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Clinical Application of the New HPLC Method for Fatty Acid Analysis

(1) Comparative Nutritional Assessment of Enteral Nutrients

TOMONOBU SATO and HIROSHI TANIMURA

Second Department of Surgery, Faculty of Medicine, Kyoto University

(Director: Prof. YORINORI HIKASA)

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Introduction

The administration of enteral nutrients to postoperative patients¹⁶⁾ is widely accepted to maintain satisfactory nutritional state over a long period of time^{3,9,10,33,39)}. However, it is well known that serious metabolic side-effects, such as essential fatty acid (EFA) deficiency occurs^{6,10,20,34,35)}, when an elemental diet (ED, Elental®) is administered for even a short period, because it contains only small amounts of EFA.

Therefore, the concomitant administration of fat emulsions should be considered when Elental is administered. Ensure® is one of the enteral nutrient modules in which linoleic acid comprises 20% of the calories. Its general clinical usefulness was already described.²⁸⁾

In this study we investigated whether Ensure which is rich in linoleic acid, can prevent EFA deficiency, from the viewpoint of changes in fatty acid composition, triene/tetraene (t/t) ratio and a new *omega*-9/*omega*-6 ratio which was proposed by the authors in the serum phospholipid fraction.

Fatty acid analysis was carried out by our newly devised HPLC method. Its expected usefulness in the clinical application was previously mentioned³¹⁾. The advantages of the new HPLC method such as rapid and accurate determination of eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6), which is impossible by the routine gas-liquid chromatography (GLC) and other HPLC method, and the rapid assessment of the EFA status are also discussed.

Therefore, this paper describes both clinical application of the new HPLC method, especially to polyunsaturated fatty acids (PUFA) analysis, and a comparative nutritional study between two enteral nutrients, Elental and Ensure, from the viewpoint of EFA status and prevention of EFA

Key Words: High-performance liquid chromatography (HPLC), Essential fatty acid (EFA) deficiency, Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), Enteral nutrients.

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Present address: Second Department of Surgery, Faculty of Medicine, Kyoto University Sakyo-ku, Kyoto, 606, Japan.

deficiency.

Materials and Methods

Enteral nutrients were administered for 7 consecutive days to 14 patients undergoing major abdominal surgery. The patients were divided into two groups of seven patients each, and received Ensure or Elental. The administration was done via a feeding tube to the jejunum, beginning with a concentration of 0.8 Cal/ml on the first postoperative day, increased to 1.0 Cal/ml by the third postoperative day and maintained at this concentration for four days. Both nutrients were administered from 8:00 a.m. to 12:00 p.m. for 16 hours.

The patients were given intravenous administration of glucose and amino acid solutions, but not fat emulsion, 25% at maximum of the total calories a day.

Blood was withdrawn before the operation and the next day after the termination of the administration (the 8th day). Fatty acid analysis was performed by HPLC in the serum phospholipid fraction. The details of the pretreatment procedures were described previously^{3,1)}.

The major compositions of the two synthetic enteral nutrients are shown in Table 1. A notable difference is found in fat content. Linoleic acid in Ensure comprises 53% of total fat content.

Table 1. Composition of Elental and Ensure

	Elental	Ensure
Fat	soy bean oil (1.5%)	corn oil (31.5%)
Nitrogen source	amino acids (16.9%)	casein from milk protein from soybean (14.0%)
Carbohydrate	dextrin (81.7%)	corn syrup sucrose (54.5%)

(Calorie %)

Table 2. Background parameters of patients receiving enteral nutrients

		Elental	Ensure
Number of patients		7	7
Age		57.4 ± 6.5	52.6 ± 14.6
Sex (M:F)		1:7	4:3
Calories/day		1134 ± 125	1032 ± 239
Maximum calories/day		1500 ± 300	1364 ± 413
Basal diseases	Esophageal cancer	1	0
	Gastric cancer	6	5
	Gastric ulcer	0	2
Operation performed	Total resection of esophagus	1	0
	Total gastrectomy	6	5
	Gastrectomy	0	2

mean ± SD

There were no significant differences in the background parameters such as operative procedures, age, calories a day or the maximum provided calories a day, between the two groups (Table 2).

Values are expressed as mean $\pm$ standard errors and the statistical analysis was carried out by Student's "t" test.

Results

i) Individual cases

Fatty acid composition in individual cases of the Elental group and the Ensure group are given in Tables 3 and 4, respectively. There were no changes in total fatty acid levels before and after the administration in both groups as shown in Fig. 1.

However, in the Elental group all cases showed a marked decrease in linoleic acid, whereas, in the Ensure group all cases showed a marked increase compared to the level before the administration (Fig. 2).

On the other hand, no significant change was observed in arachidonic acid content in either group.

ii) Mean levels of fatty acids

Mean levels of each fatty acid before and after the administration are given in Table 5. The level of representative fatty acids which are of interest because of their physiologic importance, are shown in Figs. 3-8.

1. Omega-6 fatty acids (Fig. 3)

Linoleic acid content decreased in the Elental group from 23.32 ± 3.73 mg/dl to 13.55 ± 1.74 mg/dl ($p < 0.05$). On the contrary, in the Ensure group it increased from 21.34 ± 4.51 mg/dl to

Table 3. Fatty acid levels in the serum phospholipid fraction before and after the administration of Elental (mg/dl)

