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Review

Critical review of acylation-stimulating protein physiology in humans and rodents

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Abstract

In the last few years, there has been increasing interest in the physiological role of acylation-stimulating protein (ASP). Recent studies in rats and mice, in particular in C3 (−/−) mice that are ASP deficient, have advanced our understanding of the role of ASP. Of note, the background strain of the mice influences the phenotype of delayed postprandial triglyceride clearance in ASP-deficient mice. Administration of ASP in all types of lean and obese mice studied to date, however, enhances postprandial triglyceride clearance. On the other hand, regardless of the background strain, ASP-deficient mice demonstrate reduced body weight, reduced leptin and reduced adipose tissue mass, suggesting that ASP deficiency results in protection against development of obesity.

In humans, a number of studies have examined the relationship between ASP, obesity, diabetes and dyslipidemia as well as the influence of diet, exercise and pharmacological therapy. While many of these studies have small subject numbers, interesting observations may help us to better understand the parameters that may influence ASP production and ASP action.

The aim of the present review is to provide a comprehensive overview of the recent literature on ASP, with particular emphasis on those studies carried out in rodents and humans.

Keywords: C3adesArg; Complement C3; Obesity; CAD; Diabetes

1. Introduction

In recent years, it has been widely recognised that adipose tissue constitutes not only a storehouse for energy in the form of triglyceride, but is also a metabolically active organ producing numerous secreted proteins, enzymes and hormones. Within the ever-proliferating list of autocrine and endocrine hormones produced by adipose tissue, acylation-stimulating protein (ASP) has been recognised as a member of this family of proteins. In fact, a number of recent reviews on adipose tissue hormones highlight the major functional properties of ASP in regulation of adipose tissue triglyceride storage [1–5].

The aim of the present review is to present a comprehensive summary of the physiological studies on ASP and the related proteins: complement C3, factor B and adipsin in humans and rodents. We will review the physiological studies available in rodents (rats and mice), with a particular emphasis placed on studies in C3 knockout (KO) (ASP-deficient) mice. Finally, we will attempt to integrate the physiological data available in humans for each of these plasma proteins in relation to metabolic disorders: obesity, cardiovascular disease, diabetes and dyslipidemia.

1.1. Identity of ASP

ASP is identical to C3adesArg [6,7], and is produced through a two-step process (see Fig. 1) involving three proteins of the alternate complement system: C3, factor B and adipsin, all of which are synthesised and secreted by adipocytes [7,8]. ASP is generated through interaction of C3 with factor B and the enzyme adipsin (a.k.a. complement

Abbreviations: ASP, acylation-stimulating protein; AUC, area under the curve; BMI, body mass index; CAD, coronary heart disease; CVF, cobra venom factor; FCHL, familial combined hyperlipidemia; FIAT, fatty acid incorporation into adipose triglyceride; KO, knockout; NEFA, non-esterified fatty acid; PLD, partial lipodystrophy; TZD, thiazolidinediones

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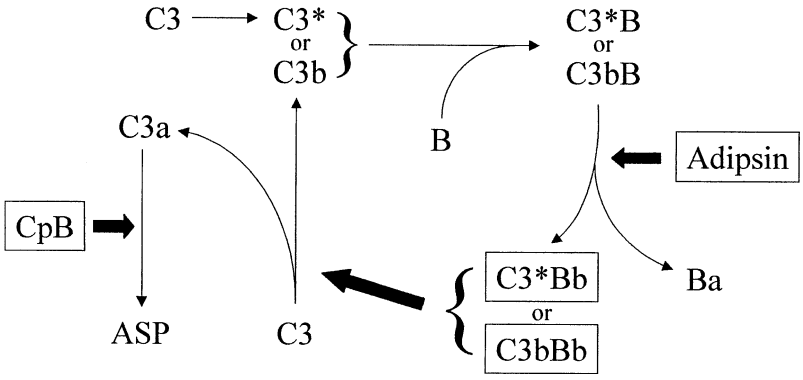

Fig. 1. Conversion of C3 to ASP: C3 converts spontaneously to activated C3*, a C3b analog which then combines with B to form C3*B. Adipsin then cleaves the bound B to generate C3*Bb and Ba. C3*Bb is the active convertase that cleaves C3 to C3a and C3b. Carboxypeptidase B (CpB) cleaves the N-terminal arginine of C3a to produce ASP. The generated C3b can combine with B to start another cycle. The enzymes adipsin, C3*Bb (or C3bBb) convertase and CpB are indicated in boxes.

factor D) resulting in (i) cleavage of the parent protein, complement C3 to generate C3a followed by (ii) desarginination of the carboxyl terminus to generate C3adesArg (ASP). While the function of C3a as an immune modulator in myeloid systems has been well documented, C3adesArg (ASP) appears to be inactive in those systems [9]. Carboxypeptidase B, produced by many cells (including fibroblasts), is present at high levels in plasma and only ASP (and not C3a) is found circulating. On the other hand, because C3a as well as ASP both appear to be potent stimulators of triglyceride synthesis in adipocytes and preadipocytes, conversion is not necessary for action [7,9].

While X-ray crystallography and NMR analysis of C3a and C3adesArg (ASP) did not detect any major differences in those areas for which resolution was possible, analysis of the carboxyl terminal flexible tail of the molecule (wherein lies the amino acid difference) was not possible [10,11]. In a recent study, we demonstrated that there were clearly differences in the structural requirements of the protein which were important in producing the immunologic effects on myeloid cells vs. the triglyceride synthetic effects on fat-storing cells [9,12]. For example, while the carboxyl terminal arginine appears to be essential for immune function of C3a, there appears to be no requirement for the terminal arginine for triglyceride synthesis [9]. Moreover, the tightly coiled core region of the molecule, which contains three disulfide bridges, is important for triglyceride synthetic activity; by contrast, this core region is not required for immunologic activity [12].

These studies suggest that the potential differences may well lie in the nature of the receptor interaction. While a C3a receptor has recently been identified [13,14] and has now been cloned in human, mouse and rat [13–16], neither C3a receptor protein nor mRNA appears to be present in adipose tissue as determined by Northern blot and immunofluorescence (unpublished data). On the other hand, ASP/C3adesArg has been shown to bind specifically and saturably to subcutaneous and omental adipocytes [17], preadipocytes [9] and fibroblasts [18] in human and murine cells, suggesting the presence of an alternate, as yet uncharacterised, receptor mediating the effects of ASP.

1.2. Role of ASP

ASP increases triglyceride synthesis in fat-storing cells [19]. The effect is achieved through stimulation of *f*atty acid *i*ncorporation into *a*dipose *t*riglyceride (FIAT) and increases in glucose transport. Glucose transport is increased through enhanced translocation of glucose transporters (Glut1, Glut3 and Glut 4) to the plasma membrane surface. While our studies have focused on humans and mice, there is now additional evidence that ASP-like proteins may influence fat storage in cows [20], pigs [21] and even plants [22]. A recent paper by Van Harmelen et al. [23] contributes importantly to understanding ASP function. Not only does ASP increase lipogenesis in adipocytes, but ASP also inhibits hormone-sensitive lipase (HSL)-mediated lipolysis.

For both FIAT and glucose transport, the effects of ASP are additive and independent of those of insulin [24–26], a well-recognised lipogenic hormone. In the studies by Van Harmelen et al. [23], in the presence of both insulin and ASP, the combined effect was a reduction in net fatty acid output from the adipocytes, with almost complete (97%) re-esterification and storage of the fatty acid available. Thus, in combination with insulin, ASP can provide a powerful drive for fat storage, at least in isolated cells. These functional data have led to increased interest in ASP, in particular in evaluation of plasma ASP in humans and rodents.

1.3. Regulation of adipose tissue production of C3 and ASP

While it has traditionally been assumed that the liver is the primary site of synthesis for C3 [27], it has since been demonstrated that a number of cells are competent for both C3 and factor B [28] synthesis. Recently, it has been clearly demonstrated that human adipose tissue can also synthesise and secrete C3 and factor B in a differentiation dependent

manner [7,8]. It is widely recognised that in humans, adipsin is primarily made in adipose tissue [29], although recently shown that liver [30,31] and macrophages [32] may produce small amounts. Thus, while the C3 cleaved to generate ASP may not be derived from adipose tissue, the adipsin required for enzymatic action almost certainly is. Considering, however, the strong relationship between C3 and ASP with obesity (see below), adipose tissue may well produce a considerable amount of total body ASP.

