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Osteoprotegerin increases in metabolic syndrome and promotes adipose tissue proinflammatory changes

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ABSTRACT

Background: Inflammation is believed to link obesity to insulin resistance, as in the setting of metabolic syndrome (MetS). Osteoprotegerin (OPG) is a soluble protein that seems to exert proatherogenic and prodiabetogenic effects. This study aims at determining OPG levels in MetS and whether OPG might contribute to MetS development and progression.
Methodology/principal findings: Circulating OPG was measured in 46 patients with MetS and 63 controls, and was found significantly elevated in those with MetS. In addition, circulating and tissue OPG was significantly increased in high-fat diet (HFD) fed C57BL6 mice, which is one of the animal models for the study of MetS. To evaluate the consequences of OPG elevation, we delivered this protein to C57BL6 mice, finding that it promoted systemic and adipose tissue proinflammatory changes in association with metabolic abnormalities.
Conclusions/significance: These data suggest that OPG may trigger adipose tissue proinflammatory changes in MetS/HFD-induced obesity.

1. Introduction

Recent studies show that type 2 diabetes mellitus (T2DM) is reaching epidemic proportions and that its prevalence is strongly associated with overall obesity and central obesity, mainly due to unhealthy changes in lifestyle (Yang et al., 2010). It has been shown that obesity is in fact an independent predictor of insulin resistance and T2DM (Collins et al., 2011), which on the other hand is prevented by weight loss (Tuomilehto et al., 2001). Among the multiple mechanisms linking obesity to T2DM, inflammation is a common feature that has been implicated in the pathology of both diseases. Several evidences (Esposito et al., 2003; Bastard et al., 2000; Ryan and Nicklas, 2004) have in fact proven the existence of an association between obesity, low-grade inflammation, and metabolic disturbances, such as insulin resistance and T2DM.

Metabolic syndrome (MetS) is a condition that clusters obesity, low-grade inflammation, and insulin resistance and predicts the future risk of diabetes and cardiovascular diseases (CVD) (Kahn et al., 2005). Although insulin resistance was initially believed to be the major underlying pathological process (Reaven, 1988), it has then been shown that the etiology of MetS was related to abnormalities in adipose tissue, or to an altered inflammatory state (Carr et al., 2004; Alberti et al., 2006); consequently obesity is now considered the first step in the etiological cascade leading to the

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other MetS disturbances. In particular, the hypothesis of what links obesity to MetS development relies on the understanding that the white adipose tissue (WAT) is an endocrine organ, whose secretory pattern changes in obese subjects as it gets inflamed (Guilherme et al., 2008) and releases more proinflammatory molecules that would impair insulin sensitivity. Experimental evidence shows that in obesity WAT contains an increased number of macrophages, which are obviously a potential source of proinflammatory factors that influence adipocyte biology and systemic insulin resistance (Weisberg et al., 2003; Xu et al., 2003; Di Gregorio et al., 2005). However, whilst it is becoming clear that obesity-related insulin resistance is, at least in part, a chronic inflammatory disease initiated in the WAT, the molecular signals that turn the increased adiposity into an inflamed adiposity, thereby triggering macrophage infiltration and promoting metabolic disturbances, are less clear (Neels and Olefsky, 2006).

Osteoprotegerin (OPG) is a soluble protein acting as decoy receptor of RANKL (receptor activator for nuclear factor κB ligand) and TRAIL (TNF-related apoptosis-inducing ligand) (Zauli et al., 2009) and it is exactly for its ability to block RANKL that OPG was initially identified as a key regulator in bone turnover (Boyle et al., 2003; Simonet et al., 1997). Not only is OPG secreted by osteoblasts (Hofbauer and Schoppet, 2004) but it is also produced by a wide range of tissues, such as the endocrine pancreas (Schrader et al., 2007), as well as different types of cells, including endothelial (Malyankar et al., 2000), smooth muscle cells (Zhang et al., 2002), and adipocytes (An et al., 2007). Interestingly, experimental evidence would suggest that the RANKL-OPG-TRAIL pathway is implicated in the regulation of glucose homeostasis (Bernardi et al., 2012b; Browner et al., 2001; Kiechl et al., 2013; Secchiero et al., 2006). Nevertheless, conflicting data have been found so far on the relationship between OPG and MetS.

In this study, we aimed at evaluating whether OPG circulating levels change in MetS. We hypothesized that OPG could increase in the setting of MetS contributing to its development and progression. Therefore, a further aim of this study was to evaluate whether an increase in OPG could contribute to adipose tissue proinflammatory changes, which seem to be fundamental to the pathogenesis of MetS.