Patient	1		2		3		4		5		6		7	
12:0	1.09	0.49	0.32	0.57	0.28	N.D.	N.D.	N.D.	0.18	0.14	N.D.	N.D.	N.D.	tr.
14:0	0.85	1.28	0.95	1.18	1.35	0.22	0.30	0.29	0.09	0.25	tr.	0.09	0.61	0.55
16:0	31.62	34.94	33.01	31.27	33.83	30.83	31.74	31.15	31.67	27.15	58.79	59.77	65.16	57.88
16:1	0.57	0.43	1.91	3.16	2.88	4.14	1.03	2.66	1.79	2.00	5.89	7.90	3.13	6.12
18:0	14.37	18.88	15.89	16.86	15.07	14.83	16.01	14.80	15.49	15.16	32.36	36.41	31.32	33.27
18:1	11.30	21.07	9.54	11.93	13.05	17.03	10.62	12.92	10.90	12.77	32.34	42.53	21.78	29.84
18:2	21.74	13.98	17.96	10.54	13.72	7.14	16.64	14.63	18.67	10.95	38.88	16.17	35.62	21.42
18:3	tr.	0.11	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
20:3	1.28	3.88	1.79	3.64	4.92	3.21	1.88	2.71	2.28	4.03	5.71	6.01	3.63	10.57
20:4	6.28	4.88	8.00	7.86	8.55	8.09	5.96	7.57	8.43	6.97	21.99	21.41	18.53	21.06
20:5	1.37	1.20	2.04	1.91	1.32	1.06	2.36	2.20	3.52	1.99	2.85	2.13	3.37	3.73
22:6	11.98	11.10	13.44	12.35	11.33	8.64	10.84	14.50	14.39	10.93	31.58	24.49	18.82	22.87
unidentified	102.50	112.21	104.90	101.32	106.29	95.20	97.38	103.38	107.40	92.34	230.40	216.88	202.00	184.42
total	105.00	114.01	106.00	102.02	107.99	96.01	98.00	103.98	108.00	91.00	232.00	219.98	203.00	211.02

mean $\pm$ SE, N.D.: not detected, tr.: trace

Table 4. Fatty acid levels in the serum phospholipid fraction before and after the administration of Ensure (mg/dl)

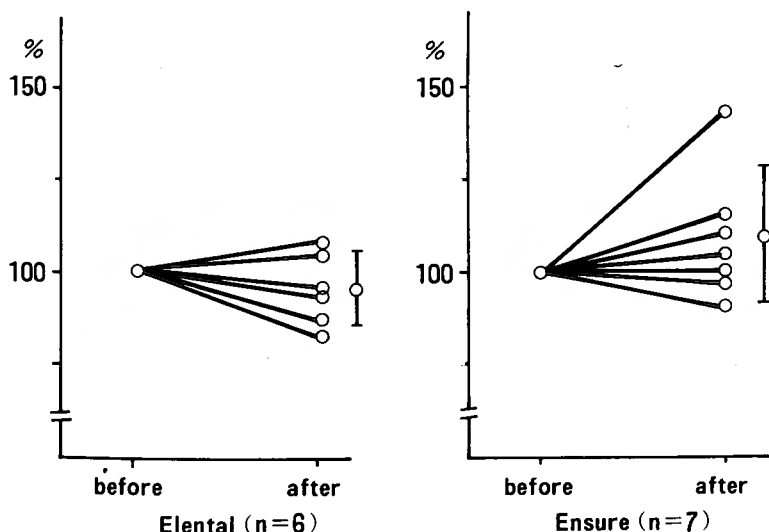
Patient	1		2		3		4		5		6		7	
12:0	0.41	0.19	0.43	0.73	1.98	0.71	tr.	N.D.	N.D.	N.D.	N.D.	tr.	N.D.	N.D.
14:0	1.13	0.55	1.23	0.94	0.61	0.52	0.38	0.18	0.22	0.22	0.19	0.48	0.62	tr.
16:0	35.19	38.81	42.20	42.10	50.19	55.31	31.68	27.90	30.07	29.30	28.61	27.04	18.82	60.95
16:1	3.38	2.62	0.41	0.58	0.20	0.26	2.10	0.56	2.40	0.30	2.46	0.90	5.69	3.15
18:0	16.66	14.53	18.82	23.47	21.65	23.77	19.07	18.69	11.63	12.68	15.36	17.07	6.06	36.12
18:1	17.71	12.61	15.37	15.25	16.93	18.23	10.55	9.31	12.25	10.67	13.44	10.63	26.63	21.11
18:2	9.68	18.86	25.47	39.44	29.70	40.72	16.20	21.70	15.43	24.89	10.37	19.01	42.54	45.95
18:3	tr.	tr.	tr.	tr.	0.17	tr.	tr.	tr.	tr.	N.D.	tr.	tr.	tr.	tr.
20:3	3.05	2.52	2.92	6.17	2.73	3.49	2.33	2.94	2.00	2.86	2.39	2.46	5.70	6.27
20:4	7.66	10.17	6.08	10.73	10.48	11.59	9.00	6.32	5.81	4.36	6.85	4.88	22.50	16.80
20:5	3.14	2.87	2.70	0.79	3.51	3.20	2.20	0.55	1.26	0.50	2.19	0.47	4.68	2.82
22:6	11.59	12.12	13.64	8.03	15.74	13.68	11.79	7.57	10.75	6.71	8.32	5.35	28.69	19.92
unidentified	0	0.20	1.70	3.70	1.90	1.50	0.70	0.30	0.20	0.52	0.82	0.01	0.10	2.10
total	109.60	116.10	131.10	151.90	155.80	173.00	106.10	96.02	92.02	93.01	91.00	88.30	162.00	215.20

mean $\pm$ SE, N.D.: not detected, tr.: trace

30.08 ± 4.36 mg/dl ($p < 0.05$). Therefore, a marked difference was observed in linoleic acid content after the administration of Elental and Ensure ($p < 0.01$). However, no change in arachidonic acid was observed in either group.

2. Omega-9 fatty acids (Fig. 4)

No significant change in palmitoleic acid was observed in the Elental group, on the other

**Fig. 1.** Total fatty acid content in the serum phospholipid fraction before and after the administration of enteral nutrients.

The levels before the administration is expressed as 100%.