It should be pointed out that ASP (C3adesArg) is not only produced through the interaction of C3, factor B and adipsin in the alternate complement pathway, but ASP could, potentially, be generated through the two other complement pathways: the classical and the lectin pathway. While adipsin was first recognised based on its expression in murine adipose tissue, multiple proteins from the alternate complement pathway are also produced by adipose tissue [33–36]. These include C3, B, factor H, factor I, properdin, Crry and complement receptors CR1 and CR2. Some of these are increased during differentiation of adipocytes (C3, adipsin), others decrease (factor H and Crry), and some remain constant (factor I, properdin, CR1). Any of these changes might influence ASP production, but this remains to be examined in future studies. However, because adipocytes do not appear to produce either C2 or C5 (both essential for the classical pathway) or any other complement factors required for the cell lysis pathway [34], it appears that the adipocyte possesses exclusively the proximal components of the alternate pathway, and ASP is likely to be formed in the microenvironment of the adipocytes [19].

The production of C3 and ASP was further examined in human studies. While the conversion of C3 to ASP may be regulated by posttranslational regulatory factors (as discussed later), the level of ASP may also be influenced by the concentration of its precursor, C3 or the initiating enzyme adipsin (see below). Three very recent studies have demonstrated the presence of C3 mRNA in subcutaneous [37–39] and omental [38] adipose tissue. C3 expression has been reported to be increased in older vs. younger subjects ($n=29$ subjects, Ref. [37]) and in omental vs. subcutaneous tissue ($n=9$ subjects, Ref. [38]). Dusserre et al. [38] suggested that ASP (in fact C3) gene expression was increased in omental fat as a compensatory phenomenon to counteract the greater lipolytic capacity of the visceral cells. A similar argument is used by Imbeault et al. [37] to explain the apparent contradictory finding that HSL activity was lower in older vs. younger subjects, in spite of increased expression of HSL mRNA. They suggested that increased C3 mRNA will result in increased production of ASP, which would inhibit HSL activity (as discussed above) and explain the apparent decreased lipolytic activity in the face of increased mRNA for HSL.

Surprisingly, although ex vivo secretion of adipsin from human adipose tissue was described a decade ago [40], we could only find a single study examining adipsin in human adipose tissue [41]. In that study ($n=12$ subjects), also surprisingly, there was no correlation of adipsin mRNA with body mass index (BMI), no difference between omental and subcutaneous tissue, and no difference between males and females. However, considering the limited data available, our knowledge would benefit from a more thorough study of all factors (C3, factor B and adipsin) in an expanded population.

We have examined direct ASP production in human subcutaneous adipose tissue [42,43]. In control subjects, while ASP in the general circulation remained relatively stable, ASP efflux from an abdominal subcutaneous adipose tissue site increased postprandially from 3 to 5 h, over the same time period when chylomicron triglyceride clearance and fatty acid flux into adipose tissue were maximal [42]. In fact, when FIAT over the 6 h was calculated, it correlated directly with the production of ASP. Interestingly, while insulin levels also increased over this same postprandial period, and certainly contributed to adipose tissue FIAT, insulin did not correlate with FIAT, while ASP did [42]. At fasting, however, there was no net production of ASP. This contrasts with ASP production in obese subjects which was greater than control at all time points and was increased even at fasting [43].

With the many factors that change postprandially or in insulin-resistant states such as diabetes, it is difficult to pinpoint exactly which component might be responsible for the stimulation of C3 and ASP production. Experiments with cultured human adipocytes have demonstrated that insulin (to a moderate degree), but especially chylomicrons, appear to stimulate both C3 and ASP production [44–46]. On the other hand, Koistinen et al. [39] showed that a euglycemic (4 h) hyperinsulinemic clamp had no effect on C3 mRNA levels. C3 mRNA did correlate with BMI, glucose disposal rate, plasma triglyceride, non-esterified fatty acid (NEFA) and leptin ($n=12$ subjects) [39]. We speculate, therefore, that ASP production may be up-regulated in insulin-resistant states, perhaps by one of the factors listed above.

A recent article demonstrated that diets containing differing types of fat (polyunsaturated vs. *trans* fatty acids) have been shown to influence fasting ASP levels after several weeks feeding in hypercholesterolemic women [47]. While the exact mechanism is unknown, several components of chylomicrons may have an effect, one of these being the amount of retinoic acid being delivered to the cell which increases C3 mRNA, C3 secretion and ASP production [46]. While we have focused on the effects of postprandial components on C3 and ASP production, many factors, especially cytokines (TNFα, IL-6) and glucocorticoids have been shown to influence many of these proteins including C3, B and adipsin and are proposed as potential factors influencing C3 and adipsin in studies with anorectic [48], obese [49] or coronary heart disease (CAD)-prone [50] subjects.

2. ASP, C3 and adipsin as circulating hormones

2.1. Human studies

Consequent to the publications on ASP function in cultured adipocytes, increased interest has prompted a number of studies on the plasma levels of ASP, C3 and/or adipsin and the potential interactions with obesity, insulin resistance, diabetes, hyperlipidemia and CAD. Before discussing these studies, one caveat to note is that other than four of the studies, which range from 200 to 1000 subjects [50–53], most of the studies have small sample sizes (average 26, range 10–60 per group). Secondly, if ASP has a paracrine, rather than an endocrine function, circulating levels may not always be sufficiently informative. Certainly, while more detailed studies will clarify the potential physiological significance of ASP and C3 in related metabolic disorders (diabetes, hyperlipidemia and CAD), these data are nonetheless thought provoking and informative (as described in detail below).

While different assays (ELISA and RIA) have been used for ASP determination, plasma concentrations in normal healthy adults were similar, ranging from 10.3 to 58.1 nM and, when all these studies were taken into account, the weighted average was 28.3 nM (253 ng/ml) ($n=376$ subjects in 13 studies, author's calculations, see Table 1 and references). This contrasts strikingly with the concentration of the precursor protein, complement C3, which is present at 225-fold greater molar concentration than ASP. In the 15 studies reviewed, plasma C3 in normal healthy adults ranged from 4.6 to 8.1 μM with a weighted average of 6.4 (author's calculations, $n=2812$ subjects). Thus, at any given time, only a relatively small proportion of C3 has been converted to circulating ASP. Surprisingly, ASP levels were even lower than those of adipsin, which is the initiating enzyme in the cleavage process. Normal adipsin concentrations range from 49.0 to 66.7 nM (weighted average = 62.9 nM, $n=284$, author's calculations, see Table 1).

Table 1
Fasting plasma ASP, C3, B and adipsin normal ranges

ASP	C3	B	Adipsin
28.3 nM (10.3–58.1)	6.4 μM (4.6–8.1)		62.9 nM (49.0–66.7)
253 ng/ml (92–518)	1.15 mg/ml (0.83–1.45)	0.3 mg/ml	1.5 μg/ml (1.2–1.6)
$n=13$ studies (376 subjects) [18,39,42,43, 48,53,119,124, 125,147,152, 180,210]	$n=15$ studies (2812 subjects) [39,48,50–52, 55,56,118,122, 124,151,160, 211,212]	$n=1$ study [48]	$n=5$ studies (284 subjects) [40,48,53, 128,204]
M = F [53]	F>M [51,54], M>F [55,56]		M>F [40], M = F [53]

Fasting plasma ASP, C3, factor B and adipsin were measured by immunoassay (as indicated). Averages presented are author's calculations where M = male and F = female.

While it has been suggested that there are gender differences in C3 and adipsin, the differences were small, and contradictory from study to study perhaps due to the phenotype of the subjects chosen [40,51,53–56]. In normal weight subjects, there did not appear to be any difference in plasma ASP between males and females [53].

2.2. Rodent studies

There is relatively little information on plasma levels of any of the alternate complement proteins in rodents, and even less information available regarding associations with obesity, insulin or lipids. Certainly, C3 [57,58] and ASP [33] levels all appear to be considerably lower in mice than in humans. No quantitative assay of mouse adipsin has been published. C3 levels vary from strain to strain [59], and while they increase with age, there did not appear to be any gender effect [57], again similar to humans. High fat diet [59], cytokines [60] and obesity [61] were all associated with increased plasma C3. While not statistically significant, ASP (C3adesArg) was 50% higher in obese vs. lean mice, and correlated to plasma triglyceride concentration [33].

