Renal Effects of Different Types of Protein in Healthy Volunteer Subjects and Diabetic Patients

HIROSHI NAKAMURA, MD
SEIKI ITO
NAOKO EBE
AKIRA SHIBATA

OBJECTIVE— To evaluate the effects of acute loading of protein from different sources on the glomerular filtration rate.

RESEARCH DESIGN AND METHODS— A total of 6 healthy volunteer subjects and 6 diabetic patients with normoalbuminuria were studied before and after ingestion on separate days of tuna fish containing 0.7 g/kg body wt of protein, boiled egg white containing the same amount of protein as the tuna fish, or boiled egg white containing 1.4 g/kg body wt of protein. Furthermore, to study the possible role of prostaglandins and amino acids in the response of GFR to protein loading, urinary excretion of prostaglandins, and plasma levels of amino acids were measured during these tests.

RESULTS— In normal subjects, the GFR rose significantly ($P < 0.01$) after ingestion of tuna fish. No significant differences were found between GFR before and after ingestion of the different amounts of egg white. The GFRs of the diabetic patients after ingestion of each of the meals were similar to the responses in healthy volunteers. Plasma levels of Gly and Ala (amino acids known to induce glomerular hyperfiltration) were higher after ingestion of tuna fish than after administration of egg white in all subjects. No differences were found in the plasma concentrations of any amino acids except Gly and Ala after loads of tuna fish and egg white containing 1.4 g/kg of protein. Urinary 6-keto-PGF_{1a} excretion increased significantly ($P < 0.01$) after tuna fish loading, but did not change after egg white challenge.

CONCLUSIONS— These findings could be explained either by differences in renal vasodilatory prostaglandin secretion or by increased plasma levels of Gly and Ala, which were increased only after ingestion of tuna fish. Thus, egg white has renal effects on GFR different from those of tuna fish, independent of the quantity of protein ingested.

FROM THE FIRST DEPARTMENT OF INTERNAL MEDICINE, NIIGATA UNIVERSITY SCHOOL OF MEDICINE, NIIGATA, JAPAN.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO HIROSHI NAKAMURA, MD, FIRST DEPARTMENT OF INTERNAL MEDICINE, NIIGATA UNIVERSITY SCHOOL OF MEDICINE, ASAHIMACHI 1–754, NIIGATA, 951 JAPAN.

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GFR, GLOMERULAR FILTRATION RATE; BMI, BODY MASS INDEX; TYPE II DIABETES, NON-INSULIN-DEPENDENT DIABETES MELLITUS; AER, ALBUMIN EXCRETION RATE; OHA, ORAL HYPOGLYCEMIC AGENT; FBG, FASTING BLOOD GLUCOSE; PG, PROSTAGLANDIN; PGE_1, PROSTAGLANDIN E_1; 6-KETO-PGF_{1A}, 6-KETO-PROSTAGLANDIN F_{1A}; TXB_2, THROMBOXAN B_2; HPLC, HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY; RIA, RADIOIMMUNOASSAY; CHO, CARBOHYDRATE.

Protein intake has a profound effect on renal function. It has been suggested that high protein intake may have a deleterious effect on the kidney (1). Although restriction of dietary protein may prevent the progression of renal failure in diabetic individuals (2,3), treatment with a low-protein diet should be approached with caution because hypoproteinemia has often been observed in protein-restricted rats with renal failure (4). Meat protein has been used mainly to study the effects of acute protein loading on renal function. It has been reported that GFR increases after ingestion of meat proteins (5,6). In a previous report, we examined the influence of proteins from different sources on renal function in healthy volunteer subjects and diabetic patients (7,8). Although our study showed that ingestion of egg white, cheese, or cooked soybean protein did not affect GFR, we could not rule out the possibility that these findings might be attributable to differences in the intestinal absorption of meals. Therefore, we have investigated the renal effects of acute loading of large amounts of egg white.

The exact mechanism of protein-induced glomerular hyperfiltration remains to be elucidated. Our previous study showed that this renal response may not be induced directly by glucagon (9), growth hormone (10), atrial natriuretic peptide (11), or kallikrein-kinin system (12). Viberti et al. (13) reported that glomerular hyperfiltration in diabetes is related to renal PG production. Therefore, another aim of this study was to examine the role of PGs in mediating glomerular hyperfiltration.

