Short reports

PLASMATIC LEVEL OF THROMBIN-FIBRINOGEN INTERACTION MARKERS IS CONNECTED WITH LIPID PEROXIDATION IN THROMBOCYTES, LEUCOCYTES AND ERYTHROCYTES (PART IV)

Byshevsky A.Sh., Galyan S.L., Zabara Ye.V., Zvereva I.V. Rudzevich A.Yu., Shapovalov P.Ya., Shapovalova Ye.M.
Tyumen State Medical Academy
Tyumen, Russia

Blood cells supposedly implement the connection between lipid peroxidation (LPO) and hemostasis [1, 2]. For the participation degree valuation of thrombocytes, erythrocytes and leucocytes - the cells, the role of which in the relation between LPO and continuous intravascular blood clotting has been established [3, 4], we studied the dynamics of the LPO shifts' nascence and intensity in these cells in vivo, and found out if the LPO shifts in the cells were connected with the shifts of thrombin-fibrinogen interaction (TFI) markers' level, practically, the markers of continuous intravascular blood clotting (CIBC) [5-9].

While planning the experiments, we chose the actions promoting a slowly developing effect, that allows solving two problems: 1) to elicit the fact of the change nascence priority order, 2) to evaluate the degree of changes taking place in various cells under the same conditions of the experiment (i.e. in one experimental group of animals). Taking into account the fact that a large volume of blood is needed for the cells' separation, there were so many rats included into the groups, that the number of laboratory definitions (n) after pulling of individual blood samplings were not less than six: the groups usually including 6 rats were expanded to 30 (in the group of 30 rats the blood of 5 rats was combined into one batch, the value of n was equal to 6). Totally 600 nonlinear white rats (170±10 g) were used.

Table 1. The content of plasmatic markers of CIBC, LPO and AOP in thrombocytes, monocytes, neutrophils and erythrocytes in different terms after 6-MTU administration, 150 mg/kg (n = 6)

Factors	Control	20th day	25th day	30th day	35th day
P_3, %	85.2±2.0	87.1±2.1	85.2±1.4	79.1±1.2*+	74.7±1.3*+
P_4, sec	3.3±0.06	3.1±0.03	2.8±0.06	2.1±0.04*+	1.8±0.04*+
FSP, mg%	16.8±1.1	16.2±0.5	15.5±0.07	13.0±0.04*+	12.1±0.04*+
SFMC, mcg/ml	25.4±1.1	24.2±1.1	23.2±1.0	20.1±0.4*+	18.3±0.3*+
D-D, mcg/ml	0.20±.0.007	0.18±0.010	0.17±0.007	0.15±0.002*+	0.13±0.006*
DC, A/mg LP	0.045±0.004 0.032±0.003 0.027±0.002 0.021±0.002	0.044±0.004 0.029±0.003 0.030±0.003 0.022±0.003	0.031±0.0005*+ 0.0.27±0.003*+ 0.029±0.002 0.022±0.019	0.024±0.0005*+ 0.019±0.0003*+ 0.022±0.0002*+ 0.017±0.0001*+	0.020±0.0003*+ 0.016±0.0002*+ 0.019±0.0003*+ 0.016±0.0004*+
TBA, units/mg LP	0.76±0.056 0.54±0.004 0.33±0.003 0.29±0.002	0.71±0.029 0.55±0.005 0.35±0.005 0.39±0.003	0.55±0.031*+ 0.49±0.004*+ 0.30±0.003 0.27±0.003	0.48±0.012*+ 0.43±0.007*+ 0.27±0.004*+ 0.20±0.002*+	0.39±0.024*+ 0.38±0.025*+ 0.23±0.006*+ 0.19±0.03*+
IP, min/ml	48.5±2.3 48.5±2.3 46.4±2.1 47.3±2.0	53.9±1.0* 48.1±2.1 47.4±2.2 49.9±2.1	59.9±1.3*+ 50.9±1.9*+ 49.4±2.3 51.9±2.3	64.8±1.4*+ 56.7±1.5*+ 54.1±2.1*+ 52.8 ±2.0*+	67.2±1.3*+ 59.3±1.4*+ 56.1±2.0*+ 53.9 ±1.8*+
OR, мм^3/min	0.69±0.04 0.64±0.03 0.61±0.04 0.63±0.05	0.65±0.03* 0.62±0.05 0.59±0.03 0.60±0.03	0.60±0.02* 0.58±0.03* 0.57±0.04 0.59±0.04	0.48±0.03*+ 0.56±0.03*+ 0.55±0.04* 0.57±0.04*	0.39±0.02*+ 0.54±0.03*+ 0.51±0.02*+ 0.56±0.03*

