THE RELATION BETWEEN SERUM FATTY ACIDS AND SOIL-TRANSMITTED HELMINTHIASIS IN THE PHILIPPINES

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Abstract. The purpose of this study was observe the effects of soil-transmitted helminthiasis on the amounts of fatty acids in the serum. The subjects were 32 females with age ranging from 15 to 55 years old (22.6 ± 12.0). The intensity of infection was light based on the criteria of the WHO Expert Committee. Seven fatty acids and serum cholesterol were determined, with the pentadecanoic acid (C15 : 0) as internal standard. Serum fatty acid levels were correlated with age-group, dietary habit and body mass index. These factors were however not related with the levels of the serum fatty acids. But the amounts of palmitic acid and stearic acid in the infected group were markedly reduced as compared with the non-infected group.

INTRODUCTION

It is well known that fatty acid intake can affect health and disease. Studies have shown relationship between fat intake and occurence of colonary heart disease (Kromhout *et al*, 1995). On the other hand, study have reported relationship between serum lipids and mosaic skin or xeroderma in children (Christiansen *et al*, 1995). The etiology of the disease is unknown, it has been discussed that the skin condition brought attention to a possible deficiency of essential nutrients, especially essential fatty acids. Fatty acids play a role in maintaining membrane integrity of cells and they provide precursors for eicosanoid development. These eicosanoids participate in the inflammatory process. The effect of n-3 fatty acid supplementation was studied in man with weight loss associated with acquired immune deficiency syndrome (Hellerstein *et al*, 1996). The *in vitro* production of interleukins (IL) and tumor necorsis factor (TNF) by the peripheral blood mononuclear cells (PBMC) was markedly reduced by n-3 fatty acid supplementation. This result seemed to indicate that these cytokines are involved in bringing about metabolic disturbances among human immunodeficiency virus (HIV) victims. This may indicate that fatty acids can modify the sequelae among HIV infected individuals.

In terms of soil-transmitted helminthiasis, there are strong indications that there is a synergistic interaction between malnutrition and intestinal helminths. But very few studies have been conducted to determine this relationship. Most of the studies related intestinal helminthiasis to anemia, levels of plasma proteins or growth retardation. Very limited studies have looked into fatty acid levels among patients with intestinal helminthiasis. It was therefore the aim of this study to determine the effect of food behavior pattern and helminthic infection on the level of serum fatty acids.

MATERIALS AND METHODS

Subjects

The subjects were 32 females with age ranging from 15 to 55 years old. They live in the municipality of Siniloan, Province of Laguna, Republic of the Philippines. All subjects had no significant exposure to environmental pollutants or disease. A questionnaire was used to obtain information regarding age, medical history and food behavior pattern. Sera were separated from blood samples and kept in the frozen state until used.

Analytical methods

Serum samples (0.5ml) were saponified with 1.5ml of 1N-KOH solution in ethanol and 0.5ml of 1 mg/ml pentadecanoic acid ethanol solution and

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heated at 75°C for 1 hour. After saponification, 1 ml of 3N HCl was added and the fatty acids were extracted with n-hexane, dried under nitrogen stream, and redissolved in 0.5ml of methano-benzene (3 : 7). The fatty acids were esterified with 50µl of 2M (trimethylsilyl)-diazomethane solution in n-hexane. After esterification, the esters were dried under N2 stream, and redissolved in n-hexane for GC analysis. GC analysis (GC-6A Shimadzu) was performed on 30m × 0.54mm bonded fused silica DB-17 column (J & W Scientific) programmed from 80°C to 280°C at 5°C/minute.

Identification of esters was made by comparing with authentic standards. Peak areas were calculated by Chromatopac C-R6A (Shimadzu).

Stool examination

All subjects were requested to submit fecal samples for examination. The compliance rate was 78.0% with 32 out of 41 submitted the samples. Using the Kato-Katz quantitative technique, the eggs were counted on all positive samples of *Ascaris lumbricoides* and *Trichuris trichiura*. The intensity of infection was determined following the criteria given by the WHO Expert Committee (WHO, 1987).

Statistical analysis

The data were analyzed by the Mann-Whitney test and Kruskal-Wallis 1-Way ANOVA, with the level of significance set at $p < 0.05$.

