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SAA is Found on ApoB-Containing Lipoproteins in Obese Diabetic Humans

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Abstract

In murine models of obesity/diabetes there is an increase in plasma SAA levels along with redistribution of SAA from high density lipoprotein (HDL) to apo-B containing lipoprotein particles, namely low density lipoprotein (LDL) and very low density lipoprotein (VLDL). The goal of this study was to determine if obesity is associated with similar SAA lipoprotein redistribution in humans. Three groups of obese individuals were recruited from a weight loss clinic: healthy obese (n=14), metabolic syndrome obese (n=8) and obese with type 2 diabetes (n=6). Plasma was separated into lipoprotein fractions by fast protein liquid chromatography (FPLC) and SAA was measured in lipid fractions using enzyme-linked immunosorbent assay (ELISA) and western blotting. Only the obese diabetic group had SAA detectable in apoB-containing lipoproteins, and SAA reverted back to HDL with active weight loss. In human subjects, SAA is found in apo-B containing lipoprotein particles only in obese subjects with type 2 diabetes, but not healthy obese, or obese subjects with metabolic syndrome.

Keywords

Serum Amyloid A; Obesity; Lipoprotein distribution; Weight loss

Introduction

The positive correlation between markers of inflammation and atherosclerosis is now established. Serum amyloid A (SAA) is a major acute phase protein shown to be predictive of cardiovascular events[1]. SAA is carried in plasma primarily on high density lipoprotein

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(HDL) particles. Chronic, modest elevations in SAA concentrations have been demonstrated in obesity, metabolic syndrome (MetS), or diabetes. Weight loss tends to decrease these levels.

Despite data suggesting that SAA is positively correlated with both obesity and cardiovascular diseases, the biochemical and molecular mechanisms underlying this association are not clear. It is hypothesized that SAA may be a molecular link between obesity and atherosclerosis independent of changes in lipids and lipoprotein levels. We and others have shown that in animal models of obesity/diabetes there is an increase in plasma SAA levels along with redistribution of a large concentration of SAA from HDL to apo-B containing lipoproteins, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) independent of lipid profiles[2–5]. These studies have led to speculation that increases in SAA levels and its association with pro-atherogenic lipoprotein particles may contribute causally to atherosclerotic lesion formation. The goal of this study was to investigate whether lipoprotein redistribution of SAA also occurs in obesity and diabetes in human subjects.

Methods and Procedures

Subjects

Obese individuals participating in a Health Management Resources (HMR®) weight loss clinic who met inclusion criteria were invited to participate. HMR is a medically supervised weight loss program that incorporates diet, exercise, behavioral and life-style modification teaching. Fasting blood was drawn at the baseline visit and after 12 weeks of weight loss. Participation included collection of an extra tube of blood for analysis of SAA, as well as review of medical records for body weight and height, medications, blood pressure, and blood tests including lipid levels and hemoglobin (Hb)A1c. Subjects who lost at least 10% of initial body weight at the 12 week visit provided a second tube of blood for SAA analysis. All plasma samples were stored at −80ºC. Exclusion criteria included acute inflammation such as fever or elevated white cell counts, chronic medical problems that could affect SAA levels such as rheumatoid arthritis, other chronic inflammatory diseases, pregnancy, or lactation. Additional exclusion criteria included use of medications that could affect SAA levels or lipid levels: thus subjects using statins, fibrates, niacin, thiazolidinediones, blood pressure agents, non-steroidal anti-inflammatory agents and steroids were excluded. All subjects provided informed consent as approved by the University of Kentucky Institutional Review Board. Three groups of subjects were recruited: healthy obese subjects (without MetS), obese subjects with MetS without diabetes (at least 3 of the 5 criteria per ATP-III), and obese subjects with diabetes (patients reporting physician diagnosed diabetes).

