Alterations in endothelium-associated proteins and serum thyroid hormone concentrations in anorexia nervosa

BY GEN KOMAKI, HAJIME TAMAI, TOSHIO MUKUTA, NOBUYUKI KOBAYASHI, KENJI MORI AND TETSUYA NAKAGAWA

Department of Psychosomatic Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan

AND LINDY F. KUMAGAI

Department of Internal Medicine, School of Medicine, University of California, Davis (LFK), Sacramento, CA, USA

(*Received 2 January 1991 – Accepted 5 July 1991*)

Plasma concentrations of endothelium-associated proteins (EAP) (plasma fibronectin (PFN), angiotensin-converting enzyme, factor VIII-related antigen (F VIII-R:Ag)) and tissue plasminogen activator and serum thyroid hormone concentrations were studied in nine patients with anorexia nervosa (AN), before and after weight gain. Before weight gain (−35·9 (SE 2·3)% of standard body-weight) PFN was significantly reduced and F VIII-R:Ag was significantly increased in AN patients compared with the concentrations in control subjects (211·5 (SE 14·9) *v.* 274·7 (SE 16·6) μg/ml, $P < 0·05$; 129·2 (SE 14·1) *v.* 88·2 (SE 9·7)%, $P < 0·05$ respectively). Serum triiodothyronine (T3) and free T3 levels were also significantly lower before weight gain in AN patients (0·85 (SE 0·07) *v.* 1·53 (SE 0·08) nmol/l, $P < 0·001$; 2·57 (SE 0·23) *v.* 5·31 (SE 0·34) pmol/l, $P < 0·001$ respectively), although serum thyroxine (T4), free T4, and thyrotropin concentrations were within the normal range throughout the study periods. Following weight gain, PFN and F VIII-R:Ag concentrations normalized as did the thyroid hormone levels. The incremental changes in PFN levels correlated significantly with those in serum thyroid hormone concentrations (T3, r 0·79, $P < 0·01$; free T3, r 0·84, $P < 0·01$). These findings suggest that PFN levels may be directly related to serum T3 concentrations in AN patients.

Endothelium-associated proteins: Serum thyroid hormone: Anorexia nervosa

Malnutrition is the major feature in anorexia nervosa (AN) patients (Newman & Halmi, 1988). Protein intake, however, is usually adequate and vitamin deficiency is rare (Curran-Celentano *et al.* 1985), so the condition is different from protein-deficient malnutrition such as marasmus (Waterlow, 1972). The endothelial vessel wall is a metabolically active tissue (Saba, 1970) which synthesizes proteins such as collagen, fibronectin (PFN), and factor VIII-related antigen (F VIII-R:Ag), as well as other compounds like prostaglandins. Although PFN, a high-molecular-weight glycoprotein, is mainly produced by the liver (Tamkun & Hynes, 1983), a contribution from endothelial cells is also possible (Saba & Jaffe, 1980; Yamada, 1983) and factors regulating PFN pool have not yet been clearly defined (Tamkun & Hynes, 1983). Energy restriction itself alters these endothelium-associated protein (EAP) levels, as has been found during acute starvation (Scott *et al.* 1982; Howard *et al.* 1984; Komaki *et al.* 1988) and in patients undergoing parenteral nutrition (Horowitz *et al.* 1985). On the other hand, the changes in concentrations of EAP have been related to concurrent alterations in serum thyroid hormone levels (Rogers *et al.* 1982; Brent *et al.* 1984; Graninger *et al.* 1985, 1986; Smallridge *et al.* 1985; Azuma *et al.*

Table 1. *Clinical features of nine anorexia nervosa patients and normal controls*

Patient no.	Sex	Age (years)	Duration of illness (months)	Wt (kg)	% decrease of standard body-weight in phase: 1	2	3
1	F	15	12	30·50	−42·66	−33·92	−9·58
2	F	24	54	33·50	−30·35	−25·67	−10·60
3	F	17	33	28·65	−45·22	−28·29	−19·69
4	F	17	12	31·85	−36·42	−25·84	(-)
5	F	25	7	35·65	−26·55	−16·19	−5·43
6	F	19	24	34·05	−31·71	−20·37	−9·84
7	F	30	72	35·50	−32·95	−25·23	−17·52
8	M	16	12	44·60	−31·14	−22·74	−10·93
9	F	17	47	30·50	−46·32	−40·41	−22·13
Mean		20·0	30·3	33·77	−35·92	−26·45	−13·21
SE		1·7	7·6	1·48	2·38	2·39	2·05
Controls (*n* 8)							
Mean		21·0		48·11	−6·41		
SE		0·5		0·84	2·20		