RESULTS

The results of stool examination are shown in Table 1. The positivity rate was 18.8%; 6.3% in double infections with *Ascaris* and *Trichuris* and 12.5% in single infections with *Trichuris*. The intensity of infection was classified as light in all of the positive subjects.

In this study, 7 fatty acids, namely mylstic acid, C14 : 0; palmitic acid, C16 : 0; stearic acid, C18 : 0; linoleic acid, C18 : 2; arachidonic acid, C20 : 4; eicosapentaenoic acid, C20 : 5; docosahexaenoic acid, C22 : 6 and cholesterol (CHOL) were analyzed

Table 1

Summary of stool examination.

Age	Intensity *Ascaris* egg/gram	Intensity *Trichuris* egg/gram
15	0	184
15	1,909	345
19	0	92
20	1,587	391
21	0	138
35	0	46
Mean ± SD	Prevalence	Prevalence
20.8 ± 7.4	2/32 (6.3%)	6/32 (18/8%)

in the serum using pentadecanoic acid (C15 : 0) as internal standard. The serum fatty acid content in all subjects and for each age-group is shown in Table 2. Fatty acid content was not significantly different among the four age-groups, however, total fatty acids and cholesterol had higher values in the group 40 years old and above.

Table 3 shows the concentration of serum fatty acids according to body mass index (BMI) using the normal value of 22. The study subjects were divided into three groups: slim when the BMI was less than 20.9, medium when the BMI ranged from 20.9 to 23.1 and fat when the BMI was greater than 23.1. The fatty acids and CHOL contents were not significantly different among the three groups.

The relationship between fatty acid content and dietary habit is shown in Table 4. Rice was considered the most important staple food by all of the subjects, and it was generally consumed more than once a day. The subjects were divided according to the main viand dish taken 4X or more a week. The majority (66%) ate a combination of meat and fish followed by 22% meat only, 6% fish only and another 6% vegetables only. There was no significant difference in the serum fatty acids among the four groups. It is likewise shown that the level of serum C20 : 5 of those who ate a combination of meat and fish and fish only was lower compared to those who ate meat only. This result does not agree with the findings of other studies, where level of serum C20 : 5 was found to be generally high in those who eat fish.

Table 2

Concentration of serum fatty acids and cholesterol on each age-groups.

Fatty acid and cholesterol mg/dl	Total	Age-group			
		< 19	20 < 29	30 < 39	40 <
C14:0	8.5 ± 4.7	8.0 ± 2.9	10.0 ± 7.2	6.3 ± 1.7	9.2 ± 3.5
C16:0	82.3 ± 23.2	79.3 ± 20.2	81.7 ± 30.2	76.8 ± 15.9	96.3 ± 20.8
C18:0	69.8 ± 16.8	68.2 ± 18.3	65.1 ± 12.5	68.2 ± 17.5	84.8 ± 16.6*
C18:2n-6	78.7 ± 15.5	77.7 ± 20.6	77.5 ± 7.0	77.6 ± 13.5	84.8 ± 18.7
C20:4n-6	19.7 ± 4.4	19.1 ± 4.4	18.3 ± 4.4	20.6 ± 2.3	22.7 ± 5.2
C20:5n-3	2.8 ± 0.6	2.7 ± 0.6	2.9 ± 0.7	2.7 ± 0.5	3.1 ± 0.6
C22:6n-3	8.6 ± 3.1	8.2 ± 3.0	9.4 ± 3.5	8.4 ± 3.8	8.3 ± 2.4
FA total	271 ± 58	263 ± 63	265 ± 56	261 ± 47	309 ± 62
CHOL	124 ± 33	120 ± 35	117 ± 21	129 ± 17	140 ± 59
Total lipid	394 ± 85	384 ± 93	382 ± 73	390 ± 63	449 ± 111
AGE	26.0 ± 12.0	15.9 ± 1.4	22.2 ± 2.6	35.4 ± 2.3	48.2 ± 4.1
BMI	21.5 ± 2.6	20.2 ± 2.2	21.4 ± 1.5	23.3 ± 2.9	22.9 ± 3.7

Value listed mean ± SD

*Kruskal-Wallis 1-Way ANOVA $p < 0.05$

Table 3

Concentration of serum fatty acid by body mass index.