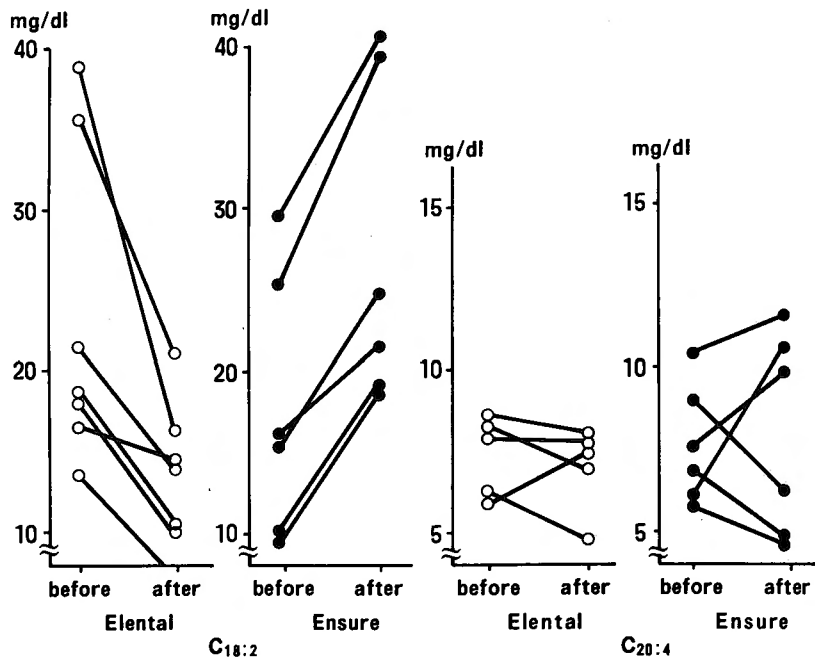


Fig. 2. Fatty acid content in the serum phospholipid fraction before and after the administration of enteral nutrients.

hand, a moderate decrease was observed in the Ensure group ($p<0.05$). Therefore, a significant difference in palmitoleic acid was observed after the administration of Elental and Ensure ($p<0.05$).

Table 5. Fatty acid composition in the serum phospholipid fraction before and after the administration of enteral nutrients

	(mg/dl)			
	Elental (n=7)		Ensure (n=7)	
	before	after	before	after
12:0	0.27± 0.15	0.17± 0.09	0.40± 0.27	0.23± 0.13
14:0	0.59± 0.19	0.55± 0.18	0.63± 0.16	0.41± 5.14
16:0	40.83± 5.51	39.00± 5.19	33.82± 3.81	40.2 ± 5.14
16:1	2.46± 0.67	3.77± 0.96	2.38± 0.70	1.20± 0.45
18:0	20.07± 3.05	21.46± 3.51	15.65± 1.99	20.90± 2.98
18:1	15.65± 3.15	20.16± 4.29	16.13± 2.00	13.98± 1.66
18:2	23.32± 3.73	13.55± 1.74	21.34± 4.51	30.08± 4.36
18:3	tr.	0.02± 0.02	0.02± 0.02	tr.
20:3	3.07± 0.65	4.86± 1.03	3.02± 0.47	3.82± 0.63
20:4	11.25± 2.40	11.12± 2.64	9.77± 2.21	9.26± 1.67
20:5	2.40± 0.34	2.03± 0.33	2.81± 0.42	1.60± 0.49
22:6	16.05± 2.78	14.98± 2.35	14.36± 2.54	10.48± 1.93
Total	137.1 ±21.0	134.0 ±21.2	121.1± 11.0	133.4 ±18.2

mean±SE

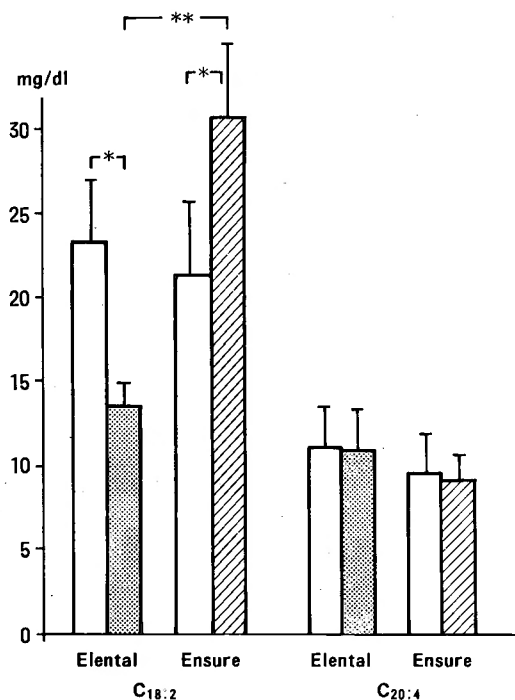


Fig. 3. Fatty acid content in the serum phospholipid fraction before (blank bar) and after (dotted and shaded bars) the administration of enteral nutrients. mean $\pm$ SE, *: $p < 0.05$, **: $p < 0.01$

Although a slight increase in oleic acid was noted in the Elental group and a slight decrease was noted in the Ensure group, they were not statistically significant.

Eicosatrienoic acid increased in the Elental group, from 3.07 ± 0.65 mg/dl to 4.86 ± 1.03

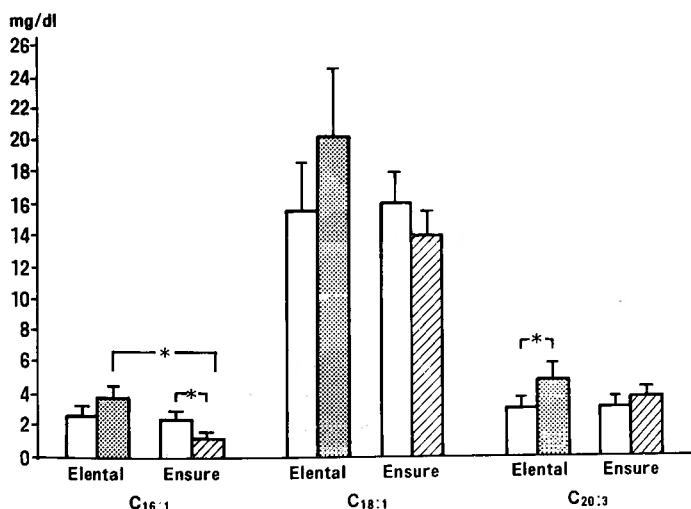


Fig. 4. Fatty acid content in the serum phospholipid fraction before (blank bar) and after (dotted and shaded bars) the administration of enteral nutrients. mean $\pm$ SE, *: $p < 0.05$.

mg/dl ($p < 0.05$), whereas, in the Ensure group, no statistically significant increase was observed.