Symbols: P_3 – P_3 factor, FSP – fibrin split products, SFMC – soluble fibrin-monomeric complexes, TBA – products reacting with thiobarbituric acid, IP – induction period, OR – oxidation rate; * - authentic difference from the control, + - authentic difference of the 4th, 5th and 6th columns from the 3rd one. **Note**: the LPO factors represented in the 2-6th columns were determined in thrombocytes (upper line), monocytes (2nd line), neutrophils (3rd line) and erythrocytes (4th line).

For the LPO inhibition 6-methylthiouracil (6-MTU) and an antioxidant dimephosphon (DM) were used, for the LPO activation thyroxine (T_4) and lead acetate were used (sampling dosage and terms – in the tables). The TFI markers (factors P_3, P_4 FSP, SFMC and D-dimers) were defined according to the description [10, 11], the LPO and antioxidant potential (AOP) were controlled as it is shown [12, 13]. DM, 6-MTU, thyroxine (T_4) or lead acetate were administered to the rats daily.

From the table 1 it is clear that the LPO activity is gradually decreasing and the antioxidant potential (AOP) is growing in thrombocytes. The TFI markers' level decrease happens later: only on the 30th and more noticeable – on the 35th day the level of FSP, SFMC and D-dimers fell down. In thrombocytes and monocytes there appeared a tendency to the LPO inhibition and AOP growth on the 20th day; on the 25th day these shifts became authentic; on the 30th day they enhanced and by the 35th day became still more significant.

In neutrophils the changes were of the same directivity (gradual LPO lowering and AOP growth), but authentic shifts appeared by the 30th day only. In erythrocytes, monocytes and neutrophils the shifts are the same, but by the 35th there were no shifts registered.

Similar experiments were carried out with DM – the LPO inhibitor varying from 6-MTU by the action mechanism. On the data of table 2, on the 5th day of the DM administration the AOP (IP elongation and OR reduction) was increased in thrombocytes and to a less extent – in monocytes. In the other cells the factors didn't differ from the control values. On the 10th day the LPO inhibition and AOP growth were detected in thrombocytes and monocytes. On the 15th day these shifts were enhanced in thrombocytes and monocytes, and in neutrophils and erythrocytes the LPO slowed down and the AOP grew (the DC and TBA level decreased, IP elonged and OR reduced).

Table 2. The content of plasmatic markers of TFI, LPO and AOP in thrombocytes, monocytes, neutrophils and erythrocytes after DM administration, 1.0 g/kg, (n = 6)

Factors	Control	5th day	10th day	15th day	20th day
P_3, %	84.9±1.7	85.2. ±1.2	81.9±1.9	77.2±1.1*$^+$	72.1±1.2*$^+$
P_4, sec	3.3±0.05	3.1±0.05	2.9±0.02*	2.0±0.03*$^+$	1.6±0.03*$^+$
FSP, ml%	15.9±1.1	15.8±1.1	16.5±0.08	13.1±0.02*$^+$	11.0±0.03*$^+$
SFMC, mcg/ml	26.0±1.2	24.7±1.2	22.8±1.1	19.0±0.3*$^+$	16.7±0.3*$^+$
D-D, kg/ml	0.18±.0.007	0.21±0.012	0.17±0.012	0.15±0.003*$^+$	0.12±0.008*
DC, A/mg LP	0.044±0.003 0.034±0.003 0.028±0.002 0.020±0.003	0.043±0.005 0.034±0.006 0.030±0.003 0.022±0.003	0.029±0.0006*$^+$ 0.0.27±0.004*$^+$ 0.026±0.004 0.021±0.019	0.019±0.0007*$^+$ 0.021±0.0003*$^+$ 0.020±0.0002*$^+$ 0.016±0.0001*$^+$	0.016±0.0003*$^+$ 0.016±.0004*$^+$ 0.018±0.0002*$^+$ 0.015±0.0003*$^+$
TBA,units/mg LP	0.73±0.050 0.54±0.003 0.32±0.003 0.31±0.002	0.72±0.030 0.52±0.006 0.32±0.004 0.33±0.004	0.51±0.032*$^+$ 0.47±0.003*$^+$ 0.28±0.004 0.26±0.004	0.46±0.011*$^+$ 0.42±0.004*$^+$ 0.28±0.003*$^+$ 0.21±0.003*$^+$	0.36±0.022*$^+$ 0.39±0.026*$^+$ 0.22±0.004*$^+$ 0.18±0.02*$^+$
IP, min/ml	48.1±2.2 48.4±2.2 47.8±2.1 47.2±2.3	54.6±1.1* 48.4±2.2 47.9±2.3 49.1±2.0	61.3±1.2*$^+$ 53.8±1.1*$^+$ 48.9±2.4 50.1±2.2	65.9±1.3*$^+$ 56.9±1.3*$^+$ 55.3±2.0*$^+$ 52.9 ±1.9*$^+$	69.3±1.4*$^+$ 59.9±1.3*$^+$ 56.5±1.9*$^+$ 54.8 ±1.7*$^+$
OR, mm^3 /min	0.71±0.03 0.65±0.02 0.60±0.03 0.62±0.04	0.63±0.03* 0.57±0.06* 0.58±0.04 0.61±0.04	0.58±0.03* 0.56±0.02* 0.57±0.03 0.58±0.05	0.48±0.03*$^+$ 0.54±0.02*$^+$ 0.53±0.03* 0.54±0.03*	0.36±0.03*$^+$ 0.52±0.03*$^+$ 0.50±0.03*$^+$ 0.54±0.04*

