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Linkage between C-reactive protein and triglyceride-rich lipoprotein metabolism

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ABSTRACT

Objective. Inflammation plays an important role in atherosclerosis. Elevated C-reactive protein (CRP) levels are associated with a greater risk of cardiovascular disease. Our goal was to study CRP metabolism, and to determine its relationship with lipoprotein metabolism using stable isotope methodology.

Material/Methods. Eight subjects with combined hyperlipidemia underwent a 15-h primed-constant infusion with deuterated leucine. CRP was purified from the plasma density fraction greater than 1.21 g/ml by affinity chromatography. Lipoprotein fractions were separated by sequential ultracentrifugation. Isotope enrichment was determined by gas chromatography/mass spectrometry.

Results. The subjects had mean LDL-C levels of 147.5 mg/dl and mean CRP levels of 3.4 mg/l. The mean CRP production rate (PR) was 0.050±0.012 mg/kg/day and the mean CRP fractional catabolic rate (FCR) was 0.343±0.056 pools/day (residence time 2.92 days). CRP pool size (PS) was significantly related to production ($r=0.93$; $p<0.001$), but not FCR. CRP PS was also related to body mass index ($r=0.79$; $p=0.02$). There was a significant association between CRP FCR and TRL apoB-100 FCR ($r=0.74$, $p=0.04$), as well as between CRP PS and TRL apoB-48 FCR ($r=-0.90$, $p=0.002$), indicating linkage between CRP and TRL metabolism.

Conclusion. The main determinant of plasma CRP levels was CRP production rate. Moreover a significant linkage between CRP metabolism and both TRL apoB-100 and apoB-48 catabolism was noted.

1. Introduction

C-reactive protein (CRP) is a member of the pentraxin family of proteins. It contains five identical, non-convalently associated protomers arranged symmetrically around a central pore. CRP is a major acute phase reactant produced by hepatocytes. The concentration rises rapidly (4–6 h), and markedly (as much as 1000 folds) after acute tissue injury or inflammation [1]. It is widely accepted that inflammation plays a clinically significant role in the development and progression of atheroscle-

Abbreviations: CRP, C-reactive protein; PR, production rate; FCR, fractional catabolic rate; PS, pool size; TRL, triglyceride-rich lipoprotein; apoB, apolipoprotein B; apoA-I, apolipoprotein A-I.

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rosis [2]. Clinical studies also support a link between chronic inflammation and coronary heart disease [3,4]. As a marker of inflammation, CRP has been included in some cardiovascular risk stratification and treatment guidelines [5]. In a 2010 meta-analysis, high sensitivity CRP (hs-CRP) was found to be an independent predictor of cardiovascular disease [6]. CRP has also been linked to insulin resistance, obesity, and metabolic syndrome [7,8]. Weight loss has been shown to decrease hs-CRP levels in obese and diabetic patients [9,10]. In addition, weight loss improves cardiovascular risk factor and reduces triglyceride (TG) levels [11]. Studies show that weight loss reduces VLDL–TG secretion rate [12] and intrahepatic triglyceride (IHTG) content [13–15]. Moreover several lipid lowering drugs such as statin, fibrates, and ezetimibe decrease both CRP and lipid levels. Our goal was to investigate the potential interrelations between CRP metabolism, and the metabolism of lipoprotein apolipoproteins B-100, B-48, and A-I using stable isotope methodology [16]. The detail of CRP isolation method was also shown in this study.