3. T/t ratio (Fig. 5)

The ratio increased, except for one case each, in the Elental group and the Ensure group, and the mean values increased from 0.29 ± 0.05 to 0.48 ± 0.06 ($p < 0.05$), and from 0.33 ± 0.03 to 0.45 ± 0.06 , respectively as shown in the left half of Fig. 8. The increase in the Ensure group was not statistically significant. In four cases in the Elental group and three cases in the Ensure group, the ratios were elevated from the non-deficient level to the deficient level. But no clinical symptoms such as dermatitis, fatty liver, anemia, thrombocytopenia, infection, dehydration were observed.

4. Sum of *omega*-9 fatty acids and sum of *omega*-6 fatty acids (Fig. 6)

There was no statistical difference in the sum of *omega*-9 fatty acids, which consist of palmitoleic, oleic and eicosatrienoic acids, in the same group between the levels before and after the administration. In the Elental group, a change from 21.17 ± 4.35 mg/dl to 25.48 ± 5.20 mg/dl, on the other hand, a change from 21.52 ± 2.95 mg/dl to 18.98 ± 2.40 mg/dl in the Ensure group were observed. However, the levels after the administration were significantly different ($p < 0.05$) between the two groups.

The changes in the sum of *omega*-6 fatty acids between the two groups also contrasted markedly. In the Elental group, a significant decrease from 34.42 ± 6.05 mg/dl to 24.54 ± 4.07 mg/dl ($p < 0.05$) was observed, while, an increase from 31.10 ± 6.44 mg/dl to 39.35 ± 5.78 mg/dl was observed in the Ensure group, but not statistically significant. Thus, the levels between the two groups after the administration were markedly different ($p < 0.01$).

5. *Omega*-9/*omega*-6 ratio (Fig. 7)

This ratio was calculated from the equation of the sum of palmitoleic acid, oleic acid and

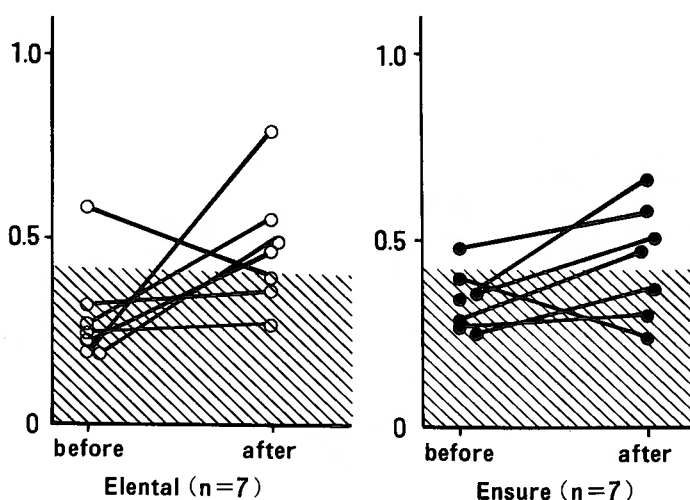


Fig. 5. Triene/tetraene ratios in the serum phospholipid fraction before and after the administration of Elental and Ensure.

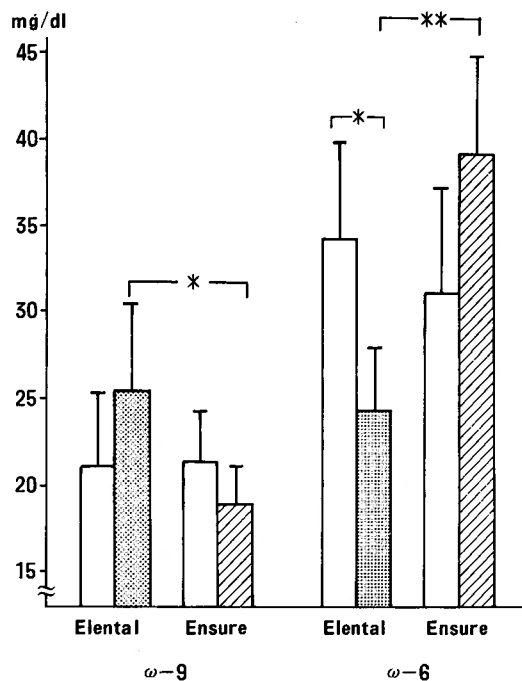


Fig. 6. Fatty acid levels before and after the administration of Elental and Ensure. $\omega-9 = C_{16:1} + C_{18:1} + C_{20:3}$, $\omega-6 = C_{18:2} + C_{20:4}$ mean $\pm$ SE, *: $p < 0.05$, **: $p < 0.01$.

eicosatrienoic acid divided by the sum of linoleic acid and arachidonic acid.

