Acylation-Stimulating Protein Precursor Proteins in Adipose Tissue in Human Obesity

Zhunan Xia and Katherine Cianflone

Recent reports have suggested a link between acylation-stimulating protein (ASP) and complement C3 with obesity, insulin resistance, coronary artery disease, and hyperlipidemia. Our aim was to examine the mRNA expression of C3 and other factors related to ASP production (such as factor B and adipsin) in adipose tissue. The influence of gender and obesity was examined in subcutaneous (SC) and omental (OM) tissues from 16 males and 16 females with body mass index (BMI) from 20 to 54 kg/m^2. The results demonstrate that factor B mRNA expression is higher in males than females in both SC and OM tissues. In female SC tissue, C3 and adipsin mRNA decrease with increasing BMI ($r = 0.557$, $P = .025$ and $r = 0.717$ $P = .002$, respectively), with no change in factor B. By contrast, in males there was a pronounced increase in C3, adipsin, and factor B in OM tissue with increasing BMI ($r = 0.759$ $P = .001$, $r = 0.650$ $P = .006$, and $r = 0.568$ $P = .022$, respectively). Of note, however, in both men and women there was a marked increase in the OM/SC ratio of C3 and adipsin with increasing BMI. These results suggest that in female SC adipose tissue, there is downregulation of factors related to ASP production in obesity, perhaps to limit further expansion of adipose tissue. In males, there is increased expression in OM tissue. In addition, relative OM/SC expression increases with obesity and these changes may contribute to the development of visceral adipose tissue.

IN RECENT YEARS there has been increasing interest in complement C3 in relation to obesity, insulin resistance, coronary heart disease and dyslipidemias. A number of recent studies in humans have demonstrated that the concentration of plasma C3 correlates to body mass index (BMI)[1-9] and plasma C3 levels are increased in obesity.[7,9,10] With weight reduction or in subjects with anorexia nervosa, plasma C3 is decreased.[7,10-12] While 2 studies suggest that females have higher plasma C3 than males, when adjusted for percent body fat there is no difference.[1,3] On the other hand, other studies suggest the converse: that plasma C3 levels in males are higher than females.[2,13]. Hypocomplementemia is a feature in many cases of acquired partial lipodystrophy.[14-17]

Beyond the association of C3 with body weight, there are also a number of studies that demonstrate that C3 is increased in type II diabetes,[18,19] coronary artery disease patients[3,8,20] hypertension,[21,22] and in dyslipidemic subjects.[2,23] In these studies, C3 was shown to correlate positively with insulin[2,4,6,8,9] and glucose,[2,4,6,8,9,24] and changed with pharmacologic treatment of diabetics with thiazolidinedione or sulfonylurea treatment.[1,5] C3 was also strongly associated with lipid parameters such as plasma apolipoprotein B (apoB),[2,4,8] total cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride,[2-4,8,9,13,22,23] and nonesterified fatty acids,[2,4] and negatively with high-density lipoprotein (HDL) cholesterol,[2,4,9] even when adjusted for BMI.[2] This relationship of C3 with plasma lipids also appears to influence the effectiveness of hyperlipidemic treatment.[25] Muscari et al demonstrated that C3 was predictive of myocardial infarction and was more significant than any other association of traditional risk factors.[3,9] In this study, of the primary variables associated with C3, insulin was the main covariate.

While it has traditionally been assumed that the major source of plasma C3 is the liver,[26] we and others have demonstrated that with differentiation, cultured human and murine adipocytes express mRNA for C3 and secrete C3.[27-29] When quantitated by competitive reverse-transcriptase polymerase chain reaction (RT-PCR), the expression level was shown to be intermediate, lower than lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL), but comparable to insulin receptor, insulin receptor substrate-1 (IRS-1), and uncoupling protein-2 (UCP-2).[30] Quantitatively, however, the values range widely from 15 to 450 amol/μg total RNA.[31-33] However, in spite of the strong associations of plasma C3 with disease as described above, there are little data available on C3 mRNA levels in adipose tissue, and no study has examined the influence of gender, obesity, or adipose tissue region.

C3 is cleaved to generate acylation-stimulating protein (ASP, or C3adesArg), a potent stimulator of triglyceride synthesis in adipocytes. As with C3, ASP is also increased in obesity,[33-36] diabetes,[33] and hyperlipidemia,[4,37] and in subjects with coronary heart disease.[37] With weight loss there is a decrease in plasma ASP.[34] Plasma levels of ASP correlate with BMI, insulin, and lipids (apoB, triglyceride, and nonesterified fatty acids).[4,33-39] While ASP is generated from C3, plasma ASP levels are many-fold lower than C3. There are only a few studies (n = 6) that have measured both ASP and C3.[4-6,33,40,41] The correlation between the 2 factors ranges from not significant[5,41] to highly correlated,[40] suggesting that other factors, such as the proteins associated with conversion of C3 to ASP (including factor B and adipsin), may influence ASP levels.

In vitro, ASP is generated through the interaction of precursor C3, cofactor B, and the serine protease enzyme, adipsin. Adipocytes express mRNA for all 3 factors in a differentiation-dependent manner and secrete all 3 factors.[27-29] Production of

From the Mike Rosenbloom Laboratory for Cardiovascular Research, McGill University Health Centre, Montreal, Quebec, Canada.

Submitted January 15, 2003; accepted April 28, 2003.