2. Methods

2.1. Study Subjects and Design

Eight subjects with combined hyperlipidemia, five men and three postmenopausal women without hormonal replacement therapy, were recruited for the study. Plasma lipid criteria for enrollment were LDL-C levels ≥160 mg/dl, TG levels ≥150 mg/dl, and low high density lipoprotein cholesterol (HDL-C) levels (≤40 mg/dl in men, and ≤50 mg/dl in women). Exclusion criteria were as follows: age<40 years, smoking, thyroid dysfunction, liver or kidney disease, diabetes mellitus, stroke, myocardial infarction in the past 6 months, and current use of medications known to affect lipid metabolism. Subjects were instructed to follow the therapeutic lifestyle changes diet as recommended by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) throughout the study. After 11 weeks on the therapeutic lifestyle diet, subjects had 12 h fasting blood samples drawn for the measurement of plasma lipids and CRP concentrations. Plasma was separated at 1000 *g* for 30 min at 4 °C and stored at −70 °C until used. At this time subjects were admitted to the General Clinical Research Center of Tufts Medical Center and underwent a stable isotope study. Subjects were fed hourly for 20 h with small identical meals starting 5 h before and continuing throughout the infusion. A primed-constant infusion of 10 μmol/kg body weight/h deuterated leucine ([5,5,5-$^{2}H_3$] L-leucine; C/D/N Isotopes, Pointe-Claire, Quebec, Canada) was carried out for 15 h. Blood samples were collected in EDTA tubes just before the infusion (0 h) and at 30, 35, 45 min and 1, 1.5, 2, 3, 4, 6, 9, 12, 14, 15 h during the infusion. The details of the study design and apolipoprotein metabolism results were published previously [17]. All study participants provided written informed consent, and the study protocol was approved by the Institutional Review Board of Tufts University-School of Medicine and Tufts Medical Center.

2.2. Lipid and Apolipoprotein measurements

Plasma total cholesterol (TC) and TG were measured by automated enzymatic assays standardized through the Centers for Disease Control [18]. Plasma LDL-C and HDL-C concentrations were measured directly with kits from Equal Diagnostics (Exton, PA) and Roche Diagnostics (Indianapolis, IN), respectively. Sequential density ultracentrifugation in a Beckman ultracentrifuge (Beckman, Palo Alto, CA) was used to separate each lipoprotein fraction from 5 ml of plasma from each infusion time point as follows; triglyceride rich lipoproteins (TRL) d <1.006 g/ml, intermediate density lipoprotein (IDL) d= 1.006–1.019 g/ml, low density lipoprotein (LDL) d=1.019–1.063, and high density lipoprotein (HDL) d=1.063–1.21 g/ml. Apolipoprotein B (apoB) concentrations in plasma, and in the TRL and IDL fractions were measured by ELISA using polyclonal antibodies from BioDesign (Saco, ME). LDL apoB concentrations were calculated by subtracting TRL and IDL apoB from total plasma apoB levels. ApoB-48 concentrations were assessed by running TRL fractions on SDS-PAGE gels, stained with 0.1% Coomassie blue R-250, and the relative proportion of apoB-48 was assessed by densitometric scanning. Plasma CRP concentrations were measured using a high-sensitivity immunoturbidimetric assay (Kamiya Biomedical Company, Seattle, WA). The averages of 8 infusion time points (1, 2, 3, 4, 6, 9, 12, and 15 h) were used in the calculation.

2.3. CRP isolation

CRP was isolated from the d>1.21 g/ml fraction of each time point by affinity chromatography and gel electrophoresis. Briefly, EDTA was removed from the samples by an overnight dialysis against 0.05 M Tris-buffered saline, pH 8 at 25 °C. The EDTA-free d>1.21 g/ml protein fractions were then incubated with immobilized *p*-aminophenyl phosphoryl choline resin (Thermo Scientific, Rockford, IL) in a chromatography column for 1 h at room temperature. The bound fraction, which contained CRP, was eluted with Tris buffer containing 2 mM EDTA. The CRP monomer unit was separated by 12% monogradient SDS-PAGE gel electrophoresis for 17 h at 50 V, transferred to polyvinylidene fluoride membranes, and visualized with 0.1% Coomassie blue R250. Purified human CRP (Meridian Life Science, Inc, ME) and a molecular weight standard (Biorad, Hercules, CA) were used to identify the isolated CRP monomer bands. CRP immunoblotting and protein identification by LC/MS/MS, using in-gel digestion and A Sequest search of the NCBI non-redundant protein database, confirmed the presence and purity of the isolated CRP proteins. Antibody to human CRP was obtained from Meridian Life Science, Inc, ME.

During the process of CRP monomer isolation on the SDS-PAGE gel; we found a protein band running just above the CRP band (Fig. 1). This protein has a size of approximately 25 kDa according to the molecular weight control standards. We used the in-gel digestion LC/MS/MS method, and A Sequest search of the NCBI non-redundant protein database, to identify and confirm that this protein was serum amyloid P. This protein has a molecular weight similar to CRP and also binds to phosphoryl choline; therefore it elutes with CRP during chromatography column isolation. For this reason it is vital