The $\omega-9/\omega-6$ ratio of each case and the mean values for both groups before and after the administration are shown in Table 6 and Fig. 7. The ratio increased in all cases in the Elental

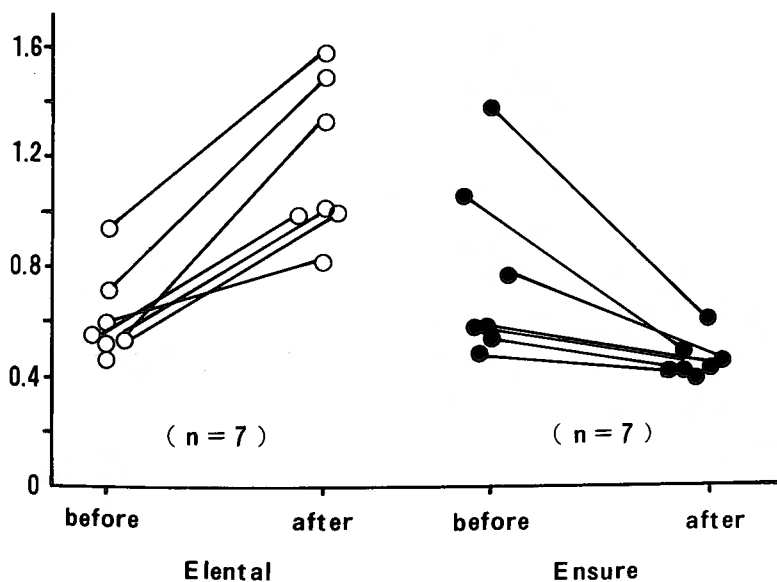


Fig. 7. $\omega-9/\omega-6$ ratio before and after the administration of Elental and Ensure.

Table 6. The ratios of triene/tetraene and ω -9/ ω -6 before and after the administration of Elental and Ensure

Elental group					Ensure group				
Patient	t/t ratio		ω -9/ ω ratio		Patient	t/t ratio		ω -9/ ω -6 ratio	
	before	after	before	after		before	after	before	after
1	0.22	0.79	0.47	1.35	8	0.40	0.25	1.39	0.61
2	0.22	0.46	0.51	1.02	9	0.48	0.58	0.59	0.44
3	0.58	0.40	0.94	1.60	10	0.26	0.30	0.49	0.42
4	0.32	0.36	0.60	0.82	11	0.26	0.47	0.59	0.46
5	0.27	0.55	0.55	1.05	12	0.34	0.66	0.79	0.46
6	0.25	0.28	0.72	1.50	13	0.35	0.50	1.06	0.95
7	0.20	0.50	0.53	1.10	14	0.25	0.37	0.58	0.49
mean ±SE	0.29± 0.05	0.48± 0.06	0.62± 0.06	1.21± 0.11		0.33± 0.03	0.45± 0.06	0.78± 0.12	0.50± 0.03

group, but, decreased in all cases in the Ensure group.

The ω 9/ ω 6 ratio of each case and the mean values for both groups before and after the administration are shown in Table 6 and Fig. 7. The ratio increased in all cases in the Elental group, but, decreased in all cases in the Ensure group.

The mean value of the ratios is shown in the right half of Fig. 8. In the Elental group, it increased from 0.62 ± 0.06 to 1.21 ± 0.11 ($p<0.01$), while, in the Ensure group, it decreased from

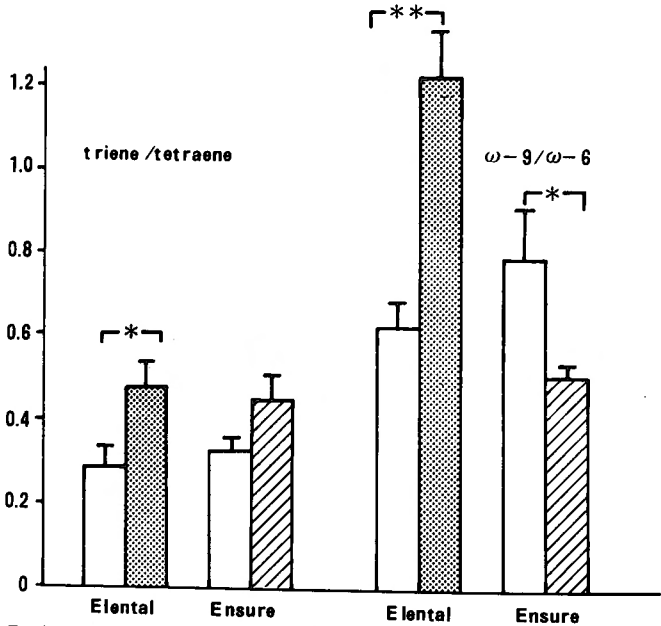


Fig. 8. Ratios of triene/tetraene and ω -9/ ω -6 before (blank bars) and after (dotted and shaded bars) the administration of Elental and Ensure mean $\pm$ SE, *: $p<0.05$, **: $p<0.01$

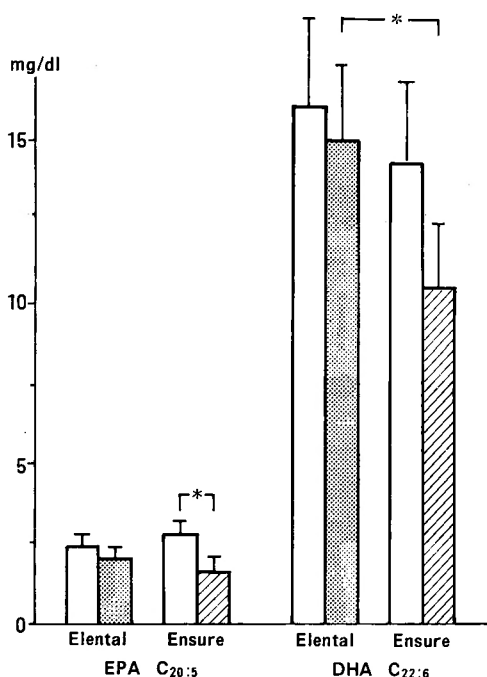


Fig. 9. Fatty acid content in the serum phospholipid fraction before (blank bar) and after (dotted and shaded bars) the administration of enteral nutrients. mean $\pm$ SE, *: $p < 0.05$

0.78 ± 0.12 to 0.50 ± 0.03 ($p < 0.05$).