Phase 1 (*n* 9), shortly after admission; phase 2 (*n* 9), at the time of 5 kg body-weight increase *v.* phase 1; phase 3 (*n* 8), at the time of 10 kg body-weight increase *v.* phase 1.

1987). However, the interrelationships between the changes in thyroid hormone and EAP concentrations are not identical in those conditions. Furthermore, the relationship between the hormonal profile of AN patients and the degree of weight reduction and undernutrition is still unclear, although most endocrine alterations recover with re-feeding and weight gain (Newman & Halmi, 1988). Therefore, serum thyroid hormone and EAP levels were measured in AN patients, when they were initially diagnosed and following restoration to normal body-weight, to gain further insight into these alterations and interrelationships.

SUBJECTS AND METHODS

Subjects

Nine hospitalized patients (eight females and one male) who met the criteria for AN according to DSMIII-R (American Psychiatric Association, 1987) were studied. Briefly, they fulfilled the following conditions: (1) refusal to maintain body-weight over a minimal normal weight-for-age and height (15% below that expected), (2) intense fear of gaining weight or becoming fat, even though underweight, (3) disturbance in the way in which one's body-weight, size, or shape is experienced, (4) in females, absence of at least three consecutive menstrual cycles when otherwise expected to occur (primary or secondary amenorrhoea). As shown in Table 1, the mean age was 20·0 (SE 1·7) years, and the mean duration of illness was 30·3 (SE 7·6) months. On admission, mean body-weight was 33·77 (SE 1·48) kg which is −35·92 (SE 2·38)% of standard body-weight for the same age, sex and height, as derived from tables of the Japanese Ministry of Health and Welfare (1986). Patients had not taken any medication for at least 2 weeks before the study. They had been continuously underweight for 6 months or more, being 25% below the standard body-weight and with no evidence of other metabolic or endocrinological disease. Renal and hepatic function were normal and no infectious diseases were noted. There were no significant electrolyte abnormalities at the time of study in these patients. The control subjects consisted of eight age-matched healthy female volunteers. Their average age was

21·0 (SE 0·5) years and their average body-weight was 48·11 (SE 0·48) kg which is 6·41 (SE 2·20)% below the standard body-weight.

Study protocol

All patients were in-patients on the ward of the Department of Psychosomatic Medicine at Kyushu University Hospital. They all gave informed consent for the study. All AN patients were treated by refeeding without medications. Weight gain was reinforced by cognitive-behaviour therapy and with continued psychotherapy. Their average energy intake commenced at 3·35–4·19 MJ/d, consisting of mixed food and tube feeding of elemental diet (Morishita Pharmaceutical Co. Ltd., Osaka), shortly after admission to the hospital. The daily diet was increased by 0·84 MJ every 2–3 weeks until they were ultimately eating 9·21–10·04 MJ/d of ordinary mixed food.

As noted in Table 1, the study was divided into three phases: phase 1 was shortly after admission; phase 2 was at the time of 5 kg body-weight increase; and phase 3 was when they had attained an increase of 10 kg. Patients were weighed daily at 06.30 hours after they had voided. Most patients gained approximately 0·7–1·0 kg/week so phases 2 and 3 occurred at about 2 and 4 months respectively after admission to the hospital.