Analytical methods

Total cholesterol, triglyceride and HDL cholesterol was measured using the enzymatic colorimetric analysis (LabCorp, Lexington KY) and LDL cholesterol was calculated using the Friedewald equation. Total plasma SAA concentration was measured using an enzyme-linked immunosorbent assay (ELISA) (Invitrogen, Carlsbad, CA; sensitivity <4ng/ml per manufacturer). Plasma from individual subjects was separated using fast protein liquid

chromatography (FPLC). Individual fractions from the peaks of each lipoprotein compartment (VLDL, LDL and HDL) were analyzed for SAA content by ELISA and western blot (ab687 Abcam, Cambridge, MA). Only the acute phase SAA isoforms SAA1 and SAA2 are recognized by this antibody. Blots were stripped and re-probed for apolipoprotein (apo)B (K23300R, Biodesign, Saco, ME) and apoA-I (ab7613, Abcam).

Statistical analysis

Values are expressed as mean ± sem. Two-tailed Student's paired t test was used to evaluate differences in SAA before and after weight loss. One way analysis of variance was used to compare the three groups. Values of $P<0.05$ were considered significant.

Results

Subject characteristics

The study comprised 14 healthy obese subjects, 8 obese MetS subjects and 6 obese subjects with type 2 diabetes (supplementary Table 1). Five of the 6 subjects with diabetes met criteria for MetS. All subjects were at or near current clinical targets for lipids and blood pressure and the diabetes group had HbA1c of 7.3 ± 0.4%. The obese diabetic subjects were significantly older than the other two groups, and the MetS subjects had significantly higher triglyceride levels. Systolic blood pressure was lower in the obese healthy group. There were no differences between groups in BMI, total cholesterol, LDL cholesterol, or HDL cholesterol.

Total plasma SAA did not differ significantly between groups, although there was a trend towards lower levels in the MetS group (supplementary Table 1). Only the diabetic group had significant amounts of SAA present in VLDL or LDL fractions (Figure 1). In diabetics, SAA was present in apoB-containing lipoproteins (Figure 1), but only in specific fractions. SAA was detected in the same fractions by both ELISA and Western blot. SAA content in apoB-containing lipoproteins correlated with HbA1c ($R=0.89$; $P=0.017$). There were no significant correlations between non-HDL SAA and VLDL TG content or any lipoprotein component. After 10% weight loss, plasma SAA declined from 78 ± 20 to 30 ± 7 mg/L in the obese diabetic group ($P=0.02$), and only one of the 6 diabetic subjects had SAA present in apoB-containing fractions, with the other 5 subjects having SAA exclusively in the HDL fraction (data not shown).

Discussion

In obese humans SAA was found in apoB-containing lipoproteins only in subjects with diabetes. This study confirms previous reports that SAA levels decrease with weight loss, but also demonstrates that SAA lipoprotein distribution shifts away from apoB-containing lipoproteins to HDL with weight loss. We and others have previously demonstrated that in murine models of diabetes/obesity there is lipoprotein redistribution of SAA compared to lean non-diabetic mice[2–4]. Elevated SAA levels are known to correlate with cardiovascular events[1, 6, 7], raising the question of whether SAA may contribute mechanistically to atherosclerosis, or merely reflect the underlying burden of atherosclerosis in existence.

SAA has been found within atherosclerotic lesions in close association to lipoproteins and proteoglycans[3, 8]. SAA is expressed by a number of cell types pertinent to atherosclerosis[9], thus atherosclerotic lesion SAA could result from entrapment of locally synthesized SAA, or from deposited circulating SAA. We previously demonstrated that SAA stimulates the synthesis of vascular proteoglycans, and augments LDL binding affinity of the vascular proteoglycans[8]. Thus, chronic elevations in SAA in individuals with obesity, MetS or diabetes may lead to alterations in the subendothelial space of blood vessel walls leading to enhanced entrapment of atherogenic lipoproteins, as outlined in the “response to retention” hypothesis of atherogenesis[10]. Furthermore, SAA itself can bind proteoglycans, and recent studies suggest that lipoproteins carrying SAA have enhanced retention by proteoglycans compared to lipoproteins lacking SAA[3, 11]. Results of the present study demonstrating SAA on apoB-containing lipoproteins in individuals with diabetes suggest a mechanism to account for accelerated atherosclerosis observed in individuals with diabetes compared to those with MetS or simple obesity.

