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Age-related differences in inflammatory markers in men: contribution of visceral adiposity

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Abstract

As visceral adipose tissue (AT) accumulation and inflammatory markers are known to increase with age, we examined whether this age-related change in regional AT distribution could contribute to the increase in the concentration of some inflammatory markers found with age. Two hundred eight healthy men aged 18.6 to 72.2 years and covering a wide range of adiposity values (body mass index, 18.5-39.3 kg/m^2) were studied. Plasma C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor–α (TNF-α) levels were measured by enzyme-linked immunosorbent assay. Anthropometric characteristics such as height, weight, and waist girth were measured; and body mass index was calculated. Cross-sectional areas of abdominal AT were obtained at L4-L5 by computed tomography. Fasting blood samples were collected to determine a complete lipoprotein lipid profile, and a 75-g oral glucose tolerance test was performed. Overall, visceral AT accumulation was positively correlated with age ($r = 0.51$, $P < .0001$) as well as with plasma CRP ($r = 0.39$, $P < .0001$), IL-6 ($r = 0.32$, $P < .0001$), and TNF-α ($r = 0.14$, $P < .05$) levels. A significant positive relationship was also observed between age and CRP ($r = 0.36$, $P < .0001$), IL-6 ($r = 0.39$, $P < .0001$), or TNF-α ($r = 0.15$, $P < .05$) concentrations. As middle-aged men were characterized by higher CRP (1.32 [25th percentile, 0.71; 75th percentile, 2.71] vs 0.66 [0.36, 1.62] mg/L, $P < .0001$) and IL-6 (1.60 [1.09, 2.28] vs 1.12 [0.77, 1.60] pg/mL, $P < .0001$) levels as well as by a greater amount of visceral AT ($P < .0001$) than young men, we have individually matched 43 young men (age, 28.6 ± 5.82 years) with 43 middle-aged men (age, 57.6 ± 5.15 years) on the basis of their visceral AT. Matching for visceral AT eliminated the difference between middle-aged men and younger adult men in inflammatory markers. These results suggest that the age-related variation in CRP and IL-6 is largely explained by differences in visceral AT.

1. Introduction

There is with age an increased prevalence of chronic diseases, such as type 2 diabetes mellitus and cardiovascular disease (CVD). A central phenomenon in the age-related increased prevalence of these metabolic diseases is the change in body composition, including an increase in fat mass (FM), in particular abdominal fat, and a decrease in fat-free mass [1]. A decrease in physical activity [2] and a decline in energy expenditure [3] may be the factors responsible for the age-related increase in adiposity. Another possible mechanism that could explain the gain in body FM with aging is reduced whole-body fat oxidation rates [4].

It is important to keep in mind that obesity is a heterogenous condition [5]. For instance, numerous studies have shown that equally obese patients do not share the same risk factor profile and that body fat distribution is a key correlate of obesity-related risk of type 2 diabetes mellitus and CVD risk. In this regard, a number of reports have demonstrated that intraabdominal (visceral) adipose tissue (AT) accumulation is closely related to the development of type 2 diabetes mellitus, dyslipidemia, hypertension, and CVD [6,7]. It is also well known that abdominal obesity, particularly visceral obesity, is associated with a cluster of atherogenic metabolic abnormalities that are now often

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referred to as the metabolic syndrome [8]. The latter includes an atherogenic dyslipidemia, insulin resistance, a prothrombotic state, and an inflammatory profile. It is more and more recognized that inflammation is an important component not only of CVD [9] but also of type 2 diabetes mellitus [10].

In recent years, studies on inflammation and CVD risk have focused predominantly on C-reactive protein (CRP). One of the key correlates of CRP is adiposity and more specifically abdominal adiposity [11,12]. In this regard, it is well established that AT is much more than an organ specialized in triglyceride storage and mobilization because it is also an endocrine organ secreting adipokines involved in the atherogenic/diabetogenic metabolic risk profile of abdominal obesity [13]. Among the wide variety of bioactive substances called adipokines, it is the secretion of proinflammatory cytokines such as tumor necrosis–α (TNF-α) and interleukin-6 (IL-6) that is responsible for the induction of the acute-phase response including the production and secretion of CRP by the liver [14]. It has been recently suggested that this overexpression of proinflammatory molecules by AT in the context of obesity could be due to macrophage infiltration [15]. Dysregulation of adipocytokine production and secretion in the context of abdominal obesity could be a link with the development of metabolic and CVD.