Assays

Serum triiodothyronine (T3) and thyroxine (T4) were measured by radioimmunoassay (RIA) (Dainabot, Matsudo City); free T3 and free T4 by RIA (Amersham International Plc, Amersham, Bucks., UK); thyroid-stimulating hormone (TSH) was measured by a highly sensitive IRMA (immuno-radiometric assay; Dainabot, Matsudo City). Thyroxine-binding protein (TBG) was measured by RIA, and assay for prealbumin was determined by the method of radial immunodiffusion. Total protein, albumin and blood chemistry studies were measured by an automated multiple-analysis system (Special Reference Laboratory, Tokyo). PFN was estimated by a kinetic immunoturbidimetric assay using rabbit antiserum against human PFN (Saba *et al.* 1981). Serum angiotensin-converting enzyme (ACE) was measured by a colorimetric assay (Kasahara & Ashihara, 1981) using *p*-hydroxybenzoyl-glycine-L-histidyl-L-leucine as substrate (Fujebiro Pharmaceutical Co. Ltd, Tokyo). The F VIII-R: Ag concentration was measured by immunoelectrophoresis (Laurell, 1966) using anti-F VIII-R:Ag immunoglobulin G. Tissue plasminogen activator antigen (t-PA) in plasma was determined by the use of enzyme-linked immunosorbent assay (ELISA) (Bergsdorf *et al.* 1983).

For all assays, the intra- and interassay coefficients of variation were less than 5 and 10% respectively.

Statistical analysis

All values are expressed as means with their standard errors. Results were evaluated by means of paired and unpaired non-parametric tests (all two-tailed). Correlations between variables were determined by Spearman's correlation coefficients. The level of significance was set at 5%.

RESULTS

Table 2 shows mean plasma protein and metabolite concentrations throughout the study periods. All were within the normal range and no significant changes were observed.

Table 3 shows changes in concentrations of serum thyroid hormones and TSH in AN patients during the period of study and in normal controls. Serum T3 and free T3 levels were significantly lower during the initial phase 1 compared with normal controls ($P <$

Table 2. *Mean plasma protein and metabolite levels in nine anorexia nervosa patients before and after weight gain**

(Mean values with their standard errors)

	Serum urea nitrogen (mmol/l)		Creatinine (μmol/l)		Total protein (g/l)		Albumin (g/l)		Prealbumin (g/l)		TBG (nmol/l)	
Phase	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1 (*n* 9)	5·19	0·38	62·8	3·4	70	2	44	1	0·27	0·02	244·5	16·8
2 (*n* 9)	4·99	0·21	57·9	3·9	71	2	42	1	0·29	0·02	277·4	17·3
3 (*n* 8)	5·04	0·37	60·7	3·5	74	2	44	1	0·29	0·01	260·6	10·2

TBG, thyroxine-binding protein.

Phase 1, shortly after admission; phase 2, at the time of 5 kg body-weight increase *v.* phase 1; phase 3, at the time of 10 kg body-weight increase *v.* phase 1.

* For details of patients, see Table 1; for details of procedures, see p. 69.

Table 3. *Changes in serum triiodothyronine (T3), thyroxine (T4), free T3, free T4 and thyroid-stimulating hormone (TSH) in nine anorexia nervosa (AN) patients before (phase 1) and during weight recovery (phases 2 and 3) and eight normal controls (NC)*‡

(Mean values with their standard errors)

	AN							
Phase...	1		2		3		NC	
n...	9		9		8		8	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
T3 (nmol/l)	0·85***	0·07	1·24*††	0·09	1·51††	0·11	1·53	0·08
T4 (nmol/l)	79·9	8·0	90·5	4·3	91·0	5·1	99·5	5·9
Free T3 (pmol/l)	2·57***	0·23	3·89*††	0·31	5·18††	0·40	5·31	0·34
Free T4 (pmol/l)	13·7	1·0	12·8	0·5	13·4	0·7	14·8	0·7
TSH (mU/l)	2·3	0·50	2·28	0·30	2·17	0·31	2·91	0·47

Phase 1, shortly after admission; phase 2, at the time of 5 kg body-weight increase *v.* phase 1; phase 3, at the time of 10 kg body-weight increase *v.* phase 1.

Mean values were significantly different from those of NC: *$P < 0·05$, ***$P < 0·001$.

Mean values were significantly different from those of phase 1: ††$P < 0·01$.

‡ For details of patients, see Table 1; for details of procedures, see p. 69.