Most of the results on adipsin derive from data on genetically obese rodents. In *ob/ob* and *db/db* mice as well as Zucker rats (all leptin pathway defective), and MSG-treated mice, adipsin was decreased with obesity in most [62–65] but not all [66] reports, although the adipsin deficiency did not appear to be the cause of obesity [63]. Insulin, corticosteroids and cytokines, all of which are increased in obesity, have been reported to suppress adipsin [36,67,68], and may explain the decreased adipsin in rodent obesity. On the other hand, lipodystrophic mice also have reduced adipsin [69]. Decreased adipsin, however, does not occur in gluttony-based obesity [70], and both a high-fat diet [66] and caffeine [71] increased adipsin levels.

Based on the demonstrated cellular effects of ASP on triglyceride synthesis, we examined the direct effect of ASP on triglyceride clearance following a fat load in lean and obese mice. In lean C57Bl/6 mice, intraperitoneal injection of ASP reduced the triglyceride area under the curve (AUC) by 40% [72]. Similarly, in *ob/ob* and *db/db* mice models which are hypertriglyceridemic, hyperinsulinemic and obese, ASP administration enhanced triglyceride clearance by 2-fold [73]. There was also an overall decrease in the plasma levels of NEFAs during the fat load, consistent with increased uptake and storage into adipose tissues. Based on these encouraging studies, we turned to examination of murine models deficient in ASP.

3. ASP deficiency and KO models

3.1. ASP deficiency

ASP deficiency could result from a lack of C3, through a genetic mutation, or, assuming that adipocyte ASP is only

generated through the alternate complement pathway, through an absence of any of the associated proteins important in initiating the proximal step of the pathway. Thus, a lack of factor B, adipsin (factor D) or properdin, the convertase stabiliser, could also result in ASP deficiency. Any of these genetic deficiencies, however, appear to be extremely rare. According to Singer et al. [74], a total of 20 people with C3 deficiency has been reported from 1972 to 1992, 14 of them female. Deficiencies of properdin and factor D are rare and no deficiency of factor B has as yet been reported. By contrast, other complement protein deficiencies, such as C2 deficiency, are "relatively common" [75].

Within the last year, there have been two reports of families with alternate pathway deficiencies: properdin deficiency [76] and factor D (adipsin) deficiency [77]. In the family with factor D (adipsin) deficiency, most (60%) of the homozygous subjects and all of those with partial deficiency (heterozygous) had no history of infections. Unfortunately, in both reports, there is no information provided beyond that related to immunological function. Consequently, it is difficult to ascertain whether the paucity of cases recognised worldwide is a consequence of the mutations being lethal or benign. The lack of human studies and the availability of mice KOs has led to studies of ASP/C3 in rodents, as described below.

3.2. Adipsin and factor B KO mice

With recent technological advances, there has been a surge of murine models developed that present with a deficiency (KO) or an excess (overexpression) of specific proteins. A recent review highlights the phenotypes of complement KOs [78], including, of interest to the present discussion, factor C3- and B-deficient strains. Because ASP is produced via the alternate pathway, deficiencies in either C3, B or adipsin should result in lack of ASP.

It had been proposed that factor B mutation might be lethal, since no human factor B deficiency has yet been described. In fact, factor B-deficient mice are viable, fertile and while manifesting absent alternative pathway activation and decreased classical pathway activation, no overall abnormalities of the immune system were detected [79], but factor B appeared to play an unexpected role in autoimmune disease [80]. Very recently, factor D/adipsin-deficient mice have been produced [81]. No apparent abnormal development or large change in body weight was detected and serum concentrations of triglyceride, cholesterol and NEFAs were reportedly not different. However, serum C3 levels were increased by 63% in D (adipsin)-deficient mice, and cobra venom factor (CVF) treatment (a C3b analogue) permitted the formation of an active C3 convertase containing intact B (instead of Bb), causing a profound and reproducible cleavage of C3 [81] (and, theoretically, a generation of ASP). The authors conclude that factor D is not an absolute requirement for the generation of a CVF-factor B convertase. By contrast, no such effect was documented in factor B-deficient mice [81].

3.3. Strain differences in C3 KO ASP-deficient mice

Two groups have created C3-deficient mice [82,83] and they have been studied in infection, humoral immune response, endotoxic shock and glomerulonephritis (for review, see Ref. [78]). We have been particularly interested in examining these C3 KO mice (obligate ASP deficient) for disturbances in lipid metabolism, especially postprandially. Our initial study, published in 1999, demonstrated a delayed triglyceride clearance in male C3 KO mice [84]. No delay in triglyceride clearance was noted in the female mice, who manifested more rapid triglyceride clearance than males overall [even in wild-type (WT) mice]. In a subsequent study, we confirmed and extended those results, demonstrating increasingly delayed triglyceride clearance as the mice aged, even on a high-fat diet [85].

By contrast, Wetsel et al. [86] demonstrated no such difference in postprandial triglyceride in their breeding colony of C3 KO mice, and in a review shortly afterwards attempted to address these differences [87]. Their critical evaluation was based on the premise that the two states, ASP deficiency and ASP resistance should present with a similar phenotype: hyperapoB. However, in the first instance, mice do not normally manifest hyperapoB (increased hepatic apolipoprotein B production) as discussed elsewhere [85], and, as discussed below, ASP deficiency and ASP resistance will not necessarily present with the same phenotype.

We speculated that, because both studies used mice derived from a mixed breeding colony (C57Bl/6 and 129Sv), it was possible that the differing results were a consequence of background strain. To resolve that question, C3 KO mice were backcrossed seven generations to each of the individual strains (C57Bl/6 and 129Sv). Plasma variables tested in each of the strains are shown in Table 2. There were no pronounced differences between KO and WT in either strain for fasting plasma cholesterol or

Table 2
Characteristics of ASP-deficient (C3 knockout) mice according to strain

	129Sv		C57Bl/6	
	WT	KO	WT	KO
n:	(37)	(52)	(38)	(26)
AGE (weeks)	20.9 ± 1.9	21.2 ± 2.1	28 ± 2.2	25.2 ± 2.9
Weight (g)	28.0 ± 1.0	26.1 ± 0.5*	31.0 ± 1.6	27.5 ± 2.1*
Cholesterol (mg/dl)	133 ± 4.2	127 ± 4.0	99 ± 4.9	91 ± 5.2
Triglyceride (mg/dl)	67.3 ± 5.5	56.9 ± 2.4*	65.3 ± 4.7	52.9 ± 4.2*
NEFA (mM)	0.90 ± 0.05	0.79 ± 0.02	1.22 ± 0.07	1.61 ± 0.11*
Glucose (mg/dl)	95.8 ± 5.2	83.3 ± 3.8*	156.8 ± 11.7	112.5 ± 6.1*
Insulin (ng/ml)	0.23 ± 0.03	0.24 ± 0.02	0.37 ± 0.09	0.33 ± 0.08

Fasting plasma was obtained and lipids and insulin measured as described elsewhere [116]. Results are presented as average ± S.E.

*$P<0.05$ for C3 knockout (KO) vs. wild type (WT).

NEFA, other than a slight increase in fasting NEFA in C57Bl/6 KO mice and small decreases in triglyceride. Similarly, there was no difference in fasting insulin, but a significant reduction in plasma glucose in KO in both strains of mice.

On the other hand, there were pronounced strain differences in the postprandial triglyceride response, as shown in Fig. 2. 129Sv male mice demonstrated a marked delay in triglyceride clearance that was not seen in either Blk6 mice, nor in 129Sv female mice (Table 3). This delay was almost completely normalised with ASP administration (Fig. 1). As this was the major difference between the two studies [85,86], and the only strain difference that we found in our parameters tested, we feel that strain difference likely explains this. One might hypothesise that the C57Bl/6 mice have a more efficient system for clearing dietary fat, perhaps due to increased insulin sensitivity, lipoprotein activity, or endogenous adipose tissue triglyceride synthetic capacity. It is likely that ASP is not the only mechanism that influences NEFA uptake, and redundant adaptive measures probably compensate when ASP is absent.