Another major characteristic of the aging process is increased levels of circulating inflammatory components in the blood including elevated concentrations of TNF-α [16,17], IL-6 [18,19], and acute-phase proteins such as CRP [20]. Hence, both aging and visceral adiposity are associated with increased circulating levels of inflammatory markers [16,21-23].

Thus, the aim of this study was to examine whether the age-related changes in regional AT distribution contribute to the increase in the concentration of some inflammatory markers observed with age in asymptomatic and healthy men.

2. Methods

2.1. Subjects

Subjects were male participants in phases 2 and 3 of the Québec Family Study, a cohort of French-Canadian families living in and around the Québec city area, recruited through the media and selected to cover a wide range of body weight values (body mass index [BMI] range, 18.5-39.3 kg/m^2). This study was designed to investigate the role of genetic factors in the etiology of obesity and its comorbidities [24]. This sample of 208 men includes parents (middle-aged men >40 years old) and adult male offsprings (young adult men ≤40 years old). Middle-aged men were on average in their mid-fifties (age range, 40.2-72.2 years), whereas young men were in their mid-twenties (age range, 18.6-40.0 years). Subjects were healthy, asymptomatic, and nondiabetic men between 18 and 72 years old who were not under treatment of coronary heart disease, diabetes, dyslipidemias, or endocrine disorders. No subjects were on anti-inflammatory drugs either before or at the time of the study or were using aspirin as a long-term medication. Finally, all participants signed an informed-consent document; and the study was approved by the Medical Ethics Committee of Université Laval. As suggested by the Centers for Disease Control and Prevention/American Heart Association, individuals with CRP level greater than 10 mg/L were excluded [25].

2.2. Anthropometric measurements

The hydrostatic weighing technique [26] was used to measure body density, which was obtained from the mean of 6 valid measurements. Pulmonary residual volume was measured before immersion in the hydrostatic tank with the helium dilution method of Meneely and Kaltreider [27]. Percentage body fat was derived from body density using the equation of Siri [28]. Fat mass was obtained by multiplying body weight by percentage body fat. Height, body weight [29], and waist circumference [30] were measured following standardized procedures.

2.3. Computed tomography

Visceral AT accumulation was assessed by computed tomography, which was performed with a Somatom DRH scanner (Siemens, Erlanger, Germany) using previously described procedures [31]. Briefly, each subject was examined in the supine position with both arms stretched above the head. The scan was performed at the abdominal level (between L4 and L5 vertebrae) using an abdominal scout radiograph to standardize the position of the scan to the nearest millimeter. Total AT area was calculated by delineating the abdominal scan with a graph pen and then computing the AT surface with an attenuation range of −190 to −30 Hounsfield units [31]. The abdominal visceral AT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was calculated by subtracting the visceral AT area from the total abdominal AT area.

2.4. Determination of CRP, TNF-α, and IL-6 concentrations

Concentrations of CRP were assessed on deeply frozen plasma samples (−80°C) and were measured with a highly sensitive immunoassay that used a monoclonal antibody coated with polystyrene particles; the assay was performed with a BN ProSpec nephelometer (Dade Behring, Marburg, Germany) according to the methods described by the manufacturer [32]. Plasma IL-6 and TNF-α concentrations were measured with a high-sensitivity enzyme-linked immunosorbent assay for human TNF-α and IL-6 (R&D Systems, Minneapolis, MN). The run-to-run coefficients of variation were less than 5%, less than 10%, and less than 10% for CRP, IL-6, and TNF-α, respectively.

