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Effect of body weight and caloric restriction on serum complement proteins, including Factor D/adipsin: studies in anorexia nervosa and obesity

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SUMMARY

Complement plays important roles in host immune defences, and recent studies suggest that adipose tissue is an important site of production for some complement proteins. Starvation has been associated with low complement levels, but studied populations have usually had concomitant opportunistic infections or other conditions which might affect complement levels. To determine the impact of body weight and changes in body weight on serum complement, we investigated levels of complement proteins in otherwise healthy patients with a wide range of body weights, including patients with anorexia nervosa before and after treatment, obese dieters before and after weight loss, and normal weight controls. We found that complement proteins of the alternative pathway (C3, B, and D), alternative pathway haemolytic activity (AP50) and the inhibitors H and I were low in starving anorectics and normalized with weight gain. C3a levels were comparable in anorectics at low weight and after weight gain, indicating that low serum complement levels were attributable to hypoproduction and not complement cascade activation with consumption. Further, levels of C3, B, AP50, H and I, but not D, were higher than controls in obese patients and decreased toward normal after weight loss. Overall, percentage of ideal body weight, changes in body weight, and serum transferrin were each highly correlated with serum levels of complement proteins. We conclude that levels of alternative pathway complement components are determined in part by factors that influence body weight and by weight changes, possibly due to changes in production in adipose tissue or at other sites.

Keywords human complement body weight anorexia nervosa obesity

INTRODUCTION

Complement plays a major role in host defence against invading microbes, via either the classical or alternative pathway [1–3]. Most complement proteins are synthesized by the liver and by macrophages [1–3]. Recent observations that adipose tissue is the site of production of some complement components, especially Factor D, suggest previously unsuspected links between the systems of energy balance and immunity [4–8].

Studies of complement in patients with low or changing body weight can provide insights regarding production of complement components and the mechanisms regulating their synthesis that operate *in vivo*. For example, starvation in third world children with protein-energy malnutrition has been associated with decreased complement levels [9–12]. However, it remains unclear if the low complement levels are due predominantly to impaired production, or if consumption due to chronic activation of the complement cascade by recurrent infections may play a role [12,13]. It would be desirable to study complement in subjects who severely restrict calories but are otherwise healthy. Patients with the eating disorder anorexia nervosa and obese dieters are two such populations.

Anorexia nervosa is a serious eating disorder in which patients, usually girls and young women, severely restrict calorie intake; body weight subsequently declines and may progress to a body weight far below ideal. Reports on a few patients have suggested that some complement components, especially those of the alternative pathway, may be deficient in patients with anorexia nervosa, but this has not been systematically studied [14–16]. Complement proteins have not been previously studied in patients with obesity.

Therefore, to delineate further the impact of caloric restriction and body weight on complement, we studied complement components in otherwise healthy women with anorexia nervosa. In order to distinguish effects of low body weight *per se* from effects of caloric restriction and changing body weight, we also studied

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complement levels in a group of obese women being treated with severe caloric restriction on a very low calorie diet.

PATIENTS AND METHODS

Subjects

Patients were recruited from the Eating Disorders Programme in the Department of Psychiatry at the University of Minnesota Medical Centre. For patients with anorexia nervosa, subjects were identified at admission to the in-patient treatment unit. Women over the age of 16 years were eligible for the study if they met DSM-IIIR criteria for anorexia nervosa [17], were < 80% of ideal body weight, and were able to provide informed consent (including parental consent for minors). Anorectic patients were treated with an intensive, standardized programme utilizing cognitive behavioural techniques. A trained dietitian prescribed caloric intake necessary for the desired weight gain and to ensure adequate protein intake. For obese patients, subjects were identified at admission to an out-patient treatment programme which utilized a very low calorie diet that consisted of liquid supplements that supplied essential nutrients, including protein. Some of these subjects were also randomized to receive cognitive behavioural therapy. Women over the age of 18 years were eligible for the study if they weighed at least 140% of ideal body weight and provided informed consent. For both groups, patients were excluded if they were receiving psychotropic medications or medications known to interfere with immune function, such as corticosteroids. Ideal body weights were determined from the 1983 Metropolitan Insurance Tables. Nineteen anorectic patients and 12 obese subjects met eligibility criteria and were enrolled in the study.

Normal weight women ($n = 18$) were recruited from laboratory personnel ($n = 12$) and the general population by newspaper advertisement ($n = 6$) to serve as controls. Control subjects had to be 90–125% of ideal body weight. Subjects were excluded if they had a past history of an eating disorder or other major psychiatric illness, or currently had any other significant medical condition, or were receiving psychotropic medications or medications known to interfere with immune function.

Calorie counts were performed by the dietitian for all anorectics at baseline and during treatment. Although caloric intake was dramatically reduced in anorectics at baseline, protein intake for all subjects met the USA required daily allowance standards. Adequate protein intake was ensured during in-hospital therapy. Obese dieters had adequate protein intake delivered by the liquid protein supplements prescribed during treatment.

Serum samples were obtained from anorexia nervosa patients at the time of hospital admission (baseline) and after achieving their goal weight, usually 90% of ideal body weight (final). Serum samples were obtained from obese subjects at the time of enrolment in the out-patient study (baseline) and at a follow-up visit 3 months later (final), when they had just completed the very low calorie diet.

Body weights

Patients with anorexia nervosa weighed only about two-thirds of their ideal body weight at enrolment, and with therapy re-gained almost 20% of their ideal body weight (Table 1), with an average weight increase of $10{\cdot}8 \pm 5{\cdot}5$ kg ($P < 0{\cdot}0001$). Obese patients weighed an average of 1·7 times their ideal body weight (Table 1). After the 3-month diet period, obese patients' weights had decreased to an average of 1·4 times their ideal body weight (Table 1); obese dieters lost an average of $15{\cdot}7 \pm 3{\cdot}5$ kg ($P < 0{\cdot}0001$).