RESEARCH DESIGN AND METHODS

— A total of 6 healthy female volunteer subjects (40.5 ± 4.7 yr of age, BMI 22.7 ± 3.0 kg/m^2) and 6 female type II diabetic subjects (42.9 ± 5.2 yr of age, BMI 23.3 ± 2.9 kg/m^2) were recruited for this study. All subjects were normotensive and had nor-

mal renal function, as evidenced by normal serum creatinine concentrations and urinalysis. The overnight urinary AER, measured three times in the 1–2 mo preceeding the study, was <10 μg/min in all subjects. Two diabetic patients were given OHAs, and the others were treated with diet therapy. All of the diabetic patients were under reasonable metabolic control (FBG 99.4 ± 8.3 mg/100 ml, HbA_{1c} 5.6 ± 0.4%, normal range 4.6–5.7%). The duration of diabetes was 3.8 ± 1.4 yr. One patient had background retinopathy. None of the patients had autonomic neuropathy.

Protein loading test

Protein loading tests were performed in the fasting state. After an overnight fast, the test was begun by oral hydration with 400 ml of water. An intravenous catheter was inserted for blood sampling, and loading and maintenance infusions of iothalamate in normal saline were administered. Urine volume was measured 1 h later, and the subjects ingested 0.7 g/kg body wt of protein in the form of cooked tuna fish together with 100 ml of water. Urine volume was recorded, iothalamate concentration in urine and plasma were measured hourly, and GFR was calculated. Plasma amino acid concentrations also were measured before and after protein loading. On a separate day, the protein loading test was performed using egg white, 0.7 or 1.4 g/kg body wt. The composition of each test meal is shown in Table 1.

To study the effect of protein loading on renal PG production, urinary concentrations of PGE_1, 6-keto-PGF_{1a}, and TXB_2 were measured.

Methods of measurement

Iothalamate was measured by HPLC. Plasma amino acid concentrations were measured with an auto-analyzer (System 6300; Beckman, Tokyo, Japan). Urinary concentrations of PGE_1, 6-keto-PGF_{1a}, and TXB_2 were measured by RIA (PEG method).

Table 1—*Composition of test meals*

	Tuna fish	Egg white
CHO (g/g protein)	0.14	0.09
NaCl (g/g protein)	0.11	0.10
Water (g/g protein)	2.96	8.43
Phosphorus (g/g protein)	15.4	1.1
Amino acid (mg/g protein)		
Asp	92	110
Thr	43	45
Ser	44	64
Glu	140	140
Pro	32	36
Gly	54	37
Ala	66	62
Val	48	73
Cys	10	31
Met	29	39
Ile	48	56
Leu	80	89
Tyr	37	40
Phe	40	60
His	93	25
Trp	13	16
Lys	87	69
Arg	66	59

Statistical analysis

All data are expressed as means ± SD. The significance of differences between the means was calculated using Wilcoxon's test.

RESULTS

Renal response to acute protein loading

The results of the protein loading tests are shown in Fig. 1. In healthy volunteer subjects, GFR increased significantly ($P < 0.01$) after ingestion of tuna fish from 98.7 ± 8.3 ml · min^{-1} · 1.73 m^{-2} during the baseline period to 134.5 ± 9.2 ml · min^{-1} · 1.73 m^{-2}. In diabetic patients, GFR also increased significantly after tuna fish ingestion (99.6 ± 10.4 to 140.2 ± 12.3). In healthy volunteer subjects, no significant differences were observed between GFR before and after ingestion of egg white, 0.7 g/kg body wt

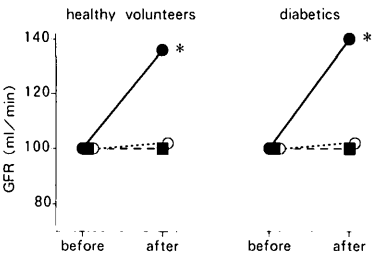

Figure 1—*Changes in GFR before and 3 h after ingestion of 0.7 g/kg of tuna fish (———), 0.7 g/kg of egg white (– – –), and 1.4 g/kg of egg white (· · · ·). GFR increased significantly after ingestion of tuna fish in both healthy volunteer subjects and diabetic patients (*P < 0.01 vs. baseline).*

(from 97.8 ± 9.4 to 102.4 ± 10.7 ml · min^{-1} · 1.73 m^2). No significant differences were detected between GFR before and after ingestion of egg white, 1.4 g/kg body wt, from 97.9 ± 12.0 to 103.0 ± 13.4 ml · min^{-1} · 1.73 m^2 after ingestion. In the diabetic patients, no significant differences were found between GFR before and after ingestion of egg white, 0.7 or 1.4 g/kg body wt. Mean blood pressure and blood glucose levels did not change in any subjects during this study.

