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Circulating IL-18 concentration is associated with insulin sensitivity and glucose tolerance through increased fat-free mass

Received: 25 January 2005 / Accepted: 19 April 2005 / Published online: 29 July 2005
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Abstract *Aims/hypothesis:* Knowledge of the factors which simultaneously contribute to insulin-resistance-related inflammation may contribute to early therapeutic targeting. IL-18 has recently been described as one of the factors which, in addition to insulin resistance, may also contribute to atherosclerosis. However, the source of IL-18 is not well characterised. *Materials and methods:* We aimed to study body composition (bioelectric impedance), glucose tolerance (OGTT) and insulin sensitivity (minimal model method) in relation to serum IL-18 (ELISA) concentration in 144 otherwise healthy men aged 51.9 ± 12.5 years. *Results:* In contrast to previous observations in women, circulating IL-18 was not significantly associated with BMI ($r=0.12$, $p=0.1$) or WHR ($r=0.08$, $p=0.3$). IL-18 was also not associated with absolute or percent fat mass (bioelectric impedance, $p>0.20$) but, interestingly, it was significantly linked to fat-free mass ($p=0.03$). Serum IL-18 increased with each quartile of fat-free mass, corresponding to values of ≤ 64.2 ; >64.2 to ≤ 71.6 ; >71.6 to ≤ 80.9 ; and ≥ 80.9 kg (ANOVA, $p<0.0001$). IL-18 was more closely associated with postload glucose during an OGTT ($p=0.04$) rather than with fasting glucose ($p=0.1$). HbA_{1c} ($p=0.03$), HDL-cholesterol ($p=0.04$) and serum triglycerides ($p=0.03$) and parameters of systemic inflammation (C-reactive protein, $p=0.02$) were also significantly associated with circulating IL-18. Insulin sensitivity (minimal mod-

el analysis) was linked to circulating IL-18 ($p=0.01$). In a multiple linear regression analysis this relationship remained significant after controlling for BMI, age and glucose tolerance status. In another model, both fat-free mass and insulin sensitivity contributed to 10% of IL-18 variance. *Conclusions/interpretation:* Fat mass does not seem to influence circulating IL-18, as initially proposed. In contrast, the fat-free mass compartment (a well-known confounder in the evaluation of insulin sensitivity) may significantly contribute to the relationship between IL-18 and insulin action.

Keywords Cytokines · Fat-free mass · Fat mass · Inflammation · Insulin sensitivity · Muscle · Oral glucose tolerance

Abbreviations CRP: C-reactive protein · sTNFR2: soluble fraction of TNF- α receptor-2

Introduction

IL-18 is a pleiotropic proinflammatory cytokine that induces the production of TNF- α [1], and IL-6 [2], which in turn regulates the synthesis of C-reactive protein (CRP) in the liver [3]. Like IL-6 and CRP, IL-18 is considered a strong risk marker for cardiovascular death [4].

Serum IL-18 concentrations were increased and correlated positively with WHR and fasting insulin levels in 40 obese women [5, 6]. Other authors also found relationships between serum IL-18 levels and obesity and with oral glucose-derived insulin resistance in women with polycystic ovarian syndrome [7]. However, a detailed study of body composition was not performed in these studies [5–7].

Serum IL-18 levels might be a sensitive marker of the chronic inflammatory process underlying insulin resistance, in contrast to other cardiovascular risk markers such as serum CRP or IL-6 concentrations that depend mostly on obesity [7].

IL-18 secretion from adipose tissue has never been reported. The increase in serum IL-18 levels might also

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result from altered glucose tolerance [8–11]. We aimed to study body composition, glucose tolerance, insulin action and components of the metabolic syndrome in relation to circulating IL-18 in otherwise healthy men.

Subjects, materials and methods

One hundred and forty-four men, aged 51.9 ± 12.5 years, reported that their body weight had been stable for at least 3 months before the study and all were normotensive and normolipaeamic. Recruitment methodology and anthropometric measurements have been previously reported [12, 13]. Fat mass and percent fat mass were calculated using bioelectric impedance (Holtain BC Analyzer, Crymych, UK) [12]. A standard OGTT, according to ADA criteria, was performed on all subjects. Insulin sensitivity was measured using the frequently sampled IVGTT, as previously described [12, 13]. Informed consent was obtained from all subjects. The Local Ethics Committee approved the study.

Analytical methods

The serum glucose and insulin concentrations, total serum cholesterol, serum triglycerides, serum CRP (Beckman, Fullerton, CA, USA), and soluble fraction of TNF- α receptor-2 (sTNF-R2) (BioSource Europe SA, Zoning Industriel B-6220, Fleures, Belgium) were determined as previously described [12, 13]. Serum IL-18 was measured using a commercial immunoassay (MBL Medical & Biological Laboratories, Nagoya, Japan), with CV values lower than 10%.