0·001) and increased significantly to be within the normal range at phases 2 and 3. Serum T4, free T4 and TSH concentrations were within the normal range throughout the study periods (the normal ranges: T3 1·2–2·8 nmol/l, T4 63–160 nmol/l, free T3 4·6–7·9 pmol/l, free T4 10·3–27·1 pmol/l, TSH 0·6–5·1 mU/l).

Fig. 1 shows changes in concentration of EAPs. PFN was significantly decreased at phase 1 ($P < 0·05$) and subsequently increased to be within the normal range following weight gain. On the other hand, F VIII-R:Ag was significantly increased at phase 1 ($P < 0·05$) and tended to decrease during restoration of body-weight. ACE levels were not

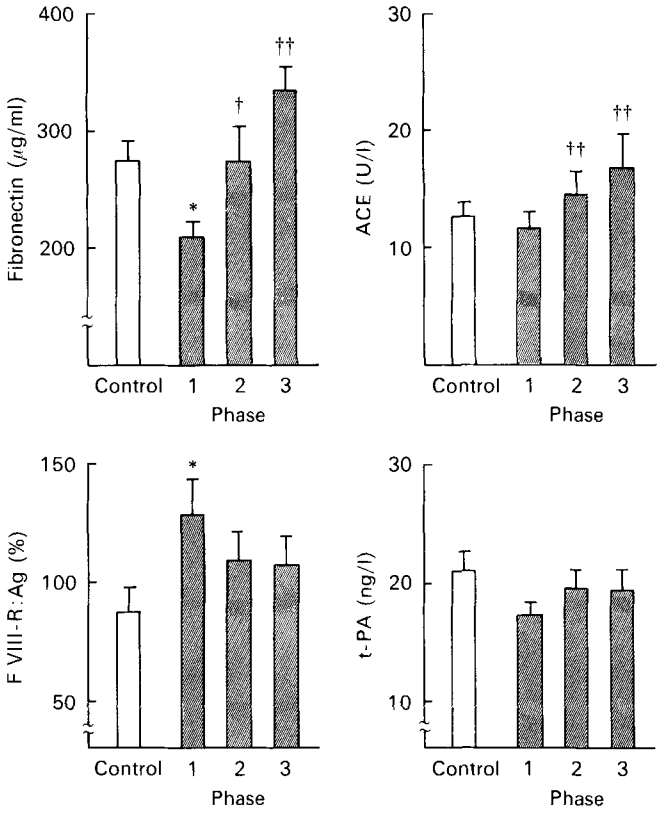

Fig. 1. Changes in plasma fibronectin, angiotensin-converting enzyme (ACE), tissue plasminogen activator (t-PA), and factor VIII-related antigen (F VIII-R:Ag) levels in nine anorexia nervosa (AN) patients before (phase 1) and during weight recovery (phases 2 and 3) and eight normal controls. AN patients: 1, shortly after admission; 2, at the time of 5 kg body-weight increase *v.* 1; 3, at the time of 10 kg body-weight increase *v.* 1. Mean values were significantly different from those of normal controls: $*P < 0{\cdot}05$. Mean values were significantly different from those of phase 1: $\dagger P < 0{\cdot}05$, $\dagger\dagger P < 0{\cdot}01$. For details of patients, see Table 1. For details of procedures, see p. 69.

significantly different from control subjects throughout the study periods, although they increased significantly following weight gain. t-PA concentrations remained normal in AN patients throughout the period of study (the normal ranges: PFN 250–460 μg/ml, ACE 8·3–21·4 U/l, F VIII-R:Ag 50–155%, t-PA ⩽ 7·6 ng/ml).

Significant correlations were found with PFN and thyroid hormone concentrations in patients with AN (phase 1) and normal controls (n 17, T3, r 0·75, $P < 0{\cdot}01$: T4, r 0·59, $P < 0{\cdot}05$; free T3, r 0·72, $P < 0{\cdot}01$). Fig. 2 shows the relationships between PFN and serum T3 concentrations in them. Other EAPs showed, however, no significant correlations with thyroid hormones. Incremental changes (phase 2 − phase 1/phase 1) in EAPs and thyroid hormones during weight gain in AN patients are shown in Table 4. The changes of PFN correlated significantly with those of thyroid hormones (n 9, T3, r 0·79, $P < 0{\cdot}01$; free T3, r 0·84, $P < 0{\cdot}01$). The same was found in the incremental changes from phase 1 to phase 3. No significant correlations were observed with the other EAPs and thyroid hormone concentrations. The change of ACE was only significantly correlated with that of body-weight (r 0·69, $P < 0{\cdot}05$).