There are many examples of strain influence on phenotypic expression of genetically modified mice (KO mice or overexpressor mice). These differences likely arise because of basic metabolic differences in the monogenic WT strains of mice. Loss-of-function mutations, such as fibronectin, apoE, eNOS, nNOS, leptin receptor (*db/db*), have all been shown to demonstrate strain-specific phenotypic expression ranging from minor to severe (neonatal death) effects. This issue has been carefully reviewed recently by Sigmund [88]. Lipid and adipose tissue metabolism were different between C57Bl/6 and AKR mice (both of which are obesity prone) and obesity-resistant strains C3H, A/J, I and KsJ [89–100]. C57Bl/6 mice, for example, are particularly susceptible to atherosclerosis, diabetes and obesity (http://www.informatics.jax.org). In studies that have compared C57Bl/6 and 129Sv strains directly, there are differences in genetic taste traits, diet preference, behaviour, electrophysiology and cerebral anatomy [101–110]. With respect to lipoproteins, 129Sv have

Table 3
Postprandial triglyceride and NEFA AUC in ASP-deficient (C3 knockout) vs. WT in 129Sv and C57Bl/6 mice

	129Sv		C57Bl/6	
	WT	KO	WT	KO
Triglyceride				
Male	602 ± 96	1012 ± 160*	412 ± 41	411 ± 32
Female	465 ± 90	399 ± 17	359 ± 26	403 ± 14
NEFA				
Male	5.91 ± 0.27	6.37 ± 0.32	5.91 ± 0.71	6.03 ± 0.18
Female	5.63 ± 0.23	5.99 ± 0.30	5.24 ± 0.31	9.73 ± 0.90*

Postprandial triglyceride (mg/dl) and (NEFAs) (mM) were measured over 6 h following administration of a fat load [85] and AUC calculated.
* $P < 0.05$.

increased LDL cholesterol [111] and different LDL composition [112] compared to C57Bl/6. Apo AIV, Apo AI, HDL cholesterol and HDL size are all increased in 129Sv vs. C57Bl/6 [111,113–115].

It is important to note that we ascribe the differences seen between KO and WT to the absence of ASP (and not C3) for several reasons. First, although these mice lack both C3 and ASP, there is no evidence to suggest that C3 plays a direct role in lipid metabolism or fat storage, at least functionally [6], while ASP has been shown to directly stimulate triglyceride synthesis and storage [19]. Second, we have previously shown that direct administration of ASP during a fat load enhanced triglyceride clearance in normal mice [72], as well as obese dyslipidemic mice such as *ob/ob* and *db/db* [73]. Administration of ASP normalises triglyceride and NEFA clearance in 129Sv male KO mice as shown here in the present study. Thus, deficiency of ASP results in delayed triglyceride clearance, and acute administration reestablishes it.

3.4. ASP deficiency induces repartitioning of energy storage and energy metabolism

Although we have also examined glucose, insulin, leptin, growth curves, food intake and body composition in our

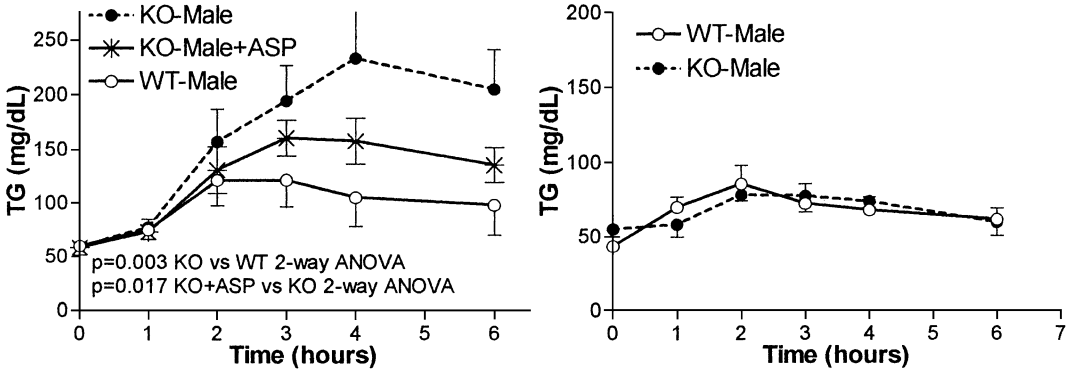

Fig. 2. Postprandial triglyceride clearance in ASP-deficient (C3 −/−) and WT in 129Sv (left panel) and C57Bl/6 (right panel) male mice. A fat load was administered and blood samples taken serially over 6 h to measure plasma triglyceride [85]. In 129Sv ASP-deficient C3(−/−) mice, an additional fat load was conducted with ASP injected intraperitoneally at the start of the fat load.

studies [84,85,116], this was not done in the study by Wetsel et al. [86]. Interestingly, however, a deficiency in ASP appears to have interesting and unanticipated effects on adipose tissue mass, glucose metabolism and energy partitioning. While the mechanism of these effects remains to be elucidated in future studies, these three surprising findings should be commented on.

First, ASP-deficient mice have reduced fat mass. This has been documented indirectly, as reflected by reduced body weight and decreased levels of plasma leptin (which reflects adiposity) as well as by direct measurement of adipose tissue mass [116]. Total fat mass was moderately reduced in males [85], but markedly reduced by up to 60% in females in all fat pads examined [116]. When tested over a wide range in ages, body weight was consistently lower in KO as was plasma leptin in both males and females [85,116]. Again, this characteristic is pronounced in both backcrossed strains of KO as clearly shown in Fig. 3. Finally, even when placed on a high-fat diet, the C3 (−/−) ASP-deficient mice were resistant to diet-induced obesity, and their body weight and plasma leptin remained consistently lower than their WT littermates. The changes in adipose tissue mass may be explained by reduced cell size or a decreased number of adipocytes.

Based on fasting glucose, insulin and oral glucose tolerance tests, ASP-deficient mice appear to be more insulin sensitive [85,116], although this could be a consequence of the relative leanness and protection against diet-induced obesity. The reduced glucose levels in both 129Sv and C57Bl/6 backcrossed strains of KO are also supportive of that (Table 2).

Finally, the most intriguing finding was that although the KO mice were leaner, food intake was much greater (up to 18% more caloric intake) with a normal fat absorption [85], raising the question: Where does all this energy go? How does the lack of ASP result in repartitioning and disposal of excess energy? The balance of body energetics would predict that any calories ingested, and not stored in some form, would have to be expended as energy. The major options are increased activity or enhanced thermogenesis. Preliminary results presented at a recent meeting (late breaking abstract, NAASO 2001) demonstrated increased oxygen consumption in KO mice, but no changes in overall activity, suggesting alternate disposal routes in the absence of efficient adipose tissue storage.

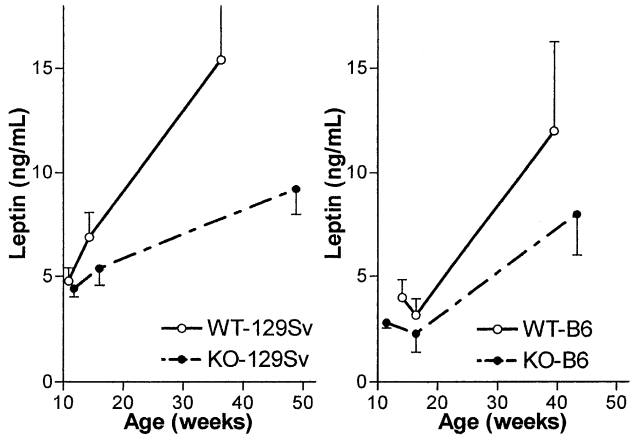

Fig. 3. Plasma leptin in ASP-deficient (C3 −/−) and WT in 129Sv (left panel) and C57Bl/6 (right panel) mice. Plasma leptin was measured by RIA as described previously [116] at the indicated ages and is expressed as nanograms per milliliter ± S.E.

While we ascribe the differences in the KO mice to the absence of ASP rather than C3 (for the reasons outlined above), it would be important to test this further in a mouse that has C3, but not the capacity to generate ASP (such as in a factor B or adipsin KO mouse). One fascinating area to probe will be the control mechanisms between energy storage and energy utilisation, and the mechanisms by which lack of ASP results in a shift to increased oxidation.

