Decrease of Circulating SAA is Correlated with Reduction of Abdominal SAA Secretion During Weight Loss

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Objective: The study goal was to determine the effect of weight loss (WL) alone and with aerobic exercise (WL + AEX) on serum amyloid A (SAA) levels and adipose SAA secretion from gluteal and abdominal depots.

Methods: Ninety-six overweight or obese postmenopausal women undertook a 6-month WL alone (n = 47) or with AEX training (n = 49) (6 months WL and WL + AEX are considered WL when groups were combined). Their serum SAA levels, body weight, and adipose SAA secretion ex vivo from gluteal and abdominal depot were measured before and after WL interventions.

Results: The participants lost an average of 8% body weight with a 10% decrease of serum SAA. Serum SAA levels remained significantly correlated with body weight before and after WL. However, the changes of serum SAA level did not correlate with changes of body weight. The gluteal adipose tissue secreted ~50% more SAA than the abdominal tissue, but the changes of abdominal, but not gluteal, SAA secretion correlated ($R^2 = 0.19, p < 0.01$) with those of serum SAA levels during WL.

Conclusions: No linear correlation between the decrease in systemic SAA and WL was found. There is a depot-dependent difference in adipose SAA secretion and abdominal SAA secretion, which may partially account for the systemic SAA reduction during WL.

Introduction

Obesity is characterized by an elevation of local adipose tissue as well as systemic low-grade systemic inflammation (3,4), which contributes to its associated comorbidities such as insulin resistance, type 2 diabetes, and cardiovascular diseases (CDV) (5,6). Whether and how these two inflammatory processes relate in humans is not well understood. Acute-phase protein serum amyloid A (SAA) is selectively expressed in adipose tissue and its tissue expression and circulating levels increase in obese subjects (7-9), suggesting that SAA may serve as a molecular link between adipose tissue and systemic inflammation. Several studies show that SAA plays an active role in regulating the inflammation process (7-9), and suggest that SAA is a pro-inflammatory cytokine that may be responsible for macrophage infiltration in the adipose tissue (10). A recent study shows that elevations in systemic SAA by transgenic overexpression increases circulating serum IL-6 and TNFz and significantly promotes atherosclerosis in mice (11), thus providing direct evidence that SAA is a causative factor for systemic inflammation and CVD in animals.

Weight loss (WL) via life-style change with or without aerobic exercise (AEX) is an effective regimen for the prevention and treatment of obesity and its associated metabolic disturbances by lowering circulating SAA levels (12,13) and adipose SAA expression (13,14). However, few studies have examined the effects of WL + AEX on adipose SAA secretion; thus, the relationship between changes in adipose SAA secretion and systemic SAA levels during WL remains unknown.

Adipose tissues of different depots have distinctive molecular, cellular, and metabolic properties (15-17) with discrete systemic metabolic and endocrine consequences (18). Indeed, the gene expression of fatty acid amide hydrolase (FAAH), an enzyme participating in endocannabinoid synthesis and implicated in adipocyte dysfunction (19), is higher in the abdominal than gluteal adipose tissue and WL by hypocaloric feeding decreases the gene expression of gluteal, but not abdominal, cannabinoid receptor 1, and FAAH. These observations suggest that abdominal and gluteal adipose tissue respond to metabolic and nutritional challenges differently; this study examines whether there are differences in SAA secretion between abdominal and gluteal subcutaneous fat depots to WL with and without AEX.

Considering that SAA plays a pivotal role in mediating inflammation and that the reduction of circulating SAA may be responsible for the reduced systemic inflammation in lifestyle change-induced WL, it will be valuable to understand the effects of WL on systemic inflammation and its associated comorbidities.
and adipose SAA levels. As the adipose tissue is a prominent organ that expresses and releases SAA, the goal of this study was to determine 1) whether there is a relationship between changes of systemic SAA levels and body weight/fat mass, and 2) whether there are differences in SAA secretion between gluteal and abdominal depots and if these changes are related to circulating SAA during WL.

