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Original Article

Cytokine-mediated inflammation is independently associated with insulin sensitivity measured by the euglycemic insulin clamp in a community-based cohort of elderly men

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Abstract: Both clinical and experimental studies suggest a close relation between an inflammatory state and insulin resistance. We investigated the association between cytokine-mediated inflammation (high sensitivity C reactive protein [hsCRP] and interleukin [IL] 6) and insulin sensitivity (insulin-mediated glucose disposal rate, assessed by the euglycemic insulin clamp) in a community-based cohort, with subgroup analyses of normal weight individuals without diabetes mellitus and metabolic syndrome (NCEP). hsCRP and IL-6 were inversely associated with insulin sensitivity (multivariable-adjusted regression coefficient for 1-SD increase of hsCRP -0.12 (-0.21-(-0.03), p=0.01) and of IL-6 -0.11 (-0.21-(-0.02), p=0.01) in models adjusting for age and components of the metabolic syndrome (systolic and diastolic blood pressure, antihypertensive drugs, HDL-cholesterol, triglycerides, fasting plasma glucose, waist circumference). The multivariable-adjusted association between hsCRP, IL-6 and insulin sensitivity were of a similar magnitude in normal weight individuals without diabetes and without the metabolic syndrome. Our data show that cytokine -mediated subclinical inflammation is independently associated with decreased insulin sensitivity also in apparently metabolically healthy normal weight individuals, indicating that the interplay between inflammatory processes and insulin resistance is present already in the early stages of the development of glucometabolic disease.

Keywords: Euglycemic insulin clamp. insulin sensitivity. inflammation. cytokines. metabolic syndrome. diabetes

Introduction

Insulin resistance and chronic inflammation have been suggested to be key triggering factors in the progressive development of both cardiovascular disease and diabetes mellitus. Both clinical and experimental studies suggest a close relation between an inflammatory state and insulin resistance [1-5]. However, most previous community-based studies have been limited by the use of surrogate markers of insulin sensitivity, such as fasting plasma insulin or HOMA insulin resistance index [2, 5]. Moreover, previous studies have included participants with obesity and prevalent metabolic disturbances, making it difficult to fully evaluate whether the association between inflammation and insulin sensitivity is independent of obesity and metabolic risk factors. We hypothesized that cytokine -mediated inflammation would be associated with insulin sensitivity in the general population independently of lifestyle factors and metabolic risk factors. Accordingly, we investigated the association between cytokine-mediated inflammation, as evaluated by serum levels of high-sensitivity C-reactive protein (hsCRP) and interleukin 6 (IL-6), and insulin sensitivity, as measured by the gold standard euglycemic insulin clamp method, in a community-based cohort of elderly men. We also used pre-specified subgroup analyses in normal weight individuals without diabetes and without prevalent meta-

bolic syndrome (ATP III-NCEP).

Materials and methods

Subjects

A reinvestigation of Uppsala Longitudinal Study of Adult Men (ULSAM, (www.pubcare.uu.se/ulsam/)) was performed 1990-94 when all subjects were ~70-years-old (n=1221). All subjects gave written consent and the Ethics Committee of Uppsala University approved the study. Data on insulin sensitivity, hsCRP and all covariates were available in 1052 participants. Of these, IL-6 data was available in 968 participants.

Assays

All participants were investigated in the morning after an overnight fast. HsCRP was measured by latex-enhanced reagent (Dade Behring, Deerfield, IL) with the use of a Behring BN-ProSpec analyzer (Dade Behring) [6]. IL-6 was analyzed by an ELISA kit (IL-6 HS, R&D Systems, Minneapolis, MN). The euglycemic insulin clamp technique according to DeFronzo was used for estimation of *in vivo* sensitivity to insulin as previously described [7, 8].

The oral glucose tolerance test, and assessment of body mass index (BMI), waist circumference, blood pressure, serum triglycerides and HDL-cholesterol, leisure time physical activity, and smoking have been described previously [8]. Diabetes mellitus was diagnosed according to current WHO-criteria and the metabolic syndrome was defined using the ATP III-NCEP-definition.

Statistical analysis

If necessary, logarithmic transformation was performed to achieve normal distribution (hsCRP, IL-6, fasting plasma glucose, serum triglycerides).