Supported by a grant from the Heart and Stroke Foundation of Canada to K.C. K.C. is a research scholar of the Fonds de Recherche en Sante de Quebec (FRSQ). Z.X. was supported by an internal scholarship (Division of Experimental Medicine, McGill University).

Address reprint requests to Katherine Cianflone, PhD, Mike Rosenbloom Laboratory for Cardiovascular Research, Room H7.30, McGill University Health Centre, Royal Victoria Hospital, 687 Pine Ave W, Montreal, Quebec, H3A 1A1, Canada.

0026-0495/03/5210-0001$30.00/0

doi:10.1016/S0026-0495(03)00254-3

ASP by cultured adipocytes[27-29] has been demonstrated. As with C3, plasma concentration of both factor B and adipsin has been shown to be increased in obesity and diabetes[7,36,42-45] and decreased with weight loss or in anorexia nervosa.[7,42,45] Plasma adipsin correlates with BMI,[36,43-45] insulin,[43-45] triglyceride,[44] and apoB.[36] However, there is no information available on either factor B or adipsin in subjects with coronary heart disease or hyperlipidemia. Surprisingly, although ex vivo adipose tissue production of adipsin was described a decade ago,[45] only one study has been published on adipsin mRNA expression in human adipose tissue in lean subjects only,[46] and there are no data on factor B mRNA variation in adipose tissue. In that study, Montague et al demonstrated that the copy number of adipsin in human adipose tissue was extremely low, lower than leptin and peroxisome proliferator-activated receptor gamma (PPARγ), contrasting with the data in mice that demonstrate that adipsin is abundant.[47]

Therefore the aim of the present study was to measure mRNA of C3, factor B, and adipsin in human adipose tissue, and to determine the effect of gender, obesity, and adipose tissue site on expression of C3, factor B, and adipsin.

MATERIALS AND METHODS

Subjects

Subjects were patients undergoing elective abdominal surgery at the Royal Victoria Hospital for one of the following procedures: hysterectomy, cholesystectomy, hernia repair, or gastroplasty. None of the subjects had diabetes, cancer, or other wasting disease, and the women were all premenopausal. Samples of subcutaneous (SC) and omental (OM) adipose tissue were excised under general anesthesia. The protocol was approved by the Ethics Committee (Royal Victoria Hospital, McGill University).

Isolation of Tissue and RNA Preparation

Adipose tissue samples were immediately frozen in liquid nitrogen and kept at −80°C until used. Tissue pieces (400 μg) were homogenized with Trizol reagent (procedure provided by the supplier) and the upper fat cake was removed. Chloroform was added at 0.2 mL/mL Trizol reagent (Invitrogen, Burlington, Canada) and centrifuged at 4°C, 10,000 × *g* for 15 minutes. Isopropanol (0.5 mL/mL Trizol reagent) was added to the supernatant with 0.5 μg glycogen added as carrier to precipitate RNA, followed by centrifugation at 4°C, 10,000 × *g* for 15 minutes. The pellet was washed with 75% ethanol (1 mL/mL Trizol reagent) and centrifuged at 4°C, 6,000 × *g* for 10 minutes. The pellet was air-dried, redissolved in diethyl pyro carbonate (DEPC)-treated water (100 μL), and heated at 55°C for 10 minutes. The RNA concentration was determined by OD 260/280 ratio and the sample stored at −80°C.

Semiquantitative RT-PCR Analysis

The methodology is described in detail elsewhere.[28,48] Briefly, 3 μg RNA was lyophilized, redissolved in 10 μL denaturing solution (10 U RNasin, 300 pmol Pd(N)6), and incubated at 65°C for 5 minutes. Reverse transcription reaction solution was added (4 μL of 5X buffer, 10 U Rnasin, 100 U murine Moloney leukemia virus (MMLV), 0.01 mmol/L dithiothreitol [DTT], 0.5 mmol/L of each dNTP), incubated at 37°C for 2 hours, and the reaction stopped by heating at 95°C for 5 minutes. The cDNA was diluted up to 100 μL in DEPC water and the samples stored at −80°C. For PCR analysis, 4 μL cDNA was added to 16 μL PCR reaction solution (Taq polymerase 0.5 U, 1X buffer, 2.0 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 0.01 mmol/L tetra methylammonium chloride (TMAC), and 1 μmol/L of each primer). The primers for factor B, adipsin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are as described previously.[28,48] For C3, the primers were *Sn: TGC AAG AAG GTC TTC CTG G, Asn: GTA ATT GTA GAG AAC GGC TCG G*. For C3, factor B, and adipsin, PCR was performed for 35 cycles of 1 minute at 94°C, 1 minute at 60°C, and 1 minute at 72°C. For GAPDH, 30 cycles were performed. A 7-minute extension at 72°C ended the reaction, which was then quenched at 4°C. For all mRNA assayed, the cycle numbers chosen were demonstrated to be in the linear range for amplification, and the signal produced was directly related to the amount of cDNA used for the reaction.[28,48]

Following PCR, a 4-μL sample was added to 6X sample loading buffer, and separated on 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with piperazine diacrylamide (PDA) gel[28,48] with New England BioLab (Mississauga, Canada) quantifiable 100-bp Ladder (N3231s), and silver-stained (BioRad Silver Stain kit, Mississauga, Canada). The dried gel was scanned with BioRad Imaging Densitometer (GS-670), and the relative amount of PCR product measured using the generated standard curve (BioRad Molecular Analyst software) where the standard curve was linear from 9 ng to 194 ng DNA and all samples fell within that range. Factors are expressed as a ratio to GAPDH. Overall, there were no changes in GAPDH mRNA expression.

