Clinical Nutrition xxx (2016) 1–8

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Effects of glucose ingestion on circulating inflammatory mediators: Influence of sex and weight excess

Héctor F. Escobar-Morreale*, M. Ángeles Martínez-García, Rafael Montes-Nieto, Elena Fernández-Durán, Sara Temprano-Carazo, Manuel Luque-Ramírez

Diabetes, Obesity and Human Reproduction Research Group, Department of Endocrinology & Nutrition, Hospital Universitario Ramón y Cajal, Universidad de Alcalá, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Carretera de Colmenar km 9,1, E-28034, Madrid, Spain

ARTICLE INFO

Article history:
Received 4 November 2015
Accepted 24 January 2016

Keywords:
Sexual dimorphism
Weight excess
Low-grade chronic inflammation
Cytokines
Chemokines

Abbreviations:
AUC
area under the curve
BMI
body mass index
E_2
total estradiol
hsCRP
high sensitivity C-reactive protein
hsIL-6
high-sensitivity interleukin-6
IL-18
interleukin-18
MCP1
monocyte chemotactic protein 1
MIF
macrophage migration inhibitory factor
MMP9
matrix metallopeptidase 9
PTX3
pentraxin-3
oGTT
oral glucose tolerance test
SHBG
sex hormone-binding globulin
T
total testosterone

SUMMARY

Background & aims: Low-grade chronic inflammation is involved in the pathophysiology of obesity. However, little is known about the influence of sex and sex hormones on surrogate inflammatory markers and mediators, particularly after glucose ingestion.
Design: Observational study.
Methods: We measured the circulating concentrations of interleukin-6, interleukin-18, macrophage migration inhibitory factor, matrix metallopeptidase-9, monocyte chemotactic protein-1 and pentraxin-3, in the fasting state and during a 75 g oral glucose tolerance test, in 24 women and 25 men. Eleven men and 11 women were lean whereas 14 men and 13 women had weight excess.
Results: Anti-inflammatory cytokines (interleukin-6 and interleukin-18) were increased in the fasting state and/or decreased in some women during the oral glucose tolerance test, as opposed to inflammatory mediators such as macrophage migration inhibitory factor and matrix metallopeptidase-9 that increased during the oral glucose tolerance test especially in subjects with weight excess. Body mass index and waist circumference were the main determinants of these changes. Fasting pentraxin-3 levels were especially increased in lean women in parallel to a decrease in free testosterone levels, and decreased during the oral glucose tolerance test as opposed to the increase in insulin concentrations.
Conclusions: The circulating concentrations of markers of low-grade chronic inflammation in young healthy adults are not only influenced by obesity but also by abdominal adiposity, fasting and glucose ingestion and, in some cases, by sex and sex hormones. These influences should be considered when these markers are used as surrogate markers of the inflammatory milieu associated with obesity.

* Corresponding author. Department of Endocrinology & Nutrition, Hospital Universitario Ramón y Cajal, Carretera de Colmenar km 9,1, E-28034, Madrid, Spain. Tel./fax: +34 91 336 9029.
E-mail address: hectorfrancisco.escobar@salud.madrid.org (H.F. Escobar-Morreale).

http://dx.doi.org/10.1016/j.clnu.2016.01.015
0261-5614/

1. Introduction

Low grade chronic inflammation plays a central role in the development of obesity-associated insulin resistance and its association with cardiovascular disease [1]. The inflammatory milieu associated with obesity is especially dependent on abdominal adiposity [2] because visceral adipose tissue is a secretory organ that releases many inflammatory mediators among other molecules [3].

Despite the evident sexual dimorphism in the distribution of body fat in humans, with women showing a predominantly peripheral and subcutaneous deposition of adipose tissue compared with the predominantly central and visceral adipose depots characteristic of men [4,5], the possibility that sex and sex hormones influence the relationship between obesity and low-grade chronic inflammation has been recognized only recently [6].

Furthermore, most current knowledge about the association of obesity and adipose tissue dysfunction with chronic inflammation in humans has been obtained in the fasting state, even though the relationship of circulating markers of inflammation with surrogate markers of abdominal adiposity and insulin resistance may not be the same in the fasting and post-meal states [7]. Moreover, the studies addressing inflammatory markers after oral intake focused mostly on the possible pro- or anti-inflammatory roles of dietary fats and antioxidants [8–10] even though, nowadays, the search for the main dietary culprit of obesity and related metabolic disorders has shifted its focus towards dietary sugars [11,12]. Glucose ingestion, in addition to alter the concentrations of many circulating inflammatory mediators [13], has also been shown to increase reactive oxygen species generation and intranuclear nuclear factor-kappaB in mononuclear cells [14,15] and, at least in women, such effects appear to be modulated by androgens, in a manner that is independent of abdominal adiposity [16,17].