4. Body weight, insulin resistance and ASP in humans

4.1. ASP, C3, B and adipsin in obesity and diabetes

Table 4 summarises studies in humans examining the relationship between plasma ASP and the precursor proteins C3, B and adipsin. Recently, there has been increasing interest in ASP and C3, but the studies on factor B and adipsin remain relatively few. Interestingly, linkage analysis on factor B demonstrated an association of obesity to the locus where factor B is found [117]. In a number of independent studies, each of these proteins has been shown to be increased in obesity, although not in all cases (for details, see Table 4). For example, the increase in ASP ranges from 58% to 400% normal. Overall, the increases in C3 and adipsin were significant but modest, while the relative increases in ASP tended to be greater. This is consistent with the hypothesis that it is ASP that is the key bioactive molecule, and the changes in C3 and adipsin serve to influence the production of ASP. It should be pointed out that the suggested link between C3 and obesity is not new [118]. This idea has greater impact, however, with the knowledge that adipose tissue may be an important source of C3 and the recognition of the bioactivity of ASP.

ASP was increased to a greater extent in obese Caucasians as compared to obese Pima Indians, although BMI were comparable between the two groups [119]. In fact, obese Pima also had lower plasma triglyceride and cholesterol [119], but higher insulin [119] and adipsin [40]. The ethnic difference in ASP remained even after adjustment for triglyceride, NEFAs and percentage body fat. In Pima, fasting C3 concentration is also closely related to adiposity, fasting insulin and especially insulin sensitivity and may link obesity, insulin resistance and possibly atherosclerosis [49]. In multiple regression analysis with C3 as the dependent variable, body fat, insulin action, gender and glucose tolerance status were all independent determinants explaining a total of 80% of the variance in C3 [49]. The low prevalence of dyslipide-

Table 4
Effects of weight gain and weight loss on ASP, C3, B and adipsin

	ASP	C3	B	Adipsin
Obesity	↑ 58% to 4 × normal [39,53,119,210] nc [43] Pima < Caucasian [119] F>M [53]	↑ 11–25% [48,50,118] nc [39,211,212]	↑ (48%) [48]	↑ 14–64% [40,53,128,204] nc [48] Pima>Caucasian [40]
NIDDM	↑ 25% [39] NIDDM = Obese [39,121]	↑ [211,213] nc [39]	↑ [214]	↑ 36% [204,214]
IDDM		↑ 24% [122,213,214]	↑ [214]	↑ [214]
Weight loss (in obese)	↓ −45% [210] post-obese = normal [125]	↓ −12% to −32% [48,126,127,118]	↓ −12% [48]	nc [48,214]
Anorexia nervosa	nc [48]	↓ −20% [48]	↓ −17% [48]	↓ −19% to −40% [40,48]
Weight gain	nc [48]	↑ 16% [48]	38% [48]	↑ 22% [40,48] ↓ −28% AIDS [40]
Lipodystrophy (LD)		↓ acquired LD [131,133–135] nc hereditary LD		nc partial LD [40] ↓ −46% total LD [40]

Fasting plasma ASP, C3, factor B and adipsin were measured by immunoassay. Abbreviations are: NIDDM (non-insulin-dependent diabetes mellitus), IDDM (insulin-dependent diabetes mellitus), F (female), M (male), LD (lipodystrophy), nc (no change vs. control).

mia in Pima has been linked to a region on chromosome 19p which includes genes not only for lipid transport proteins, but also includes the gene for C3 [120].

All of these factors (ASP, C3, B and adipsin) have also been shown to be significantly increased in Type II (NIDDM) diabetes and also in some studies of Type I diabetes (Table 4). As diabetes is often associated with obesity, this may be a confounding factor. For example, ASP is increased in NIDDM [39], but only to a level comparable to non-diabetic subjects of matched weight [39,121]. However, even in non-obese Type I diabetics, plasma C3 levels are increased, and this is further exacerbated by hypertension [122]. ASP correlated with whole-body glucose and lipid metabolism in nondiabetics, but not in diabetics [39]. It was suggested that the metabolic disturbances in diabetes overcame the regulatory role of ASP, explaining the loss of association [39].

4.2. Effect of weight loss, weight gain and exercise

Considering the increases in ASP, C3, adipsin and factor B with obesity, it is perhaps not surprising that these factors changed with weight loss (Table 4) or exercise. In two separate studies with moderately trained athletes, ASP/C3adesArg levels were 2.7–6.1 nM [123] and 21.3 nM [124] at baseline, and increased acutely by 67% [123] and 29% [124] during a timed run (0.5–2.5 h), then returned to baseline shortly afterwards. While there was no exercise-induced change in C3, baseline values were lower in the athletes compared to controls (frequency distribution was significantly shifted to the left) [124]. In a much larger study that included 1090 middle-aged men, there was an inverse relationship between physical activity and C3, an association that was independent of the relationship to other plasma variables (lipids, glucose, insulin) [50].

ASP decreased with weight loss in obese subjects [56], and normal weight post-obese women had normal levels of ASP [125]. Similarly, plasma C3 decreased with weight loss [48,118,126,127] and was decreased in subjects with anorexia nervosa [48]. Both adipsin and factor B decreased with weight loss [48,128], and were decreased in anorexia nervosa [40,48] and increased toward normal levels with weight gain in subjects with anorexia nervosa [40,48]. One study examined classical complement pathway components as well and concluded that there was little change in the components of the classical pathway, and that alternative complement components were preferentially affected by weight loss in both anorectics and obese dieters [48]. Thus, overall, all of these factors change in concert with changes in body weight (and accordingly fat mass), suggesting that at least one major influence on plasma levels of ASP and C3 is adipose tissue mass.

4.3. Lipodystrophy

Related to the issue of weight loss, there are some interesting changes in ASP pathway proteins in partial lipodystrophy (PLD). PLD can be divided into congenital and acquired forms. Congenital PLD appears to be hereditary, is X-linked and appears to be lethal in the hemizygous state [129]. Congenital PLD is associated with increased insulin, NEFAs, insulin resistance and sometimes diabetes [130]. On the other hand, acquired PLD develops later, but still in childhood and occurs more often in females than males [131]. Acquired PLD has consistently been reported to be associated with striking decreases in C3 and the presence of C3 nephritic factor (C3NeF) which is not found in normal individuals [132] (Table 4). For example, Ipp [133] reported 6 of 7 patients had reduced C3 and Sissons et al. [131] reported the same phenomenon in 17 of 21 patients. More recently, Tanuma et al. [134] reported that all patients had only 10% of normal C3 levels and Skattum et al. [135] reported that 20 of 25 had reduced C3. On the other hand, other plasma complement proteins (such as B, proper-

din, C2, C4, C5 and C6) appeared to be normal [131,134,136]. While there is little information on insulin and plasma lipids in acquired PLD, it has been shown in one case to be associated with fatty liver [136].

C3 nephritic factor has been shown to be an IgG autoantibody reacting with a neoantigen exposed on the surface of the alternative pathway convertase, C3bBb, which is stabilised and rendered resistant to the physiological control proteins, factors H and I [137]. This results in hypercatabolism and chronic depletion of C3 [138]. This reaction is specific to the alternative pathway and does not occur in the classical pathway [133]. A recent report has shown that adipocytes are lysed by C3NeF-containing serum, but not normal serum, suggesting that local production of C3/B/adipsin by adipocytes (which produces ASP) in the presence of C3NeF results in acute dysregulation of alternate complement activation in the microenvironment of the adipocytes, and these cells are targeted for lysis by the terminal complement components [139].

4.4. Effect of insulin sensitizer pharmacological therapy on ASP and C3

Three recent studies have examined the changes in ASP and/or C3 with pharmacological treatment in diabetics with either sulfonylurea drugs (glibenclamide) [121,140] or with thiazolidinediones (TZD) (troglitazone and pioglitazone) [54,140]. In a study by Ebeling et al. [54], following treatment for 16 weeks of older diabetic subjects, troglitazone decreased C3 as well as glucose and insulin requirements. This effect was selective for C3, as other acute-phase proteins (alpha-1 antitrypsin, ceruloplasmin, C-reactive protein and haptoglobin) were not affected. There were no significant changes in any plasma lipids or apolipoprotein B, but apolipoprotein A1 decreased. While baseline C3 correlated with BMI, C3 decreased with treatment while BMI increased significantly.