2.5. Statistical analyses

The normality of distribution of each variable was tested; and logarithmic transformations were applied to CRP, TNF-α, and IL-6 data. Pearson correlation coefficients were used to quantify the univariate associations between variables. The unpaired Student *t* test was used to compare mean values between young and middle-aged men. After individual matching of young vs middle-aged men on the basis of abdominal visceral AT cross-sectional area (within 5 cm^2), the 2 age groups were compared by Student paired *t* tests. Multiple regression analyses were used to define the contributions of age, FM, visceral AT area, age/FM interaction, and age/visceral AT interaction to the variance of plasma CRP, IL-6, and TNF-α. Normality and variance assumptions were verified using the Shapiro-Wilk test and Brown and Forsythe test, respectively. Results were declared significant at the .05 level. All analyses were performed with the statistical package SAS (SAS Institute, Cary, NC).

3. Results

Table 1 shows physical and metabolic characteristics of young and middle-aged men. Middle-aged men had significantly higher indices of total body fatness such as BMI and FM and also higher levels of abdominal adiposity as reflected by the waist circumference compared with young men ($P < .05$). As for depot-specific AT variables such as visceral and subcutaneous AT, middle-aged men had a greater amount of visceral AT ($P < .0001$) than young men; but there was no difference in subcutaneous AT.

Table 1
Physical and metabolic characteristics of young and middle-aged men of the study

Variables	Young men	Middle-aged men
	≤40 y old	>40 y old
N	102	106
Age (y)	28.1 ± 5.51	55.8 ± 6.7*
BMI (kg/m^2)	25.8 ± 4.5	27.4 ± 3.9*
FM (kg)	16.8 ± 9.6	21.3 ± 7.6*
Weight (kg)	79.4 ± 15.6	79.8 ± 13.6
Waist girth (cm)	88.3 ± 13.2	95.7 ± 10.9*
AT accumulation (cm^2)		
Visceral	93.3 ± 55.7	156 ± 70.3*
Subcutaneous	210 ± 137	222 ± 102
Total	303 ± 183	378 ± 153*
Cholesterol (mmol/L)	4.54 ± 0.89	5.46 ± 0.92*
LDL cholesterol (mmol/L)	2.83 ± 0.78	3.55 ± 0.82*
HDL cholesterol (mmol/L)	1.11 ± 0.23	1.12 ± 0.31
Cholesterol/HDL cholesterol	4.26 ± 1.20	5.15 ± 1.32*
Triglycerides (mmol/L)	1.11 (0.80, 1.71)	1.69 (1.17, 2.20)*
Fasting insulin (pmol/L)	50 (37, 75)	59 (47, 85)
Fasting glucose (mmol/L)	5.1 (4.9, 5.3)	5.6 (5.1, 5.9)*
Insulin area ($[pmol\ L^{-1}\ min^{-1}] \times 10^{-3}$)	58.2 (39.4, 92.3)	65.1 (49.1, 93.4)
Glucose area ($[mmol\ L^{-1}\ min^{-1}] \times 10^{-3}$)	1.10 (1.00, 1.27)	1.27 (1.08, 1.48)*
CRP (mg/L)	0.66 (0.36, 1.62)	1.32 (0.71, 2.71)*
IL-6 (pg/mL)	1.12 (0.77, 1.60)	1.60 (1.09, 2.28)*
TNF-α (pg/mL)	1.64 (1.41, 1.95)	1.75 (1.36, 2.14)

Data are presented as mean ± SD or as median (interquartile range) for skewed variables. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein.

* Significant difference between young vs middle-aged men: $P < .05$.

When univariate correlation coefficients were computed in the overall sample, it was found that plasma CRP ($r = 0.36$, $P < .0001$), IL-6 ($r = 0.39$, $P < .0001$), and TNF-α ($r = 0.15$, $P < .05$) levels were positively correlated with age. A significant and positive relationship was also observed between age and visceral AT ($r = 0.51$, $P < .0001$) as well as visceral AT accumulation with plasma CRP ($r = 0.39$, $P < .0001$), IL-6 ($r = 0.32$, $P < .0001$), and TNF-α ($r = 0.14$, $P < .05$) levels.