Changes in plasma amino acids after protein loading

The results of plasma amino acid analysis are shown in Table 2. Almost all amino acids increased in plasma after ingestion of each meal. Ingestion of tuna fish caused significantly greater increases in total plasma amino acid concentration than egg white containing the same amount of protein. No significant differences were observed between the total plasma amino acid concentration after tuna fish loading and after ingestion of twice the amount of egg white.

Urinary excretion of PGs

Urinary 6-keto-PGF_a excretion increased significantly ($P < 0.01$) only after ingestion of tuna fish (Table 3). No significant differences were observed be-

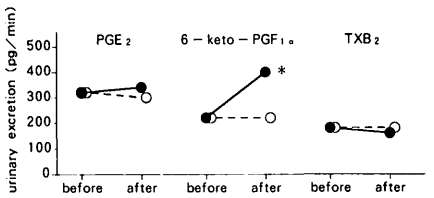

Figure 2—*Changes in urinary excretion of PGs after protein loads. Urinary 6-keto-PGF_{1a} excretion increased significantly after ingestion of tuna fish (*P < 0.01 vs. baseline), but did not change after egg white challenge.*

tween urinary excretion of PGE_1 or TXB_2 before and after ingestion of tuna fish. No significant differences were found between urinary excretion of PGs before and after administration of egg whites.

CONCLUSIONS— This study shows that independent of the quantity of protein ingested, administration of egg white has renal effects on GFR different from those of tuna fish. In our study, plasma levels of Gly, Ala, and Arg (amino acids known to induce glomerular hyperfiltration) increased significantly only after ingestion of tuna fish, which caused an increase in the GFR. We therefore believe that the difference in the increase of these plasma amino acids caused a difference in the renal responses to proteins.

Previously, it has been reported that dietary protein restriction prevents the progression of diabetic nephropathy (2,3). However, a low-protein diet may cause hypoproteinemia, muscle wasting, or malnutrition (4). The beneficial effect of a low-protein–essential amino acid diet has been reported (14,15). An essential amino acid diet, however, sometimes produces intolerable nausea. On the other hand, in our previous study, glomerular hyperfiltration was not induced after administration of certain kinds of protein, i.e., boiled egg white, cheese, or tofu (bean curd) (7,8). Our findings suggest that these foods can be excepted from dietary protein restriction. If these proteins need not be restricted, protein restriction may not cause malnutrition.

Intravenous administration of Ala, Gly, and Arg increased GFR. In our previous study, plasma levels of these amino acids increased significantly only after ingestion of tuna fish, which caused an increase in GFR (8). This finding suggests that the increase in GFR after ingestion of tuna fish may be caused by an increase of these amino acids in plasma. In contrast, ingestion of egg white, cheese, or bean curd did not produce increased plasma levels of these amino acids or glomerular hyperfiltration, de-

Table 2—*Changes in plasma amino acids concentrations after protein loading in healthy volunteer subjects and diabetic patients*