A more recent study by this same group has examined the effects of a TZD (pioglitazone) and a sulfonylurea drug (glibenclamide) on both ASP and C3 in diabetics [140]. In contrast to the previous study, this TZD did not have an effect on plasma C3; however, there was a significant decrease in C3 with glibenclamide treatment. Both treatments produced a decrease in glucose and HbA_{1c}, but a slight increase in plasma cholesterol with no effect on other lipids. Again, while C3 correlated with BMI, this did not explain the change because the decrease in C3 with treatment was accompanied by an increase in BMI. There was a trend for changes in ASP, but the individual group effects were not significant. However, the change in HbA_{1c} with treatment, which reflects glycemic control, correlated positively with the change in ASP. In fact, the change in HbA_{1c} was best explained by changes in ASP and insulin (negative) that together explained 33% of the variation.

Rieusset et al. [141] examined the acute effects of rosiglitazone on gene expression in human adipocytes. Although a short-term (4 h) incubation increased PI3 kinase, UCP2 and decreased leptin mRNA, there was no effect on ASP (C3 mRNA). Similarly, there was no effect on IRS1, Glut4, lipoprotein lipase, HSL or PPARγ. Thus, the effects may be different for each TZD, or may require longer incubation times.

Another study has also reported effects of glibenclamide on plasma ASP [121]. Following 4 months of treatment in poorly controlled diabetic obese women, there was a significant decrease in glucose, HbA_{1c}, leptin and plasma cholesterol but, by contrast, a significant 30% increase in ASP. Neither ASP nor leptin correlated with any of the fasting plasma variables examined (glucose, insulin or lipids). Only leptin correlated with BMI. While fasting ASP values increased with improved glycemic control, the lack of correlation with measured variables suggests that other factors may play a role in influencing ASP levels. The authors suggest that glibenclamide, through interaction with the sulfonylurea receptor 1, which is expressed in human adipose tissue [142], could potentially increase ASP production. This receptor influences lipogenesis and lipolysis through phosphoinositide specific phospholipase C and tyrosine phosphorylation [143,144]. Thus, this effect on ASP could be mediated by (i) increasing insulin sensitivity because insulin increases C3 production and thus ASP [44,45]; or by (ii) inhibiting TNFα production [145], preventing TNFα suppression of adipsin secretion [146], a key enzyme for cleavage of C3 to ASP; or (iii) as a result of a primary drug effect on ASP production.

4.5. Correlations of ASP, C3, B and adipsin with body size and glucose/insulin

Many of these studies have examined the correlations of ASP, C3 and adipsin with parameters that reflect body size or insulin/glucose homeostasis as summarised in Table 5. A number of studies have demonstrated that plasma ASP correlates positively with various indices of body size. This includes BMI and percentage ideal body weight [39,53,119,147], waist to thigh or waist to hip ratio [39,147], total fat mass or percentage body fat [39,49,119]. Not all studies, however, have demonstrated this relation [121]. In the same way, there are a number of studies that have demonstrated similar associations with C3 ($n=9$ studies) and adipsin ($n=5$ studies), while only 1 study [48] has examined this with factor B (see Table 5). Certainly, however, the evidence is very consistent.

Studies with obese, poorly controlled diabetic and treated diabetic subjects lend themselves to correlations with insulin, glucose and associated parameters. As summarised in Table 5, ASP was associated with insulin and glucose disposal rate in some [39,147] but not all studies [49,121]. ASP was also associated with changes that reflect diabetic control following treatment [140]. Three studies with adipsin also suggested a correlation with insulin. Similarly, C3 correlated significantly with insulin, glucose and glucose

Table 5
Correlation of ASP, C3, B and adipsin with body size indices and glucose homeostasis

	ASP	C3	Adipsin
BMI or percentage ideal body weight	+ve [39,53,119,147] ns [49,121]	+ve [48–51,54,55,140,147,160]	+ve [40,53,128,204] also +ve factor B [48]
Waist/hip or waist/thigh	+ve [39,147] ns [49,121]	+ve [49,147,160]	+ve [204]
Percentage fat or total fatness	+ve [39,49,119]	+ve [49]	+ve [40,128]
Change in body weight		+ve change [48]	+ve change in adipsin and factor B [48]
Leptin			+ve [128]
Age			ns [40]
Insulin or insulin AUC	+ve [39,147] ns [49,121]	+ve [49,50,55,147,160]	+ve [40,128,204]
Glucose or glucose AUC	ns [49,121,147]	+ve [49,50,55,147,160,215]	ns [204]
GDR or M	−ve [39] ns [49]	−ve [49]	
Other	+ve HbA_{1c} [140] +ve Antitrypsin [140] C-reactive protein [140]	+ve HbA_{1c} [54] +ve Antitrypsin [140]	+ve PAI-1, tPa [204]

ASP, C3, factor B and adipsin were measured in the indicated studies by immunoassay and correlated to the various indices. Abbreviations are: +ve (significant positive correlation), −ve (significant negative correlation), ns (no significant correlation), GDR (glucose disposal rate), M (HOMA index of insulin sensitivity).

disposal rate, as well as factors related to diabetic treatment. As commented on by Weyer et al. [49], it remains unknown whether the strong association of C3 with obesity is a consequence of increased adipose tissue production or due to secondary mechanisms involving hepatic C3 production. The association of increased C3 with insulin resistance may be a cause or consequence of effects mediated through cytokines such as TNFα or IL-6 [148] or glucocorticoids [149]. Interestingly, while glucocorticoids stimulate C3 production, they inhibit adipsin production [67].

5. ASP resistance and cardiovascular disease

5.1. Associations of ASP and C3 with cardiovascular disease, hypertension and hyperlipidemia

Only C3 and to a lesser extent ASP have been examined with respect to CAD, hypertension or hyperlipidemia. In two studies with hypertensive subjects, it was demonstrated that C3 specifically, but none of the other immunologic factors tested, was higher in hypertensive subjects [150]. In a larger study with 350 women with no known CAD, divided according to hypertensive and diabetic status, increased C3 was shown to be associated with hypertension and diabetes (with an additive effect) [122].

In two large studies by Muscari et al. [50,51] ($n=860$ and 1090 subjects), C3 was shown to be a powerful predictor of myocardial infarction, especially in men [51]. None of the other immunologic variables examined was found to be independently associated with ischemic events. This relationship persisted retrospectively and, as noted by the authors, was more significant than any other association of traditional risk factors with myocardial infarction [50]. Of the many variables associated with C3 (Tables 5 and 6), insulin was the main covariate, suggesting that C3 is a marker of metabolic imbalance coinciding with insulin resistance [50].

C3 is also associated with various forms of hyperlipidemia, even in the absence of atherosclerosis [151]. Specifically, Type IIb (but not IIa), Type IV hyperlipidemia [151] and familial combined hyperlipidemia (FCHL) [55,147], all of which are characterised by increased fasting plasma triglyceride and/or cholesterol, have been shown to have increased C3. While most of these studies have demonstrated an association of C3 with BMI [50,51,55,147], the C3 increases were independent of the effect of BMI [50,55,151] and were strongly associated with plasma triglycerides, cholesterol, LDL cholesterol [50,51,55,147,151] and an especially striking correlation with apolipoprotein B ($r=0.64$ and 0.77) [55,147].

There are only two studies examining ASP in similar subjects: one in patients with CAD [152] and one in subjects with FCHL [147]. ASP was increased overall in subjects with CAD, especially in those with increased plasma triglyceride and/or cholesterol as characterised by increased plasma apolipoprotein B levels (HyperapoB) [152]. While subjects with FCHL have increased ASP compared to normal healthy controls [147], there appeared to be no difference between hypertriglyceridemic FCHL and normotriglyceridemic FCHL [147]. In contrast, with C3, there was a further increase in hypertriglyceridemic FCHL compared to normotriglyceridemic FCHL [55].