For comparison, anorectic patients at baseline weighed an average of 30·6% below their ideal body weight, while obese patients lost an average of 27·3% of their ideal body weight during treatment. Nevertheless, obese patients remained an average of 41·5% greater than ideal body weight at the end of the study period.

Estimated nutritional status

Transferrin was measured as an indicator of nutritional status. Anorexia nervosa patients had significantly lower transferrin levels at baseline than controls (Table 1). In anorectics, transferrin levels increased an average of $86{\cdot}2 \pm 63{\cdot}5$ mg/dl, returning to normal with successful treatment ($P < 0{\cdot}0001$, Table 1). Transferrin levels in obese patients were similar to those of controls at baseline and dropped an average of $43{\cdot}5 \pm 31{\cdot}9$ mg/dl after dieting (Table 1, $P = 0{\cdot}001$). However, transferrin levels in untreated (starving) anorectics were significantly lower than the levels observed in obese patients after 3 months on the very low calorie diet (259 *versus* 281 mg/dl, $P < 0{\cdot}05$).

Complement assays

Serum was immediately separated and stored at −70°C. All complement assays were performed in a single laboratory in

Table 1. Characteristics of anorexia nervosa, obese, and control study populations

		Anorexia nervosa ($n = 19$)	Obesity ($n = 12$)	Control ($n = 18$)	*P*
Age (years)		24·9 ± 7·9	40·5 ± 7·2‡§	28·2 ± 7·0	< 0·0001
Weight (kg)	Baseline	39·9 ± 7·4‡	97·6 ± 15·7‡§	59·9 ± 6·4	< 0·0001
	Final	50·7 ± 5·4*	81·9 ± 13·6*‡§		< 0·0001
% ideal body wt	Baseline	69·4 ± 10·0‡	168·8 ± 22·9‡§	107·3 ± 10·9	< 0·0001
	Final	88·5 ± 4·9*‡	141·5 ± 19·1*‡§		< 0·0001
Transferrin (mg/dl)	Baseline	259·3 ± 67·1‡	324·5 ± 66·0§	321·2 ± 33·8	0·002
	Final	345·4 ± 45·5*	281·0 ± 58·4†§		0·002

* $P < 0{\cdot}0001$ *versus* baseline; † $P < 0{\cdot}001$ *versus* baseline; ‡ $P < 0{\cdot}05$ *versus* control subjects; § $P < 0{\cdot}05$ anorexia nervosa *versus* obesity.

batched groups. Serum C3, C4, and Factor B levels were determined by standard nephelometry techniques using the Beckman Array Protein System. For CH_{50} assays, serum samples in serial dilution were mixed with sensitized sheep erythrocytes and the OD_{414} of the supernatant was determined after incubation at 37°C for 60 min. Results are expressed as the reciprocal of the dilution required to lyse 50% of the erythrocytes [18,19]. To assess function of the alternative complement pathway, AP50 assays were performed by mixing dilutions of serum samples with rabbit erythrocytes and determining the OD_{414} of the supernatant after incubation at 37°C for 60 min. Results are expressed as the reciprocal of the dilution required to lyse 50% of the erythrocytes [20]. Serum levels of C1q, C2, C5, C1-inhibitor and Factors H and I were measured by radial immunodiffusion using commercially available kits (The Binding Site Inc., San Diego, CA).

Factor D was measured by a haemolytic assay [21]. Factor D-depleted serum was prepared by passing human serum over a Bio Rex-70 cationic exchange resin (BioRad Labs, Hercules, CA). Dilutions of serum samples were then added to the D-depleted human serum and incubated with rabbit erythrocytes in Mg-EGTA for 1 h at 37°C, and supernatants were read at OD_{414}. Concentrations of D in μg/ml were determined by comparison with a standard curve using known amounts of purified D (Quidel, San Diego, CA).

To determine if low serum complement levels were attributable to hypoproduction or to activation of the complement cascade with consumption, we performed additional studies of the activation peptide C3a. C3a serum levels were determined by radioimmunoassay. A positive control using zymosan-activated serum was 79 749 ng/ml.

Statistical analysis

Differences between groups in study population characteristics (age, weight, percentage of ideal body weight, transferrin) at baseline and at final assessment were compared using one way analysis of variance with Tukey's HSD *post hoc* comparisons. Changes from baseline to final assessment within each eating disordered group were examined separately using repeated measures analysis of variance.

Analysis of complement components of the alternative pathway (C3, Factor B, AP50), the classical pathway and membrane attack complex (C1q, C4, C2, CH_{50} and C5), and complement inhibitors (C1-inhibitor, Factor H, Factor I) was performed in a similar manner with the following exceptions: (i) a multivariate analysis of variance was performed separately for each group of complement levels to limit the number of comparisons; univariate analyses for individual components were considered significant only when the multivariate analysis was significant; and (ii) age was used as a covariate to control for baseline differences. A separate analysis of variance was performed for Factor D.

Correlations of baseline complement levels with transferrin and percentage of ideal body weight across all groups were examined using Pearson correlations. Correlations of baseline to final assessment change in complement and body weight for the anorectic and obese groups were evaluated using Pearson correlations.

