Effect of freeze-dried soybean curd (tofu) on various bodily functions

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Abstract : The present study was designed to examine the effect of freeze-dried soybean curd (tofu) on various bodily functions. The dietary experiment consisted of 4 days of a non-prescribed ordinary diet, 10 days of an experimental diet that contained 190 g of meat contributing about 38 g of protein (hereinafter referred to as the "meat period"), and 39 days of an experimental diet that contributed vegetable protein, i.e., freeze-dried tofu, corresponding to the protein that 190 g of meat would provide (hereinafter referred to as the "freeze-dried tofu period"). Three eggs (about 150 g) and 180 ml of cow's milk were prescribed for the daily diet, and staple and other foods were dispensed freely during both the meat and the freeze-dried tofu periods. The results showed that serum cholesterol levels and diastolic blood pressure significantly decreased in the freeze-dried tofu period as compared with the meat period. Freeze-dried tofu was found to be a valuable food for preventing lifestyle-related chronic diseases. J. Med. Invest. 46 : 67-74, 1999

Key words **:** *freeze-dried soybean curd, vegetable oil, serum cholesterol, blood pressure*

INTRODUCTION

With the recent developments in dietetics concerning proteins, amino acids, and fatty acids, the value of soybean protein, as well as animal protein that contains a healthy balance of essential amino acids, is receiving much attention. On the other hand, it has been shown that a large intake of animal fat contained in animal food can contribute to arteriosclerosis (1-3). Thus, much attention is being paid to the nutritional value of soybean products that contain vegetable protein (4-5). Fueling this interest, Michi *et al*. (6) report that freeze-dried soybean curd (tofu), a soybean product, decreases serum cholesterol levels.

In the present study, a dietary experiment was performed to determine the effect of freeze-dried tofu on various bodily functions.

Received for publication November 26, 1998 ; accepted January 6, 1999.

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EXPERIMENTAL METHOD

A. Experimental Period and Subjects

The periods of the experiment were enacted over a total of 71 days in the following order : ordinary diet period I (4 days), meat period (10 days), freeze-dried tofu period I (19 days), freeze-dried tofu period II (20 days), and ordinary diet period II (18 days). The subjects were 4 healthy male and 2 female adults (Table 1).

Table 1. Age, sex and physical characteristics of subjects

Subject (No.)	Age (years)	Sex	Height (cm)	Body weight (kg)
1	62	male	155.4	55.0
2	45	male	162.4	66.7
3	40	male	170.2	68.5
4	32	male	174.6	58.0
5	40	female	157.8	55.0
6	27	female	157.6	46.0

B. Measurement Items and Nutrient intakes

1. Body weight, condition of fatigue, and blood pressure

Body weight was measured before breakfast once a day. The condition of fatigue was assessed before lunch once a day by determining flicker values (obtained using Ohshima's measurement apparatus (7), blinker values obtained using Fukui's measurement apparatus) (7), and grasp values (obtained using dynamometer of Smedlay's method) (7). Blood pressure was measured with a sphygmometroscope.

2. Blood studies

Blood samples were collected before breakfast. Whole blood gravity (Gb), hemoglobin (Hb) and hematocrit (Ht) levels were measured using the copper sulfate method, the cyanomethemoglobin method, and the capillary method, respectively.

3. Liver function tests and measurement of serum protein levels

Serum was separated from the blood samples collected before breakfast to measure serum levels of total protein (TP) and liver function under the parameters such as GOT, GPT, ALP, albumin, and ZTT. A kit for liver function tests was supplied by Chugai Pharmaceutical Co., Ltd., and a specific apparatus was used for the tests.

4. Measurement of serum lipids

Serum was separated from the blood samples collected early in the morning before the subjects had ingested any food, to measure serum total cholesterol and serum free cholesterol levels. A kit for measuring serum lipids was supplied by Chugai Pharmaceutical Co., Ltd., and a specific apparatus was used for the test. Blood studies were performed on Day 3 of ordinary diet period I, on Day 8 of the meat period, on Days 9 and 18 of freeze-dried tofu period I, on Day 18 of freeze-dried tofu period II, and on Day 18 of ordinary diet period II.