A very recent study has examined both C3 and ASP in subjects with nephrotic syndrome [153]. Nephrotic syndrome is characterised by increased hepatic protein and lipoprotein synthesis. Interestingly, in a group of 25 subjects, fasting ASP was increased 4.5-fold, with no corresponding increase in either C3 or leptin. This was accom-

panied by a striking increase in total cholesterol, triglycerides, apolipoprotein B and LDL cholesterol. The increase in ASP cannot be simply a consequence of up-regulated hepatic protein secretion, because the precursor C3, which can be synthesised by the liver, was not significantly increased.

5.2. Correlations of C3 and ASP to lipids

As summarised in Table 6, a number of studies have shown associations of C3 and ASP with lipids in varied subject groups including normal healthy controls, obese, diabetic and hyperlipidemic subjects. Overwhelmingly, C3 correlates positively with plasma triglycerides, total cholesterol, LDL cholesterol, apolipoprotein B and NEFAs, with significant negative correlations with HDL cholesterol. Several studies show similar associations with ASP (Table 6), although not in all studies [121]. There are only four studies with adipsin. A genome wide linkage analysis in a study with a large number of subjects suggested an association of blood pressure to adipsin locus [154].

Table 6
Association of ASP and C3 with dyslipidemia and CAD

	ASP	C3	Adipsin
CAD	↑ [152]	↑ [51,52,160] +ve [39,51]	
Hypertension (blood pressure)	ns [121]	↑ [122,150] +ve [50,51,150, 160]	linkage analysis +ve [154]
Hyperlipidemia	↑ H apoB [152] ↑ FCHL [147] HTg FCHL= NTg FCHL [147]	↑ Type II, IV [151] ↑ FCHL [55] HTg FCHL> NTg FCHL [55]	
Triglyceride	+ve [39,53,119, 147,152] −ve [216] ns [121]	+ve [50,55,56, 147,150,151,160]	+ve [128] ns [204]
Non-esterified fatty acids	+ve [39,53,119, 210] ns [147] −ve [216]	+ve [55,147]	+ve [53]
Apolipoprotein B	+ve [147] ns [39,121]	+ve [55,147,160]	+ve [53]
Total cholesterol	ns [119,121,147]	+ve [50,51,55,56, 147,151,160]	
LDL cholesterol	−ve [216] ns [121]	+ve [55,160] +ve Lp (a) [52]	
HDL cholesterol	−ve [39,147] ns [121]	−ve [50,55,147]	
Correlation of ASP with C3: +ve $r=0.38$ [147], 0.43 [49], 0.58 [39], 0.68 and 0.92 [153] and ns [140,180]			

ASP and C3 were measured by immunoassay and correlated to the various indices. Abbreviations are: +ve (significant positive correlation), −ve (significant negative correlation), ns (no significant correlation), CAD (coronary artery disease), HapoB (hyperapobetalipoproteinemia), FCHL (familial combined hyperlipidemia), HTg (hyper-triglyceridemia), NTg (normo-triglyceridemia), Lp(a) (lipoprotein (a)).

5.3. HyperapoB: in vivo to in vitro functionality

While the dyslipidemia which is common to insulin resistance, syndrome X and CAD likely has multiple causes [155–157], it has been proposed that abnormal fatty acid fluxes to the liver may be one driving component [158]. One interesting link supporting the notion of ASP resistance has been demonstrated in HyperapoB subjects tested for ASP response. Cells from HyperapoB subjects with increased plasma ASP appear to have a reduced response to ASP [18], those with normal plasma ASP responded normally to ASP when tested in cell culture [18], suggesting that a high plasma ASP might be a marker of ASP resistance. In some patients, reduced adipose tissue response to ASP could result in increased hepatic fatty acid flux, which stimulates increased apolipoprotein B production (both VLDL and LDL) [159], explaining the associations between C3, ASP, lipids and insulin parameters. In fact this hypothesis of "ASP resistance" in adipose tissue, which we have proposed [159], is seconded by several authors in their studies [39,121,153,160] although not by all [147].

6. Relationship of C3 with ASP

Surprisingly, considering the number of studies on C3 and ASP in the last few years, there are few studies that have actually examined both proteins, and even fewer that have directly compared the levels of ASP to C3, especially considering that ASP is the product of C3 cleavage. In a study by Ylitalo et al. [147], a strong familial component to both C3 and ASP was identified. Thus, C3 demonstrated a significant sibling–sibling association, and ASP a significant parent–offspring correlation. However, in spite of the association of C3 with CAD, there was no association between C3 and familial history of myocardial infarction, suggesting that the association of C3 to CAD is not solely due to hereditary linkages [50]. In those studies that have compared both C3 and ASP (Table 6), while one study showed strong correlations (up to $r=0.92$) [153], most are not at all strong. In fact, in two studies, there was no significant correlation at all, suggesting that determination of ASP concentration will depend not only on production of C3, but also on factors which regulate conversion of C3 to ASP.

6.1. Conversion of C3 to ASP and C3 polymorphisms

The conversion of C3 to ASP requires multiple factors. Adipsin is needed to cleave factor B to generate the membrane-attached active convertase (C3bBb), which in

turn is stabilised by properdin, but destabilised by factors H and I [161]. Thus, the concentration of any one of these factors may determine the overall kinetics of the reaction.

However, other than the concentration of any one of these factors, especially C3, the structure of the proteins themselves may well influence ASP production. It has long been recognised that C3 polymorphisms occur predominantly in two forms: C3F and C3S, termed fast and slow according to migration on electrophoretic gels [162]. The molecular basis for this difference was resolved by Botto et al. [163] who demonstrated a C to G shift at bp 364 in exon 3 leading to an Arg (+) to Gly (neutral) shift in amino acid 102. The frequency of C3S is much more common than C3F, especially in non-Caucasian populations with frequencies of 0.79, 0.95, 0.97 and 0.99 in Caucasian, African American, SA Indian and Asian, respectively [74]. A similar polymorphism distribution has been noted in mice (C3A and C3B), as in humans (C3S and C3F), and this is associated with differences in functional activation [164] and, possibly, with strain differences [165].

There is no evidence that the variant of C3 influences plasma C3 levels [162,166–168], but it has been suggested that C3F may have increased activation rates [162,164,169] which might result in increased plasma ASP. C3F also has increased binding to its clearance receptor (CR) [163,164,166] although both these differences have not been confirmed in all studies [166,169,170].

Correlation studies suggest that the C3F variant has an increased association with atherosclerosis and hypertension [171–174] and with hypercholesterolemia [175,176] but not with Type I or Type II diabetes [177]. In addition, C3F is associated with PLD [74,163,178] and in one study, it was demonstrated that PLD was associated with a change in the distribution with proportionally less serum C3F present in affected individuals [179]. It remains to be seen in future studies whether the generation of ASP is influenced not only by C3 levels, but also by C3 polymorphisms, as well as other factors.

6.2. Postprandial ASP and C3

Considering the functional role proposed for ASP, and the correlation between ASP and C3 with plasma triglycerides and other lipids (Table 6), examination of postprandial ASP is natural. To date, there are five studies on postprandial ASP and three on postprandial plasma C3 in human subjects. While the fat load meal from study to study varies in daily calorie allotment (20–60%), and in fat content (28–100 g fat), the results are similar.

Two studies on C3 [39,180] reported no change, and another study reported a small (24%) but significant increase in C3 at 2–6 h postprandially [160]. As the authors point out [160], because systemic ASP levels do not change postprandially, suggesting that ASP production is a local phenomenon, they have focused on postprandial C3 changes. While our initial study reported much higher levels of plasma ASP which increased postprandially [181], we later realised that this was due to antibody cross-reaction with C3 [182] and, in retrospect, would be consistent with the present findings by Halkes et al. on C3.