When circulating inflammatory markers were compared between the 2 age groups, middle-aged men were characterized by higher CRP ($P < .0001$) and IL-6 ($P < .0001$) levels; but no difference for plasma TNF-α concentrations could be found (Fig. 1A). To further investigate the contribution of adiposity to age-related differences in circulating inflammatory markers, we individually matched 53 young men with 53 middle-aged men on the basis of their total FM (Fig. 1B). After this matching procedure, differences in CRP and IL-6 levels were attenuated; but there was a trend for differences in CRP ($P = .1$) and IL-6 ($P = .07$) levels. When 43 young men were individually matched for visceral AT to 43 middle-aged men (Fig. 1C), differences in CRP and IL-6 levels were completely eliminated.

Moreover, to investigate the potentially additional contribution of visceral AT to the variance in inflammatory markers beyond age, the total sample was classified on the basis of age (<40 vs ≥40 years, which also corresponded to the median of the age distribution) and further stratified on the basis of the 50th percentile of visceral AT (<111 vs ≥111 cm^2). Fig. 2 shows that although visceral AT contributed to the variation in CRP and IL-6, age also had a significant contribution to the variation of these inflammatory markers. In addition, the interaction term between age and visceral AT was highly significant, suggesting that the relationship between visceral AT and CRP is different depending on the age group. In fact, younger men with higher visceral AT (≥111 cm^2) had significantly increased circulating CRP and IL-6 levels. In middle-aged men, there were no differences for both inflammatory markers between the high– vs low–visceral AT groups. Moreover, middle-aged men with low visceral AT (<111 cm^2) had significantly higher CRP and IL-6 levels than younger men. However, in the subgroups with high visceral AT, there was no "effect" of age on circulating CRP and IL-6 levels. As IL-6 is a key driver of CRP production by the liver, CRP levels across tertiles of IL-6 concentrations were compared in the 2 age groups (data not shown). This procedure revealed that the IL-6–CRP relationship was not affected by age.