5. Nutrient intakes

Based on individual food intake data that was recorded for a selected two-day span during each experimental period, nutrient intakes were calculated according to the Standard Tables of Food Composition in Japan (8).

C. Conditions for Dietary Intake in Experimental Periods

During the ordinary diet period, the subjects were given an unregulated diet. During the meat period, they were required to ingest meat (190 g), three eggs, and 180 ml of cows' milk per day, but the subjects were also allowed other foods, including drinks and staple food, without any restrictions. During the freeze-dried tofu period, the previously administered meat protein was replaced by vegetable protein, i.e., freeze-dried tofu (about 20 g/tofu x 4 units). Similarly, three eggs (about 150 g) and 180 ml of cows' milk were prescribed during the tofu period, but other animal protein intake was forbidden. Staple food, other foods, and drinks were allowed without any restrictions.

The aim of this study was to examine the effect of freeze-dried tofu on various functions of the body. To measure this effect, during the experimental portion of testing, while a sufficient total protein intake was prescribed throughout testing, animal protein was replaced by vegetable protein, i.e., freeze-dried tofu, and animal protein intake, other than the prescribed three eggs and 180 ml of cows' milk, was forbidden.

RESULTS

1. Nutrient intakes

Table 2 shows the mean nutrient intakes of the six subjects in each experimental period. The meat period was associated with the highest protein intake (108.4 g), which was about 38% higher than that in the ordinary diet period I. Protein intake was about 90 g in freeze-dried tofu periods I and II, which was slightly higher than that in ordinary diet period I. All subjects had consumed an amount of protein that satisfied the protein requirement in all experimental periods. As for the nature of protein consumed, animal protein alone increased by about 70% in the meat period as compared with the ordinary diet period. In the freeze-dried tofu period, however, it decreased to about half that in the ordinary diet period, and there was a two-fold increase in vegetable protein. Consequently, the animal/vegetable protein ratio was about 3 : 7 in the freeze-dried tofu periods I and II, as opposed to 7 : 3 in the meat period. Animal fat intake was 2.5 times higher in the meat period than in the ordinary diet period I, reflecting changes in the nature of protein. On the other hand, whereas animal fat intake was only slightly lower in freeze-dried tofu periods I and II than in the ordinary diet period I, there was a two-fold increase in vegetable fat intake during freeze-dried tofu periods. In the freeze-dried tofu periods, calcium intake was also particularly high, reaching 1.6 times (980 mg) the calcium requirement. Cholesterol intake was the highest (1100 mg) in the meat period in

which animal protein was predominant, followed by the freeze-dried tofu period in which eggs were given to supply animal protein (over 800 mg). In the ordinary diet periods I and II, cholesterol intake was about half that in the meat period.

2. Changes in body weight and blood pressure

The mean body weight and blood pressure of the six subjects are shown according to the experimental period of measurement in Table 3. Slight body weight gain was seen in the shift from the ordinary diet period I to the meat and freeze-dried tofu periods. This may be attributable to an increase in caloric intake. There were almost no differences in systolic blood pressure between ordinary diet period I and the meat period, but it was lower in the freeze-dried tofu periods than in the meat period. On the other hand, there was a statistically significant decrease in diastolic blood pressure from the