Methods

Human Subjects
The Institutional Review Board of the University of Maryland approved all human studies, and each volunteer provided written informed consent to participate. All subjects were relatively healthy, non-diabetic by fasting glucose (<126 mg/dl), but overweight or obese (body mass index [BMI] >25 kg/m², range 25-48 kg/m²) women between the ages of 49 and 76 years. The women were post-menopausal and had not menstruated for ≥1 year. Details about this WL program have been described elsewhere (20). In brief, all women in WL and WL + AEX attended weekly WL classes led by a registered dietitian. Women were instructed to reduce their caloric intake by 300-500 kcal/day. For the WL + AEX intervention, women exercised three times per week for 6 months using treadmills and elliptical trainers as described (20). Fat mass was determined by dual-energy X-ray absorptiometry (Prodigy; Lunar Radiation Corp., Madison, Wisconsin). Blood samples were obtained from an antecubital vein after a 12-h overnight fast, 36-48 h after the last bout of exercise in WL + AEX, and stored at −80°C until analysis. Fasting serum levels of SAA were measured before and after the intervention in all participants.

Adipose Tissue Culture and SAA Measurement
Some of the participants underwent adipose tissue biopsies before and after the WL regimen. Biopsies were performed 36-48 h after the last bout of exercise in the WL + AEX group. Abdominal and gluteal adipose tissue samples were obtained from overnight fasted subjects by aspiration with a 3 mm cannula under local anesthesia with lidocaine as previously described (21). Adipose tissue fragments were incubated for 3 h in M199-1% BSA (~100 mg/ml) and the media was collected and stored at −80°C as previously described (22), until SAA assay.

Serum SAA levels were measured with an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer’s instructions (BioSource, Camarillo, California, USA). For high sensitive measurement of SAA levels in a small volume of conditional media of the acute human adipose culture, a Meso Scale Discovery (MSD) multiplex electrochemiluminescence (ECL) assay (Meso Scale Discovery, Gaithersburg, Maryland, USA) was employed. The SAA detection sensitivities by ELISA and MSD were 5 ng/ml and 8 pg/ml, respectively. For ELISA, the intra- and inter-assay coefficients of variation (CV) were 5 and 8%, respectively, whereas for MSD, the intra-assay CV was 4%. The inter-assay CV was not determined since all samples were assayed in one time. The correlation coefficient between the two assays was 0.87. All samples were assayed in duplicate and the mean was used for data analysis.

Statistical Analysis
Data are expressed as mean ± SEM unless otherwise specified. Variables that were not normally distributed were logarithmically trans-
p < 0.001) and after (R = 0.74, p < 0.001) WL (Figure 3b). Notably, there was a larger variation of up to 100-fold difference in SAA secretion before and after WL. Thus, although the overall adipose SAA secretion decreased by 30% (p = 0.09) and 22% (p = 0.6) in abdominal and gluteal depots, respectively, after WL, the reduction after WL did not reach statistical significance.

**FIGURE 1** Change of serum SAA levels and its relationship with BMI/fat mass before and after WL. (A) Relationship between BMI and % fat mass and serum SAA pre-WL. Linear correlation analysis of serum SAA levels (µg/ml, log-transformed and presented) with BMI and percentage of fat mass (% of fat mass) pre-WL in 96 overweight or obese postmenopausal women. (B) Changes in body weight (BW) and SAA after WL and WL + AEX. 47 subjects underwent a WL program and 49 subjects received WL + AEX. SAA levels (µg/ml) and body weight (kg) changes were compared before and after WL (”**p < 0.01, paired t test”) with the mean % reduction of SAA and body weight shown. No difference was detected between WL vs. WL + AEX treatment by two-way ANOVA. (C) Relationship between BMI and % fat mass and serum SAA post-WL. Linear regression analysis of serum SAA levels (log-transformed) with BMI and percentage of fat mass post-WL in the 96 participants.
Effects of WL on Relationships Between Changes of Adipose SAA Secretion and Serum SAA Levels

We examined the relationship between changes in systemic SAA levels and adipose secretion from abdominal and gluteal regions after WL. Spearman analysis revealed a moderate correlation of the changes of serum SAA levels with changes in SAA secretion in abdominal ($R = 0.43$, $p < 0.01$), but not in gluteal, adipose tissues (Figure 4).
Discussion