Table 1. mRNA Expression of C3, Factor B, and Adipsin in Women and Men

	Female		Male		
	SC	OM	SC	OM	*P* (ANOVA)
C3	6.6 ± 1.4	7.6 ± 0.9	5.2 ± 0.4	5.5 ± 0.7*	NS
Adipsin	4.6 ± 0.7	5.1 ± 0.9	6.1 ± 1.0	5.8 ± 0.7	NS
Factor B	3.4 ± 0.3	7.1 ± 0.9	7.9 ± 1.5*	9.0 ± 1.1*	$P < .002$

NOTE. Results are presented as mean mRNA ± SEM where n = 16 for each group.

Abbreviation: NS, not significant.

*$P < .05$ for males *v* females.

Statistical Analysis

All results are expressed as the mean ± SEM. Groups were compared by 2-mean *t* test or by paired *t* test for SC to OM comparisons. Pearson correlation was used to determine relationships between 2 factors.

RESULTS

Basic Subject Characterization

Adipose tissue samples were obtained from 16 female and 16 male subjects at the time of elective abdominal surgery. BMI ranged from 20 to 54, with a mean of 35.9 ± 2.7 in females and 33.6 ± 2.7 in males. Mean ages were 42.3 ± 1.5 in females and 38.9 ± 1.5 in males. There was no difference in average BMI or age between females and males.

mRNA Expression in SC and OM Adipose Tissue in Females and Males

C3, adipsin, and factor B were measured for all subjects in both SC and OM adipose tissue (Table 1). There was no overall difference among the 4 groups (female SC, female OM, male SC, and male OM) for either C3 or adipsin. However, there were marked differences in factor B ($P < .002$ by analysis of

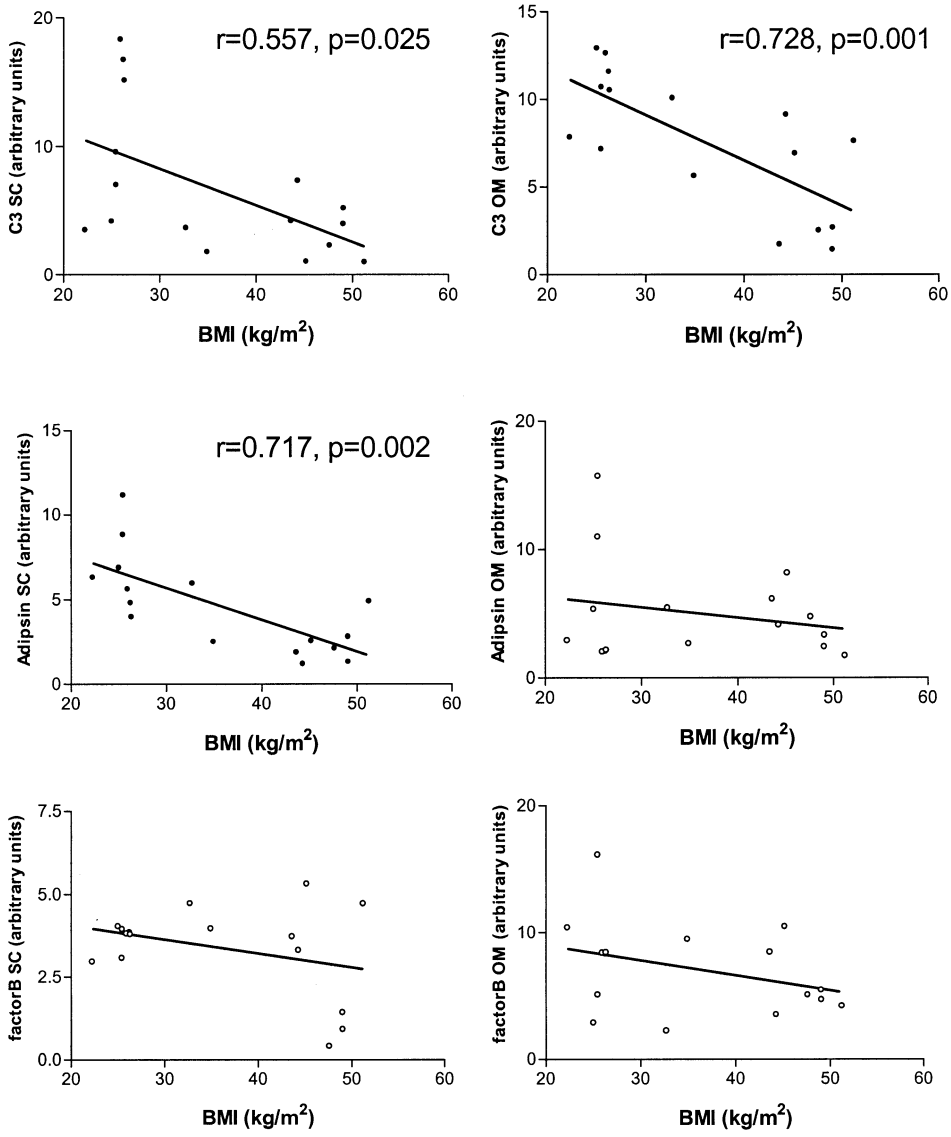

Fig 1. Correlation of C3, adipsin, and factor B *v* BMI in SC and OM adipose tissue in women calculated by linear regression for 16 women; data provided for significant correlations only.

variance [ANOVA]), such that the values in males overall were increased relative to the females in both adipose tissues.