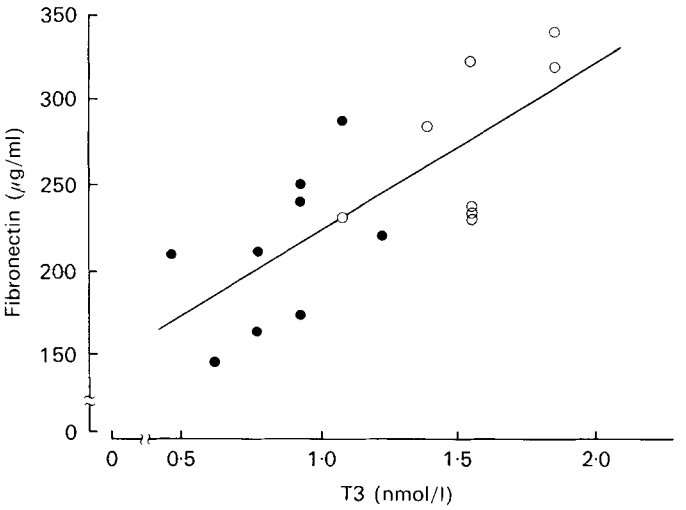

Fig. 2. The relationship between serum triiodothyronine (T3) and plasma fibronectin concentrations in nine patients with anorexia nervosa (phase 1; shortly after admission) (●) and eight normal controls (○). The regression line (r 0·75, $P < 0·01$): fibronectin = 98·33 × T3 + 125·78. For details of patients, see Table 1. For details of procedures, see p. 69.

Table 4. *Correlations between incremental changes (phase 2 − phase 1/phase 1) in serum thyroid hormone and endothelium-associated protein (EAP) concentrations in nine anorexia nervosa patients during weight gain*†

	PFN	ACE	F VIII-R:Ag	t-PA
T3	0·79**	0·12	0·49	0·39
T4	0·52	0·06	0·03	−0·34
Free T3	0·84**	0·05	0·21	0·30
Free T4	0·53	−0·01	0·01	−0·07

PFN, plasma fibronectin; ACE, angiotensin-converting enzyme; F VIII-R:Ag, factor VIII-related antigen; t-PA, tissue plasminogen activator; T3, triiodothyronine; T4, thyroxine.

**$P < 0·01$.

† For details of patients, see Table 1; for details of procedures, see p. 69.

DISCUSSION

In the present study, thyroid function in AN patients with severe weight loss demonstrated a 'low T3' state. This finding is confirmed by previous reports on AN patients (Newman & Halmi, 1988). The nutritional state of AN patients, however, appears to be different from protein–energy malnutrition (Ingenbleek *et al.* 1972; Waterlow, 1972), as indicated by concentrations of total protein and albumin as well as other rapid turnover hepatically-synthesized proteins. Serum protein concentrations are seldom below normal in AN, and plasma aminograms have a normal appearance, which is quite unlike that observed in kwashiorkor or marasmus (Waterlow, 1972).

With regard to EAPs, PFN was significantly decreased and F VIII-R:Ag was increased when the AN patients were markedly underweight. The reductions in PFN are confirmed

observed. This finding is similar to that reported in patients with diabetic ketoacidosis (Alexander *et al.* 1983), while no information was obtained regarding the correlations between PFN and serum T4 levels in those patients with a low T4 state. Based on the findings of the present study, the decreased T3 concentration can be explained by the concept 'low T3' state rather than hypothyroidism (Chopra *et al.* 1975). The 'low T3' state is attributed to the impairment of extravascular binding of T4 and its transport across the cell membrane, as well as to the defect of peripheral T4 conversion to T3 by 5′-deiodinase (*EC* 3.8.1.4) (Van Der Hyden *et al.* 1986). PFN concentrations increased to be within the normal range in association with restoration of body-weight and normalization of serum T3 concentrations. Accordingly, rather than PFN being an indicator of thyroid state, the parallel changes appear to be a reflection of the nutritional and metabolic state of AN patients during weight recovery.