Numerous studies demonstrate that SAA is an adipokine that is elevated in the circulation in obesity and SAA has been considered a mediator between obesity and associated insulin resistance, low-grade inflammation, and risk for CVD (23-25). To better understand the relationship between adipose and systemic SAA levels, we conducted this study and examined the changes of adipose SAA secretion \textit{ex vivo} in relationship to its circulating level in a cohort of well-characterized postmenopausal women who went through WL via WL or WL + AEX (20). Our results show that serum SAA levels correlate with BMI and fat mass in overweight or obese subjects before WL. An average loss of 8% body weight resulted in a comparable reduction of systemic SAA after the 6-month WL program alone or with AEX. Moreover, the relationship of systemic SAA and body fat mass remained after WL. These observations further support that BMI/fat mass is a determinant of circulating SAA levels. However, no correlation was detected between the changes of BMI vs. changes of serum SAA at the individual level (Figure 2). This is a somewhat unexpected finding, considering that serum SAA levels are well correlated with BMI before and after WL (Figure 1c). A possible explanation is that a marked variation of serum SAA relative to the narrow changes of body weight during the WL period significantly reduced the statistical power to detect such a correlation. Another explanation is that fat mass accounts for ~19 and 26% of systemic SAA variation in pre- and post-WL states (Figure 1), and fat mass-independent mechanisms (e.g., the rate of adipose SAA secretion and non-adipose SAA secretion) have a greater weight in determining systemic SAA levels.

Since there are regional differences in gene expression and regulation in the subcutaneous fat depot (26-28), we examined the changes of SAA release \textit{ex vivo} of adipose samples from abdominal and gluteal regions. Results show that adipose tissue in the gluteal depot secretes ~50% more SAA than that of the abdominal, both before and after WL. A strong correlation is observed in the amount of SAA secretion between the two depots, suggesting a similar regulatory mechanism for adipose SAA production between the depots. Remarkably, a large individual variation in SAA secretion was observed during WL, indicating that the rate of SAA secretion is highly variable among individuals before and after WL. As a result, the dramatic ~25% reduction in SAA secretion after WL did not reach statistical significance.

This study finds a moderate correlation between the reduction of serum SAA levels and that of abdominal SAA secretion ($R = 0.43$, Fig. 4), suggesting that the decrease in abdominal SAA secretion could contribute to the lower SAA in circulation after WL. It is intriguing to note that although gluteal adipose tissue releases more SAA than abdominal adipose tissue, only abdominal adipose tissue SAA secretion is significantly related to circulating SAA levels. One possible explanation is that the local abdominal condition facilitates the release of SAA at the whole body level. This is supported by the finding that the blood flow rate in abdominal depot is three times that in the gluteal depot (29). Moreover, the abdominal skin temperature is 1°C higher than the gluteal (33.3 vs. 32.2°C), which may enhance SAA release from abdominal depot into the circulation. Another possibility is via an indirect mechanism, wherein abdominal adipose tissue releases more pro-inflammatory fatty acids systemically (29). The local SAA reduction would lower lipolysis and the output of fatty acids and inflammatory cytokines into the circulation, resulting in a systemic reduction in inflammatory status and SAA production from non-adipose tissue, such as the liver.

Collectively, our data further support the fact that BMI and fat mass are a determinant of systemic SAA levels. However, the post-WL systemic SAA reduction appears not to be the direct consequence of the reduction in BMI or fat mass alone and may be attributable to other chronic conditions like stress, hypertension, and osteoarthritis (30-32). Such a large variation might have obscured or overwhelmed the contribution of fat mass changes to systemic SAA levels during WL. Reasons for the greater individual pre- vs. post-WL variation in \textit{ex vivo} SAA secretion from adipose tissue are unclear. Possible explanations include that SAA is a highly and rapidly regulated protein; hence, the large SAA variation in the acute explant study may reflect the actual state of rapid dynamics of SAA production and secretion as well as newly synthesized SAA at the time of fat biopsy. Further, the finding that in the same individual, the gluteal and abdominal SAA secretion correlate well pre- and post-WL (Figure 3), suggests similar regulatory mechanisms may exist within the two fat regions in response to the loss of body weight.
In summary, these findings show that BMI and fat mass are determinants of the circulating SAA levels. The overall extent of serum SAA decrease is comparable to that of WL with and without exercise in the cohort, but only collectively do the changes in SAA directly relate to the reduction of fat mass. We also find that the gluteal adipose tissue secretes ~50% more SAA than the abdominal adipose tissue, and that adipose SAA secretion tends to decrease after WL; however, only the reduction of abdominal SAA secretion correlated with that of systemic SAA. Thus, both reduced fat mass and decreased abdominal adipose SAA secretion, along with other yet unidentified factors, contribute to the reduction in systemic SAA levels after WL.

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