Table 2. Nutrient intakes in each experimental diet

	Ordinary I	Meat	Freeze-dried tofu		Ordinary II
			I	II	
Energy (kcal)	2139.7±388.0	2685.2±349.3	2268.7±381.5	2068.8±267.8	1994.8±469.6
Protein total (g)	78.8±12.7	108.4±10.4	90.0±9.6	88.2±5.3	84.6±16.2
Animal (g)	46.3±10.9	78.3±5.4	26.0±0.1	23.6±2.4	54.9±12.3
Animal protein ratio (%)	58.4±8.2	72.4±3.1	29.1±2.8	26.8±3.4	64.7±5.8
Lipid total (g)	51.5±19.3	101.1±12.8	71.8±8.5	63.3±4.5	48.9±21.2
Animal (g)	31.9±18.1	78.6±5.3	30.5±3.1	26.1±0.5	34.6±18.7
Animal lipid ratio (%)	60.0±14.6	78.2±5.5	42.6±2.3	41.3±4.2	69.6±12.4
Carbohydrate (g)	314.9±67.3	302.4±54.0	298.0±50.4	280.9±53.2	272.0±73.1
Minerals (mg)					
Ca	559.2±121.4	64.0±89.4	991.2±124.0	968.5±71.4	480.0±191.1
Fe	12.2±2.3	18.7±1.7	18.3±2.1	18.2±1.8	12.7±2.5
Vitamins A (IU)	1425.5±521.4	3258.3±526.0	2519.5±597.8	3035.2±668.5	1809.8±486.8
B_1 (mg)	1.47±1.05	2.33±0.35	1.66±0.28	1.21±0.09	1.30±1.14
B_2 (mg)	1.19±0.24	1.72±0.14	1.21±0.11	1.21±0.09	1.12±0.27
C (mg)	59.2±25.9	87.0±20.5	86.7±28.9	103.7±29.1	128.2±57.1
Cholesterol (mg)	479.11±112.4	1111.1±40.7	848.0±13.0	796.8±113.3	510.6±221.8

Table 3. Changes in body weight and blood pressure in each experimental diet

	Ordinary I	Meat	Freeze-dried tofu		Ordinary II
			I	II	
Body weight (kg)	55.5±14.7	56.8±7.7	58.6±7.6	58.8±7.5	58.1±8.8
SBP (mmHg) 1)	114.3±15.4	114.4±11.0	111.7±10.6	111.2±11.0	115.8±12.0
DBP (mmHg) 2)	71.4±6.0	72.9±7.6	69.1±7.1**	69.9±6.2*	72.2±6.3

1) Systolic blood pressure
2) Diastolic blood pressure
Values are expressed as mean±standard deviation of six subjects.
*$p<0.05$, **$p<0.01$ Significantly different from the Meat diet.

meat period to the freeze-dried tofu periods I ($p<0.01$) and II ($p<0.05$), as opposed to the increase in diastolic blood pressure that occurred between the ordinary diet period to the meat period. Both systolic and diastolic blood pressures were lower in the freeze-dried tofu periods than in the ordinary diet period I.

3. Measurement of fatigue

Mean flicker values, blinker values, and grasp values in six subjects are shown according to the experimental period of measurement in Fig. 1. There were minimal differences in all measured values among the experimental periods.

4. Changes in blood findings and liver function findings

As shown in Table 4, there were almost no differences in Gb, Hb, or Ht among the experimental periods. On the other hand, there were slight differences in serum proteins and liver function findings among the periods, which may have been attributable to the different quantity of protein intake. However, these findings were all within physiologically normal limits.

5. Changes in serum total cholesterol and serum free cholesterol

Table 5 shows the mean levels of serum total cholesterol and serum free cholesterol in the six subjects according to the experimental period of measurement. There were significant increases in both serum total cholesterol ($p<0.01$) and serum free cholesterol levels ($p<0.05$) in the meat period as compared with ordinary diet period I. However, both levels decreased between the meat period and freeze-dried tofu periods. In particular, there was a statistically significant difference in total cholesterol levels ($p<0.05$) and free cholesterol levels ($p<0.01$) between the freeze-dried tofu period II and the meat period. Free cholesterol levels were lower in freeze-dried tofu periods I and II than in the other experimental periods, and the ester rate tended to be high in the freeze-dried tofu periods.