Fatty acid and cholesterol mg/dl	Body mass index		
	< 20.9	20.9 < 23.1	23.1 <
C14:0	7.9 ± 3.0	10.3 ± 7.6	7.7 ± 2.6
C16:0	81.9 ± 19.1	83.0 ± 31.5	82.4 ± 22.7
C18:0	70.4 ± 16.7	66.0 ± 14.0	73.0 ± 21.1
C18:2n-6	79.6 ± 16.4	74.2 ± 12.0	82.3 ± 17.8
C20:4n-6	19.6 ± 3.9	18.6 ± 5.0	20.9 ± 4.8
C20:5n-3	2.7 ± 0.6	2.8 ± 0.6	3.0 ± 0.7
C22:6n-3	8.0 ± 3.1	9.6 ± 3.3	8.7 ± 3.0
FA total	270 ± 55	265 ± 62	278 ± 68
CHOL	123 ± 32	116 ± 24	133 ± 46
Total lipid	393 ± 81	381 ± 82	411 ± 105
AGE	23.1 ± 11.3	20.6 ± 5.6	37.5 ± 11.6

Value listed mean ± SD

Table 4

Concentration of serum fatty acid by dietary habit group.

Fatty acid and cholesterol mg/dl	Dietary habit			
	Meat	Fish	Meat and fish	Vegetable
C14:0	8.5 ± 3.9	8.1 ± 0.6	8.8 ± 5.3	5.6 ± 1.4
C16:0	85.5 ± 25.2	84.1 ± 18.2	83.6 ± 23.5	56.3 ± 5.3
C18:0	73.8 ± 23.2	83.1 ± 17.0	69.2 ± 13.9	48.7 ± 3.9
C18:2n-6	83.9 ± 19.0	79.7 ± 3.5	79.0 ± 14.5	57.7 ± 6.4
C20:4n-6	20.4 ± 3.2	20.6 ± 4.0	19.8 ± 4.8	14.9 ± 0.7
C20:5n-3	3.0 ± 0.6	2.8 ± 0.8	2.8 ± 0.6	2.1 ± 0.4
C22:6n-3	7.6 ± 1.5	10.3 ± 4.2	9.0 ± 3.3	6.6 ± 5.0
FA total	283 ± 70	289 ± 48	272 ± 54	192 ± 20
CHOL	122 ± 39	131 ± 29	125 ± 35	103 ± 9
Total lipid	405 ± 102	420 ± 77	398 ± 82	295 ± 29
AGE	25.6 ± 14.2	26.0 ± 11.3	26.9 ± 12.2	18.0 ± 1.4
BMI	20.4 ± 3.0	22.3 ± 1.4	21.7 ± 2.6	21.5 ± 2.3

Value listed mean ± SD

Table 5 shows the relationship between the amounts of serum fatty acids and helminthic infection. C16 : 0 and C18 : 0 were reduced in the infected groups; the amount of the other fatty acids and CHOL were also lower in the infected group compared with the non-infected group.

Table 6 shows the comparison of the amount of serum fatty acid between the Siniloan residents and the Japanese female students. The C18 : 0 was three times higher among the Siniloan residents than among the Japanese students; while the C18 : 2 was 1.3 times higher among the Japanese students.

DISCUSSION

The most abundant naturally occuring lipids are the fatty acids which serve as building blocks for several classes of lipids. The parasitic helminths depend on the host diet for their fatty acid requirements. The major fatty acids contain 16 or 18 carbon atoms which in this study were markedly reduced in the infected group. A lowered fatty acid level is expected if the subjects are vegetables only, but the significance of the results was not changed when the vegetable group free data set was used.

Table 5

Comparison of serum fatty acid by helminthic infection.

Fatty acid and cholesterol mg/dl	Helminthic-egg	
	Negative	Positive
C14:0	9.1 ± 5.0	6.2 ± 1.2
C16:0	86.4 ± 23.8	64.5 ± 6.1*
C18:0	72.9 ± 17.0	56.5 ± 6.3*
C18:2n-6	80.4 ± 15.3	71.7 ± 15.6
C20:4n-6	20.1 ± 4.4	17.8 ± 3.9
C20:5n-3	2.9 ± 0.6	2.4 ± 0.3
C22:6n-3	8.8 ± 3.4	7.9 ± 1.5
FA total	281 ± 59	227 ± 26*
CHOL	126 ± 36	115 ± 14
Total lipid	406 ± 89	342 ± 39
AGE	27.2 ± 12.6	20.8 ± 7.4
BMI	21.5 ± 2.7	21.4 ± 2.4

Value listed mean ± SD
*Mann-Whitney U-Test $p < 0.05$

Table 6

Comparison of serum fatty acids on Siniloan residents and Japanese female students.