RESULTS

Alternative complement pathway

Because a few small studies had reported that the alternative pathway might be preferentially affected in anorexia nervosa [14–16], we first studied components of this pathway including C3 (which also participates in the classical pathway), Factor B and the functional assay, AP50. In untreated subjects, serum levels of these alternative pathway components (C3, Factor B, AP50) were significantly different among the three study groups (multivariate $F = 8{\cdot}98$, $P < 0{\cdot}0001$) (Fig. 1). Univariate comparisons and *post hoc* tests revealed that C3 ($F = 13{\cdot}2$, $P < 0{\cdot}0001$), Factor B ($F = 12{\cdot}3$, $P < 0{\cdot}0001$) and AP50 ($F = 27{\cdot}9$, $P < 0{\cdot}0001$) were significantly lower in untreated anorectics and significantly higher in untreated obese patients at baseline in comparison with normal controls (Fig. 1). Serum levels of alternative complement pathway proteins were 1·5–2-fold higher in obese patients at baseline than in

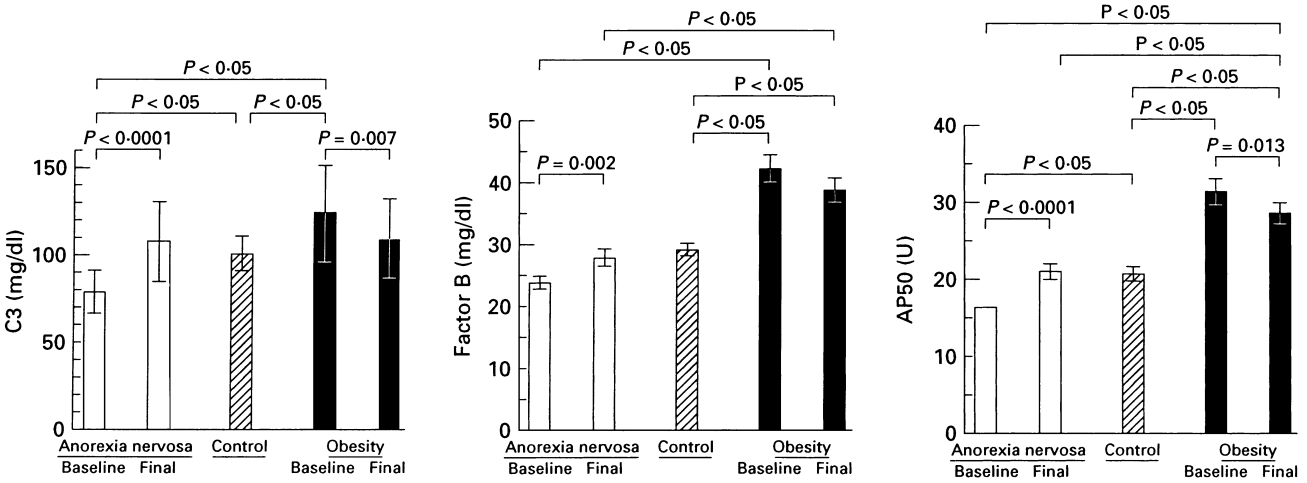

Fig. 1. Serum levels of complement components of the alternative pathway in patients with anorexia nervosa and obesity before and after treatment (mean ± s.e.m.). Serum C3, Factor B, and AP50 levels were significantly lower in untreated patients with anorexia nervosa and significantly higher in obese patients than in normal-weight controls. Treatment resulted in normalization of serum levels of alternative complement pathway components, with increased C3, Factor B, and AP50 levels in anorectics and decreased C3 and AP50, but not Factor B, levels in obese subjects. At study end, levels of C3, Factor B, and AP50 were comparable in treated anorectics and controls; levels of C3 were comparable in dieting obese subjects and controls, but differences in Factor B and AP50 levels between obese subjects and controls persisted.

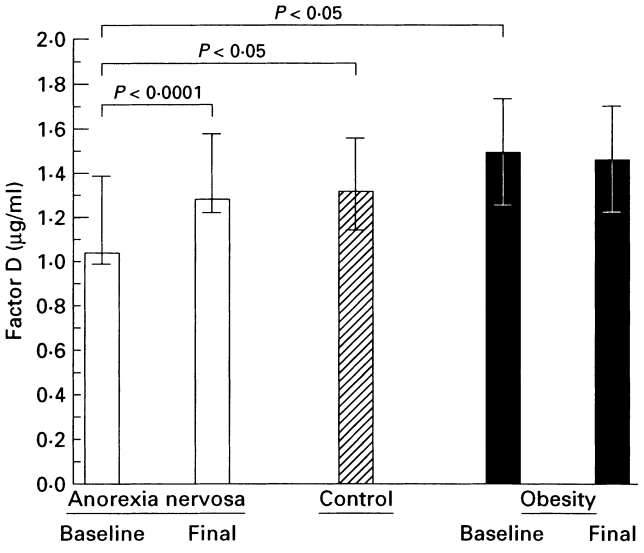

Fig. 2. Serum levels of Factor D/adipsin in patients with anorexia nervosa and obesity before and after treatment (mean ± s.e.m.). Factor D levels were significantly lower in untreated anorectics than normal-weight controls and, after treatment, increased significantly to levels comparable to controls. In contrast, obese patients had Factor D levels comparable to controls both before and after dieting.

untreated anorectics. Thus, a strong association between serum levels of alternative complement pathway proteins and body weight was observed.

For anorectics, treatment and consequent weight gain were associated with significant increases in serum components of the alternative pathway (multivariate $F = 11{\cdot}8$, $P < 0{\cdot}0001$). Univariate comparisons revealed significant increases after treatment in C3 ($F = 34{\cdot}1$, $P < 0{\cdot}0001$), Factor B ($F = 13{\cdot}1$, $P < 0{\cdot}002$) and AP50 ($F = 25{\cdot}9$, $P < 0{\cdot}0001$). C3 increased by 36%, B increased by 19%, and AP50 by 28%. Conversely, for obese patients, dieting and weight loss were associated with significant changes in serum components of the alternative pathway (multivariate $F = 7{\cdot}6$, $P = 0{\cdot}008$). Dieting obese patients experienced significant decreases in C3 ($F = 10{\cdot}8$, $P = 0{\cdot}007$) and AP50 ($F = 8{\cdot}8$, $P = 0{\cdot}013$), but not Factor B ($F = 1{\cdot}8$, $P = 0{\cdot}21$). C3 decreased by 11% and AP50 by 10%.