DISCUSSION

The present study of nutrient intakes revealed that approximately 80 g of protein was consumed and the animal/vegetable protein ratio was 6 : 4 during the ordinary diet periods. In the meat period, during which the volunteers had to take 190 g of meat, the prescribed three eggs (about 150 g), 180 ml of cows' milk, and freely distributed staple and

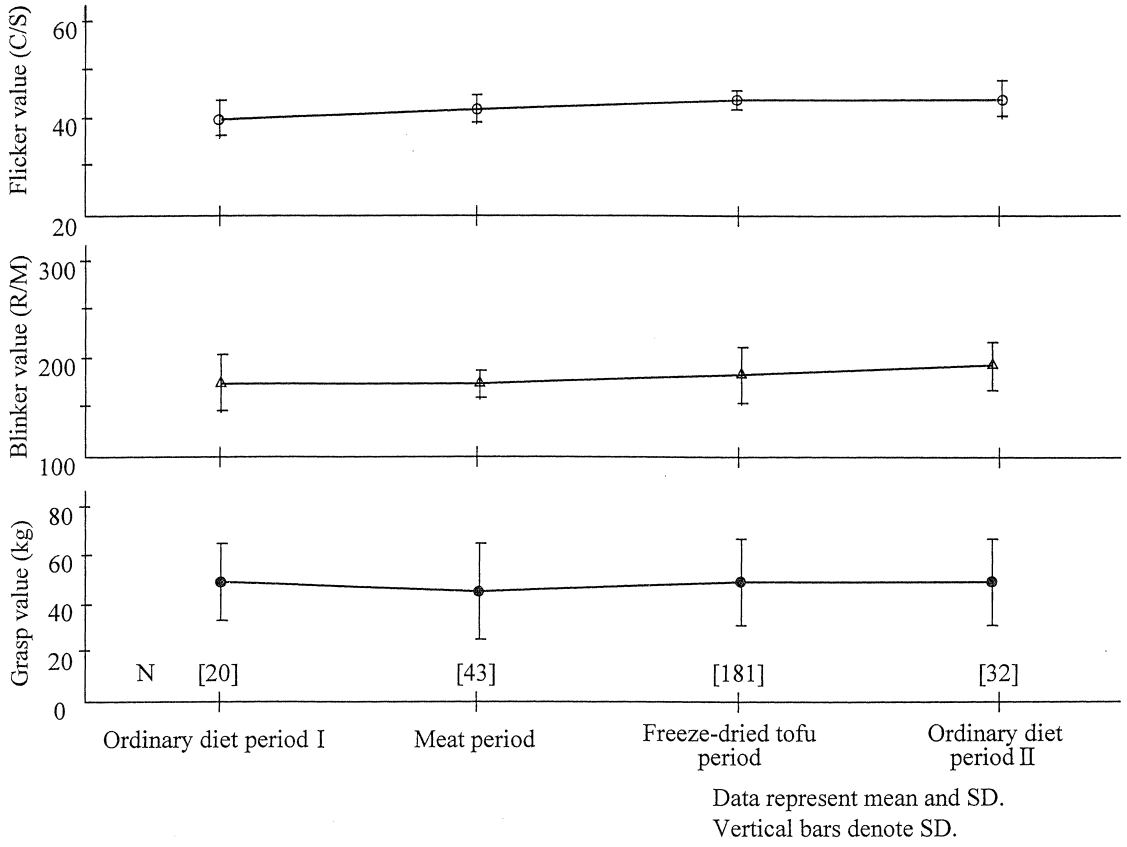

Fig. 1. Flicker, Blinker, and Grasp values in each experimental diet

Table 4. Changes in blood parameters in each experimental diet

	Ordinary I	Meat	Freeze-dried tofu		Ordinary II
			I	II	
Blood examinations GB	1.0567±0.0046	1.0567±0.0035	1.0558±0.0032	1.0557±0.0031	1.0555±0.0024
Hb (g/dl)	14.6±1.3	14.3±1.5	13.9±1.3	14.5±1.3	14.3±1.4
Ht(%)	43.8±3.9	43.8±4.0	43.4±3.2	43.1±3.6	43.2±3.4
TP (g/dl)	7.5±0.6	7.9±0.5	7.5±0.5	7.8±0.2	7.7±0.5
Liver function tests GOT (Karmen Unit)	14.8±12.4	16.2±8.5	17.5±7.2	10.7±8.0	14.3±11.9
GPT (Karmen Unit)	11.8±8.2	14.3±6.4	15.2±5.0	10.2±4.3	9.5±6.4
ALP (King-Armstrong Unit)	8.7±1.2	9.0±1.5	9.3±1.8	10.5±2.1	11.8±2.7
ZTT (Kunkel Unit)	6.9±7.0	5.8±1.9	7.6±2.8	6.5±2.6	5.0±2.2
Albumin (g/dl)	4.2±0.4	4.4±0.3	4.1±0.3	4.4±0.3	4.4±0.2

Values are expressed as Mean±Standard deviation of 6 subjects.

Table 5. Changes in serum cholesterol in each experimental diet

	Ordinary I	Meat	Freeze-dried tofu		Ordinary II
			I	II	
Total cholesterol (mg/dl)	184.8±15.4	220.0±19.5♦♦	199.0±20.6	188.3±19.0*	201.4±14.0
Free cholesterol (mg/dl)	57.7±5.8	70.5±9.1♦	56.8±7.9*	54.3±3.5**	62.2±4.7▲▲
Cholesterol ester (%)	68.9±3.6	68.1±1.7	71.5±1.1**	71.1±1.4**	69.3±2.8