Linear regression analyses were used to assess the associations between cytokine-mediated inflammation (hsCRP and IL-6; independent variables) and insulin-mediated glucose disposal (dependent variable). The independent variables were modeled as a continuous variable expressed as 1 standard deviation increase. The following multivariable models were used:

Model A: age-adjusted;

Model B: adjusted for age and the components of the metabolic syndrome (systolic and diastolic blood pressures, antihypertensive drugs, HDL-cholesterol, triglycerides, lipid lowering drugs, fasting plasma glucose and waist circumference)

Model C: adjusted for age, BMI (as a proxy for an unhealthy lifestyle), leisure time physical activity and smoking

The analyses were also performed in the following pre-specified subgroups: 1) Without diabetes (n=991); 2) Without diabetes and without metabolic syndrome (n=824); and 3) Normal weight individuals without diabetes and without metabolic syndrome (n=382)

A two-sided p-value<0.05 was regarded as significant. The statistical software STATA 10.0 (Stata Corp College Station, TX, USA) was used for all analyses.

Results

Baseline characteristics of the study population and the different sub-samples are presented in **Table 1**.

In the whole cohort, higher serum hsCRP and higher serum IL-6 were both significantly associated with lower insulin sensitivity in age adjusted models (model A), when adjusting for the components of the metabolic syndrome (Model B) or lifestyle factors (Model C). These associations remained essentially unaltered after exclusion of participants with diabetes, prevalent metabolic syndrome and overweight/obese individuals (**Table 2**)

In secondary analyses, the addition of steady state insulin levels or serum adiponectin to all multivariable models, the addition of BMI to multivariable model B or the addition of dietary intake of tea, coffee, fibers and alcohol to multivariable model C did not substantially alter the association between hsCRP, IL-6 and insulin sensitivity (data not shown).

Discussion

Our study, using the gold standard euglycemic hyperinsulinemic clamp technique, confirms

Table 1 Baseline characteristics of the whole study population and different sub-samples

Variable	Total cohort (n=1052)	Without diabetes (n=991)	Without diabetes, without metabolic syndrome (n=824)	Without diabetes, without metabolic syndrome and normal weight (n=382)
Age (years)	71.0±0.6	71.0±0.6	71.0±0.6	71.0±0.6
Serum hsCRP (mg/l)	3.3±4.7	3.3±4.8	3.2±4.8	3.1±5.1
Serum IL-6 (ng/l)	5.8±8.9	5.8±9.0	5.6±9.0	5.7±9.9
Glucose disposal rate (M, mg/kg bw/min)	5.3±2.0	5.4±2.0	5.8±1.8	6.6±1.7
Fasting plasma glucose (mmol/l)	5.5±1.0	5.4±0.6	5.3±0.5	5.2±0.5
Systolic blood pressure (mmHg)	147±19	146±18	145±18	144±18
Diastolic blood pressure (mmHg)	84±9	84±9	83±9	81±10
Antihypertensive drugs	353 (34)	311 (31)	235 (29)	94 (25)
Serum HDL-cholesterol (mmol/l)	1.3±0.3	1.3±0.3	1.4±0.3	1.4±0.4
Serum triglycerides (mmol/l)	1.4±0.7	1.4±0.7	1.2±0.5	1.1±0.5
Lipid lowering drugs	91 (9)	86 (9)	73 (9)	36 (9)
Diabetes mellitus	61 (6)	0 (0)	0 (0)	0 (0)
MetS (NCEP)	210 (20)	167 (17)	0 (0)	0 (0)
Body mass index (kg/m^2)	26.2±3.4	26.0±3.2	25.3±2.8	23.0±1.4
Waist circumference (cm)	94±9	94±9	92±8	87±6
Smoking	218 (21)	208 (21)	177 (21)	91 (24)
Leisure time physical activity				
- sedentary	36 (4)	31 (3)	22 (3)	8 (2)
-moderate	315 (33)	294 (32)	237 (31)	103 (29)
-regular	545 (57)	524 (58)	448 (59)	212 (60)
-athletic	60 (6)	58 (6)	55 (7)	29 (8)

Date are mean±SD for continuous variables and n (%) for dichotomous variables, Metabolic syndrome (MetS) was defined using the ATP III-NCEP-definition