Multivariate comparisons revealed that significant differences remained among the three groups in alternative pathway complement components after treatment (multivariate $F = 8{\cdot}9$, $P < 0{\cdot}0001$). Despite normalization towards control values, differences in Factor B ($F = 7{\cdot}4$, $P = 0{\cdot}011$) and AP50 ($F = 10{\cdot}0$, $P = 0{\cdot}004$) levels remained among groups at study end (Fig. 1). The difference was due to persistent differences between levels in obese subjects and controls, possibly corresponding to persistent obesity (i.e. at study end, obese patients remained an average of 34% of ideal body weight heavier than controls). In contrast, serum levels of Factor B and AP50 in treated anorectic patients were comparable

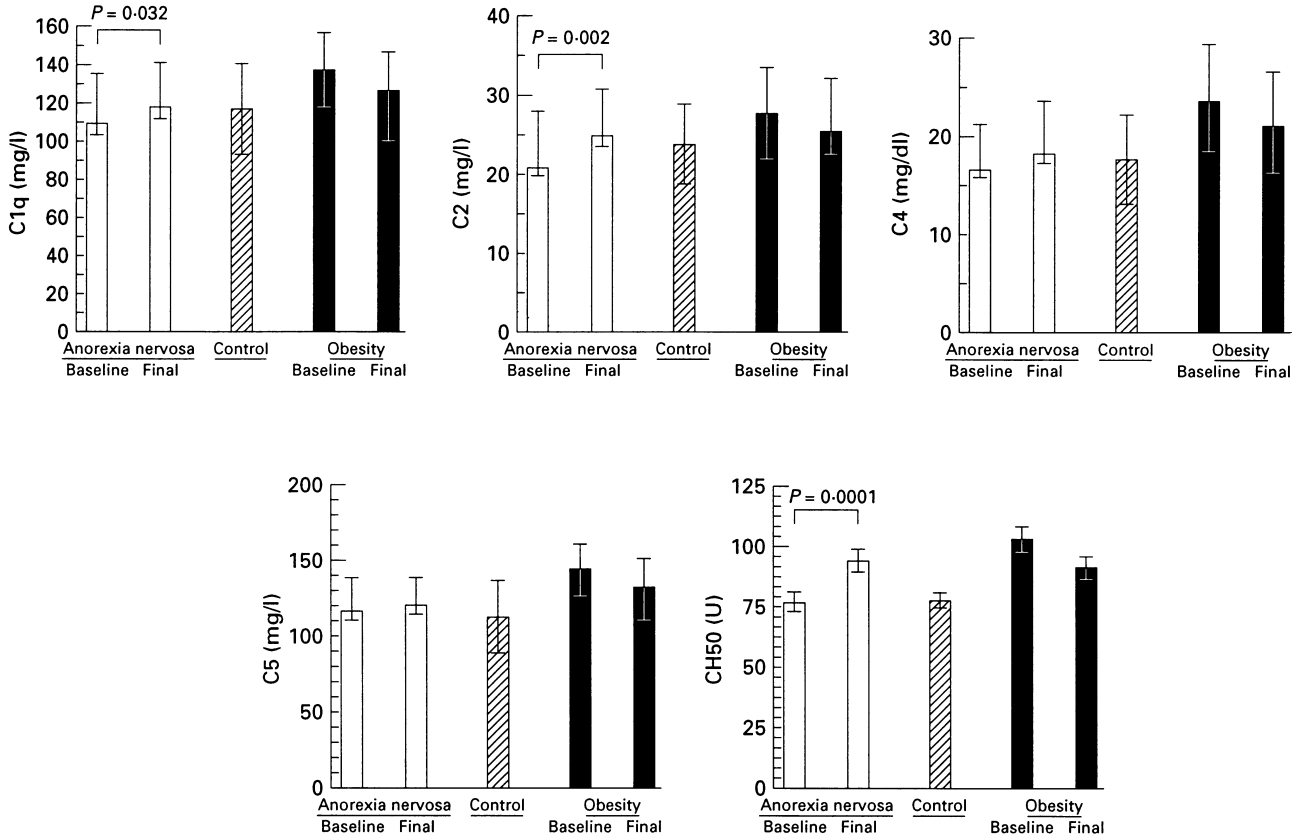

Fig. 3. Serum levels of complement components of the classical pathway and C5 in patients with anorexia nervosa and obesity before and after treatment (mean ± s.e.m.). Complement components of the classical pathway did not differ significantly among groups at baseline or after therapy. Levels in anorexia nervosa patients increased significantly from baseline to study end due to increases in C1q, C2 and CH_{50}. All complement components of the classical pathway and C5 were unchanged during dieting in obese patients.

to those of control subjects (Fig. 1). Further, serum levels of C3 did not differ significantly among the three study groups by study end ($F = 0{\cdot}1$, $P = 0{\cdot}74$) (Fig. 1). These results suggest that weight-associated variations in components of alternative complement pathway are at least partially correctable with normalization of weight.

Factor D (adipsin)

Factor D of the alternative complement pathway is now recognized to be identical with adipsin in both mice and humans, and is considered to be the rate-limiting step in the alternative complement cascade. In normal weight control subjects, D levels averaged $1{\cdot}30 \pm 0{\cdot}25$ μg/ml; these results are comparable to those previously reported by investigators using an ELISA assay [22].