To provide new insights into these controversial and still unsolved issues we aimed to study several circulating inflammatory mediators and markers, both in the fasting state and during an oral glucose challenge, in men and women presenting with or without weight excess from whom surrogate indexes of abdominal adiposity and insulin resistance had been adequately recorded [18].

2. Materials and methods

2.1. Subjects

We studied 25 young adult men and 24 premenopausal women. Subjects were classified according to their body mass index (BMI) into lean (BMI < 25 kg/m^2, 11 men and 11 women) and weight excess (BMI ≥ 25 kg/m^2, 14 men and 13 women) subgroups. The groups were composed by patients reporting to the Department of Endocrinology and Nutrition of Hospital Ramón y Cajal because of weight excess and by healthy non-obese volunteers. Men and women were selected as to be similar in terms of age and were matched for BMI. Before enrollment, the participants had no history of obesity-associated comorbidities including disorders of glucose tolerance, hypertension, cardiovascular disease, androgen excess or sleep apnea. The subjects had not received treatment with oral contraceptives, antiandrogens, insulin sensitizers, statins, antihypertensives or drugs that might interfere with clinical/biochemical variables or influence body fat depots for at least 6 months. The local ethics committee of Hospital Universitario Ramón y Cajal approved the study and all participants gave their written informed consent.

2.2. Study protocol and assays

A complete clinical evaluation that included BMI, waist circumference and waist-hip ratio was performed in all subjects. We obtained serum and plasma samples after a 12 h overnight fasting, during the follicular phase of the menstrual cycle in women. These samples were used for the measurement of total testosterone (T), total estradiol (E_2), and sex hormone-binding globulin (SHBG). A complete lipid profile was also obtained and a 75 g oral glucose tolerance test (oGTT) was performed, assaying samples taken at 0, 30, 60, 90 and 120 min for serum insulin, plasma glucose and circulating inflammatory markers. Patients were instructed to follow a diet unrestricted in carbohydrates for 3 days before sampling the in order to avoid false positive results in the oGTT. Blood samples were immediately centrifuged, and serum and plasma were separated and frozen at −30 °C until assayed. The assays used for these measurements have been described in detail elsewhere [19,20].

Free T and free E_2 concentrations were calculated from total levels and SHBG concentrations [21]. Fasting glucose and insulin levels were used for homeostasis model assessment of insulin resistance [22] and the composite insulin sensitivity index was estimated from the glucose and insulin concentrations measured during the oGTT [23]. We used the trapezoidal rule to calculate the areas under the curve (AUC) of glucose, insulin and cytokines during the oGTT.

2.3. Assays used to measure circulating inflammatory markers

Plasma or serum concentrations of high-sensitivity C-reactive protein (hsCRP), high-sensitivity interleukin-6 (hsIL-6), interleukin-18 (IL-18), macrophage migration inhibitory factor (MIF), matrix metallopeptidase 9 (MMP9), monocyte chemotactic protein 1 (MCP1) and pentraxin-3 (PTX3) levels were assayed with commercial immunochemiluminescence or enzyme-linked immunosorbent assay kits (Immulite 2000 high-sensitivity CRP, Siemens Healthcare, Los Angeles, CA; R&D Systems, UK for hsIL-6, PTX3, MIF and MMP9; MBL, Japan for IL-18; and BioVendor, Czech Republic for MCP1). The lower limits of detection and intra- and inter-assay coefficients of variation were 0.1 mg/l and <5% at 0.2 mg/l of hsCRP; 0.039 pg/ml, 7.4% and 7.8% for hsIL-6; 12.5 pg/ml, 7.3% and 7.7% for IL-18; 0.025 ng/ml, 3.9% and 5.1% for PTX3; 0.016 ng/ml, 5.3% and 9.1% for MIF; 2.3 pg/ml, 4.7% and 8.7% for MCP1; and 0.156 ng/ml, 2.3% and 7.5% for MMP9.

2.4. Statistical analysis

Continuous variables are reported as mean ± standard deviation (text and tables) and means and 95% confidence interval or means ± standard error of the mean (figures). We used the Kolmogorov–Smirnov statistic to check continuous variables for normality, and applied logarithmic or square root transformations as needed to ensure their normal distribution.

We used univariate two-way general linear models to evaluate within a single analysis the influence of sex, weight excess and the interaction between both factors on continuous variables such as fasting circulating inflammatory markers and their AUCs. Circulating levels of glucose, insulin and inflammatory mediators were also submitted to univariate repeated-measures general linear models introducing their concentrations at the different time points of the oGTT as within-subjects effect and sex and weight groups as between-subjects effect. Mauchly's test served to assess sphericity and we applied Greenhouse-Geisser correction when required.

Please cite this article in press as: Escobar-Morreale HF, et al., Effects of glucose ingestion on circulating inflammatory mediators: Influence of sex and weight excess, Clinical Nutrition (2016), http://dx.doi.org/10.1016/j.clnu.2016.01.015