6. Omega-3 fatty acids (Fig. 9)

Although a significant decrease in EPA was observed in the Ensure group ($p < 0.05$), no significant decrease in EPA was observed in the Elental group, from 2.81 ± 0.42 mg/dl to 1.60 ± 0.49 mg/dl, and 2.40 ± 0.34 mg/dl to 2.03 ± 0.33 mg/dl, respectively. A slight decrease in DHA from 16.05 ± 2.78 mg/dl to 14.98 ± 2.35 mg/dl was observed in the Elental group and from 16.05 ± 2.78 mg/dl to 14.98 ± 2.35 mg/dl was observed in the Elental group and from 14.36 ± 2.54 mg/dl to 10.48 ± 1.93 mg/dl in the Ensure group, but neither was statistically significant. However, the levels in DHA between the two groups after the administration was statistically significant ($p < 0.05$).

Discussion

Since the introduction of elemental diets (ED) in the 1970's, a drastic improvement in nutritional support for nutritionally depleted patients has been achieved. Moreover, patients maintained on ED escape pneumothorax, hemothorax, and sepsis which are the most frequent and serious complications encountered during total parenteral nutrition (TPN) maneuver.

However, it has been found that a serious metabolic side-effect, namely, EFA deficiency occurred among patients who were maintained on ED. Moreover, the EFA deficiency pattern in serum fatty acid was noted as early as a few days after the initiation of ED when no symptoms

are detected or no abnormal laboratory findings except for compositional changes in serum fatty acids are observed. EFA deficiency occurs earlier than expected, especially in nutritionally depleted patients such as those with inflammatory bowel disease who are candidates for ED or TPN²⁶⁾.

At present, concomitant administration of fat emulsions, with linoleic acid comprising 50% of the calories is widely accepted for prevention and treatment of EFA deficiency³⁶⁾. However, the required dose was not determined for individual cases, and 200 ml of 10% fat emulsion every day or 500 ml of it every other day is administered empirically. This dose is dependent on the recommendation that EFA content required for prevention of EFA deficiency is 4% of total calories, but it is still under debate^{4,7,14)}. The optimal dose should be ideally determined for individual cases by fatty acid analysis.

Thus, it is necessary to carry out fatty acid analysis in patients receiving enteral nutrients³⁰⁾. However, because the current methods are laborious and tedious, and their accuracy is less reliable, it has not yet been fully applied clinically.

Therefore, a rapid, precise and reliable method to carry out fatty acid analysis has been awaited. The author devised a new HPLC method and its future clinical application was discussed³¹⁾.

In the Elental group a decrease in linoleic acid and an elevation of t/t ratio were observed as expected, because Elental contains only a small amount of linoleic acid, namely, less than 1.0% of total calories.

In the Ensure group, a marked increase in linoleic acid was observed but not in arachidonic acid or eicosatrienoic acid. Thus, suppression in the elevation of t/t ratio was also observed in the Ensure group in good contrast to the significant increase in t/t ratio in the Elental group. From these observations it was concluded that Ensure elevated linoleic acid and suppressed the increase in eicosatrienoic acid compared with Elental but did not induce an increase in arachidonic acid in the serum phospholipid fraction from the early postoperative patients during a short-term (7 days) period. However, an increase in arachidonic acid in the serum phospholipid fraction might be observed if longer administration of Ensure (months?) is performed.

The finding of an increase in linoleic acid with no increase in arachidonic acid implies that enterally administered linoleic acid contained in corn oil as triglyceride was well absorbed from the intestinal lumen and converted into chylomicrons and transferred into the liver where it was incorporated into the serum phospholipid fraction. However, the conversion from transferred linoleic acid to arachidonic acid was not demonstrated, although arachidonic acid should have been synthesized by chain elongation and desaturation in liver mitochondria in the presence of abundant linoleic acid^{12,25,27,32)}. It is probable that total calories supplied was not enough initially, thus the administered linoleic acid might have been consumed as an energy source to spare arachidonic acid. However, a marked increase in linoleic acid, the level of which had not been deficient before the administration, refutes this hypothesis. Moreover, in the Ensure group, a decrease in arachidonic acid was observed in three cases in spite of an increase in linoleic acid.

The eight plausible factors that inhibit arachidonic acid synthesis from linoleic acid are as follows:

1. Inadequate supply of substrate; Arachidonic acid is not synthesized in a substrate (linoleic acid) deficient state. This is the state of EFA deficiency where the chain elongation and desaturation enzymes act in *omega*-9 fatty acids, namely, increases in palmitoleic acid, oleic acid and eicosatrienoic acid are observed¹⁴⁾. The *omega*-9 fatty acids and *omega*-6 fatty acids compete for the same chain elongation and desaturation enzyme systems^{2,19,24,32)}. But the affinity of the *omega*-6 fatty acids for the enzyme systems is much higher than that of the *omega*-9 fatty acids in the presence of linoleic acid^{1,5,13)}. Thus, if linoleic acid is abundant, no increase in *omega*-9 fatty acids are observed. If linoleic acid supply is insufficient, the enzyme system favours the *omega*-9 fatty acids, thus increasing *omega*-9 fatty acid levels.

2. Inadequate caloric supply; With an insufficient supply of calories, the administered linoleic acid is consumed as an energy source to spare protein. In this study, the daily calorie was 1800 Cal even at maximum, and this level is considered to be insufficient in the early postoperative period. However, if it were true, no increase or only a slight increase in linoleic acid would have been observed because linoleic acid had been consumed as an energy source as another fatty acids and not spared as an EFA. However, a marked increase in linoleic acid was observed. Thus, it was concluded that caloric supply was not insufficient for the cases studied in this trial.