Baseline levels of D were significantly different between the three study groups ($F = 9{\cdot}63$, $P = 0{\cdot}0003$). Levels of D in starving anorectics were significantly lower than in controls (Fig. 2). Factor D levels increased by 23% with refeeding ($F = 27{\cdot}2$, $P < 0{\cdot}0001$) (Fig. 2). Furthermore, D levels in untreated anorectics were 1·5-fold lower than in obese patients, suggesting that low body weight is associated with low D levels (Fig. 2). Although baseline D levels tended to be slightly higher in obese patients than in controls, the difference was not statistically significant. D levels did not decrease in obese patients during caloric restriction ($F = 1{\cdot}1$, $P = 0{\cdot}32$), i.e. at a time of decreasing body weight and low transferrin but persistent obesity. Thus, no significant differences in Factor D levels among the three groups were observed after treatment ($F = 1{\cdot}6$, $P = 0{\cdot}21$).

Classical complement pathway

We also studied components of the classical complement cascade (C1q, C2, C4, CH_{50}) and the membrane attack complex (C5). Overall, anorectics, obese patients and controls had comparable levels of these complement components both at baseline (multivariate $F = 1{\cdot}5$, $P = 0{\cdot}15$) and at the final assessment (multivariate $F = 0{\cdot}259$, $P = 0{\cdot}93$) (Fig. 3). These results suggest that there is no significant effect of body weight on complement components of the classical pathway, contrasting with the striking effect observed for alternative pathway components.

Treatment of patients with anorexia nervosa was associated with significant changes in classical complement pathway components (multivariate $F = 4{\cdot}48$, $P = 0{\cdot}012$). Univariate tests revealed significant increases in C1q ($F = 5{\cdot}4$, $P = 0{\cdot}032$), C2 ($F = 12{\cdot}6$, $P = 0{\cdot}002$) and CH_{50} ($F = 24{\cdot}5$, $P < 0{\cdot}0001$), but not C4 ($F = 2{\cdot}3$, $P = 0{\cdot}15$) or C5 ($F = 0{\cdot}98$, $P = 0{\cdot}34$). For obese patients, there was no significant change in any of the classical pathway complement components during dieting (multivariate $F = 0{\cdot}909$, $P = 0{\cdot}52$) (Fig. 3).

Inhibitors of complement activation

To explore complement further in patients with different body weights, we studied levels of factors responsible for negative regulation of the complement cascade. C1-inhibitor binds to and removes C1r and C1s from C1 complex; Factor H inhibits assembly and accelerates decay of C3bBb and is a cofactor for C3b cleavage by Factor I; and Factor I causes proteolytic inactivation of C4b and C3b. Low Factor H and/or I levels have been associated with uncontrolled activity of the alternative pathway, and thus, low C3 levels.

In our study, baseline serum levels of inhibitors of complement activation were significantly different among the three study groups (multivariate $F = 6{\cdot}1$, $P < 0{\cdot}0001$) (Fig. 4), suggesting a significant association between body weight and serum levels of these inhibitors. Univariate tests and *post hoc* comparisons revealed that Factor H ($F = 9{\cdot}6$, $P < 0{\cdot}0001$) and Factor I ($F = 11{\cdot}5$, $P < 0{\cdot}0001$) were significantly lower in starving anorectics and significantly higher in obese patients at baseline in comparison with normal controls. At baseline, levels of Factor H and Factor I were more that 1·5-fold higher in obese subjects than in starving anorectics. Levels of these inhibitors changed significantly after treatment in anorectics (multivariate $F = 9{\cdot}5$, $P = 0{\cdot}001$), with univariate tests showing increases in Factor H ($F = 20{\cdot}0$, $P < 0{\cdot}0001$) and Factor I ($F = 26{\cdot}0$, $P < 0{\cdot}0001$), but not C1-inh ($F = 2{\cdot}5$, $P = 0{\cdot}13$) (Fig. 4). H levels increased by 24% and I levels increased by 36% during treatment and weight gain in anorectics. In contrast, obese patients demonstrated no significant change in serum levels of inhibitors of complement activation after treatment (multivariate $F = 3{\cdot}31$, $P = 0{\cdot}07$). However, serum levels of Factors H and I decreased toward normal (by 13% and 14%, respectively) with caloric restriction and weight loss. Comparisons

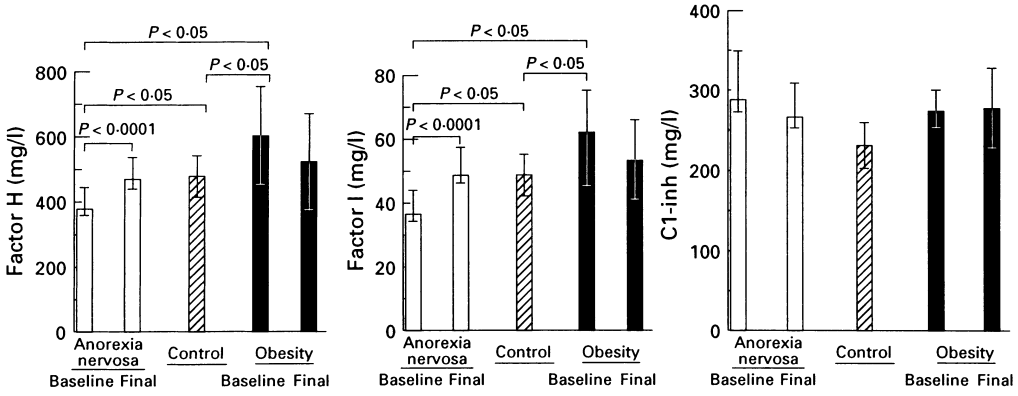

Fig. 4. Serum levels of inhibitors of complement activation in patients with anorexia nervosa and obesity before and after treatment (mean ± s.e.m.). Serum Factor H and Factor I levels were significantly lower in untreated patients with anorexia nervosa and significantly higher in obese patients than in normal-weight controls. Treatment resulted in an increase in Factor H and Factor I levels towards normal in anorectics; small decreases in Factor H and Factor I levels were observed in dieting obese subjects, but the changes did not reach statistical significance. At study end, serum levels of inhibitors of complement activation were comparable in all groups.