On the other hand, conflicting information about the influence of thyroid hormone on fibronectin synthesis was noted in several experiments. While thyroid hormones inhibit cellular-type fibronectin synthesis by cultured human skin fibroblasts (Murata *et al.* 1987), PFN levels are up-regulated by thyroid hormones (Graninger *et al.* 1985, 1986; Azuma *et al.* 1987). Certainly, as compared with PFN, cellular or tissue fibronectin is a less soluble form of fibronectin, localized in the extracellular matrix of many cells and in connective tissue matrices (Yamada, 1983). The major source of PFN appears to be the liver (Tamkun & Hynes, 1983); however, factors regulating the PFN pool still remain uncertain (Yamada, 1983; Tamkun & Hynes, 1983). In the present study it is improbable that the decreased hepatic PFN synthesis was simply attributed to the low thyroid hormone concentrations (Graninger *et al.* 1985) because there is not a simple cause-and-effect relationship between them (Alexander *et al.* 1983), and both the synthesis and catabolism of proteins are generally affected by thyroid hormones (Azume *et al.* 1987). Hypothyroid patients have shown rather normal PFN levels (Graninger *et al.* 1985). In addition, plasma concentrations of proteins known to be synthesized only by the hepatocytes were not decreased in AN patients. It seems possible that, as observed in trauma and burn patients (Saba & Jaffe, 1980), not only synthesis but also catabolism or turnover of PFN, or both, may be changed in AN patients, thereby affecting the PFN pool. Taken together, although the elevation in PFN of AN patients during recovery may be mainly a direct reflection of increased hepatic synthesis following their ability to resume feeding, it requires further investigation to clarify the exact mechanism(s) of the synthesis and metabolism of PFN in those underweight patients.

Furthermore, all physiological functions of PFN have not been identified, although it is believed to be the primary non-specific circulating opsonin (Saba & Jaffe, 1980). The reduction of PFN is reported in sepsis and multiple organ failure (Saba, 1986). Palmblad *et al.* (1977) demonstrated impaired granulocyte bactericidal capacity and reduced granulocyte adherence in AN. PFN reduction in AN may contribute, at least in part, to dysfunction in the reticulo-endothelial system, although these patients are found to be less vulnerable to infection than are those suffering from other forms of malnutrition and weight loss (Bhanji & Mattingly, 1988). Moreover none of the patients in the present study had any evidence of infection.

Other EAPs, including ACE, have also been reported to be appropriate indicators of thyroid hormone activity (Graninger *et al.* 1986); however, a direct correlation with serum thyroid hormone levels was not observed in our AN patients. Only the significant correlation with body-weight change was found. This observation is supported by our previous studies of AN patients (Matsubayashi *et al.* 1988) and acute starvation (Komaki *et al.* 1988). Other factors, such as the nutritional state and weight reduction, as well as the renin–angiotensin system might modify ACE levels.

Changes in F VIII-R:Ag activity have been also reported to correlate with thyroid function (Rogers *et al.* 1982; Graninger *et al.* 1986). In the present study, however, F VIII-R:Ag levels were increased in the 'low T3' state. Our subjects had no history of taking contraceptives. It is reported, in general, that this factor increases during the acute phase of various conditions such as malignancy, post-operative state, renal and liver disease (Lombardi *et al.* 1981), and during exercise (Prentice *et al.* 1972). Since it is recognized that many AN patients exercise vigorously (Kron *et al.* 1978; Bhanji & Mattingly, 1988), the activity of our patients was closely monitored 24 h/d on the ward to prevent hyperactivity and other various abnormal behaviours during hospitalization. However, no evidence of hyperactivity was found during this study period. Further study is necessary to clarify the mechanism of the increase in F VIII-R:Ag concentrations in AN.

In conclusion, these findings suggest that PFN concentrations may be directly related to those of serum T3 during weight recovery with adequate nutrition in anorexia nervosa, whereas other EAPs are changed with no obvious relationships.