	Healthy volunteer subjects			Diabetic patients		
	tuna fish (0.7 g/kg)	egg white (0.7 g/kg)	egg white (1.4 g/kg)	tuna fish (0.7 g/kg)	egg white (0.7 g/kg)	egg white (1.4 g/kg)
Amino acid (nmol/ml)						
Total	1200.8 ± 72.4	549.9 ± 53.6*	1150.2 ± 69.5	551.3 ± 52.7*	1114.7 ± 67.8	
Asp	4.3 ± 1.4	1.9 ± 0.7†	4.5 ± 1.8	5.0 ± 1.3	2.0 ± 1.0†	4.9 ± 1.6
Thr	52.6 ± 17.7	30.4 ± 13.4†	58.1 ± 16.5	51.9 ± 16.5	28.6 ± 12.0†	53.2 ± 13.6
Ser	45.6 ± 16.3	33.7 ± 10.3	47.2 ± 15.9	47.9 ± 17.4	31.5 ± 9.9	52.1 ± 10.3
Glu	22.3 ± 7.8	9.2 ± 4.6†	30.7 ± 15.0	24.3 ± 6.9	6.2 ± 6.0†	31.4 ± 9.6
Pro	49.3 ± 10.1	41.9 ± 14.4	52.8 ± 22.1	53.9 ± 12.9	43.2 ± 11.7	47.6 ± 19.0
Gly	79.3 ± 38.7	12.1 ± 5.4*	16.4 ± 7.2	68.2 ± 27.0	17.5 ± 6.7	21.0 ± 9.1*
Ala	85.7 ± 26.1	25.6 ± 9.3	34.7 ± 12.4‡	75.2 ± 24.8	33.4 ± 9.4‡	33.8 ± 10.1‡
Val	182.6 ± 36.3	102.4 ± 25.8‡	245.9 ± 28.6	190.4 ± 35.9	103.5 ± 20.6‡	201.8 ± 22.4
Cys	5.1 ± 3.6	2.8 ± 2.6	8.6 ± 3.5	4.5 ± 3.8	3.8 ± 2.3	7.1 ± 4.7
Met	42.9 ± 6.4	16.0 ± 5.8‡	38.1 ± 8.8	46.0 ± 8.9	15.2 ± 4.6‡	44.3 ± 8.8
Ile	112.1 ± 20.1	49.8 ± 19.6‡	128.2 ± 42.9	153.5 ± 18.4	32.5 ± 13.4‡	135.6 ± 37.1
Leu	141.6 ± 36.4	59.8 ± 14.1*	168.0 ± 18.3	128.5 ± 27.0	70.3 ± 19.6*	157.2 ± 12.6
Tyr	40.6 ± 7.1	25.6 ± 8.1†	39.4 ± 7.2	33.3 ± 7.3	20.7 ± 6.5†	35.3 ± 5.7
Phe	18.3 ± 8.2	17.7 ± 6.0	20.1 ± 7.6	29.6 ± 6.1	24.5 ± 5.3	24.3 ± 7.9
His	50.8 ± 13.4	12.6 ± 5.2‡	19.4 ± 4.9*	46.7 ± 8.5	11.5 ± 3.7*	16.5 ± 5.6*
Trp	24.6 ± 6.0	8.1 ± 3.8*	25.2 ± 11.7	23.4 ± 6.8	12.5 ± 8.0†	32.6 ± 9.2
Lys	161.8 ± 19.4	59.1 ± 19.2†	148.9 ± 17.0	157.6 ± 20.6	42.3 ± 21.8*	152.0 ± 12.4
Arg	81.3 ± 18.4	41.2 ± 15.6*	64.0 ± 16.5	70.4 ± 16.1	52.1 ± 12.8†	64.0 ± 15.1

Data are means ± SD of the plasma concentration.
*$P < 0.01$ vs. postingestion of tuna fish.
†$P < 0.05$ vs. postingestion of tuna fish.
‡$P < 0.001$ vs. postingestion of tuna fish.

Table 3—*PG urinary excretion rate before and after protein loading in healthy volunteers*

	Tuna fish				Egg white			
	Baseline	1 h after ingestion	2 h after ingestion	3 h after ingestion	Baseline	1 h after ingestion	2 h after ingestion	3 h after ingestion
PGE_2 (pg/min)	327 ± 9	354 ± 12	350 ± 10	340 ± 11	321 ± 12	348 ± 11	348 ± 14	309 ± 16
6-keto-PGF_{1a} (pg/min)	215 ± 11	414 ± 25*†	484 ± 21*†	405 ± 19*†	207 ± 18	219 ± 34	264 ± 29	216 ± 24
TXB_2 (pg/min)	178 ± 15	194 ± 19	168 ± 27	162 ± 15	182 ± 14	179 ± 21	197 ± 19	180 ± 20

Data are means ± SD.
*$P < 0.01$ vs. baseline.
†$P < 0.01$ tuna fish vs. egg white.

spite the fact that these amino acids are as abundant in the other protein sources tested as in tuna fish. Furthermore, meat contains less of these amino acids than tuna fish, even though ingestion of meat induced hyperfiltration (5,6). The mechanism behind these phenomena remains to be elucidated. We could not rule out the possibility that these findings may be attributable to differences in the intestinal absorption of meals. In this study, we demonstrated the absence of any significant differences between the total plasma amino acid concentration after tuna fish loading and after administration of twice the amount of egg white. Moreover, GFR did not rise after administration of large amounts of egg white. These facts disprove any effect of absorption on the results.

Another explanation of the different renal responses to tuna fish and egg white is likely to reside in the different PG responses elicited by the two challenges. Vasodilatory PGs are likely to be the final effectors of renal hemodynamic and proteinuric responses to ingestion of certain kinds of protein. This possibility has been demonstrated in studies in which the renal response to a meat meal was abolished by the administration of indomethacin (16).

Our study involved only acute loading, and prolonged clinical study is necessary to evaluate this possibility and safety. Phosphorus and lipid content should be taken into consideration in prolonged diet therapy, because these have an unfavorable effect on renal function (17,18).

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