2. Material and methods

2.1. Clinical study

2.1.1. Subject selection

To evaluate OPG levels in MetS, 46 patients with newly diagnosed MetS (cases) along with 63 healthy subjects matched by age and sex (controls) were consecutively selected from the subjects referring to three hospital-based specialized Internal Medicine Clinics, over a period of 18 months. The exclusion criteria were: age below 18 or above 65 years, history or clinical evidence of cardiopulmonary, renal, or hepatic diseases. MetS was diagnosed according to the International Diabetes Federation (IDF) definition (Alberti et al., 2006). After the initial screening visit at our Clinics and before blood sampling, all the subjects selected were asked to sign a written informed consent for participating in this study, whose protocol had been previously approved by the Institutional Ethics Committee of the University of Trieste (AOUTS – Azienda Ospedaliero Universitaria di Trieste).

2.1.2. Laboratory tests

Blood samples were collected at 08.00 a.m., after overnight fasting. Glucose, insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides and C-reactive protein (CRP) were measured by autoanalyzer. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedwald's formula. Insulin sensitivity was calculated according to the formula of the homeostasis model assessment (HOMA) index method: insulin resistance = fasting plasma insulin (μUI/ml) × fasting plasma glucose (mmol/l)/22. Plasma OPG was measured by ELISA, according to the manufacturer's instructions (Alexis biochemical distributed by Axxora.com; Cat#Alx-850-280A-KI01)

2.2. Experimental studies

2.2.1. Animals

Study 1. High-fat diet (HFD) fed mice represent an useful animal model for the study of MetS (Fraulob et al., 2010; Gallou-Kabani et al., 2007). So, in order to confirm OPG increase in the setting of MetS, 18 adult (8-wk-old) male C57BL6 mice were randomly allocated to a standard chow diet (C57 chow = 9), or a HFD (C57 HF = 9) for 12 weeks. The animals were kept in a temperature-controlled room (22 ± 1 °C) on a 12-h light/dark cycle with free access to food and water and they were fed *ad libitum* for the length of the study. The standard chow diet had 19.6% of protein, 4.6% of fat, and 4.5% of crude fibre, providing a digestible energy of 14.3 MJ/kg. The HFD had 22.6% of protein, 23.5% of fat, and 5.4% of crude fibre, providing a digestible energy of 19 MJ/kg. Intraperitoneal (ip) glucose and insulin tolerance tests (IPGTT and IPITT) were performed at week 6 and 12. As for the IPGTT, glucose (2 g/kg of body weight) was injected intraperitoneally after an overnight fast and bloods were collected from the tail tip at baseline, 15, 60, and 120 min after glucose injection. Blood glucose was measured by an automatic glucometer. Then blood samples were centrifuged at 6000 rpm for 6 min and serum insulin was measured by ELISA. As for the IPITT, insulin (1 unit/kg of body weight) was injected intraperitoneally after a 6-h fast, bloods were collected as before, and blood glucose was measured by an automatic glucometer. At the end of the study, total body mass and fat mass were measured by EchoMRI (Echo Medical Systems), and systolic blood pressure by tail-cuff pletismography. Then, the animals were anethestized by an ip injection of pentobarbitone (Euthal, Delvet, NSW, Australia) at a dose of 100 mg per kg of body weight. Blood was collected from the left ventricle, centrifuged and plasma was stored at −20 °C for analysis. Epididymal white adipose tissue (WAT), pancreases, and livers were collected and either snap-frozen and stored at −70 °C or fixed for histological analysis. This study was carried out at the Baker IDI Institute and was approved by the AMREP Animal Ethic Committee (ID 0796/2009).

Study 2. In order to determine the significance of OPG increase, 18 adult (8-wk-old) male C57BL6 mice were randomized to receive either human recombinant full-length OPG (C57 OPG, $n = 9$) or vehicle (C57 veh, $n = 9$) every 3 weeks for 12 weeks. Human recombinant full-length OPG (R&D Systems, Minneapolis) was delivered intraperitoneally at a dose of 1 μg per mouse in a total of 200 μl of HEPES-buffered saline. The animals were kept in a temperature-controlled room (22 ± 1 °C) on a 12-h light/dark cycle with free access to food (standard chow diet) and water and they were fed *ad libitum* for the length of the study. An IPGTT was performed at week 12, as before. At the end of the study, after measuring body weight and systolic blood pressure, all the animals were anesthetized and sacrificed for collecting their plasma and WAT, as above. This study was carried out at Cattinara University Hospital and was approved by its Animal Ethic Committee (ID 28.0.2008). In both studies, principles of laboratory animal care as well as specific national laws were followed where applicable.

2.2.2. Glucose, OPG, proinflammatory cytokine, and lipid measurement

Glucose was measured using an automatic glucometer (Accu-Check II; Roche) during the IPGTT, the IPITT, and at the end of both studies, at fasting. Insulin was measured by ELISA (Millipore,