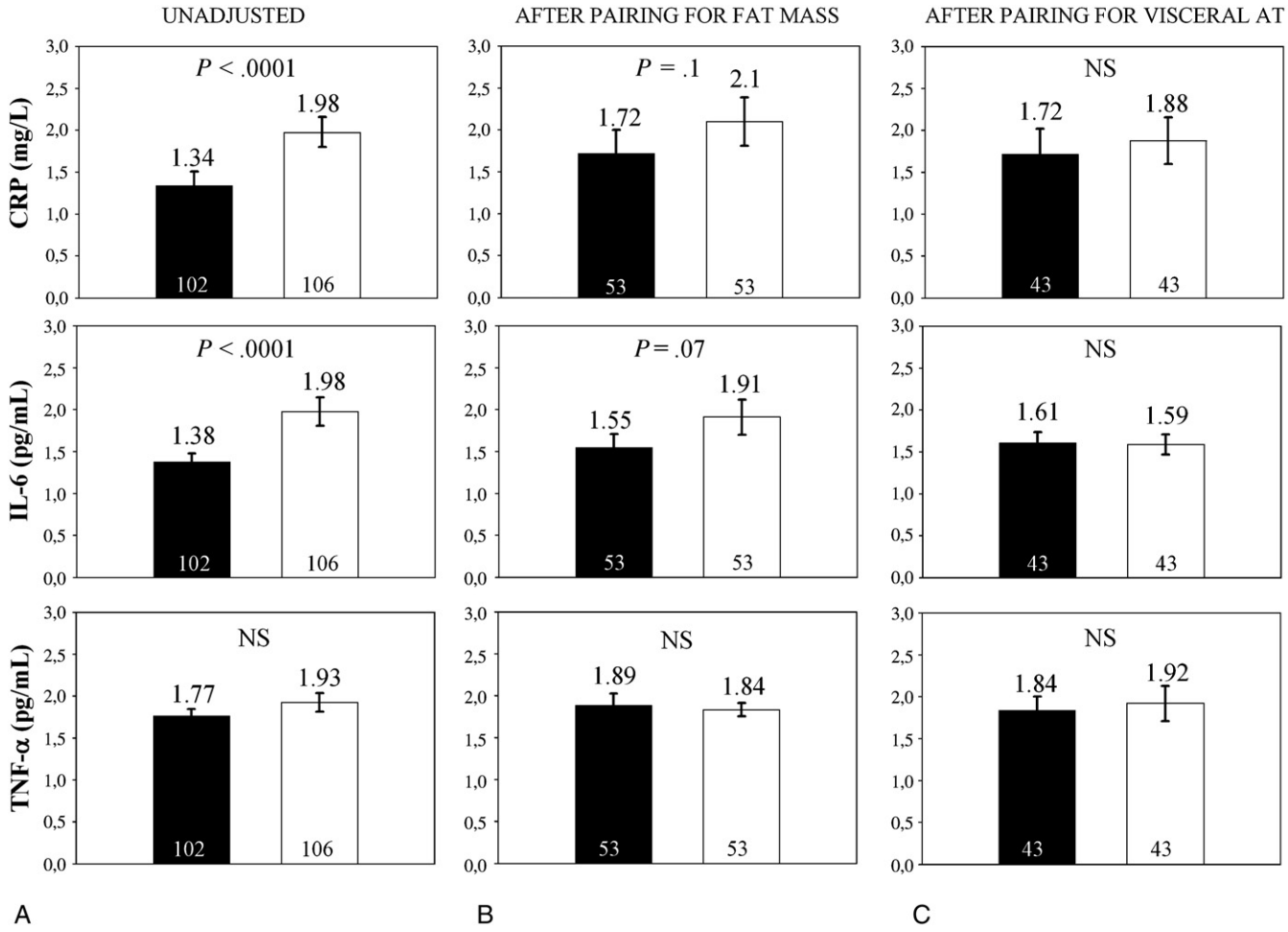

Fig. 1. Circulating inflammatory markers of young (black) and middle-aged (white) men before (A) and after individual matching for total FM (B) and for abdominal visceral AT (C). Because variables were skewed, *P* values of the log-transformed variables are presented.

Finally, multiple regression analyses were conducted to quantify the independent contributions of age, total body FM, visceral AT accumulation, and age/visceral AT and age/FM interaction terms to the variance of plasma CRP and IL-6 levels. Visceral AT (r^2 partial ×100 = 16%, $P < .0001$), age (r^2 partial ×100 = 2.3%, $P < .05$), and the age/visceral AT interaction term (r^2 partial ×100 = 5.9%, $P < .0001$) contributed independently to the variance in CRP, with 24% (R^2 total ×100) of its variance explained by the combination of these predictors, whereas only age/FM interaction term contributed independently to the variance of IL-6 levels (R^2 total ×100 = 13% of variance explained, $P < .0001$).

4. Discussion

The present study provides further evidence that changes in adiposity are related to the increase in the concentration of some inflammatory markers with age. There are, with age, noticeable changes in body composition and AT distribution [33,34]. In particular, increased visceral adiposity is a common and well-documented phenomenon [33,35-37]. Results of the present study are concordant with these findings because our middle-aged men were characterized by a greater total body FM as well as a higher accumulation of visceral AT ($P < .0001$) than younger men. However, no significant difference between the 2 age groups was noted for abdominal subcutaneous AT accumulation assessed by computed tomography. Moreover, the strongest correlation overall between total or specific adiposity parameters and age was with visceral AT accumulation ($r = 0.51$, $P < .0001$).

Middle-aged men also showed increased plasma CRP and IL-6, whereas no differences between young and middle-aged men were observed for circulating TNF-α levels. The lack of difference in TNF-α could be explained by the fact that circulating levels of TNF-α are not always representative of its activity in a context of low-grade chronic inflammation [38]. Another possibility is that our middle-aged men (mean age, 55.8 ± 6.7 years) were not old enough for their TNF-α levels to be affected [16]. Increases in circulating CRP, IL-6, and TNF-α have been reported in apparently healthy older individuals compared with younger subjects [21]. One proposed mechanism is the reduced influence of the