Symbols: like in table 1; * - authentic differences from the control; + - the difference of the 4th, 5th and 6th columns from the 2nd one. **Note**: like in table 1.

On the 20th day all the enumerated shifts in all the cells, especially in thrombocytes, enhanced.

The TFI markers' level shifts are as follows: on the 10th day only the P_4 factor level decreased, on the 15th day all the other factors decreased, the shift became still more noticeable by the end of the observation.

Hence, as in the experiment with 6-MTU, the LPO and AOP change earlier than the TFI markers' level shifts appear (according to the CIBC). The fact that according to the ability to react on the LPO changes the investigated cells are ranged in the following succession:

thrombocytes > monocytes > neutrophils > erythrocytes.

Further on, T_4 or lead in the dosages changing the LPO and AOP [14, 15] were used in the experiments, and the following was found out (table 3): on the 5th day of T_4 administration the signs of LPO acceleration in thrombocytes, monocytes and erythrocytes appeared (DC and TBA level growth), the AOP decreased (the IP shortening in thrombocytes and neutrophils, and OR growth in thrombocytes, monocytes and neutrophils). All the shifts are little, but authentic ($p < 0.05$); by the 7th day, and especially by the 9th one, the shifts enhanced (exclusive of neutrophils and erythrocytes, wherein the shifts on the 7th and 9th day were practically the same).

Table 3. The content of plasmatic markers of TFI, LPO and AOP in thrombocytes (upper line), monocytes (2nd line), neutrophils (3rd line) and erythrocytes (4th line) in various terms of T_4 administration, 8.0 mg/kg (n = 6).

Factors	Control	3rd day	5th day	7th day	9th day
P_3, %	85.4±1.4	86.0 ±1.7	89.0±1.1	91.6±1.0*	98.2±1.3*
P_4	3.2±0.02	3.4±0.06	3.5±0.05	4.1±0.05*	4.6±0.02*
FSP, mg%	15.8±1.0	16.1±0.8	16.9±0.09	18.3±0.06*	23.8±0.06*
SFMC, mcg/ml	26.4±1.5	25.9±1.2	28.1±1.3	27.9±1.5	32.4±0.6*
D-D, mcg/ml	0.21±.0.005	0.22±0.014	0.22±0.011	0.23±0.010	0.29±0.004*
DC, A/mg LP	0.050±0.002 0.031±0.002 0.024±0.003 0.021±0.003	0.051±0.003 0.033±0.004 0.025±0.002 0.020±0.003	0.064±0.001* 0.037±0.003* 0.029±0.003* 0.024±0.004	0.072±0.003* 0.040±0.004* 0.031±0.003* 0.028±0.003*	0.081±0.005* 0.044±0.003* 0.036±0.004* 0.032±0.002
TBA, units/mg LP	0.75±0.051 0.52±0.004 0.33±0.004 0.28±0.003	0.79±0.021 0.50±0.005 0.31±0.002 0.29±0.004	0.93±0.031* 0.55±0.002* 0.35±0.005 0.34±0.002*	0.99±0.039* 0.62±0.005* 0.38±0.002* 0.36±0.003*	1.21±0.039* 0.68±0.006* 0.40±0.003* 0.37±0.002*
IP, min/ml	48.1±2.2 48.4±2.2 47.8±2.1 48.2±2.3	46.1±1.5 48.0±2.0 49.7±2.2 47.6±2.4	42.0±1.3* 47.0±2.4 44.8±1.8* 46.2±2.2	40.1±1.2* 44.2±2.0* 43.1±2.2* 44.2±2.3*	36.0±1.3* 42.5±2.1* 44.1±2.0* 44.9±2.2*
OR, mm^3/min	0.71±0.03 0.64±0.03 0.59±0.03 0.64±0.04	0.76±0.07 0.66±0.03 0.61±0.04 0.65±0.03	0.82±0.03* 0.68±0.02* 0.66±0.02* 0.67±0.04	0.88±0.05* 0.71±0.03* 0.68±0.04* 0.69±0.03*	0.94±0.04* 0.72±0.02* 0.69±0.04* 0.89±0.03*