We then examined the effect of BMI on C3, factor B, and adipsin expression in males and females separately. As shown in Fig 1, in females, there was a significant decrease in C3 mRNA with increasing BMI in both SC and OM adipose tissue. There was also a significant decrease in adipsin expression in SC adipose tissue with increased BMI. On the other hand, there was no significant change in adipsin in OM tissue, or in factor B in either tissue with increasing obesity, although the trend was to decrease in all cases.

The changes in males are shown in Fig 2. In contrast to the females, the major changes in males were seen in the OM adipose tissue where the expression levels of all 3 factors (C3, adipsin, and factor B) increased significantly with increasing BMI. There was also a significant increase in factor B mRNA in SC adipose tissue. It was very striking that in females, all of the significant correlations with BMI were negative (downregulation with increasing BMI), while the opposite was true in males (upregulation with increasing BMI), with the exception of adipsin SC tissue.

Comparison of OM to SC Expression

The expression levels of OM to SC adipose tissue were compared using a ratio of OM/SC as suggested by Duserre et al.[32] A ratio of 1.0 indicates similar relative levels in both tissues (OM and SC) as shown in Table 2. While the OM/SC ratio was greater than 1 for all factors, it was significantly increased only in some cases. In females the OM/SC ratio was increased for factor B by almost 3-fold ($P < .001$). Overall in males the OM/SC ratios were not significantly different from females.

We also compared the OM/SC ratio over the range of BMI to determine if obesity induced different or similar changes within the 2 tissues using linear regression analysis. There are

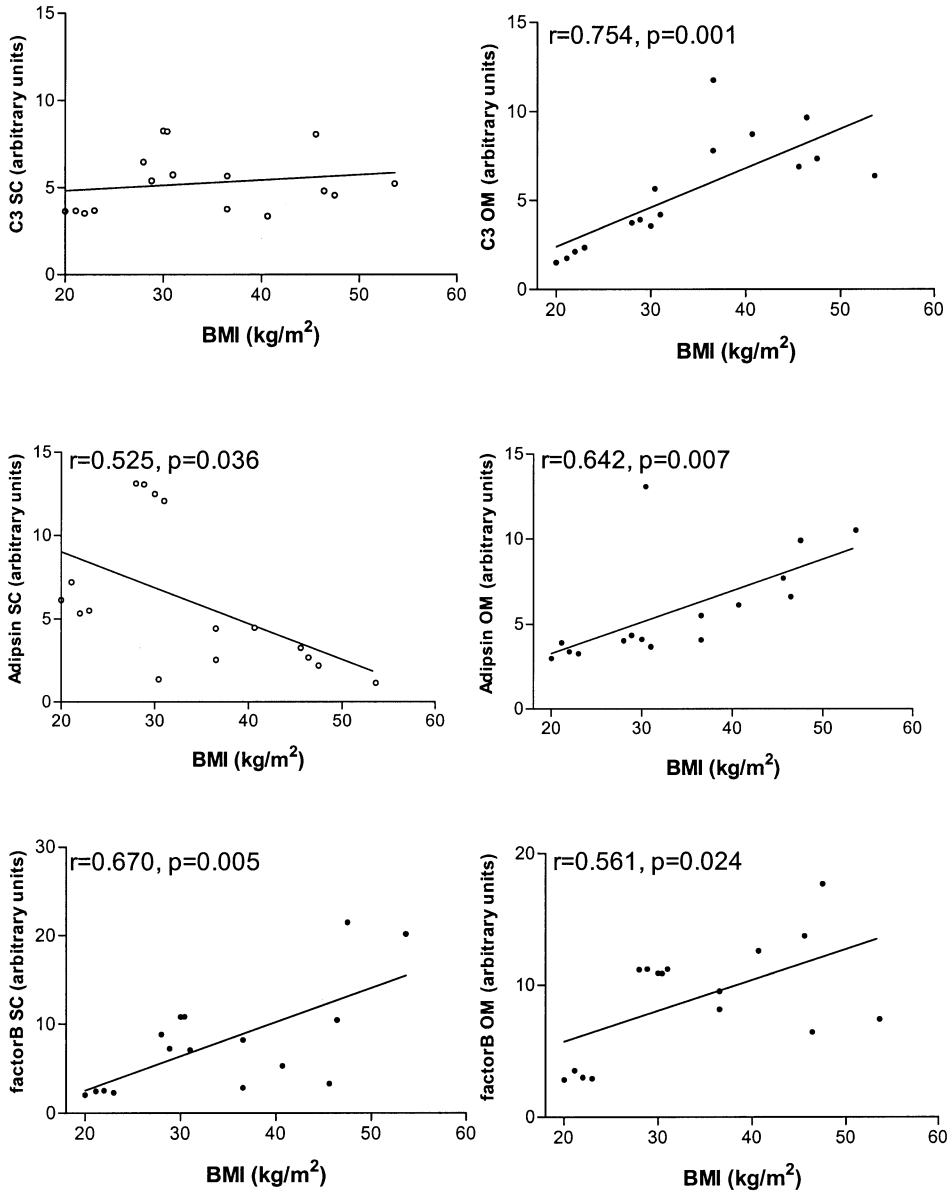

Fig 2. Correlation of C3, adipsin, and factor B *v* BMI in SC and OM adipose tissue in men calculated by linear regression for 16 men; data provided for significant correlations only.

