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ORIGINAL ARTICLE

Simple steatosis is a more relevant source of serum inflammatory markers than omental adipose tissue

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Summary
Background and aims: Serum inflammatory biomarkers are closely associated with the risk of cardiovascular disease. However, the major source of these biomarkers is not yet determined. Therefore, we aimed to assess whether simple steatosis or visceral adiposity was a more relevant predictor for serum inflammatory biomarkers.
Methods: A double approach was used: i) clinical: 50 patients with biopsy-proven simple steatosis, 50 non-simple steatosis overweight patients, and 50 controls were explored for their serum biomarkers (high-sensitivity C-reactive protein, plasminogen activator inhibitor-1 activity, tumor necrosis factor α, and fibrinogen levels) and for visceral adiposity (measured by computed tomography); ii) experimental: using a rat simple steatosis model the effect of omentectomy on inflammatory biomarkers was investigated.
Results: Serum inflammatory biomarkers were significantly higher in the simple steatosis group than in the overweight group. Using multivariate analysis, simple steatosis, visceral adiposity index and visceral adiposity were independently associated with inflammatory biomarkers. In particular, serum inflammatory biomarkers increased with the severity of liver histology ($p < 0.05$), but no with visceral adipose tissue increase. In rats with simple steatosis, the omentectomy treatment was not associated with a decrease of serum inflammatory biomarkers in rats with simple steatosis.
Conclusions: Clinical and experimental data both indicate that simple steatosis may be more associated with inflammatory biomarkers than omental adipose tissue.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CT, computed tomography; CVD, cardiovascular disease; FBG, fibrinogen fasting levels of glucose; HDL, high-density lipoprotein; HFD, high-fat diet; HOMA, homeostasis model assessment; hs-CRP, high-sensitivity C-reactive protein; LDC, low-density lipoproteins; LSR, liver—to—spleen attenuation ratio; NAFLD, Nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ND, normal diet; OM, omentectomy; PAI-1, plasminogen activator inhibitor-1 activity; SHAM, sham-surgery; TNF-α, tumor necrosis factor α; TG, Triglycerides; VAI, visceral adiposity index; VAT, visceral adipose tissue; WC, waist circumference.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease not only in Western countries [1] but also in Asia [2,3]. Approximately 15 to 30% of adults in the general population in Asia have NAFLD [4,5]. Emerging evidence suggests that NAFLD is not only a risk factor for the development of type 2 diabetes [6], but also independently associated with an increased risk of cardiovascular disease (CVD) [7]. It is well established that serum inflammatory biomarkers play a significant role in the development and progression of CVD, which is strongly associated with NAFLD and visceral adiposity [8].

Visceral adiposity is emerging as a key mediator of cardiometabolic disorders in the general population and of liver disease in NAFLD, likely through the modulation of inflammatory biomarkers secretion [9]. Several clinical studies have demonstrated that visceral adiposity and NAFLD independently predict glucose intolerance and dyslipidemia in men [10,11]. Extensive cross-sectional studies have concluded that NAFLD is associated with serum inflammatory biomarkers and increased CVD risk [7,12]. However, these studies did not consider the potential effect of visceral adiposity.

A recent study of 135 men confirmed that Nonalcoholic steatohepatitis (NASH) patients had higher serum inflammatory biomarkers than overweight men without hepatic steatosis but with similar levels of visceral adiposity, suggesting that NASH can contribute to a more atherogenic risk profile than visceral adiposity [13]. Beside NASH there is a large body of epidemiological studies, which found that men with simple steatosis also exhibit higher serum inflammatory biomarkers than healthy or overweight subjects [14,15]. However, the studies were limited in their statistical analysis, as they did not control the influence of factors such as age or BMI. Resolution of these limitations may help to uncover the specific mechanisms by which NAFLD may contribute to the development and progression of CVD, and may be of clinical importance in planning preventive and therapeutic strategies. Since both NAFLD [16] and atherosclerosis [17,18] are more common in males, we chose to study only males in the present study.

To better understand the relative contributions of liver steatosis (without NASH) and visceral adiposity to serum inflammatory biomarkers, we have comprehensively evaluated the association between hepatic steatosis, visceral adiposity and serum inflammatory biomarkers in both adult men and male rat models.

Methods

Patients and control subjects

A total of 150 adult men (50 with hepatic steatosis, 50 overweight without hepatic steatosis on computed tomography [CT], and 50 control subjects) of similar socioeconomic level were recruited. All selected participants were matched for age. Hepatic steatosis patients and overweight without hepatic steatosis subjects were also matched for visceral adiposity and BMI. Patients were included if they had a histological NAFLD diagnosis done less than 6 months before enrolment. The diagnosis of NAFLD was based on persistently (> 6 months) elevated liver enzymes, without any other liver or biliary tract disease; negative viral markers, no history of alcohol consumption (< 20 g/day), steatosis (> 5% of hepatocytes) at histology with/without necroinflammation and/or fibrosis. Exclusion:

- advanced cirrhosis or cancer;
- other causes of acquired or hereditary liver diseases;
- immunosuppressive drugs and/or regular use of steatosis-inducing drugs, as evaluated by interview;
- active intravenous drug addiction.

This study was conducted with the approval of the Ethics Committees of Third Military Medical University and Daping Hospital, and written informed consent was obtained from all participants.

Clinical and laboratory evaluation

A complete physical examination was performed on each subject. Height, body weight, and systolic and diastolic blood pressures were measured in duplicate and the results were averaged. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared. Waist circumference (WC) was measured as the mid-point between the lower costal margin and the level of the anterior superior iliac crests. A diagnosis of arterial hypertension was based on the following criteria: systolic blood pressure > 135 mmHg and/or diastolic blood pressure > 85 mmHg. A diagnosis of type 2 diabetes was based on the revised criteria (a value of fasting blood glucose > 126 mg/dL on at least two occasions) [19]. Smoking status was defined as a current smoker or a non-smoker who had stopped smoking within the last 6 months. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG) and high-density lipoprotein (HDL) were assayed using Synchron Clinical System LX20 (Beckman-Coulter Diagnosis, Fullerton, CA, USA). Serum insulin levels were measured in duplicate by the chemiluminescence method (Roche Diagnostics, Osaka, Japan). Insulin resistance was calculated by the modified homeostasis model assessment of insulin resistance (HOMA-IR) using the formula: HOMA-IR = fasting insulin (lU/mL) $\times$ serum glucose (mg/dL)/405 [20]. Serum inflammatory biomarkers, including high-sensitivity C-reactive protein (hs-CRP), plasminogen activator inhibitor-1 activity (PAI-1), tumor necrosis factor α (TNF-α) and fibrinogen were measured by the ELISA method using commercially available kits according to the manufacturer's instructions. The visceral adiposity index (VAI) score was calculated using the reported formula [21] and was gender-specific (males): VAI = (WC/39.68 + (1.88 $\times$ BMI)) $\times$ (TG/1.03) $\times$ (1.31/HDL).

Pathology studies

Liver tissues were stained with hematoxylin—eosin, reticulin, and Gomori trichrome stains. All biopsies had a minimum of 6 portal tracts and a biopsy length of at least 12 mm. All biopsies were scored and read by one hepatopathologist (B.W.). Histological features were graded according to method of Brunt et al. [22]. Steatosis was