretion of inflammatory factors from adipose tissue play a central role and link obesity to the pathogenesis of insulin resistance.[1,3,20,21] Knowledge about the mechanisms or factors involved in the dysregulated production and secretion of adipokines is of utmost importance. We recently reported that accumulation of CML in the adipose tissue can contribute to the inflammatory process in obesity.[8] By using a combination of human samples, animal, and in vitro experimentation, we demonstrated that obesity is characterized by RAGE-mediated CML trapping and activation of the CML-RAGE axis, leading to, on the one hand, lower circulating CML plasma levels and, on the other hand, induction of inflammation. These mechanisms could thus explain the significantly higher levels of inflammation, but lower levels of CML measured in plasma associated with increasing levels of obesity, particularly central obesity, as observed in the present study. Sebekova et al had also reported an inverse relationship between plasma CML levels and body fat mass in obese adolescents.[13] Likewise, Semba et al demonstrated that serum CML concentration was lower among those with increasing levels of total and regional body fat mass in adults, but the mechanisms underlying this inverse association have only recently begun to be unraveled.[8,9,14] In this line, Sebekova et al has recently reported an inverse relationship between CML and the number of metabolic syndrome traits (particularly with abdominal obesity) in young to middle-aged adults without diabetes mellitus.[12] Our findings confirm and extend these findings to a large cohort of individuals at increased risk of T2DM and cardiovascular disease. In addition, we now show that levels of CML in plasma were not only inversely associated with (central) obesity but also with inflammation, and CML explained about 10% to 20% of the association between (central) obesity and inflammation. These findings suggest that (central) obesity-related lower levels of CML in plasma may reflect CML trapping in the adipose tissue and thus indirectly, that is, as a marker, explain part of the (central) obesity-associated inflammation.

Although levels of CML in plasma significantly explained a part of the associations between obesity and inflammation—independently of and to a larger extent of a set of other related

Table 2. Associations Between Plasma Levels of Protein-Bound CML and Low-Grade Inflammation (LGI)

Model	β	95% CI	*P* Value
1	−0.132	−0.187; −0.078	<0.001
2	−0.123	−0.178; −0.068	<0.001
3a	−0.088	−0.144; −0.031	0.002
3b	−0.073	−0.130; −0.015	0.013

β indicates standardized regression coefficient; CI, confidence interval; and HDL, high-density lipoprotein. Model 1, adjusted for age, sex, cohort, serum creatinine, type 2 diabetes mellitus, and smoking status; Model 2, model 1 further adjusted for glycohemoglobin, total-to-HDL cholesterol ratio, triglycerides, and pulse pressure; and Model 3a, model 2 further adjusted for body mass index; Model 3b, model 2 further adjusted for waist circumference (and body height).

Table 3. Associations of BMI, Waist Circumference or Trunk Fat Mass With Low-Grade Inflammation and the Explanatory Role of Plasma Levels of Protein-Bound CML and Other Risk Factors Herein

	BMI (n=1270)			Waist Circumference (n=1270)			Trunk Fat Mass (n=576)		
	β	95% CI	%*	β	95% CI	%*	β	95% CI	%*
Path c	0.197	0.143; 0.251	…	0.262	0.203; 0.321	…	0.189	0.066; 0.312	…
Path c′	0.141	0.080; 0.201	…	0.202	0.138; 0.266	…	0.079	–0.053; 0.210	…
Portion explained by all risk factors (c-c′)	0.056	0.034; 0.081	28.4	0.060	0.028; 0.090	22.9	0.110	0.053; 0.174	58.2
…of which independently by†									
(M_1) HbA1c	0.016	0.008; 0.028	8.1	0.019	0.009; 0.032	7.3	0.028	0.006; 0.059	14.8
(M_2) total-to-HDL	0.020	0.006; 0.036	10.2	0.025	0.009; 0.044	9.5	0.019	–0.024; 0.067	10.0
(M_3) triglycerides	–0.007	–0.025; 0.009	–3.6	–0.012	–0.035; 0.007	–4.6	0.023	–0.031; 0.080	12.2
(M_4) pulse pressure	0.003	–0.000; 0.010	1.5	0.002	–0.001; 0.008	0.8	0.000	–0.005; 0.010	0.0
(Z) plasma CML	0.024	0.009; 0.043	12.2	0.026	0.005; 0.049	9.9	0.040	0.001; 0.092	21.2

β indicates standardized regression coefficient; BMI, body mass index; CI, confidence interval; CML, N^{ε}-(carboxymethyl)lysine; HbA1c, glycohemoglobin; HDL, high-density lipoprotein; and LGI, low-grade inflammation. Path c, adjusted for age, sex, cohort, serum creatinine, type 2 diabetes mellitus, and smoking status (and height in analysis with waist circumference or trunk fat mass as independent variables); analyses with trunk fat as independent variable are also adjusted for peripheral fat and peripheral lean mass; Path c′, further adjusted for CML, HbA1c, total-to-HDL cholesterol ratio, triglycerides, and pulse pressure.

*Portion of total effect of BMI, waist circumference, or trunk fat mass on LGI independently explained by each variable listed expressed in percentage (eg, for the BMI–LGI association independently explained by plasma CML: 0.024/0.197×100=12.2%).