Symbols: like in table 1; * - authentic difference from the control

The TFI markers' (FSP, SFMC, P_3 and P_4 factors) level increase was detected on the 7th day of the experiments already. On the 9th day the shifts were more noticeable, plus the D-dimers' level increase.

In the experiments with the administration of lead – a pro-oxidant, (table 4) differing from T_4 on the action mechanism, the following was found out: on the 10th day already the LPO and AOP shifts were detected (the TBA and DC growth in thrombocytes and monocytes, and DC growth in neutrophils as well), the IP shortening in all the cells (excepting erythrocytes) and OR increase (exclusive of neutrophils). By the 12th day the shifts became more significant and spread onto all the cells, enhancing by 15th and 18th days.

The content of TFI markers didn't change on the 10th and 12th days of the lead administration, but somehow increased (excepting SFMC and D-dimers) by the 12th day. On the 18th day the content of all the TFI markers was increased.

Thus, while being administrated in comparatively small doses, lead and T_4 cause the progressing growth LPO intensity and the AOP reduction. These shifts are ahead of the TFI markers' level change in time.

The data got allow coming to the following conclusions:

1. The LPO activation or inhibition and the conforming to them decrease (or increase) of the AOP are attended by a decrease (increase accordingly) of the TFI intensity, i.e. the intensity of the continuous intravascular blood clotting.
2. The LPO and AOP changes in thrombocytes preface the changes of continuous intravascular blood clotting intensity.
3. Simultaneously with the LPO and AOP changes in thrombocytes, while affected with pro- and antioxidants, the LPO and AOP in monocytes, heterophilic leukocytes and erythrocytes change in the same direction.

In connection with this it seems to be advisable to control the content of plasmatic TFI markers at the diseases attended with the activation of free-radical processes.

Naturally, the investigations aimed at the specification of the character of the relation between the LPO intensity in blood cells, especially in thrombocytes, and the CIBC markers' content make sense.

Table 4. The content of CIBC markers in plasma, LPO and AOP in thrombocytes, monocytes, neutrophils and erythrocytes (1st, 2nd, 3rd and 4th line accordingly) in various terms of lead acetate administration in the dosage of 50 mg/kg (n = 6 in every stage).

Factors	Control	10th day	12th day	15th day	18th day
P_3, %	85.3±1.4	86±.1.5	87.8±1.1	101±1.4*	122±1.7*
P_4, sec	3.1±0.02	3.3±0.03	3.5±0.05	4.1±0.03*	5.0±0.04*
FSP,mg %	17.0±1.0	16.9±0.8	16.8±0.1.0	19.9±0.7*	24.2±0.06*
SFMC, mcg/ml	25.4±1.3	25.9±1.2	26.4±1.3	29.1±1.4	36.8±0.7*
D-D, mcg/ml	0.18±.0.003	0.20±0.014	0.19±0.011	0.27±0.034	0.35±0.011*
DC, A/mg LP	0.048±0.003 0.033±0.002	0.057±0.003* 0.039±0.001*	0.064±0.004* 0.042±0.002*	0.072±0.003* 0.048±0.005*	0.083±0.006* 0.05±±0.002*
TBA, units/mg LP	0.76±0.052 0.51±0.003	0.83±0.022* 0.56±0.002*	0.94±0.033* 0.59±0.002*	0.98±0.032* 0.64±0.005*	1.25±0.041* 0.69±0.005*
IP, min/ml	48.5±2.1 47.1±1.9	44.1±1.1* 45.0±0.4*	41.1±1.1* 44.1±2.0*	39.6±1.2* 42.1±1.2*	35.2±1.3* 41.3±2.0*
OR, mm^3/min	0.70±0.03 0.65±0.02	0.76±0.03* 0.69±0.01*	0.83±0.04* 0.67±0.02*	0.89±0.04* 0.72±0.04*	0.96±0.04* 0.73±0.03*

Symbols: like in table 1; * - authentic difference from the control.