♦p<0.05, ♦♦p<0.01 Significantly different from the Ordinary diet I.
*p<0.05, **p<0.01 Significantly different from the Meat diet.
▲▲p<0.01 Significantly different from the Freeze-dried tofu diet II.

other foods, total protein was about 110 g. Animal protein intake alone increased by about 70% as compared with the ordinary diet period I. Consequently, the animal/vegetable protein ratio was 7 : 3. In the freeze-dried tofu periods, the 38 g of protein that originated in the previously administered 190 g of meat was replaced by about 72 g of (vegetable protein) freeze-dried tofu, and three eggs and 180 ml of cows' milk were administered to contribute animal protein. The results showed that total protein intake was slightly higher (about 90 g) in the freeze-dried tofu periods than in the ordinary diet periods, but that the animal/vegetable protein ratio was 3 : 7, opposite to that in the meat period. Fat intake was dependent on the nature of protein. In the ordinary diet periods, total fat intake was about 50 g, and the animal/vegetable lipid ratio was 6 : 4. The meat period brought about a 2.5-fold increase in animal fat intake, and the animal/vegetable lipid ratio became 8 : 2. In the freeze-dried tofu period, animal fat intake was almost similar to that in the ordinary diet periods, but vegetable protein intake was two times higher (about 40 g) than in the ordinary diet periods. As a result, the animal/vegetable lipid ratio was 4 : 6, opposite to that in the ordinary diet periods. The discrepancy in such nutrient intakes led to changes in blood-test results. There was a statistically significant increase in serum total