Table 2. Ratio of indicated Parameters and Linear Regression Analysis Versus BMI

	Female	*v* BMI	Male	*v* BMI
C3 OM/SC	2.06 ± 0.51	NS	1.13 ± 0.20	$r = 0.569$ $P = .021$
Adipsin OM/SC	1.43 ± 0.26	$r = 0.557$ $P = .025$	2.26 ± 0.73	$r = 0.546$ $P = .028$
Factor B OM/SC	2.97 ± 0.69*	NS	1.52 ± 0.24	NS

NOTE. Results are presented as OM/SC ratio for the normalized expression levels expressed as mean ± SEM where n = 16 for each group, Linear regression of individual factors vs BMI was calculated for males and females separately.

*$P < .001$ by paired *t* test for OM *v* SC.

tissue site differences, because the OM/SC ratio does not remain constant with increasing BMI (Table 2). In females, adipsin mRNA decreases in SC (Fig 1) but not OM tissue, resulting in a significant increase in OM/SC with increasing BMI (linear regression: $r = 0.557$, $P = .025$). In males, C3 and adipsin mRNA both increase in OM tissue with increasing BMI (Fig 2), with no increase in SC expression, resulting in a significant increase in OM/SC ratio (linear regression: $r = 0.569$, $P = .021$ for C3 and $r = 0.546$, $P = .028$ for adipsin). In all cases where there is an obesity-related change, in both males and females, the OM/SC ratio increases with increasing BMI.

DISCUSSION

In the present study, we examined the expression of factors involved in ASP generation: C3, factor B, and adipsin. There is

relatively little data available on expression of C3, factor B, and adipsin in adipose tissue and the present study is the first to examine the effects of gender, size, and tissue site. Our results demonstrate marked changes associated with increased obesity that were very different in men and women. Specifically, with increased BMI, in women there was a decreased expression of C3 and adipsin, while in men there was increased expression of C3, factor B, and adipsin. In women, SC tissue was the primary target, while in men OM tissue was more often affected. These results also demonstrate a greater relative expression in OM than SC tissue with development of obesity (increased OM/SC ratio) in both men and women.

That there are gender differences should come as no surprise since there are well-recognized differences in body fat distribution between men and women, where in obesity women characteristically deposit fat in SC sites (gynoid obesity), and men tend to increase abdominal and especially OM fat depots more easily (android obesity).[49-51] While gynoid obesity is often associated with hyperinsulinemia, the metabolic changes in android obesity are more deleterious, and are associated with hyperinsulinemia and lipoprotein disorders (increased apoB, hypertriglyceridemia, small dense LDL, and decreased HDL), as well as increased risk of cardiovascular disease and type II diabetes.[52,53] There are numerous molecular and biochemical studies that demonstrate important tissue site and gender differences in function at a cellular level. The general consensus is that adipose tissue from females is more effective at fat storage than males, and that SC tissue is more efficient than OM tissue. On the other hand, OM tissue (especially from males) would appear to be more lipolytically active when stimulated.[49-51] Because of these clear gender differences not only in our data, but in adipose tissue metabolism as a whole, the results in women and men will be discussed separately.

First, in women, C3 expression (in both SC and OM) as well as adipsin mRNA levels are decreased with increasing BMI. At first glance, this might seem to contrast with the increased levels of plasma C3 and adipsin, which have been reported in obesity. However, considering the massive increases in adipose tissue mass in obesity (both increased number and size of fat cells), this may more than compensate for the downregulation on a per cell basis.

Plasma adipsin, C3, and factor B increase in obesity (about 30% for C3,[7,10,54] 45% for factor B,[7] and an average of 37% for adipsin[7,36,43-45]). These changes are modest compared to the average increase in ASP of 200% in obesity[6,35,36]; however, small changes in substrate (C3) and enzyme (adipsin) may produce much larger changes in product (ASP). Although we have yet to understand what regulates the enzymatic conversion process, we do have direct in vivo evidence that the basal production of ASP by SC adipose tissue is increased in obesity.[55,56] Following production, ASP interacts specifically and saturably with an adipocyte cell surface receptor that has been recently identified[57] to stimulate glucose transport and fatty acid esterification for formation of triglyceride, as well as inhibit triglyceride lipolysis.[58-60] Initial studies indicate that ASP stimulates triglyceride synthesis to a greater extent in SC than OM adipose tissue,[61] and this is consistent with binding studies that demonstrate a higher affinity binding of ASP to SC compared to OM plasma membranes.[59] Regulation of ASP production is that much more important because neither the number of high-affinity binding sites[59] nor the response to ASP in obese adipocytes from females[62] appears to be downregulated in obesity.

While we can only hypothesize, the decreased expression of C3 and adipsin with obesity may help to restrict further expansion of an already enlarged adipose tissue by reducing (on a per cell basis) the potential for generation of ASP. This may also prevent "overstimulation" of the adipose tissue since there does not appear to be any desensitization to ASP in adipose tissue from obese women.[62]

In men, the major changes demonstrated were in OM tissue, with marked increases in obesity. Expression of C3, factor B, and adipsin all increased with increasing BMI. This is consistent with the demonstrated increases in plasma C3, factor B, and adipsin that have been noted in obesity (as reviewed above), although it is difficult to say whether this increase in OM tissue would be of sufficient magnitude to result in the increases of 30% to 45% reported. Increases in all 3 parameters would explain increased plasma levels of ASP (as has been demonstrated in obese men).[36] The circulating levels, however, may underestimate the concentrations in the adipose tissue bed of interest. Local tissue concentrations of these factors (as autocrine/paracrine effectors) and local generation of ASP are likely more important than general circulating levels. We have demonstrated that the in situ increase in ASP in the adipose tissue bed is greater than the circulating plasma ASP level, especially postprandially.[55,56] Thus, in the obese men, the local adipose tissue ASP concentration may be much higher than that seen in circulation.