†Significance was ascertained after drawing 1000 bootstraps samples to estimate bias corrected 95% CIs.

risk factors that could mediate such associations—a large portion remained unexplained. Other mechanisms triggering the proinflammatory state in obesity may thus be involved. Recent research has focused on the potential role of macrophage accumulation in adipose tissue, promoting this inflammatory process.[22] In addition, increased oxidative stress and hypoxia is associated with the increased expression of inflammation-related adipokines.[23,24]

Despite the large sample size, the use of state-of-the-art methodology for assessment of protein-bound CML plasma levels, and comprehensive adjustment for potential confounders and other mediators of the (central) obesity–LGI association, our study has some limitations. First, the hypotheses investigated herein were addressed within the context of a cross-sectional study design, which hinders definite conclusion in terms of causality. Although our prior experimental observations served as basis to the present study, further studies testing the same mechanistic hypotheses still need to be conducted among humans. For instance, assessing whether levels of CML in plasma increase and whether such increases are followed by decreases in (markers of) inflammation as a consequence of weight loss observed in the context of a randomized controlled trial could better demonstrate causality. Nevertheless, we would like to emphasize that increasing plasma levels of CML as a consequence of weight loss should not be interpreted as causally linked to LGI (risk factor) but, instead, reflect an underlying mechanism of less CML trapping in the adipose tissue, where proinflammatory mechanisms are ignited (risk marker). Second, the study of waist circumference, in addition to BMI, allowed us to address central obesity and not just overall obesity as a correlate of plasma levels of CML and LGI. The fact that waist circumference emerged as a stronger correlate of CML (inversely) and LGI (positively)

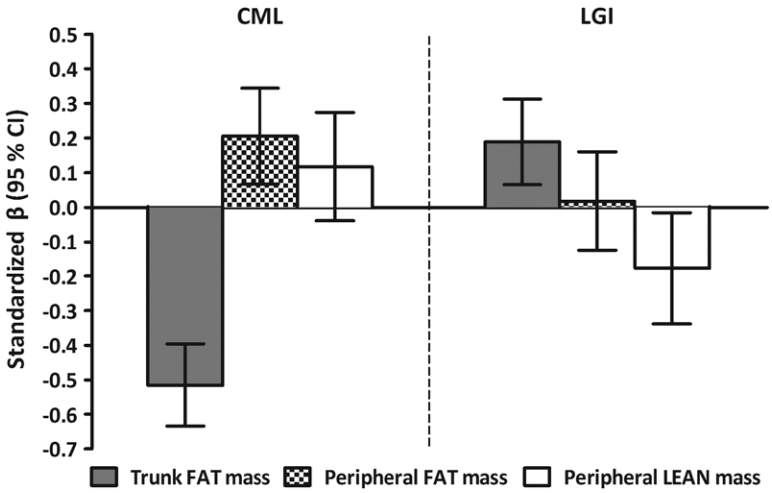

Figure 2. Associations between regional body composition, as measured by dual-energy X-ray absorptiometry (DXA), with protein-bound N^{ε}-(carboxymethyl)lysine (CML) plasma levels and low-grade inflammation (LGI); bars reflect the independent associations of trunk fat, peripheral fat, and peripheral lean masses, expressed as standardized regression coefficients, and whiskers comprise the 95% confidence intervals; all data are adjusted for age, sex, body height, serum creatinine, type 2 diabetes mellitus, and smoking status.

was further supported by analyses of body composition by DXA, where trunk fat but not peripheral fat was similarly associated with CML and LGI. Still, although trunk fat mass, as assessed by DXA, correlates highly with intra-abdominal fat,[25] it does not distinguish between subcutaneous and visceral fat in the trunk/abdominal area. Further studies are needed to shed further insight into potential differential contributions of these two adipose tissue depots to plasma CML and LGI. Third, when combining biomarkers into an overall LGI score, the underlying assumption is that each biomarker contributes, to a similar extent, to this pathophysiological process. It is, however, unknown whether this is the case for all of the biomarkers used in this study. In fact, we observed that among the 6 biomarkers investigated, associations with measures of (central) adiposity were stronger for CRP, SAA, IL-6, and ICAM-1 (Table II in the online-only Data Supplement). In addition, we observed that plasma CML was more strongly associated with CRP, SAA, and IL-6 than with the remaining inflammation markers (Table III in the online-only Data Supplement), and thus further (basic) studies need to be conducted to examine potential specific links between CML and some of the biomarkers reported in the present study. Fourth, although we calculated study-specific z-scores for the biomarkers to properly accommodate differences in methodology (ie, use of EDTA-plasma samples in the CODAM study and use of serum samples in the Hoorn study, and thus possibly different absolute mean and distribution concentrations), harmonization of the data between the 2 cohorts may still not have been optimal. Finally, our study populations consisted of middle-aged and older white individuals at higher risk for T2DM and cardiovascular disease. Caution is thus needed in the extrapolation of our findings to other populations, that is, younger and healthier individuals and of other ethnicities.

In conclusion, we showed that plasma levels of CML are inversely associated with central obesity and inflammation and significantly explain a part of the obesity-related increases in inflammation. Lower levels of CML in plasma may serve as a marker of greater accumulation/trapping of CML in the adipose tissue, where it contributes to proinflammatory processes associated with obesity.

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Disclosures

None.

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Significance

This study therefore provided, for the first time and in a large cohort, evidence that N^{ε}-(carboxymethyl)lysine plasma levels are decreased in obesity and that N^{ε}-(carboxymethyl)lysine is a significant mediator in the association between obesity and inflammation. Targeting N^{ε}-(carboxymethyl)lysine may have future therapeutic potential in the management of obesity-related complications.