cholesterol and free cholesterol levels in the meat period as compared with the ordinary diet periods. On the other hand, both parameters significantly decreased in the freeze-dried tofu period II as compared with the meat period. The proportion of animal fat intake, as well as cholesterol intake, are dietary factors influencing serum cholesterol levels, and the proportion of animal fat intake is known to influence the rate of absorption of cholesterol intake (9-10). Accordingly, the correlation between the cholesterol intake and the proportion of animal fat intake in the experiment was expressed as the following formula : cholesterol intake x animal fat intake/vegetable oil intake. As shown in Figs. 2 and 3, there was a statistically significant correlation between the proportion of animal fat intake and both serum total cholesterol and serum free cholesterol. The fact that the meat period was associated with not only higher cholesterol intake but also a higher proportion of animal fat intake appears to reflect the appearance of higher serum cholesterol levels. On the other hand, although the cholesterol intake was 1.5 times higher in the freeze-dried tofu periods than in the ordinary diet periods, and although there was little difference in cholesterol intake between the freeze-dried tofu period and the meat period, the fact that serum cholesterol decreased may be explained by a lower proportion of animal fat intake and a higher proportion of vegetable oil contained in freeze-dried tofu (11-12). Besides the vegetable oil in freeze-dried tofu, we consider that soybean protein also had an effect on lowering the serum cholesterol (13). As shown in Fig. 4, there was an inverse correlation between the proportion of animal protein and the proportion of animal fat/vegetable oil. While it is important to maintain a certain level of animal protein in terms of the quality of protein intake, an extremely high animal protein intake leads to an excessive intake of saturated fatty acid, resulting in an increase in serum cholesterol. Considering these factors, the proportion of animal protein intake is recommended to be within 40%-50% (14). In the present study, the proportion of animal protein intake to the entire nutrient intake was 60%-70%, which exceeded the recommended range, even in the ordinary diet period as well as in the meat period. This result should be carefully considered, because it may lead to an excessive intake of saturated fatty acid and an increase in serum cholesterol. On the other hand, because the proportion of animal protein intake was found to be low (about 30%) during the freeze-dried tofu periods, freeze-dried tofu appears to prevent an excessive intake of saturated fatty acid and to restrain an increase in serum cholesterol. It is also thought that limiting amino acids do not appear if there is

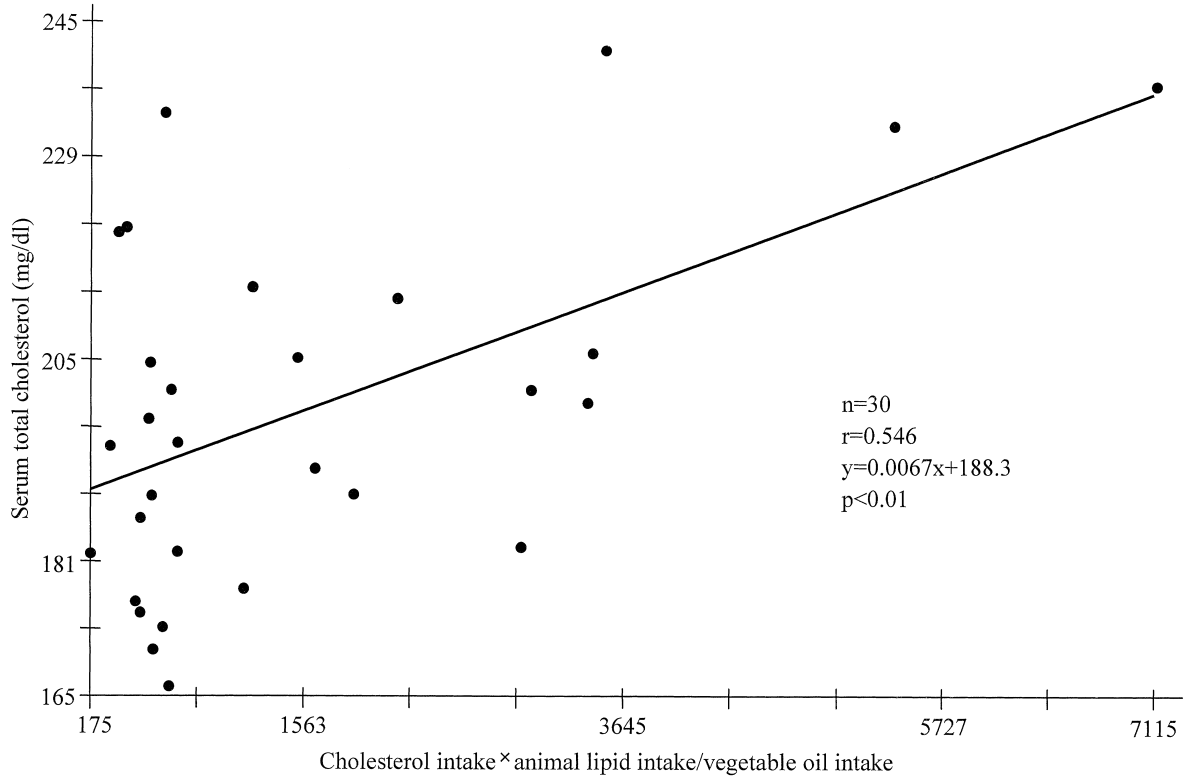

Fig. 2. Correlation between serum total cholesterol and values of multipling cholesterol intake by ratio of animal lipid intake to vegetable oil intake