Table 2. Correlation of percent ideal body weight, nutritional status (transferrin), and serum complement in patients with anorexia nervosa and obesity prior to treatment

Complement component	Correlation with transferrin	*P*	Correlation with percent ideal body weight	*P*
Alternative pathway				
C3	0·593	< 0·0001	0·720	< 0·0001
Factor B	0·465	0·001	0·686	< 0·0001
Factor D	0·248	NS (0·09)	0·589	< 0·0001
AP50	0·481	< 0·0001	0·831	< 0·0001
Classical pathway				
C1q	0·460	0·002	0·447	0·003
C2	0·457	0·002	0·339	0·026
C4	0·256	NS (0·07)	0·474	0·001
CH_{50}	0·355	0·012	0·488	< 0·0001
Membrane attack complex				
C5	0·172	NS (0·27)	0·416	0·005
Inhibitors				
C1-inhibitor	−0·229	NS (0·14)	−0·208	NS (0·18)
Factor H	0·643	< 0·0001	0·690	< 0·0001
Factor I	0·549	< 0·0001	0·752	< 0·0001

of the three groups after treatment revealed no significant differences in serum levels of inhibitors of complement activation (multivariate $F = 0{\cdot}05$, $P = 0{\cdot}71$).

Correlation of percentage ideal body weight with serum complement

Complement components of both the alternative and classical pathways correlated closely with percentage of ideal body weight. For all groups (anorexia nervosa, control, obese) at baseline, correlations of percentage ideal body weight and C3, B, D, AP50, C1q, C2, C4, CH_{50}, C5, C1-inhibitor, H and I were each statistically significant (Table 2). Transferrin was used as an indicator of nutritional status. Transferrin correlated closely with most of the complement components studied; correlation coefficients were best for the components of the alternative complement pathway (Table 2).

The strong correlation of body weight with serum complement levels is reflected in our finding that the lowest serum complement levels were found in anorectic patients with the lowest body weights. For example, the most severely affected anorectic patient

Table 3. Correlation of change in serum complement levels and change in body weight after treatment in patients with anorexia nervosa and obesity

Complement component	Change in complement component		Correlation with change in body weight	*P*
	Anorexia	Obese		
Alternative pathway				
C3	+28·1 ± 21·0	−14·1 ± 14·8	0·845	< 0·0001
Factor B	+4·4 ± 5·3	−3·0 ± 7·8	0·573	0·001
Factor D	+0·24 ± 0·2	−0·03 ± 0·1	0·688	< 0·0001
AP50	+4·6 ± 3·9	−3·0 ± 3·5	0·800	< 0·0001
Classical pathway				
C1q	+9·7 ± 8·2	−10·4 ± 21·2	0·430	0·016
C2	+4·0 ± 4·9	−2·2 ± 5·2	0·575	0·001
C4	+1·6 ± 4·7	−2·4 ± 3·5	0·535	0·002
CH_{50}	+17·3 ± 15·2	−11·6 ± 23·2	0·590	< 0·0001
Membrane attack complex				
C5	+4·6 ± 20·4	−11·2 ± 20·9	0·330	NS (0·07)
Inhibitors				
C1-inhibitor	−21·2 ± 58·5	+4·7 ± 52·5	−0·237	NS (0·20)
Factor H	+90·3 ± 88·0	−78·2 ± 113·7	0·710	< 0·0001
Factor I	+13·0 ± 11·1	−8·7 ± 9·7	0·796	< 0·0001

Table 4. Serum C3a levels in anorexia nervosa

	Serum C3a (ng/ml) + s.e.m.
Anorexia nervosa ($n = 8$)	
Baseline	280 ± 30 (range = 201–430)
Final	290 ± 30 (range = 213–461)
Control ($n = 7$)	290 ± 32 (range = 161–406)

(weighing 21 kg, i.e. 47% of her ideal body weight) had the lowest levels of C3 (55 mg/dl), AP50 (10·3 U) and D (0·59 μg/ml). With treatment, she more than doubled her body weight to 49 kg and her alternative pathway complement levels also rose markedly, including C3 (131 mg/dl), AP50 (24·1 U) and D (1·22 μg/ml). Factor H levels rose from a baseline of 276 mg/*l* to 642 mg/*l* and I levels increased from 25 mg/*l* to 67 mg/*l*. Interestingly, classical pathway components were much less affected in this patient, with a baseline C4 level of 23 mg/dl, rising only slightly to 28 mg/dl at treatment end. C5 levels actually decreased from 131 mg/*l* to 97 mg/*l* during treatment.

Correlation of changes in body weight with changes in serum complement

To determine if changes in body weight were associated with changes in serum complement, we correlated changes in these parameters over the time of the study (Table 3). The pre- to post-treatment changes in C3, B, D, AP50, C1q, C2, C4, CH_{50}, C5, C1-inhibitor, H and I were each significantly correlated with change in body weight. Successful treatment of anorexia nervosa with caloric repletion and weight gain was consistently associated with increases in serum complement components of both the alternative and classical pathways. Dieting and the subsequent weight loss in obese patients were consistently associated with decreases in serum complement values. In the latter group, the decline in serum complement was from supra-normal levels toward normal.

C3a assays

To determine if low serum complement levels were due to hypoproduction or to activation of the complement cascade with consumption, we assayed C3a. C3a is an activation peptide which would be expected to be elevated with activation of the complement cascade and would remain normal if low serum complement levels were due to hypoproduction. We found that C3a levels were comparable in anorectics at low weight and after weight gain, and did not differ significantly from controls (Table 4).