Table 2. The association of insulin sensitivity (glucose disposal rate) and cytokine-mediated inflammation: Multivariable linear regression

	hsC-reactive protein		Interleukin-6	
	β-coefficient (95% CI)	p-value	β-coefficient (95% CI)	p-value
Whole sample (n=1052)				
Model A	-0.39 (-0.51-(-0.27))	<0.001	-0.28 (-0.40-(-0.15))	<0.001
Model B	-0.12 (-0.21-(-0.03))	0.01	-0.11 (-0.21-(-0.02))	0.01
Model C	-0.13 (-0.23-(-0.03)	0.01	-0.17 (-0.28-(-0.07)	<0.001
Without diabetes (n=991)				
Model A	-0.37 (-0.49-(-0.25))	<0.001	-0.26 (-0.38-(-0.13))	<0.001
Model B	-0.13 (-0.22-(-0.04))	0.006	-0.12 (-0.22-(-0.03))	0.01
Model C	-0.13 (-0.23-(-0.03))	0.01	-0.17 (-0.28-(-0.07))	<0.001
Without diabetes and without MetS (NCEP) (n=824)				
Model A	-0.32 (-0.46-(-0.20))	<0.001	-0.22 (-0.35-(-0.09))	<0.001
Model B	-0.12 (-0.22-(-0.01))	0.03	-0.13 (-0.23-(-0.02))	0.02
Model C	-0.13 (-0.24-(-0.01))	0.03	-0.17 (-0.29-(-0.06))	0.004
Normal weight, without diabetes and without MetS (n=382)				
Model A	-0.28 (-0.45-(-0.12))	<0.001	-0.26 (-0.44-(-0.07))	0.007
Model B	-0.18 (-0.33-(-0.04))	0.01	-0.23 (-0.39-(-0.07))	0.005
Model C	-0.20 (-0.36-(-0.03)	0.02	-0.30 (-0.48-(-0.11)	0.002

Data are regression coefficients indicating the change in glucose disposal rate (mg/kg bw/min) associated with 1-standard deviation higher hsCRP or IL-6, respectively. Model A adjusted for age, Model B adjusted for the age and the components of the metabolic syndrome (systolic blood pressure, diastolic blood pressure, antihypertensive medication, HDL-cholesterol, triglycerides, lipid lowering treatment, fasting plasma glucose, waist circumference), Model C adjusted for age and lifestyle factors (Body mass index, leisure time physical activity and smoking). Number of participants in the analyses with IL-6: whole sample n=968, without diabetes n=913, without diabetes and without MetS n=755, Normal weight, without diabetes and without MetS (n=352); Metabolic syndrome (MetS) was defined using the ATP III-NCEP-definition.

findings from prior community-based investigations that have reported associations between higher circulating levels of cytokine-mediated inflammation markers and impaired insulin sensitivity [1, 2, 5, 9]. In addition to previous data, we showed that this association was consistent in normal weight individuals without diabetes and without the metabolic syndrome even after adjustment for the different components of the metabolic syndrome, lifestyle factors and age.

Several potential mechanisms may explain the association between cytokine-mediated inflammation and insulin sensitivity in the present study: The primary trigger of hepatic CRP synthesis is IL-6, a key regulator of the inflammation process which have been shown to decrease biological effects of insulin in adipocytes and skeletal muscle [10, 11]. The association between higher IL-6 and impaired insulin sensitivity found also in normal weight individuals even after adjustment for both BMI and waist circumference indicates that pathways not primarily mediated via increased fat mass could also be involved. This is further supported by a previous study where increased CRP levels were found only in a subgroup of obese subjects, those who were insulin resistant [12].

The opposite chain of events has also been proposed, i.e. that insulin resistance could induce inflammation [13]. For instance, insulin exerts significant biochemical effects on hepatic protein secretion [14], conceivably through signaling endogenous anti-inflammatory cascades. Interfering these effects may essentially cause an enhanced synthesis of hepatic CRP, and other acute phase proteins and further their release into the circulation [15]. This is also supported by *in vitro* and *in vivo* studies where insulin have been shown to possess anti-inflammatory properties by stimulating I and inhibiting intranuclear NF which are blunted in insulin-resistant individuals [16].

Strengths of our investigation include the large, homogenous and community-based study sample with detailed characterization of participants. Limitations include the unknown generalizability to women and other age and ethnic groups.

In summary, our data show that the association between cytokine-mediated inflammation and insulin sensitivity is present in the absence of obesity and of the metabolic syndrome indicating that the interplay between inflammatory processes and insulin resistance is present already in the early stages of the development of glucometabolic disease. However, in the present cross-sectional observational study, no conclusions regarding causality can be made. The complex interplay between cytokine mediated inflammation and impaired insulin sensitivity needs to be further investigated in experimental and clinical studies.

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