References:

1. Byshevsky A.Sh. Blood cells in lipid peroxidation and hemostasis relation implementation / Byshevsky A.Sh., Galyan S.L., Dementyeva I.A. and others) // Problems of physiology and hemostasis pathology (works of problem committee at Interdepartmental Research Council on hematology and transfusiology of RAMS. – Barnaul. – 2000 – pp. 156-163.
2. Galyan S.L. Influence of levonorgestrel on continuous intravascular blood clotting depending on lipid peroxidation intensity / Galyan S.L., Aptekar I.A., Alborov R.G. and others // Scientific Reporter of TSMA, 2003. – 2. – p. 31.
3. Alborov R.G. Blood cells – factor of lipid peroxidation and continuous intravascular blood clotting relation / Alborov R.G. // Materials of interregional conference of biochemists of Ural, Western Siberia and Volga Region "Biochemistry: from investigation of molecular mechanisms to introduction into clinical practice and production", Orenburg, 2003, pp. 152-154.
4. Gawaz M.P. Blood Platelets / M.P.Gawaz // Stuttg.; New York: Time. - 2001. – p. 190.
5. Zubairov D.M. Über die Thrombinzirculation in Blut / D.M. Zubairov // Folia Haematol. - 1962. – 79. - 1. – S. 62-75
6. Zwaal R.F.A. Blood, membranes and haemostasis / R.F.A.Zwaal // Haemostasis. - 1982. - 11. - Pp. 12-39.
7. Bokarev I.N. Differential diagnostics and treatment of internal diseases. Bleeding sickness, or hemorrhagic syndrome. Differential diagnostics / Bokarev I.N. // Moscow. – 2002. – p. 75.
8. Zubairov D.M. Molecular fundamentals of blood clotting and thrombosis. Kazan: FES AST. – 2000. – p. 367.
9. Byshevsky A.Sh. Vitamins, intravascular blood clotting and lipid peroxidation / Byshevsky A.Sh., Galyan S.L., Shapovalov P.Ya. // Moscow: Medicine. – 2006. – p. 105.
10. Baluda V.P. Laboratory methods of hemostasis system investigation / Baluda V.P., Barkagan Z.S., Goldberg Ye.D. and others. – Tomsk. – 1980. – p. 310.
11. Barkagan Z.S. Introduction into clinical hemostasiology / Barkagan Z.S. // M.: Newmediamed-JSC. – 1988. – p. 45.
12. Ushkalova V.N. Complex analysis of blood lipids by spectrophotometric, fluorometric and kinetic methods / Ushkalova V.N., Ioanidis N.V., Deyeva Z.M. and others // Laboratory work. – 1987. – 6. – pp. 446-460.
13. Ushkalova V.N. Free-radical lipid peroxidation in experiment and clinic. – Tyumen: TSU Publishing House – 1997. – pp. 5-21.
14. Venditti P. Antioxidant-sensitive shortening of ventricular action potential in hyperthyroid rats is independent of lipid peroxidation / P.Venditti, De T.Leo, Di S.Meo // Mol. Cell.Endocrinol. – 1998. –

142. – 1.- 2. P. 15-23

15. Venditti P. Thyroid hormone-induced oxidative stress / P.Venditti, Di Meo S. P. Venditti, S. Di Meo // Cell. Mol. Life Sci. - 2006. - 63(4). - P. 414-434.

PLASMA INTACT FIBROBLAST GROWTH FACTOR 23 LEVELS IN WOMEN WITH ANOREXIA NERVOSA

Makoto Otani, Yoshiyuki Takimoto, Junko Moriya, Kazuhiro Yoshiuchi, Akira Akabayashi

Department of Stress Sciences and Psychosomatic Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

Background: Fibroblast growth factor (FGF)23 is a novel phosphaturic factor associated with inorganic phosphate homeostasis. Previous human studies have shown that serum FGF23 levels increase in response to a high phosphate diet. For anorexia nervosa (AN) patients, inorganic phosphate homeostasis is important in the clinical course, such as in refeeding syndrome. The purpose of this study was to determine plasma levels of intact FGF23 (iFGF23) in restricting-type AN (AN-R) patients, binge-eating/purging-type AN (AN-BP) patients, and healthy controls.