There are some limitations of our study. First, the exclusion criteria meant that our subjects were relatively healthy compared to many obese subjects. Whether statins or other medications affect SAA lipoprotein distribution is not known. Although our study population was fairly small, all 6 subjects with type 2 diabetes had SAA detectable within VLDL and LDL fractions (range 24% to 83% of total SAA in apoB-containing fractions). Conversely in healthy obese individuals the maximum SAA present in apoB-containing fractions was 3.8%, with only 6 of 14 having any detectable SAA in apoB fractions. In the MetS group only 1 of 8 individuals had any detectable SAA in apoB fractions (5% of total SAA in apoB fractions). The metabolic data available was limited, and we cannot determine if any of these individuals was pre-diabetic, for example, having impaired glucose tolerance. Furthermore, the diabetic group was significantly older, thus a potential impact of age cannot be excluded. However, older individuals within the obese, healthy and obese MetS groups did not differ compared to younger individuals in terms of SAA lipoprotein distribution, suggesting that diabetes, not age, accounted for the shift in SAA to apoB-containing lipoproteins. Furthermore, with weight loss SAA shifted back towards HDL in the diabetic group.

The mechanisms underlying the SAA redistribution in diabetes, and shift back to HDL with weight loss is unknown. However, with weight loss the diabetic group had a striking improvement in glycemic control from HbA1c of 7.3 ± 0.4% to 6.4 ± 0.1%. As expected, all groups had significant reductions in total and LDL cholesterol with weight loss (not shown), so a lipid specific effect cannot be excluded. Further studies are required to evaluate the biological effects of SAA on apoB-containing lipoprotein particles, and to determine mechanisms leading to SAA redistribution.

The finding of SAA in apoB-containing lipoproteins in diabetes is provocative. Several clinical studies have reported the cardiovascular disease rates in individuals with diabetes without known cardiovascular disease to be similar to those in survivors of cardiovascular events without diabetes, illustrating the extreme cardiovascular risk of diabetes[12–14]. Adjustment for known cardiac risk factors does not fully explain this increased cardiac risk in diabetes, suggesting that other factors may play a role. Altered SAA lipoprotein

distribution in individuals with diabetes compared to similarly obese individuals without diabetes could be a contributing factor.