REFERENCES

Alexander, C. M., Lum, S. M. C., Rhodes, J., Boarman, C., Nicoloff, J. T. & Kumar, D. (1983). Rapid increase in both plasma fibronectin and serum triiodothyronine associated with treatment of diabetic ketoacidosis. *Journal of Clinical Endocrinology and Metabolism* **56**, 279–282.

American Psychiatric Association (1987). *Diagnostic and Statistical Manual of Mental Disorders*, 3rd ed. (revised) (DSM-IIIR). Washington, DC: American Psychiatric Association.

Azuma, H., Takeichi, T., Ohara, T. & Shirakami, A. (1987). Metabolism of plasma fibronectin in rabbits with experimental hyperthyroidism and hypothyroidism. *Metabolism* **36**, 777–780.

Bhanji, S. & Mattingly, D. (1988). Haematology and immunology. *Medical Aspects of Anorexia Nervosa*, pp. 55–62. London: Wright.

Bergsdorf, N., Nilsson, T. & Wallé, P. (1983). An enzyme-linked immunosorbent assay for determination of tissue plasminogen activator applied to patients with thromboembolic disease. *Thrombosis and Haemostasis* **50**, 740–744.

Brent, G. A., Hershman, J. M., Reed, A. W., Sastre, A. & Lieberman, J. (1984). Serum angiotensin-converting enzyme in severe nonthyroidal illnesses associated with low serum thyroxin concentration. *Annals of Internal Medicine* **100**, 680–683.

Chopra, I. J., Chopra, U., Smith, S. R., Reza, M. & Solomon, D. H. (1975). Reciprocal changes in serum concentrations of 3,3′,5′-triiodothyronine (Reverse T3) and 3,3′,5-triiodothyronine (T3) in systemic illness. *Journal of Clinical Endocrinology and Metabolism* **41**, 1043–1049.

Curran-Celentano, J., Erdman, J. W., Nelson, R. A. & Grater, S. J. E. (1985). Alterations in vitamin A and thyroid hormone status in anorexia nervosa and associated disorders. *American Journal of Clinical Nutrition* **42**, 1183–1191.

Graninger, W., Pirich, K., Derfler, K. & Waldhäusl, W. (1985). Plasma fibronectin and thyroid function. *Journal of Clinical Pathology* **38**, 64–67.

Graninger, W., Pirich, K. R., Speiser, W., Deutsch, E. & Waldhäusl, W. K. (1986). Effect of thyroid hormones on plasma protein concentrations in man. *Journal of Clinical Endocrinology and Metabolism* **63**, 407–411.

Horowitz, G. D., Groeger, J. S., Legaspi, A. & Lowry, S. F. (1985). The response of fibronectin to differing parenteral caloric sources in normal man. *Journal of Parenteral and Enteral Nutrition* **9**, 435–438.

Howard, L., Dillon, B., Saba, T. M., Hofmann, S. & Cho, E. (1984). Decreased plasma fibronectin during starvation in man. *Journal of Parenteral and Enteral Nutrition* **8**, 237–244.

Ingenbleek, Y., De Visscher, M. & De Nayer, P. (1972). Measurement of prealbumin as an index of protein-calorie malnutrition. *Lancet* **i**, 106–108.

Japanese Ministry of Health and Welfare (1986). *Diagnostic Tables and Figures for Obesity and Emaciation* Health Promotion and Nutrition Division, Health Service Bureau, Ministry of Health and Welfare. (In Japanese.) Tokyo: Daiichi Press.

Kasahara, Y. & Ashihara, Y. (1981). Colorimetry of angiotensin-I converting enzyme activity in serum. *Clinical Chemistry* **27**, 1922–1925.

Komaki, G., Tamai, H., Mori, T., Nakagawa, T. & Mori, S. (1988). Changes in serum angiotensin-converting enzyme in acutely starved non-obese patients: a possible dissociation between angiotensin-converting enzyme and the thyroid state. *Acta Endocrinologica* **118**, 45–50.

Kron, L., Katz, J. L., Gorzynski, G. & Weiner, H. (1978). Hyperactivity in anorexia nervosa: a fundamental clinical feature. *Comprehensive Psychiatry* **19**, 433–440.