Methods: The subjects consisted of 6 female AN-R patients, 6 female AN-BP patients, and 11 healthy female controls; both inpatients and outpatients were included. Plasma iFGF23, 1,25- dihydroxyvitamin D (1,25-$(OH)_2D$), and 25-hydroxyvitamin D (25-OHD) levels were measured. Data are presented as the median and the range. A two-tailed Mann-Whitney U-test with Bonferroni correction was used to assess differences among the three groups, and a value of $p < 0.017$ was considered statistically significant.

Results: There were no differences between AN-R patients and controls in the iFGF23 and 1,25-$(OH)_2D$ levels. In AN-BP patients, the iFGF23 level (41.3 pg/ml; range, 6.1–155.5 pg/ml) was significantly higher than in controls (3.8 pg/ml; range, not detected-21.3 pg/ml; $p = 0.001$), and the 1,25-$(OH)_2D$ was significantly lower in AN-BP patients (7.0 pg/ml; range, 4.2–33.7 pg/ml) than in controls (39.7 pg/ml; range, 6.3–58.5 pg/ml; $p = 0.015$). No differences in plasma 25-OHD levels were observed among the groups.

Conclusion: This preliminary study is the first to show that plasma iFGF23 levels are increased in AN-BP patients, and that these elevated plasma FGF23 levels might be related to the decrease in plasma 1,25-$(OH)_2D$ levels.

Findings

Fibroblast growth factor (FGF)23, a circulating 26 kDa peptide produced by osteogenic cells, is a novel phosphaturic factor. It is important for the regulation of inorganic phosphate homeostasis and for vitamin D metabolism [1]. FGF23 inhibits renal proximal tubule phosphate reabsorption, increases renal phosphate excretion, and reduces serum phosphate without affecting serum calcium. FGF23 also strongly suppresses 1,25-$(OH)_2D$ production [2,3].

Anorexia nervosa (AN) is an eating disorder characterized by decreased caloric intake, low weight, and reduced body fat. To date, two subtypes have been identified: restricting- type (AN-R); and binge-eating/purging-type (AN-BP). AN is diagnosed by weight loss and refusal to maintain a minimal normal body weight, an intense fear of gaining weight or becoming fat, a self-evaluation unduly influenced by body shape and weight, and amenorrhea [4]. AN-R patients restrict food intake, while AN-BP patients regularly engage in binge-eating and/or purging.

In patients with AN, refeeding syndrome is a well-known phenomenon that occurs during the course of nutritional rehabilitation; it is characterized by hypophosphatemia, which may result in serious consequences, such as cardiac dysrhythmia, delirium, and even sudden death [5]. Although inorganic phosphate homeostasis is important in AN patients, no previous studies have examined plasma FGF23 levels in AN. Therefore, the present study determined plasma FGF23 concentrations in AN-R patients, AN-BP patients, and healthy controls.

The subjects included 12 female AN patients who met the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV) [4] and 11 healthy female controls. The 12 AN patients included 6 patients with AN-R and 6 patients with AN-BP. No patients had a previous diagnosis of bulimia nervosa. The study's cases included outpatients and inpatients of the University of Tokyo Hospital. Except for proper doses of antidepressants, anxiolytics, hypnotics, laxatives and stomach agents, patients with AN-BP were treated with lactomin (3 g/day; n = 1), lomerizine (10 mg/day; n = 1) and pantethine (300 mg/day; n = 1), and AN-R patients and controls did not receive drug therapy. Premorbid renal dysfunction was an exclusionary criterion.

Blood samples were collected from all subjects after over- night fasting. The protocol was approved by the Institutional Ethics Committee of the University of Tokyo, and written informed consent was obtained from all subjects prior to enrollment in the study.

All blood samples were drawn into chilled tubes contain- ing EDTA-2Na (1 mg/ml) and were then immediately centrifuged at 4°C. Plasma portions were stored at -70°C prior to analysis. Plasma concentrations of intact FGF23 (iFGF23) were measured using an ELISA kit (Immutopics, San Clemente, CA, USA) (6,7), with a sensitivity of 1.0 pg/ ml, intra-assay variability of <4.4%, and inter-assay variability of <6.5%. All samples were analyzed in duplicate. Plasma 1,25-$(OH)_2D$ and 25-OHD concentrations