sufficient protein intake, even of the proportion of animal protein intake is only 30% (15). When the amount of amino acids contained in the food consumed was calculated to determine the E/T ratio and N/E ratio in the present study, there were no differences in these ratios between the freeze-dried tofu period and the ordinary diet period. This indicates that the quality of protein is not reduced by a

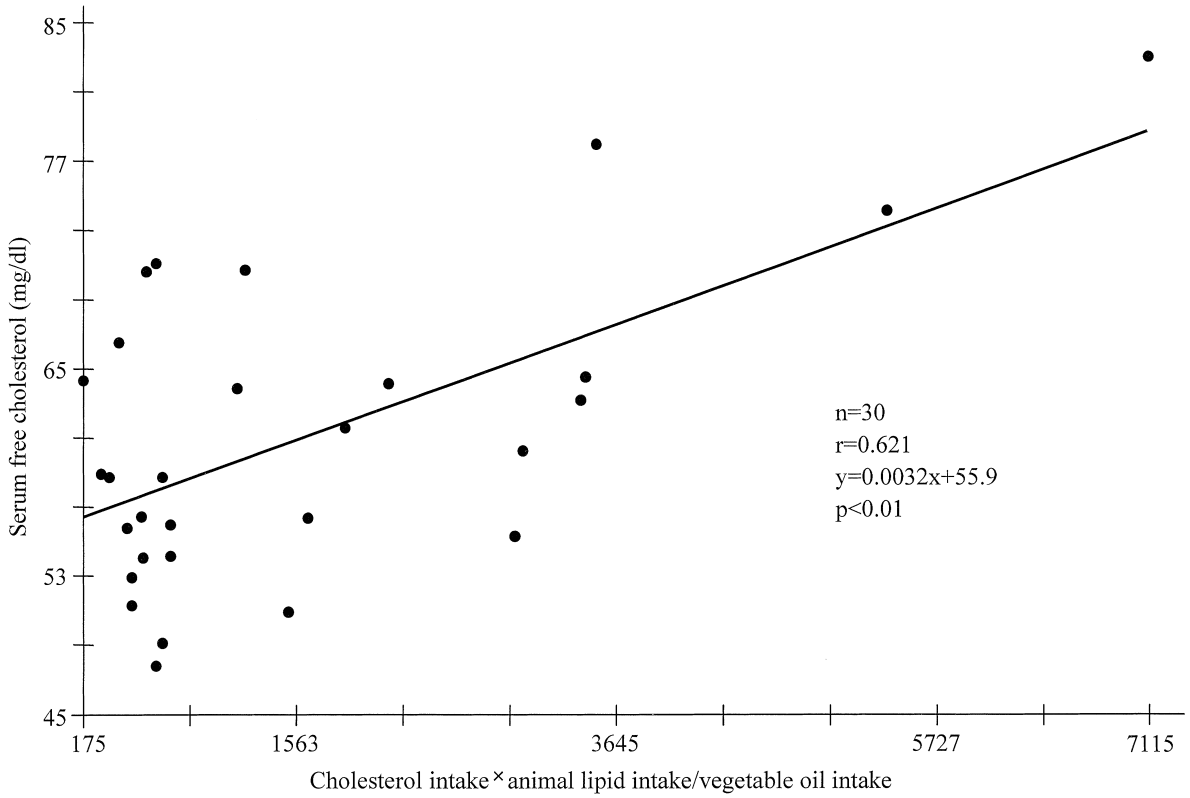

Fig. 3. Correlation between serum free cholesterol and values of multipling cholesterol intake by ratio of animal lipid intake to vegetable oil intake

