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Association between hypertriglyceridemia and protein oxidation and proinflammatory markers in normocholesterolemic and hypercholesterolemic individuals

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ABSTRACT

Background: Although hypercholesterolemia is a well-established risk factor for coronary heart disease, evidence suggests that increased triglyceride (TG) concentrations are also an independent risk factor. TG concentrations >150 mg/dl are observed nearly twice as often in subjects with atherosclerosis. We assessed the association between hypertriglyceridemia and protein oxidation and proinflammatory markers in normocholesterolemic and hypercholesterolemic individuals.
Methods: We included 127 volunteers enrolled in Cruz Alta, RS, Brazil. The patients were stratified based on total cholesterol and TG concentrations for analysis of associations with inflammation (high-sensitivity C-reactive protein — hs-CRP), endothelial dysfunction (nitric oxide — NOx) and oxidative stress (advanced oxidation protein products — AOPPs; ischemia-modified albumin — IMA). Correlations between variables were determined and multiple regression analysis was employed to investigate whether some variables correlate with TG concentrations.
Results: Hypertriglyceridemia was related to oxidative stress and proinflammatory markers in individuals independent of total cholesterol concentrations. Moreover, the results indicate a stronger association of tested biomarkers with TG concentrations than with total cholesterol. The results indicate a positive correlation between oxidative stress and TG concentrations in the sera of hypercholesterolemia subjects. AOPPs and IMA concentrations were associated with the presence of hypertriglyceridemia in a manner that was independent of age, gender, hypertension and diabetes mellitus disease, smoking habits, sedentary lifestyle, BMI, waist circumference, LDL, HDL and total cholesterol concentrations.
Conclusions: We speculate that TG concentrations can reflect the enhancement of protein oxidation and proinflammation.

1. Introduction

The triglyceride (TG) concentrations above 150 mg/dl are observed nearly twice as often in subjects with atherosclerosis [1]. For this reason, comprehensive evaluation and management of coronary heart disease (CHD) must address associated hypertriglyceridemia and dyslipidemia [2]. Although increased LDL is a well-established, major predictor of CHD risk and has been the primary target in strategies aiming to lower lipid concentrations [3], evidence suggests that increased TG concentrations are also an independent risk factor [4]. Postprandial, but not fasting, hypertriglyceridemia is already associated with an increased risk of atherosclerosis and is now considered an important risk factor for CVD compared with the fasting state, as has been thoroughly discussed in the literature [5–7].

High TG concentrations are a marker for atherogenic lipoproteins and are particularly important in patients with insulin-resistant syndromes such as type 2 diabetes mellitus and metabolic syndrome [8]. These patients are at significant risk for CHD even if LDL concentrations are within a healthy range; therefore, treatment beyond the use of statins is warranted to address the other lipid abnormalities [9].

Results from individual epidemiologic studies differ with regard to the concentration of association between hypertriglyceridemia and

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CHD. The differences are even more apparent after adjusting for the presence of associated risk factors, such as insulin resistance and high-density lipoprotein (HDL) cholesterol concentrations; variability in results is further increased after accounting for complications due to skewed distribution and the high variability of TG concentrations [10]. High serum cholesterol and TG concentrations in combination with low HDL concentrations are associated with the emergence of atherosclerosis, where the TG/HDL-C ratio is being suggested as an additional marker for the atherogenic potential of the lipid profile [11]. Therefore, meta-analysis has been a crucial tool in establishing TG concentrations as an independent risk factor as opposed to a risk marker of associated conditions, such as those in metabolic syndrome [12].

There is growing evidence that oxidative stress contributes to vascular dysfunction in the atherogenic process [13]. Several lines of evidence indicate that protein oxidation and the subsequent accumulation of oxidized proteins in cells, which potentially represents an early indication of oxygen-radical-mediated tissue damage, occurs during periods of oxidative stress and atherosclerosis [14]. The oxidative stress biomarker advanced oxidation protein products (AOPPs) are used as a measure of high concentrations of oxidized proteins, particularly albumin [15]. AOPPs have been highly correlated with carotid intima–media thickness and may even be related to atherosclerotic cardiovascular events [16]. Moreover, it has been suggested that AOPPs represent a novel class of proinflammatory mediators and act as a source of further oxidative stress and monocyte activation [17].

Furthermore, the overproduction of free radicals may produce chemical modifications of human serum albumin, resulting in increased ischemia-modified albumin (IMA) [18]. Currently, IMA is regarded as a biomarker of oxidative stress that is related to ischemia–reperfusion in a variety of clinical conditions associated with oxidative stress, such as myocardial ischemia [19], metabolic syndrome [20] and hypercholesterolemia [21]. A central feature of these conditions is impaired endothelial function. Cardiovascular risk factors such as hypercholesterolemia, hypertension, and diabetes mellitus enhance reactive oxygen species (ROS) generation and thereby result in oxidative stress. This oxidative stress leads to the impaired bioactivity of endothelium-derived nitric oxide (NO), which plays a protective role in the vasculature [22]. This impairment leads to the oxidative modification of lipoproteins and phospholipids, which contributes to atherogenesis [23].