3. Increased catabolism; In the early postoperative period, from the first until the fifth or seventh day, negative nitrogen balance is observed in spite of energetic supply of calories and amino acids by TPN. This means that even if sufficient calorie and sufficient doses of linoleic acid were given, linoleic acid was not used as a substrate for arachidonic acid synthesis. This might be the most plausible explanation. In the era when TPN was not available, negative nitrogen balance continued for seven to ten days after major abdominal surgery.

4. Latent period; WENE et al reported that fat-free regimen (tube feeding and TPN) induced an EFA deficiency pattern in blood fatty acid composition as early as three days after the initiation of experimental feedings³⁸⁾. A decrease in linoleic acid and the appearance of eicosatrienoic acid, which was considered to be absent in the nondeficient state, were observed. However, no remarkable changes in arachidonic acid were seen during such a short-term period. WENE's experiments showed no decrease in arachidonic acid in spite of a marked decrease in linoleic acid, and the appearance of eicosatrienoic acid with concomitant increases in palmitoleic acid and oleic acid seven days after the initiation of a fat-free regimen. This implies that there is a latent period for the decrease in arachidonic acid. Conversely, the latent period might explain the absence of an increase in arachidonic acid in spite of an increase in linoleic acid. Most clinical reports of EFA deficiency revealed decreases in arachidonic acid but these cases exhibited far advanced EFA deficiency.

5. Increased breakdown of arachidonic acid; It might be considered that the breakdown of arachidonic acid canceled its production. PUFA such as arachidonic acid is more prone to be oxidized to the corresponding more saturated fatty acids than the less unsaturated and saturated fatty acids. In other words, the turn-over rate of arachidonic acid is higher than those of other

fatty acids.

6. Eicosatrienoic acids; A high level of eicosatrienoic acid was considered to inhibit conversion of linoleic acid into arachidonic acid³⁷⁾. However, this explanation should be discarded, because there was no significant increase in eicosatrienoic acid in the Ensure group.

7. Olefinic acid (C18:1) isomers; C18:1-isomers is reported to inhibit arachidonic acid synthesis, aggravate EFA deficiency and affect prostaglandin synthesis^{15,22,23)}. However, this is unlikely, because no detectable amounts of C18:1-isomers were present in this study.

8. Another explanation for the absence of an increase in arachidonic acid is that an already sufficiently high level before the administration initiates some feed-back mechanisms that prevent the conversion from linoleic acid into arachidonic acid²⁾.

The present study suggests that enteral administration of fat (linoleic acid?) contained in Ensure induces decreases in EPA and DHA.

There is no appreciable *de novo* synthesis of these fatty acids in human, so they must be provided exogenously²⁹⁾. The Elental supplies only small amounts of fat, thus mobilization of these fatty acids into blood released from fat depot by lipase occurs smoothly and their blood levels are maintained. However, in abdominal subcutaneous tissue obtained during laparotomy, the EPA and DHA levels were too low to supply these fatty acids to the liver and serum (unpublished data).

It is very probable that they are mobilized from the liver. Rat liver contains large amounts of PUFA in the cellular phospholipid fraction as well as testis and heart^{21,40,41)}. It was considered that EPA and DHA was mobilized from liver to maintain serum levels in the Elental group, although no data on the EPA and DHA contents in the liver of human are available and species differences must be taken into consideration. On the contrary in the Ensure group, its high fat content prevented smooth fatty acid mobilization from the liver, thus, decreases in these fatty acids were observed.

These findings concerning the changes in EPA and DHA have not been reported due to lack of useful analytical instruments. The importance of these fatty acids were already briefly discussed in a previous study³¹⁾, showing that the new HPLC method devised by the authors³¹⁾ is a useful analytical method to investigate the metabolisms of these fatty acids.

At present, the t/t ratio is widely accepted to assess the EFA status¹¹⁾. However, as the knowledge about the PUFA, especially EPA, has increased, other parameters that reflect the EFA status more sensitively and more physiologically have been lacking. Moreover, the physiological and biochemical value of the t/t ratio has also been questioned by KINSELLA et al^{17,18)} and by HASSAM et al⁸⁾. It seems that it may not be more than just one of many parameters needed to describe the EFA.

In discussing the EFA status, EFA must be assessed by the sum of linoleic acid and arachidonic acid. For the same reason, the EFA deficiency must be judged from the sum of palmitoleic acid, oleic acid and eicosatrienoic acid all of which increase in case of EFA deficiency.

A marked difference was observed between the t/t and *omega*-9/*omega*-6 ratios in the Ensure group. In that group all cases except the patient 8, show decreases in the *omega*-9/*omega*-6 ratio,

in spite of increases in the t/t ratio, whereas, the patient 3 in the Elental group shows a marked increase in the *omega*-9/*omega*-6 ratio, in spite of a decrease in the t/t ratio.

With regard to the *omega*-9/*omega*-6 ratio, there was a marked difference in the ratios and their mean values between the Elental group and the Ensure group, although no difference was observed in the t/t ratios between the two groups. Moreover, if the EFA status is assessed only by the t/t ratio, the same trend in t/t increment might lead investigators to conclude that both enteral supplements aggravate EFA deficiency.

However, if the EFA status is judged from the *omega*-9/*omega*-6 ratio, a marked decrease in the Ensure group and a marked increase in the Elental group are observed, therefore, we conclude that Ensure did not induce EFA deficiency.

In the Ensure group, a marked decrease in the palmitoleic acid ($p < 0.05$) and a slight decrease in oleic acid (not statistically significant) were reflected in a marked decrease in the *omega*-9/*omega*-6 ratio, although no significant changes in eicosatrienoic acid or arachidonic acid were observed.

Thus, the EFA status should be assessed not only by the t/t ratio but also by the *omega*-9/*omega*-6 ratio, although its normalcy is not yet determined.