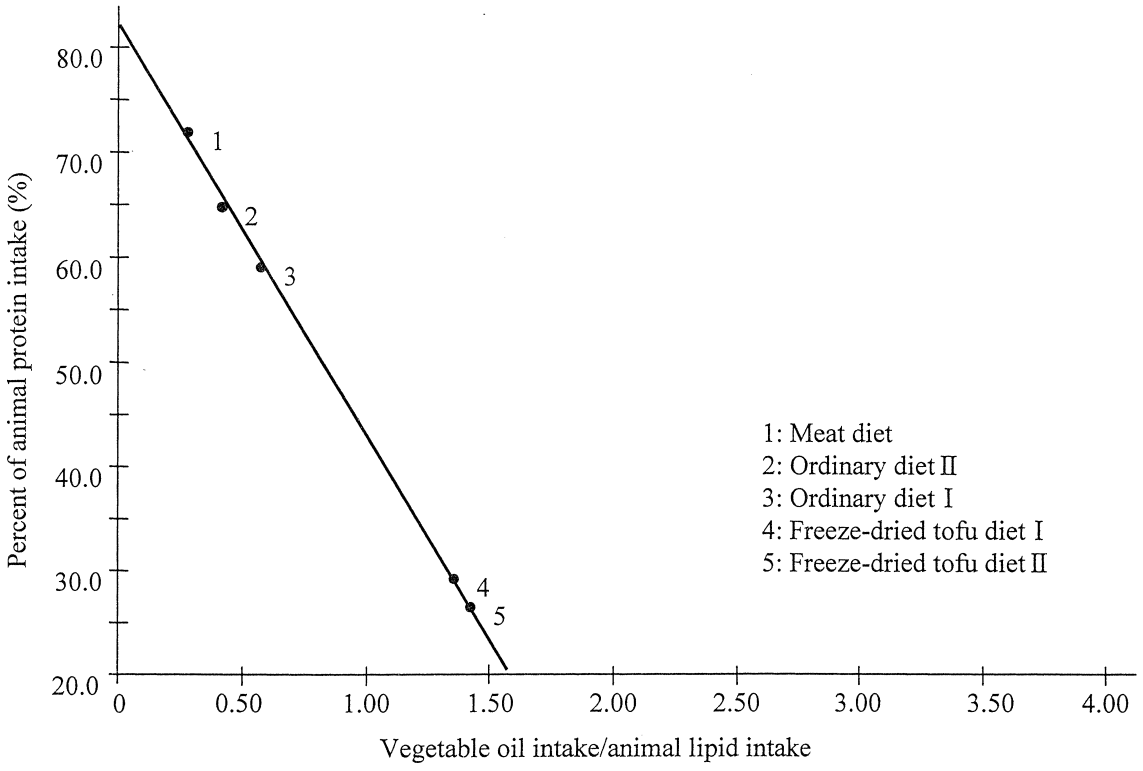

Fig. 4. Relationship between percent of animal protein intake and ratio of vegetable oil intake to animal lipid intake

freeze-dried tofu diet. This can also be explained by the fact that there were no differences in blood findings, such as Gb, Hb, Ht, and TP, or liver function findings among the experimental periods. From the standpoint of preventing lifestyle-related chronic diseases, it is important to maintain the normal limits of blood pressure. In the present study, diastolic blood pressure, which is frequently associated with high fat intakes and high cholesterol intakes, tended to increase in the meat period (16). On the contrary, freeze-dried tofu decreased both systolic blood pressure and significantly decreased diastolic blood pressure. It has already been shown that there is a correlation between blood pressure and dietary calcium and magnesium intakes (17), and as freeze-dried tofu is rich in minerals it deserves further attention. In conclusion, when animal protein intake was restricted to 30% and freeze-dried tofu administered to maintain a total protein intake similar to that of a meat-rich diet, excessive eating can be prevented, and blood findings, blood pressure, and serum cholesterol levels can be maintained within physiologically normal limits. Therefore, freeze-dried tofu appears to be a valuable food for preventing lifestyle-related chronic diseases.

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