A *p*-value of less than 0.05 was considered to be statistically significant.

Results

Men had a BMI of 27.7 ± 3.8 kg/m², WHR 0.93 ± 0.07 and fat-free mass of 72.4 ± 11.1 kg. The interquartile range of circulating IL-18 was 240.7–375.3 ng/ml, and that of insulin sensitivity was $1.19\text{--}3.32 \times 10^{-4}$ min⁻¹ mU⁻¹ l⁻¹. In contrast to previous observations in women, circulating IL-18 was not significantly associated with BMI (Pearson's $r=0.12$, $p=0.1$) or WHR ($r=0.08$, $p=0.3$). IL-18 was also not associated with absolute or percent fat mass ($p>0.20$) but, interestingly, it was linked to fat-free mass ($r=0.19$, $p=0.03$). Serum IL-18 increased with each quartile of fat-free mass (64.2, 71.6 and 80.9 kg, ANOVA, $p<0.0001$) (Fig. 1).

IL-18 was more closely associated with postload glucose after OGTT ($r=0.18$, $p=0.04$) rather than with fasting glucose ($r=0.13$, $p=0.1$). HbA_{1c} ($r=0.19$, $p=0.03$), HDL-cholesterol ($r=-0.17$, $p=0.04$), serum triglycerides ($r=0.19$, $p=0.03$), CRP ($r=0.18$, $p=0.02$) and serum sTNFR2 ($r=0.17$, $p=0.03$) were also associated with circulating IL-18.

Fig. 1 Log-transformed serum concentration of sTNFR2 (a) and IL-18 (b) according to quartiles of fat-free mass in the studied subjects

Minimal model-derived insulin sensitivity and IL-18 correlated significantly ($r=-0.25$, $p=0.01$). In a multiple linear regression analysis this relationship remained significant after controlling for BMI, age and glucose tolerance status. In another multiple linear regression analysis, both fat-free mass ($p=0.03$) and insulin sensitivity ($p=0.02$) contributed to 10% of IL-18 variance.

Discussion

Systemic levels of IL-18 were significantly associated with biochemical indices of insulin resistance (HDL-cholesterol, fasting triglycerides) and inflammation (CRP and sTNFR2) in men. Although the associations were relatively weak, the strength of the findings is substantiated by their level of statistical significance and by the fact that they were observed in apparently healthy men without severe degrees of obesity. The inclusion of patients with more severe degrees of insulin resistance would probably potentiate the slope of the relationship. Serum IL-18 was also significantly associated with insulin action independently of obesity, age and glucose tolerance status. We are unaware of previous studies using clamp or minimal

models to explore the relationship between insulin action and IL-18.

The increased levels of IL-18 in men with quartiles of fat-free mass and the positive association between IL-18 with fat-free mass itself, which persisted after controlling for insulin sensitivity, suggest that circulating IL-18 might be produced not by adipose tissue, but possibly by muscle. IL-18 was positively associated with sTNFR2, which has also been described as being associated with fat-free mass [13–15]. In fact, IL-18 mRNA transcripts have been found to be constitutively expressed in normal muscle tissues [16, 17]. Increased expression of other cytokines such as TNF- α and IL-6 have also been demonstrated in muscle tissue [18, 19]. As this latter tissue is the main target for insulin-stimulated glucose disposal, fat-free mass constitutes a determinant of insulin sensitivity. The association between fat-free mass and IL-18 might be attributed to increased production by muscle of IL-18, resulting in insulin resistance at the level of the adipocyte in a paracrine manner, and possibly at muscle in an autocrine way. This latter hypothesis would explain the differences in IL-18 in parallel with fat-free mass and insulin sensitivity. However, the contribution of other intra-abdominal organs (as other components of the fat-free mass compartment) cannot be excluded. IL-18 mRNA expression has also been demonstrated in the liver [20].

We also found that fasting IL-18 was associated with postprandial glucose after an OGTT in healthy volunteers. It has previously been suggested that serum IL-18 is increased in patients with type 2 diabetes [21], and that its concentration is influenced by macronutrient intake and the fibre content of meals in these patients [9]. Acute hyperglycaemia also led to increased plasma IL-18 in healthy volunteers [22].

In summary, fat mass does not seem to influence circulating IL-18, as initially proposed in women [5–7]. In contrast, the fat-free mass compartment may significantly contribute to the relationship between IL-18 and insulin action in middle-aged men, further substantiating the hypothesis of inflammatory activity mediated by insulin resistance [23].

Acknowledgements This work was partially supported by research grants from the Instituto de Salud Carlos III (RGDM G03/212, RGTO G03/028) and grant 00/0024-01 from the Fondo de Investigaciones Sanitarias, National Health Institute of Spain.

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