Judging from these observations with respect to fatty acid compositional changes in the serum phospholipid fraction, Ensure might be administered in the early postoperative period to patients with normally functioning bowels instead of Elental which, eventually causes biochemical EFA deficiency as early as four days.

Conclusion

Changes in the fatty acid composition and EFA status between the Elental and Ensure groups were compared, in the serum phospholipid fraction by the new HPLC method devised by the authors, and the following conclusions were obtained.

- 1) Ensure induced an increase in linoleic acid and a decrease in palmitoleic acid, whereas, Elental induced a decrease in linoleic acid and an increase in eicosatrienoic acid.

- 2) Although the triene/tetraene ratio exceeded the upper normal limit (0.4) in both groups, the *omega*-9/*omega*-6 ratio considered by the authors to be more valuable for assessing the EFA status than the t/t ratio, revealed a significant difference between the two groups; a marked increase in the Elental group and a marked decrease in the Ensure group.

- 3) Moreover, a decrease in EPA was observed in the Ensure group. A decrease in DHA was also observed in comparison with the level in the Elental group after the administration.

Judging from the above mentioned changes in fatty acid composition and the *omega*-9/*omega*-6 ratio, Ensure is a more suitable enteral nutrient module from viewpoint of preventing EFA deficiency which undoubtedly occurs even during a short-term administration of Elental without concomitant administration of fat emulsions. Moreover, the authors' HPLC method firstly demonstrated that enteral nutrients have effects on EPA and DHA metabolisms in the serum phospholipid fraction. However, the details of the effects on these fatty acids remains to be investigated.

In addition, clinical usefulness of the newly devised HPLC method was confirmed in the

following points;

- 1) Triene/tetraene ratio was calculated rapidly.
- 2) Linoleic acid, arachidonic acid contents were also determined rapidly.
- 3) Eicosatrienoic acid, palmitoleic acid and oleic acid contents were determined rapidly.
- 4) EPA and DHA were determined accurately and rapidly.

All of the above advantages will surely contribute to the early diagnosis and treatment of EFA deficiency.

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和文抄録

新 HPLC 脂肪酸分析法の臨床応用

(1): 経腸栄養剤の比較・検討

京都大学医学部外科学教室第2講座(指導: 日笠頼則教授)

佐藤 友信, 谷村 弘

外科領域においても、脂肪酸分析の必要性の認識は急激に増加しており、臨床症状の発現以前に生化学的必須脂肪酸(EFA)欠乏を早期に把握し、その防止と治療対策を講じる必要がある。そのため、臨床の実際に役立つ生体試料脂肪酸分析法として HPLC を用いる方法を開発し既に報告した。

今回、この HPLC 分析法の臨床的有用性を証明すると同時に、経腸栄養剤の臨床的有用性の判定に応用するため、必須脂肪酸をカロリー比 18.9%含有する Ensure® と 1.0%以下の Elental® とを用い、血清リン脂質分画の必須脂肪酸量の測定を行い比較・検討を行った。

腹部外科手術後の症例14名を封筒法無作為割付にて2群に分け、経鼻的に空腸に留置した feeding tube を通じて手術翌日より7日間、いずれかの経腸栄養剤を連日投与した。採血は手術前、投与終了翌日の2点で行い、著者らの開発した HPLC 法により脂肪酸分析を行った。

1) いわゆる必須脂肪酸としては、リノール酸 $C_{18:2}$ 含量が、Elental 群で減少、Ensure 群で増加したが、 $C_{18:2}$ から合成されるべきアラキドン酸 $C_{20:4}$ には変化はなかった。

2) $\omega-9$ 系脂肪酸としては、Ensure 群にて $C_{16:1}$ が減少し、Elental 群にて $C_{20:3}$ が増加した。

3) $\omega-3$ 系脂肪酸としては、Ensure 群にて EPA $C_{20:5}$ が減少した。DHA $C_{22:6}$ も減少傾向を示したが有意ではなかった。

4) 正常が0.4以下とされる triene/tetraene 比は Elental 群にて 0.29 ± 0.05 (mean $\pm$ SE) から $0.48 \pm$

0.06 へ、Ensure 群にて 0.33 ± 0.03 から 0.45 ± 0.06 へと共に増加した。しかし、著者らの提唱する $\omega-9/\omega-6$ 比は Elental 群にて 0.62 ± 0.06 から 1.21 ± 0.11 へと増加 ($p < 0.05$) したのに対し、Ensure 群にて 0.78 ± 0.12 から 0.50 ± 0.03 へと著明に減少した ($p < 0.01$)。各症例毎に検討しても t/t は両群とも1例を除き増加したのに対し、 $\omega-9/\omega-6$ 比は Elental 群で全例増加し、Ensure 群で全例低下した。

Ensure 群で $\omega-9/\omega-6$ 比とも t/t 比とが全く逆の増減態度を示したことは、Ensure 群では $\omega-6$ 系脂肪酸 ($C_{18:2} + C_{20:4}$) が増加したことに加えて、 $C_{16:1}$ が減少したことが影響していると考えられる。

以上の成績から、EFA 欠乏症の診断基準として Holman らの提唱以来、広く利用されてきた t/t 比では、両群間には差を認め難いが、著者らが提唱する $\omega-9/\omega-6$ 比を用いて比較すれば、両栄養剤の脂肪酸に及ぼす影響の相異が明確に表現されており、EFA 欠乏の判定の指標として $\omega-9/\omega-6$ 比の方が優れている。その結果、Ensure は単独で用いる経腸栄養剤として Elental より EFA 欠乏の発現防止の観点からも、優れているという。

また、EPA、DHA などの高級不飽和脂肪酸は従来正確に測定できず、無視されてきたが、著者らの HPLC 法を用いれば、正確・迅速に測定できることが証明され、さらに経腸的脂肪投与が、これら EPA、DHA の体内代謝に影響を与えていることを示唆する所見を得、今後、その方面の検討も重要課題の1つと考えられた。