This is particularly relevant as adipose tissue binding studies have indicated that the affinity of the ASP receptor is lower in OM versus SC adipose tissue, markedly so in males.[59] In fact, subjects with coronary artery disease that are characterized by increased plasma apoB (hyperapoB) have increased plasma ASP,[37] delayed triglyceride clearance,[63,64] and a reduced binding and cellular response to ASP.[65] A local increase in ASP concentration could overcome the limited cellular response due to decreased receptor affinity, particularly since physiological concentrations are within the range of receptor affinity (nanomolar range).

Imbeault et al have demonstrated that SC C3 expression was greater in middle-aged versus younger men of comparable BMI.[31] This difference was present even when corrected for differences in percent body fat between the 2 groups. They also noted an increase in mRNA of HSL, which contrasted with decreased activity of HSL. They speculated that upregulation of C3 expression may indirectly explain the impaired adipose tissue lipolytic capacity, since increased C3 may lead to increased ASP and ASP has been shown to inhibit basal and norepinephrine-stimulated fatty acid release from adipocytes through effects on re-esterification and lipolysis.[60]

Koistinen et al also demonstrated an increase in C3 mRNA in adipose tissue from obese men compared to lean men.[33] While these obese men only demonstrated a slight but not significant increase in plasma C3, they did have a substantial increase in plasma ASP. Interestingly, the level of C3 mRNA correlated inversely with glucose disposal rate but positively with BMI and postprandial triglyceride clearance.

Finally, an interesting phenomenon in the present study was that the regulation of C3, factor B, and adipsin in SC and OM tissues were distinctly different, such that changes in one tissue were not necessarily seen in the corresponding tissue from the other site. In all cases, the OM/SC ratio of all factors was greater than 1.0 (indicating greater relative expression in OM tissue). In addition, the ratio of OM/SC tended to increase with increasing BMI. Thus, in women, the factors were downregulated to a lesser extent in OM tissue (therefore OM/SC ratio increased). In men, there was a preferential upregulation in OM versus SC tissue (again OM/SC increased). Similarly, Duserre et al demonstrated that "ASP" (in fact C3) expression in OM was higher than SC at all BMI studied.[32] By contrast Montague et al found expression of adipsin to be comparable in men versus women, with no difference in OM and SC.[46] This may be because only lean subjects were examined, and the largest differences in the present study were the effects of obesity, with adipsin changing according to BMI.

In conclusion, the changes in C3, factor B, and adipsin in females are consistent with a downregulation to limit an ASP responsive tissue, while the changes in men in factor B, C3, and adipsin are consistent with an upregulation in order to compensate for decreased response or a proposed "ASP resistance" in OM tissue.

ACKNOWLEDGMENT

We appreciate the assistance of Dr Jumana Saleh.

REFERENCES

1. Ebeling P, Teppo AM, Koistinen HA, et al: Troglitazone reduces hyperglycaemia and selectively acute-phase serum proteins in patients with type II Diabetes. Diabetologia 42:1433-1438, 1999

2. Ylitalo K, Porkka KV, Meri S, et al: Serum complement and familial combined hyperlipidemia. Atherosclerosis 129:271-277, 1997

3. Muscari A, Bozzoli C, Puddu GM, et al: Association of serum C3 levels with the risk of myocardial infarction. Am J Med 98:357-364, 1995

4. Ylitalo K, Pajukanta P, Meri S, et al: Serum C3 but not plasma acylation-stimulating protein is elevated in Finnish patients with familial combined hyperlipidemia. Asterioscler Thromb Vasc Biol 21:838-843, 2001

5. Ebeling P, Teppo AM, Koistinen HA, et al: Concentration of the complement activation product, acylation-stimulating protein, is related to C-reactive protein in patients with type 2 diabetes. Metabolism 50:283-287, 2001

6. Weyer C, Tataranni PA, Pratley RE: Insulin action and insulinemia are closely related to the fasting complement C3, but not acylation stimulating protein concentration. Diabetes Care 23:779-785, 2000

7. Pomeroy C, Mitchell J, Eckert E, et al: Effect of body weight and calorie restriction on serum complement proteins, including factor D/adipsin: Studies in anorexia nervosa and obesity. Clin Exp Immunol 108:507-515, 1997

8. Halkes CJM, Kijk HV, de Jaegere PPT, et al: Postprandial increase of complement component 3 in normolipidemic patients with coronary artery disease: Effects of expanded-dose simvastatin. Arterioscler Thromb Vasc Biol 21:1526-1530, 2001

9. Muscari A, Massarelli G, Bastagli L, et al: Relationship of serum C3 to fasting insulin risk factors and previous ischemic events in middle-aged men. Eur Heart J 21:1081-1090, 2000

10. Scriba PC, Bauer M, Emmert D, et al: Effects of obesity, total fasting and re-alimentation on L-thyroxine (T4), 3,5,3′-L-triiodothyronine (T3), 3,3′,5′-L-triiodothyronine (rT3), thyroxine binding globulin (TBG), cortisol, thyrotrophin, cortisol binding globulin (CBG), transferrin, alpha 2-haptoglobin and complement C′3 in serum. Acta Endocrinol (Copenh) 91:629-643, 1979