In summary, this study demonstrates that in obese humans with diabetes, but not obese with MetS or healthy obese, SAA is redistributed from HDL to apoB containing lipoproteins. Further research is required to understand the biological implications of this redistribution, and to determine if it contributes mechanistically to accelerated atherosclerosis observed in diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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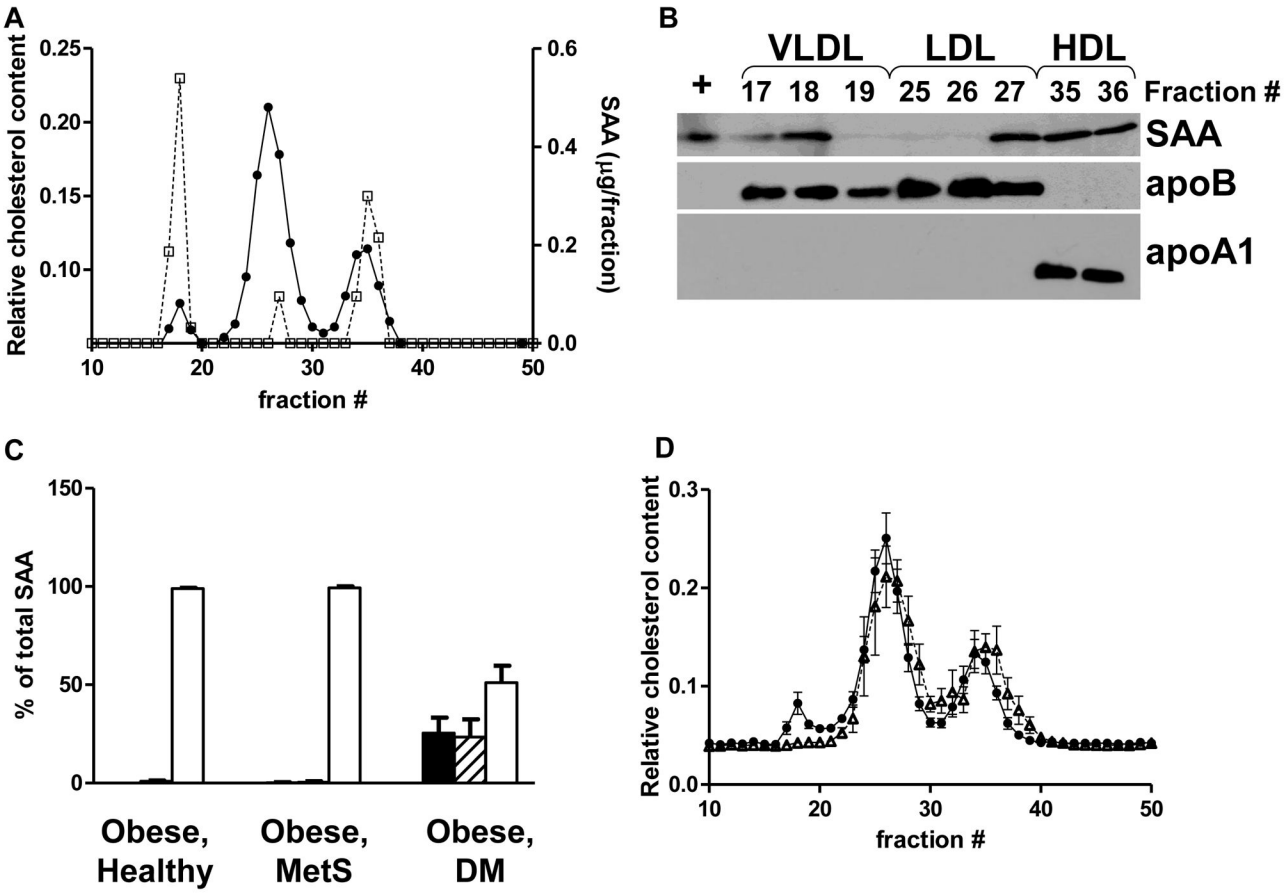

Figure 1.

SAA redistributes to apoB-containing fractions in obese diabetic subjects. A. Representative FPLC profile from an obese diabetic subject showing relative cholesterol content (solid circles, solid lines, left Y axis) and SAA content (open squares, dashed lines, right Y axis). B. Representative western blot from the same obese diabetic subject as in panel A for SAA, apoB, and apoA-I as indicated. Individual plasma fractions separated by FPLC were analyzed by western blot. The gel was loaded using equal volumes per aliquot and the same blot was stripped then re-probed for apoB and apoA-I. The "+" shows a positive loading control for SAA where 1 μg of SAA was loaded on the gel. The FPLC fractions run on the gel are indicated. C. SAA distribution (as percent of total SAA) in each lipoprotein fraction, as determined by ELISA on individual FPLC fractions. Solid bars show percent SAA in VLDL, hatched bars show percent SAA in LDL, and open bars show percent SAA in HDL. D. FPLC profiles from obese diabetic subjects before (solid circles, solid lines) and after (open triangles, dashed lines) 10% body weight loss. Shown is mean ± SEM for N=6 diabetic subjects. MetS: metabolic syndrome; DM: diabetes.