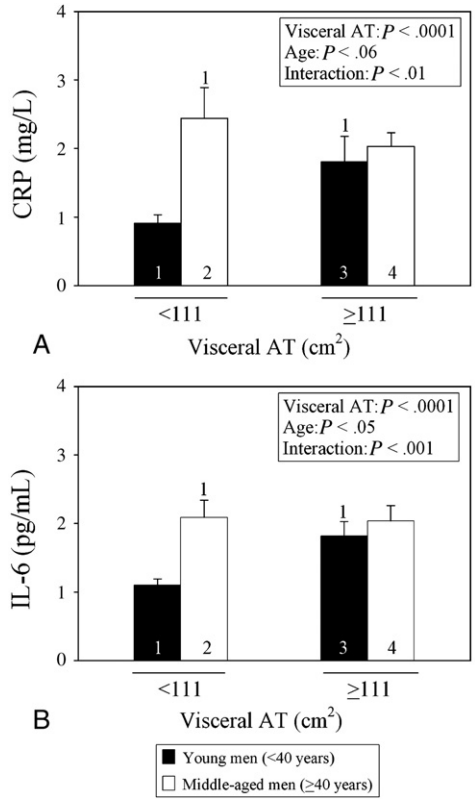

Fig. 2. Contributions of visceral AT and age to the variance in inflammatory markers (CRP [A] and IL-6 [B]) in the different subgroups (1, 2, 3, 4) classified on the basis of age (<40 vs ≥40 years) and the 50th percentile of visceral AT (<111 vs ≥111 cm^2). [1]Significantly different from the corresponding subgroup; $P < .05$ on log-transformed values.

normally inhibiting sex steroids on endogenous IL-6 and TNF-α gene expression [39]. On the other hand, the increased oxidative stress associated with aging could also have an impact on these markers of inflammation [40]. A disturbance in the redox balance produces activation of redox-sensitive transcription factors that causes the inflammation process. This phenomenon could explain why aging is associated with chronic, low-grade inflammatory activity. In our study, age was overall positively correlated with plasma CRP ($r = 0.36$, $P < .0001$), IL-6 ($r = 0.39$, $P < .0001$), and TNF-α ($r = 0.15$, $P < .05$) levels.

It is now recognized that abdominal obesity is associated with an inflammatory profile [11,12]. In the present study, significant and positive associations were found for both CRP and IL-6 levels with visceral AT. However, excess visceral AT deposition was not found to be a critical correlate of circulating TNF-α levels. The main objective of our study was to investigate whether visceral obesity was the adiposity phenotype most likely to explain the increase in inflammatory markers with age. Matching individuals for total body fatness resulted in a marked reduction in age-related differences in plasma inflammatory markers. However, the significant differences in plasma CRP and IL-6 levels initially found between young and middle-aged men were no longer observed after pairing for visceral adiposity. These results provide further evidence that visceral AT is an important correlate of circulating CRP and IL-6 levels. However, the relation between CRP and IL-6 with visceral AT was different between the 2 age groups studied. In young men, visceral AT contributed significantly to the increase in CRP and IL-6 levels, whereas in middle-aged men, the contribution of visceral adiposity to the variation in CRP and IL-6 levels seemed to be less important, suggesting a role for the other possible mediators of age-related chronic inflammation described above. On the other hand, age had a significant impact on CRP and IL-6 levels only in men with low levels of visceral AT. Results of multivariate analyses suggest that the increase in inflammatory markers found with age in men could be explained, at least in part, by the concomitant increase in visceral AT. However, age also independently contributed to the variance of both inflammatory markers.

In the present study, whereas young adult men characterized by excess visceral AT showed increased concentrations of IL-6 and CRP, middle-aged men were characterized by high CRP levels independently of the amount visceral AT. These results suggest that, at a certain age, some processes related to age could impact on the immune system in addition to the inflammatory response related to visceral adiposity. In fact, with aging, there is a decrease of circulating steroid hormone concentrations that may favor an increase of cytokines [39]. On the other hand, young men with visceral AT may be exposed to elevated inflammatory markers in early adulthood rather than later in life, which could increase their risk of diabetes and CVD.

In summary, results of the present study show that middle-aged men are characterized by higher visceral AT accumulation and by differences in circulating inflammatory markers compared with younger men. Inflammation has become a topic of interest in a number of chronic diseases including type 2 diabetes mellitus. The present study suggests that the age-related variation in circulating CRP and IL-6 levels is partly explained by differences in visceral AT. Prospective studies and experimental interventions to verify these associations are warranted.

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