11. Pasquali R, Casimirri F, Melchionda N: Protein metabolism in obese patients during very low-calorie mixed diets containing different amounts of proteins and carbohydrates. Metabolism 36:1141-1148, 1987

12. Fisler JS, Drenick EJ, Blumfield DE, et al: Nitrogen economy during very low calorie reducing diets: quality and quantity of dietary protein. Am J Clin Nutr 35:471-486, 1982

13. Muscari A, Bozzoli C, Puddu GM, et al: Correlations between serum lipids and complement components in adults without demonstrated atherosclerotic disease. Atherosclerosis 81:111-118, 1990

14. Ipp MM, Minta JO, Gelfand EW: Disorders of the complement system in lipodystrophy. Clin Immunol Immunopathol 7:281-287, 1977

15. Sissons PJG, West RJ, Fallows J, et al: The complement abnormalities of lipodystrophy. N Engl J Med 294:461-465, 1976

16. Tanuma Y, Ohi H, Hatano M: Two types of C3 nephritic factor: Properdin-dependent C3NeF and properdin-independent C3NeF. Clin Immunol Immunopathol 56:226-238, 1990

17. Skattum L, Martensson U, Sjoholm AG: Hypocomplementaemia caused by C3 nephritic factors (C3 NeF): Clinincal findings and the coincidence of C3 NeF type II with anti-C1q autoantibodies. J Intern Med 242:455-464, 1997

18. Morimoto Y, Taniguchi H, Yamashiro Y, et al: Complements in diabetes mellitus: Activation of complement system evidenced by C3d elevation in IDDM. Diabetes Res Clin Pract 5:309-312, 1988

19. McMurray DN, Beskitt PA, Newmark SR: Immunologic status in severe obesity. Int J Obesity 6:61-68, 1982

20. Ledue TB, Neveux LM, Palomaki GE, et al: The relationship between serum levels of lipoprotein(a) and proteins associated with the acute phase response. Clin Chim Acta 223:73-82, 1993

21. Mantov S, Raev D: Addictive effect of diabetes and systemic hypertension on the immune mechanisms of atherosclerosis. Int J Cardiol 56:145-148, 1996

22. Bozzoli C, Muscari A, Puddu GM, et al: Association of serum C3 and essential hypertension. G Ital Cardiol 22:1361-1366, 1992

23. Uza G, Cristea A, Cucuianu MP: Increased level of the complement C3 protein in endogenous hypertriglyceridemia. J Clin Lab Immunol 8:101-105, 1982

24. McMillan DE: Elevation of complement components in diabetes mellitus. Diabetes Metab 6:265-270, 1980

25. Nemeth A, Szakmary K, Kramer J, et al: Apolipoprotein E and complement C3 polymorphism and their role in the response to gemfibrozil and low fat low cholesterol therapy. Eur J Clin Chem Clin Biochem 33:799-804, 1995

26. Alper CA, Johnson AM, Birtch AG, et al: Human C3: Evidence for the liver as the primary site of synthesis. Science 163:286-288, 1969

27. Peake PW, O'Grady S, Pussell BA, et al: Detection and quantification of the control proteins of the alternative pathway of complement in 3T3-L1 adipocytes. Eur J Clin Invest 27:922-927, 1997

28. Cianflone K, Maslowska M: Differentiation induced production of ASP in human adipocytes. Eur J Clin Invest 25:817-825, 1995

29. Choy LN, Spiegelman BM: Regulation of alternative pathway activation and C3a production by adipose cells. Obes Res 4:521-532, 1996

30. Rieusset J, Auwerx J, Vidal H: Regulation of gene expression by activation of the peroxisome proliferator-activated receptor gamma with rosiglitazone (BRL 49653) in human adipocytes. Biochem Biophys Res Commun 265:265-271, 1999

31. Imbeault P, Vidal H, Tremblay A, et al: Age-related differences in messenger ribonucleic acid expression of key proteins involved in adipose cell differentiation and metabolism. J Clin Endocrinol Metab 86:828-833, 2001

32. Dusserre E, Moulin P, Vidal H: Differences in mRNA expression of the proteins secreted by the adipocytes in human subcutaneous and visceral adipose tissues. Biochim Biophys Acta 1500:88-96, 2000

33. Koistinen HA, Vidal H, Karonen SL, et al: Plasma acylation stimulating protein concentration and subcutaneous adipose tissue C3 mRNA expression in nondiabetic and type 2 diabetic men. Arterioscler Thromb Vasc Biol 21:1034-1039, 2001

34. Cianflone K, Sniderman AD, Kalant D, et al: Response of plasma ASP to a prolonged fast. Int J Obesity 19:604-609, 1995

35. Weyer C, Pratley RE: Fasting and postprandial plasma concentrations of acylation-stimulation protein (ASP) in lean and obese Pima Indians compared to Caucasians. Obes Res 7:444-452, 1999

36. Maslowska M, Vu H, Phelis S, et al: Plasma acylation stimulating protein, adipsin and lipids in non-obese and obese populations. Eur J Clin Invest 29:679-686, 1999

37. Cianflone K, Zhang XJ, Genest J Jr, et al: Plasma acylation stimulating protein in coronary artery disease. Arterioscler Thromb Vasc Biol 17:1239-1244, 1997

38. Matthan NR, Cianflone K, Lichtenstein AH, et al: Hydrogenated fat consumption affects acylation-stimulating protein levels and cholesterol esterification rates in moderately hypercholesterolemic women. J Lipid Res 42:1841-1848, 2001