DISCUSSION

Serum levels of complement components, especially those of the alternative pathway, varied with percentage ideal body weight and changes in body weight. As in other forms of starvation [12], complement levels were significantly below normal in low-weight anorectics, suggesting that low body weight *per se*, even in the absence of complement activation by opportunistic infections and in the absence of protein deficiency, results in decreased serum complement levels. Unexpectedly, we found that serum complement levels in obese patients were significantly higher than those in normal weight controls. Decreases in serum complement levels were seen after calorie restriction in both groups, regardless of initial weight. Taken together, these results support a significant role for body weight and changes of body weight in the determination of serum complement levels.

Complement components of the alternative pathway were preferentially affected by weight loss in both anorectics and obese dieters, while components of both the classical and alternative pathway have been reported to be decreased in protein-energy malnutrition [11]. The preservation of protein intake in the diet of anorectics and obese dieters (receiving liquid nutritional supplements) contrasts with the dramatically reduced protein intake documented by others in children with protein-energy malnutrition. These observations suggest that the components of the alternative pathway may be preferentially affected by loss of adipose tissue, while classical pathway components may be more sensitive to changes in protein status.

Decreased serum complement values that we observed could have been due to activation of the complement cascade with consumption, or alternatively due to hypoproduction. We found that the complement inhibitors H and I were significantly affected by weight loss. Since low Factor H and I levels can result in uncontrolled activation of the alternative pathway and thus low C3 levels, it has been proposed that defective H and I production is the primary defect in anorectics with low complement [16]. However, in our study, the levels of H and I in most anorectic patients did not appear to be low enough to compromise their regulatory function of the alternative pathway. Further, levels of C3a, an activation peptide, were comparable in starving anorectics, anorectics after weight gain, and controls. C3a should be formed if there is excessive spontaneous activation of the alternative pathway due to insufficient regulation. Therefore, low complement levels in anorectics cannot be attributed to activation of the alternative pathway; implying hypoproduction as the predominant etiology.

The cellular site of hypoproduction of alternative complement components during caloric restriction remains unknown. Factor D is produced predominantly in fat cells [4]; small amounts are also made in monocytes or macrophages. Therefore, decreased production of D in adipose tissue appears to be the most likely etiology of low D levels in anorectic patients. Hypoproduction of D by adipose tissue could be related to decreased synthesis per adipose cell, or due to decreased numbers of normally functioning adipose cells. In either case, a serum level proportional to adipose tissue mass, like that observed in our study, would be expected.

It is more difficult to conjecture about the cellular site of hypoproduction of the other alternative pathway components. Adipose cells also synthesize other essential components of the alternative pathway, including C3 and B [23] (though to a lesser extent than for D), but not components of the classical pathway. It is possible that low levels of C3 and B might be attributable to decreased production in adipose cells. However, the serum D levels are much lower than C3 and B levels and the turnover of C3 is quite rapid, suggesting that adipose cells would have to be producing a great deal of C3 if changes in serum C3 were due entirely, or even predominantly, to production in adipose cells. It seems more probable that the low C3 and B levels in patients with low or declining body weight may be attributable to hypoproduction at other sites, such as the liver. Therefore, the impact of changes in body weight on complement may be explained by decreased production of D in adipocytes and of other alternative pathway complement components at other sites, such as the liver.

Adipsin, now recognized as identical to human complement

Factor D [7,8], is a novel member of the serine protease family, which was first investigated in mouse models of genetic obesity. In these animals, adipsin levels were markedly reduced both in fatty tissue and in serum [5]. We found decreased levels of D in starving, low-weight anorectic patients, as had been previously reported [22], and confirmed that D increases with refeeding in these subjects. Decreased adipsin levels, as seen in starving anorectics, were initially reported in mice with genetic obesity. To explain this apparent paradox, we attempted to discern what similarities obese mice and starving anorectics might have. Genetic mouse obesity models (ob/ob and db/db) are characterized by glucocorticoid excess [5,6]; starving anorectics have marked activation of the hypothalamic–pituitary–adrenal axis with sustained hypercortisolism [24]. We hypothesize that glucocorticoid excess may be the factor responsible for low levels of D in both genetically obese mice and in starving anorectics. Regardless of the explanation, these observations reinforce that events in genetically obese mice do not necessarily correspond to events in humans. In obese patients, levels of D tended to be higher than in controls, but the increases were not statistically significant. In a previous report, adipsin levels in obese humans were significantly increased [22]. Since human obesity has a myriad of etiologies, these divergent results may reflect differences in the populations studied.

Our findings clearly demonstrate a role for body weight and changes in body weight in determining levels of alternative pathway complement proteins. Our studies were not designed to distinguish between the impact of low body weight *versus* the importance of specific nutrient deficiencies in causing the low complement levels which characterized untreated anorexia nervosa. Correction of nutritional deficiencies inevitably occurs during treatment designed to normalize body weight in anorectics. It is certainly possible that correction of nutrient deficiencies facilitates restoration of alternative complement pathway components in starving individuals, and that full restoration of adipose tissue may not necessarily be required for alternative pathway complement protein levels to normalize. Further studies would be required to clarify this issue. However, if nutritional effects *per se* were the determinants of complement levels, it would be expected that non-dieting obese patients and normal weight patients would have comparable complement levels. In fact, we found that obese patients actually had higher complement levels than did normal-weight controls. These findings would suggest that body weight itself plays a role in determining levels of alternative pathway complement components.