Fat load studies demonstrate either no change in plasma ASP [39,147,180] or a slight tendency for ASP to decrease over time [119,125]. Only one study with a very high fat load (100 g) demonstrated a significant linear positive trend vs. time [125]. In a recent study with post-obese subjects [125], plasma ASP tended to be lower in post-obese vs. controls, and there was a significant drop in ASP in both groups over the 8 h. In fat loads with obese diabetic subjects, fasting and postprandial ASP were consistently higher in obese diabetics vs. control [39,119], but not different from obese [39]. Parallel determinations of C3 in these patients showed no difference in any group [39]. While there was no postprandial change in ASP, fasting ASP did correlate with postprandial triglyceride and NEFA AUC [39]. Interestingly, while obese Caucasians had markedly increased fasting and postprandial levels compared to controls, in Pima subjects, the differences between obese and controls were less pronounced [119]. In this last study [119], there was a significant decrease in plasma ASP over the 6 h in all subject groups (control and obese, Caucasian and Pima). The authors suggest that the observed decrease in ASP concentrations reflects either an underlying diurnal rhythm as is seen with leptin [183,184] or that postprandially, ASP is shifted from the intravascular to the interstitial compartment where it exerts its biologic action.

In FCHL subjects, fasting ASP was significantly higher than controls, and there was no consistent change in ASP over the fat load. Although there was a trend to differences in incremental AUC between FCHL (average 149 ng/ml/h) and controls (26 ng/ml/h), the high variation precluded significance. The effects of hyperlipidemic treatment on fasting and postprandial C3 have been examined in an interesting study by Halkes et al. [160]. In a group of normolipidemic non-obese CAD patients, plasma C3 and postprandial C3 AUC were significantly increased vs. control subjects. Following 5 weeks of treatment with simvastatin (an HMGCoA reductase inhibitor), there were decreases in fasting plasma cholesterol, LDL cholesterol, triglyceride, and apolipoprotein B with no change in either glucose, insulin or BMI. There was also a significant drop in both fasting C3 and postprandial C3 AUC with treatment. Fasting C3 and postprandial C3 correlated with triglyceride AUC. With treatment, the drop in triglyceride AUC was the only variable that correlated with the drop in fasting C3. Plasma C3 was also significantly associated with several features of the insulin resistance syndrome (glucose, triglyceride, insulin, BMI, blood pressure and waist circumference), consistent with the studies above [50,51]. Treatment with statins not only increases LDL clearance, but also decreases VLDL production [185] and enhances chylomicron clearance [186,187]. The decreased chylomicron concentration may reduce the drive for C3 and ASP production;

at least this is a powerful stimulus to both C3 and ASP secretion in vitro from human adipocytes [44–46].

7. Integration of CAD, insulin resistance, ASP and complement

7.1. ASP deficiency vs. ASP resistance

Teleologically, then, a dysfunctional ASP pathway might result from either an absence of ASP (ASP deficiency), or a decrease in response to the biologic action of ASP (ASP resistance). This is the same argument that might apply to insulin or leptin. Both a lack of leptin (rare mutations) [188] and the proposed leptin resistance [189] are associated with obesity. Similarly, a lack of insulin (Type I diabetes) or insulin resistance (Type II diabetes) unquestionably both result in disturbances of glucose homeostasis. However, the remaining metabolic profiles are not necessarily the same. Insulin-resistant Type II diabetes is associated with obesity, especially visceral obesity, increased NEFAs, dyslipidemia and HyperapoB [190]. Type I diabetes is not. By analogy, then, although we cannot predict with certainty the phenotype of ASP deficiency vs. ASP resistance, we should not necessarily expect the metabolic profiles to be identical. Hypothetically, based on studies in C3 KO ASP-deficient mice [84,85,116], one might expect ASP-deficient subjects to be lean but with the potential for delayed triglyceride synthesis. Again, hypothetically, ASP resistance by extension from responses in HSF from hyperapoB subjects, could demonstrate reduced response to ASP coupled with increased plasma ASP and apoB [18].

7.2. Adipose tissue, liver and arterial wall

This review has focused on the association of ASP and C3 with CAD and associated risk factors, especially insulin and insulin resistance, with a focus on the links to adipose tissue metabolism. On the other hand, the interactions of C3 and complement (and thus ASP) with liver metabolism and arterial wall milieu cannot be ignored, as these may also contribute to the described associations with CAD and diabetes. Granted, it is difficult to identify the relative contributions of all of these circulating factors (C3, ASP, lipids, glucose, insulin) on individual tissues (adipose tissue, liver and arterial wall) to determine the contributions to metabolic disturbances (obesity, insulin resistance, dyslipidemia and atherosclerosis).

The cycle of association between increased C3, CAD and lipids [50,51,56] may simply reflect the fact that these lipid risk factors induce atherosclerosis. In the arterial wall, this leads to increased activated macrophages in atherosclerotic plaques which in turn can produce C3 [191]. C3 has been identified in extracts from atherosclerotic plaques [192,193], and it has been suggested that a cholesterol–albumin-rich extract from plaques may actually activate complements [194]. As pointed out by Ylitalo et al. [147], "it remains unclear whether C3 is an independent risk factor or a surrogate marker of vascular inflammation and atherosclerosis". On the other hand, although C3 appears to bind to both LDL and Lp(a) [195], neither these nor oxidised LDL, had any effect on complement activation [192,194]. While complement receptors CR1 and CR3 have been identified by immunological techniques in the arterial wall, so have many other proteins such as apolipoprotein B, albumin, α2 macroglobulin, HDL and α1 antitrypsin [192,196–198], suggesting a nonspecific infiltration of plasma proteins into damaged arterial wall.

The associations of C3 and ASP with CAD and dyslipidemia could also reflect secondary mechanisms involving hepatic C3 production. Certainly the liver has long been proposed as a major source of C3 [27]. As discussed by Muscari et al. [50], while there is a strong association of C3 with insulin, other acute-phase proteins (PAI-1, fibrinogen and C-reactive protein) also correlate with insulin [199–201]. This may be an effect of cytokines (such as TNFα) which induce both acute-phase protein synthesis in the liver and insulin resistance. In a recent commentary, Yudkin et al. [202] proposed that the source of such cytokines may actually be the adipose tissue. Insulin can inhibit the cytokine-mediated effects on hepatic synthesis of acute-phase proteins including C3 [203], an effect that might be abrogated in insulin resistance. Weyer et al. [49] also suggest direct links between insulin resistance and C3 production in hepatic and adipose tissue.

In the study by Muscari et al. [50], in multivariate analysis, the relationship between risk factors and C3 persisted after the addition of insulin to the independent variables. Blood pressure and glucose were better predicted by C3 than by insulin, leading the authors to comment that "serum C3 might be a better marker of insulin resistance than insulin itself" [50]. Similar considerations apply to adipsin and ASP. There are strong associations between adipsin and PAI-1 as well as other related factors (BMI, insulin and triglycerides). Changes following weight loss might be explained by either hepatic or adipose tissue modulation of PAI-1 production [128,204]. In the study by Ebeling et al. [140], while ASP correlated strongly with glycemic improvement in treatment of diabetics, ASP also correlated with a number of acute-phase proteins, which are characteristically increased in Type II diabetes. As the authors point out, the correlation between changes in inflammatory factors and glycemia was not stronger than the decreases in HbA_{1c} and ASP, suggesting other mechanisms of association.

One intriguing point to be addressed is the apparent overlap between the immune system and adipose biology. Which one actually originated first is an important question. Evolutionarily, C3 appears to be present in organisms that lack a complement lysis pathway. This immune/adipose cross talk is true not only for ASP, but also for adipsin (factor D), TNFα, angiotensin II, many interleukins, TGFβ, all of which are produced by adipocytes, influence adipo-

genesis [205] yet have well-recognised roles in immune (or other) systems. A number of review articles have commented on this duality [206–209]. In fact, many proteins have been shown to fulfill dual roles, depending on tissue expression. Thus, we cannot completely rule out the involvement of hepatic and arterial wall immune responses in the associations of C3/ASP with CAD, obesity and metabolic dysfunction.

8. Conclusion and future directions

In this review, we have summarised recently published data on ASP and C3, and attempted to present the cellular, rodent and human studies in an integrated physiological model. In cellular studies, ASP increases fat storage through increased triglyceride synthesis and decreased intracellular lipolysis. In rodent models, absence of ASP results in reduced adipose tissue; obesity is associated with increased ASP. Evidence is accumulating in humans which supports a role for ASP and C3 in adipose tissue function and maintenance of metabolic homeostasis. This is particularly noted in the links between ASP and C3 with diabetes and CAD and well-recognised risk factors such as lipids and glucose/insulin. While the etiology of many of these relationships is not yet understood, future studies in understanding the signalling mechanisms of ASP in health and disease will lead us in that direction.

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