39. Ozata M, Gungor D, Turan M, et al: Improved glycemic control increases fasting plasma acylation-stimulating protein and decreases leptin concentrations in type II diabetic subjects. J Clin Endocrinol Metab 86:3659-3664, 2001

40. Ozata M, Ortenli C, Culec M, et al: Increased fasting plasma acylation-stimulating protein concentrations in nephrotic syndrome. J Clin Endocrinol Metab 87:853-858, 2002

41. Charlesworth JA, Peake PW, Campbell LV, et al: The influence of oral lipid loads on acylation stimulating protein (ASP) in healthy volunteers. Int J Obes Relat Metab Disord 22:1096-1102, 1998

42. Titov LP, Korvigo GV, Kharitonik GD, et al: Levels of various components of the classical and alternative pathways of complement activation in diabetes mellitus patients. Probl Endokrinol (Mosk) 33: 9-12, 1987

43. Alessi MC, Parrot G, Guenoun E, et al: Relation between plasma PAI activity and adipsin levels. Thromb Haemost 74:1200-1202, 1995(letter)

44. Mavri A, Stegnar M, Krebs M, et al: Impact of adipose tissue on plasma plasminogen activator inhibitor-1 in dieting obese women. Arterioscler Thromb Vasc Biol 19:1582-1587, 1999

45. Napolitano A, Lowell BB, Damm D, et al: Concentrations of adipsin in blood and rates of adipsin secretion by adipose tissue in humans with normal, elevated and diminished adipose tissue mass. Int J Obes Relat Metab Disord 18:213-218, 1994

46. Montague CT, Prins JB, Sanders L, et al: Depot-related gene expression in human subcutaneous and omental adipocytes. Diabetes 47:1384-1391, 1998

47. Choy LN, Rosen BS, Spiegelman BM: Adipsin and an endogenous pathway of complement from adipose cells. J Biol Chem 267: 12736-12741, 1992

48. Cianflone K, Roncari DAK, Maslowska M, et al: The adipsin/acylation stimulating protein system in human adipocytes: Regulation of triacylglycerol synthesis. Biochemistry 33:9489-9495, 1994

49. Arner P: Differences in lipolysis between human subcutaneous and omental adipose tissues. Ann Med 27:435-438, 1995

50. Bouchard C, Despres JP, Mauriege P: Genetic and nongenetic determinants of regional fat distribution. Endocr Rev 14:72-93, 1993

51. Bjorntorp P: The regulation of adipose tissue distribution in humans. Int J Obesity 20:291-302, 1996

52. Bjorntorp P: Metabolic implications of body fat distribution. Diabetes Care 14:1132-1143, 1991

53. Lapidus L, Bengtsson C, Larsson B, et al: Distribution of adipose tissue and risk of cardiovascular disease and death: A 12 year follow up of participants in the population study of women in Gothenburg, Sweden. Br Med J 289:1257-1261, 1984

54. Boggs RD, McCumbee WD, Cobbs SL, et al: Increased expression of complement component C3 in the plasma of obese Zucker fa and LA/N fa(f) rats compared with their lean counterparts. Obes Res 6:361-367, 1998

55. Saleh J, Summers LKM, Cianflone K, et al: Coordinated release of acylation stimulating protein (ASP) and triacylglycerol clearance by human adipose tissue in vivo in the postprandial period. J Lipid Res 39:884-891, 1998

56. Kalant D, Phelis S, Fielding BA, et al: Increased postprandial fatty acid trapping in subcutaneous adipose tissue in obese women. J Lipid Res 41:1963-1968, 2000

57. Kalant D, Cain SA, Maslowska M, et al: The chemoattractant receptor-like protein C5L2 binds the C3a des-Arg77/acylation-stimulating protein. J Biol Chem 278:11123-11129, 2003

58. Cianflone K, Maslowska M, Sniderman AD: Acylation stimulating protein (ASP), an adipocyte autocrine: New directions. Semin Cell Dev Biol 10:31-41, 1999

59. Saleh J, Christou N, Cianflone K: Regional specificity of ASP binding in human adipose tissue. Am J Physiol 276:E815-E821, 1999

60. Van Harmelen V, Reynisdottir S, Cianflone K, et al: Mechanisms involved in the regulation of free fatty acid release from isolated human fat cells by acylation-stimulating protein and insulin. J Biol Chem 274:18243-18251, 1999

61. Maslowska M, Sniderman AD, MacLean LD, et al: Regional differences in triacylglycerol synthesis in adipose tissue. J Lipid Res 34:219-228, 1993

62. Walsh MJ, Sniderman AD, Cianflone K, et al: The effect of ASP on the adipocyte of the morbidly obese. J Surg Res 46:470-473, 1989

63. Castro Cabezas M, Erkelens DW, Kock LA, et al: Postprandial apolipoprotein B100 and B48 metabolism in familial combined hyperlipidaemia before and after reduction of fasting plasma triglycerides. Eur J Clin Invest 24:669-678, 1994

64. Genest J, Sniderman AD, Cianflone K, et al: Hyperapobetalipoproteinemia: Plasma lipoprotein responses to oral fat load. Arteriosclerosis 6:297-304, 1986

65. Zhang XJ, Cianflone K, Genest 444J, et al: Plasma acylation stimulating protein (ASP) as a predictor of impaired cellular biological response to ASP in patients with hyperapoB. Eur J Clin Invest 28:730-739, 1998