Although the increase in oxidative stress in hypertriglyceridemia has already been reported [24], there are few studies, to our knowledge [25,26], demonstrating some evidence in AOPPs and IMA concentrations as a biomarker of protein oxidation in hypertriglyceridemic subjects, independently of cholesterol concentrations.

2. Materials and methods

2.1. Study population

This study included 200 volunteers from the southern Brazil city of Cruz Alta, Rio Grande do Sul state, who were found through announcements in the local media to participate in this study. Patients presenting any heart, thyroid, lung, kidney and liver diseases, acute or chronic infections, pregnancy, inflammatory process or users of anti-inflammatory drugs, including acetyl salicylic acid, and those on statin therapy were excluded from this study. Based on these exclusion criteria, the study included 127 volunteers, 57 ± 12 years, who agreed to participate. The subjects were divided into three groups to assess the baseline characteristics in accordance with lipid profile reference value intervals for cholesterolemia in adults over 20 years of age [27]: patients with total cholesterol concentrations <200 mg/dl and with LDL cholesterol concentrations between 100 and 129 mg/dl (desirable concentrations: normocholesterolemic patients — NP); patients with total cholesterol concentrations between 200 and 240 mg/dl and with LDL cholesterol concentrations between 130 and 159 mg/dl (borderline concentrations: undesirable-concentration patients — UP); and patients with total cholesterol concentrations ≥240 mg/dl and with LDL cholesterol concentrations ≥160 mg/dl (high concentrations: hypercholesterolemic patients — HP). Later, in a separate analysis, patients were stratified based on the intervals used to assess cholesterolemia (NP, UP, HP) or triglyceridemia [27]: patients with TG concentrations <150 mg/dl (desirable concentrations), patients with TG concentrations between 150 and 200 mg/dl (borderline concentrations) and patients with TG concentrations between 201 and 499 mg/dl (high concentrations). These ranges were also used to assess inflammation, endothelial dysfunction and oxidative stress parameters. An additional analysis of the oxidative profile was performed on patients with and without hyperlipidemia, who were divided in accordance with the intervals used to assess cholesterolemia (NP, UP, HP) and subdivided according to the absence or presence of hypertriglyceridemia (patients with TG concentrations ≥150 mg/dl). Correlations between variables were determined and multiple regression analysis was employed to investigate whether any variables independently influence TG concentration. This protocol was approved by the Human Ethics Committee of the University of Cruz Alta (number 623.510).

2.2. Clinical measurements

Anthropometric measurements were obtained with an emphasis on clinical markers of adiposity. Waist circumference (cm) was measured as the average distance between the last rib and iliac crest around the navel [28]. All patients answered a clinical and epidemiological assessment evaluating their individual habits involving physical exercise, being considered sedentary individual who has not practiced at least three times a week any aerobic activity, smoking habits, and the presence of history of hypertension and diabetes mellitus.

2.3. Laboratory assays

Blood samples were collected from all subjects after an overnight fast by venous puncture into Vacutainer® (BD Diagnostics) tubes with either ethylenediaminetetraacetic acid (EDTA) or no anticoagulants for biochemical assays. Specimens were routinely centrifuged at 2500 × *g* for 15 min at 4 °C. Serum was used to assess the concentrations of total cholesterol, HDL cholesterol, LDL cholesterol, TGs, nitrite/nitrate (NOx), IMA and hs-CRP using standard and previously described methods on a Cobas MIRA® automated analyzer (Roche Diagnostics). Plasma EDTA was used to measure AOPPs. LDL concentration was estimated with the Friedewald equation [29].

AOPPs concentration was determined using the spectrophotometric technique as previously described by [30]; the results are expressed in units of μmol/l. Serum IMA was measured by a colorimetric assay based on the biochemical tendency of albumin to bind exogenous cobalt, as previously described [31]. IMA results are expressed in absorbance units (ABSU). Serum hs-CRP was measured by immunoturbidimetry using a Cobas Integra 400 Plus® (Roche Diagnostics); the results are expressed as mg/dl. NO concentrations in the sera of patients were analyzed by indirectly quantifying serum nitrite/nitrate (NOx) according to a technique described in detail [32]. In this procedure, the NOx concentration was measured using 50 μl of sample; the results are expressed in units of μmol/l.

2.4. Statistical analysis

The distribution of variables was tested using the Kolmogorov–Smirnov test. Data are expressed as the mean and standard deviation (SD). Data were analyzed statistically using one-way ANOVA followed by the Newman–Keuls Multiple Comparison Test or unpaired *t* test to parametric data, and Kruskal–Wallis test followed by Dunn's Multiple Comparison Test or Mann Whitney test to nonparametric data. LDL cholesterol, TGs, AOPP and NOx results were processed for analysis,