Laurell, C. B. (1966). Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Annals of Biochemistry* **15**, 45–52.

Lombardi, R., Mannucci, P. M., Seghatchaian, M. J., Garcia, V. V. & Coppola, R. (1981). Alterations of factor VIII von Willebrand factor in clinical conditions associated with an increase in its plasma concentrations. *British Journal of Haematology* **49**, 61–68.

Matsubayashi, S., Tamai, H., Kobayashi, N., Takaichi, Y., Fukata, Y., Hirota, Y., Kuma, K., Nakagawa, T. & Kumagai, L. F. (1988). Angiotensin-converting enzyme and anorexia nervosa. *Hormone and Metabolic Research* **20**, 761–764.

Murata, Y., Ceccarelli, P., Refetoff, S., Horowitz, A. L. & Matsui, N. (1987). Thyroid hormone inhibits fibronectin synthesis by cultured human skin fibroblasts. *Journal of Clinical Endocrinology and Metabolism* **64**, 334–339.

Newman, N. M. & Halmi, K. A. (1988). The endocrinology of anorexia nervosa and bulimia nervosa. *Endocrinology and Metabolism Clinics of North America* **17**, 195–212.

Palmblad, J., Fohlin, L. & Lundstrom, M. (1977). Anorexia nervosa and polymorphonuclear (PMN) granulocyte reactions. *Scandinavian Journal of Haematology* **19**, 334–342.

Prentice, C. R. M., Forbes, C. D. & Smith, S. M. (1972). Rise of factor VIII after exercise and adrenalin infusion, measured by immunological and biological techniques. *Thrombosis Research* **1**, 493–506.

Rogers, J. S. II, Shane, S. R. & Jencks, F. S. (1982). Factor VIII activity and thyroid function. *Annals of Internal Medicine* **97**, 713–716.

Saba, T. M. (1970). Physiology and physiopathology of the reticulo-endothelial system. *Archives of Internal Medicine* **126**, 1031–1052.

Saba, T. M. (1986). Plasma and tissue fibronectin: its role in the pathophysiology of the critically ill septic patients. In *Critical Care: State of the Art*, vol. 7, pp. 437–464. [B. Chernow and W. C. Shoemaker, editors]. Fullerton, CA: Society of Critical Care Medicine.

Saba, T. M., Albert, H. W., Blumenstock, F. A., Evanega, G., Staehler, F. & Cho, E. (1981). Evaluation of a rapid immunoturbidimetric assay for opsonic fibronectin in surgical and trauma patients. *Journal of Laboratory and Clinical Medicine* **98**, 482–491.

Saba, T. M. & Jaffe, E. (1980). Plasma fibronectin (opsonic glycoprotein): its synthesis by vascular endothelial cells and role in cardiopulmonary integrity after trauma as related to reticuloendothelial function. *American Journal of Medicine* **68**, 577–594.

Scott, R. L., Sohmer, P. R. & MacDonald, M. G. (1982). The effect of starvation and repletion on plasma fibronectin in man. *Journal of the American Medical Association* **248**, 2025–2027.

Smallridge, R. C., Chernow, B., Snyder, R., Zaloga, G. P. & Burman, K. D. (1985). Angiotensin-converting enzyme activity: potential marker of tissue hypothyroidism in critical illness. *Archives of Internal Medicine* **145**, 1829–1832.

Tamkun, J. W. & Hynes, R. O. (1983). Plasma fibronectin is synthesized and secreted by hepatocytes. *Journal of Biological Chemistry* **258**, 4641–4647.

Van Der Heyden, J. T. M., Docter, R., Van Toor, H., Wilson, J. P. H., Hennemann, G. & Krenning, E. P. (1986). Effects of caloric deprivation on thyroid hormone tissue uptake and generation of low-T3 syndrome. *American Journal of Physiology* **251**, E156–E163.

Waterlow, J. C. (1972). Classification and definition of protein-calorie malnutrition. *British Medical Journal* **3**, 566–569.

Yamada, K. M. (1983). Cell surface interactions with extracellular materials. *Annual Review of Biochemistry* **52**, 761–799.

Printed in Great Britain