Interestingly, the low complement values observed in starving anorectics did not predispose these extremely low-weight patients to infection [25]. Although complement levels of anorectics were significantly decreased, the levels were apparently not low enough to increase infection risk, i.e. levels were not as low as those observed in congenital deficiencies [1–3]. The differential infection risk in anorectics *versus* children with protein-energy malnutrition may be attributable to the relatively normal classical complement cascade in anorectics and/or the absence of other risk factors that are present in many third world children, such as low T-helper cells and impaired granulocyte function [26,27], as well as poor socioeconomic conditions.

Our findings demonstrate that both percentage ideal body weight and changes in body weight impact on serum complement values. Levels of alternative pathway complement protein were low in starving anorectics and higher in obese patients than in normal weight controls. Low serum complement levels in anorexia nervosa were not associated with altered risk of infection or other clinical consequences, and normalized with correction of body weight. We conclude that changes in body weight, even in the absence of secondary infections or protein deficiency, affect serum levels of complement proteins, especially those of the alternative pathway. Our findings suggest that factors that influence body weight, and changes of body weight, contribute to control of serum levels of the alternative pathway complement proteins, and that these changes are due to altered production in adipose tissue and/or other sites.

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REFERENCES

1 Colten HR. Complement deficiencies. Annu Rev Immunol 1992; **10**:809–83.
2 Dalmasso AP. Complement in the pathophysiology and diagnosis of human diseases. Crit Rev Clin Lab Sci 1986; **24**:123–83.
3 Frank MM. The complement system. In: Samter M, Talmage DW, Frank MM, Austen KR, Claman HN, eds. Immunological diseases, 4th edn. Boston: Little, Brown and Co. Inc., 1988:203–32.
4 Lowell BB, Flier JS. Differentiation dependent biphasic regulation of adipsin gene expression by insulin and insulin-like growth factor-1 in 3T3-F442 A adipocytes. Endocrinology 1990; **127**:2898–906.
5 Flier JS, Lowell B, Napolitano A *et al.* Adipsin: regulation and dysregulation in obesity and other metabolic states. Rec Prog Hormone Res 1989; **45**:567–81.
6 Spiegelman BM, Lowell B, Napolitano A *et al.* Adrenal glucocorticoids regulate adipsin gene expression in genetically obese mice. J Biol Chem 1989; **264**:1811–5.
7 Rosen BS, Cook KS, Yaglom J *et al.* Adipsin and complement factor D activity: an immune-related defect in obesity. Science 1989; **244**:1483–7.
8 White RT, Damm D, Hancock N *et al.* Human adipsin is identical to complement factor D and is expressed at high levels in adipose tissue. J Biol Chem 1992; **267**:9210–3.
9 Hafez M, Aref GH, Mehareb SW *et al.* Antibody production and complement system in protein energy malnutrition. J Trop Med Hyg 1977; **80**:36–39.
10 Haller L, Zubler RH, Lambert PH. Plasma levels of complement components and complement haemolytic activity in protein-energy malnutrition. Clin Exp Immunol 1978; **34**:248–52.
11 Keusch GT, Torum B, Johnston RB *et al.* Impairment of hemolytic complement activation by both classical and alternative pathways in serum from patients with kwashiorkor. J Ped 1984; **105**:434–6.
12 Sirisinha S, Edelman R, Suskind R *et al.* Complement and C3-proactivator levels in children with protein-calorie malnutrition and effect of dietary treatment. Lancet 1973; **1**:1016–20.
13 Suskind R, Edelman R, Kulapongs P *et al.* Complement activity in children with protein-calorie malnutrition. Am J Clin Nutr 1976; **29**:1089–92.
14 Kim Y, Michael AF. Hypocomplementemia in anorexia nervosa. J Ped 1975; **87**:582–5.
15 Palmblad J, Fohlin L, Norberg R. Plasma levels of complement factors 3 and 4, orosomucoid and opsonic functions in anorexia nervosa. Acta Paediatrica Scandinavica 1979; **68**:617–8.
16 Wyatt RJ, Farrell M, Berry PL *et al.* Reduced alternative complement

pathway control protein levels in anorexia nervosa: response to parenteral alimentation. Am J Clin Nutr 1982; **35**:973–80.
17 American Psychiatric Association. Diagnostic and statistical manual of mental disorders, revised 3rd edn. Washington, DC: American Psychiatric Association, 1987.
18 Gaither TA, Frank MM. Complement. In: Henry JB, ed. Clinical diagnosis and management by laboratory methods. Philadelphia: WB Saunders, 1984:879–92.
19 Kent JF, Fife EH Jr. Precise standardization of reagents for complement fixation. Am J Trop Med 1963; **12**:103–16.
20 Platts-Mills TAE, Ishizaka K. Activation of the alternative pathway of human complement by rabbit cells. J Immunol 1974; **113**:348–58.
21 Lesavre PH, Hugli TE, Esser AF *et al.* The alternative pathway C3/C5 convertase: chemical basis of Factor B activation. J Immunol 1979; **123**:529–34.
22 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 Obesity 1994; **18**:213–8.
23 Choy LN, Rosen BS, Spiegelman BM. Adipsin and an endogenous pathway of complement from adipose cells. J Biol Chem 1992; **267**:12736–41.
24 Gold PW, Gurstman H, Avgerinos PC *et al.* Abnormal hypothalamic–pituitary–adrenal function in anorexia nervosa. N Engl J Med 1986; **314**:1334–42.
25 Pomeroy C, Mitchell JE, Eckert ED. Risk of infection and immune function in anorexia nervosa. Int J Eat Dis 1992; **12**:47–55.
26 Chandra RK, Kumari S. Nutrition and immunity: an overview. J Nutr 1994; **124**:1433–5.
27 Chandra RK. Nutrition and immunity: lessons from the past and new insights into the future. Am J Clin Nutr 1991; **53**:1087–101.