Fatty acid and cholesterol	Siniloan residents[a]		Japanese students[b]	
	mg/dl	%	mg/dl	%
C14:0	7.8 ± 4.9	3.2	2.9 ± 2.3	1.3
C16:0	71.2 ± 14.5	29.5	63.7 ± 22.9	29.1
C18:0	59.4 ± 7.7	24.6	20.6 ± 5.9	9.4
C18:2n-6	75.2 ± 9.4	31.1	102.3 ± 26.5	46.7
C20:4n-6	17.2 ± 4.1	7.1	17.2 ± 4.7	7.8
C20:5n-3	2.7 ± 0.6	1.1	4.1 ± 2.1	1.9
C22:6n-3	8.2 ± 2.1	3.4	8.4 ± 2.6	3.8
FA total	241		219	
AGE	20.8 ± 1.0		19 to 20	

Value listed mean ± SD
[a]In this work
[b]Umemura U *et al* (1993)

Not only the C16 : 0 and the C18 : 0 were lowered: all the other fatty acids and CHOL levels were lower in the infected group. There is some evidence that the lipids found in helminths reflect the lipids of the host. This possibly indicates that the endoparasites absorb a portion of the fatty acids from the host. It is an accepted fact that helminths depend on the nutrients of the host. This result may also be related to the ready diffusion of fatty acids across the pipid bilayer of the absorptive surface of the parasitic membrane during the uptake of nutrients from the host.

The intensity of infection was light and yet the levels of fatty acids were lower in the infected group. From the results, we may be able to speculate that fatty acid level will be very much lower if infection is moderate or heavy.

Fatty acids serve a variety of functions in man. They are involved in determining the immune status of an individual, by way of supplying membrane constituents to preserve the integrity of cells. The physico-chemical properties of the lymphocyte membrane affects the interaction between a foreign substance and the receptors on the lymphocyte. Recognition of the foreign substance will require proliferation of the lymphocyte population, where rapid membrane synthesis is involved.

These fatty acids are also involved in specific uptake processes for obtaining appropriate precursors for eicosanoids. They participate in the process of inflammation. Results possibly indicate that individuals with low fatty acids level due to soil-transmitted helminthiasis may fail to elicit a good immune response. Biosynthesis of the fatty acids is related to the environmental conditions of both plants and animals (McElhaney *et al*, 1976). The results obtained in the comparison of fatty acid levels between the Siniloan residents and the Japanese female students showed a significant difference in C18 : 0 and C18 : 2 levels. This difference may possibly be due to a difference in the fish intake of the two groups. From the questionnaire, Siniloan residents take mainly freshwater fish from Laguna Bay. The Japanese female students, on the other hand take marine fish with high frequency. The difference possibly indicates that the fatty acid content of the marine and freshwater fish is not the same. Other studies show that soil-transmitted helminthiases related to the occurrence of iron-deficiency anemia (Pawlowshi *et al*, 1991; Yu and Shen, 1990) and hypoproteinemia among infected children (Bundy and Cooper, 1990). Infected women in the child bearing age had babies with low weights. All the results are strong indications to institute measures of control and prevention for these parasitic infections.

CONCLUSIONS AND LIMITATIONS

Results of this study show a possible correlation between the level of fatty acids and intestinal helminthiases. But the number of subjects used in this study is not sufficient to statistically establish the relationship. Likewise, the dietary habit concerned is limited to the study group only and does not reflect the intake pattern in the whole population of Siniloan. It is therefore suggested that further studies be conducted on a bigger scale to able to compare effects of moderate and heavy worm burden on the level of serum fatty acids.

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REFERENCES

Bundy DAP, Cooper ES. Trichuriasis. In: Warren KS, Mahmoud AAF eds. Tropical and geographical medicine. 2nd ed. New York: McGraw-Hill 1990: 400-2.

Christiansen EN, Skara BB, Sorensen E, Grav HJ. Serum lipids in children with xeroderma from an inland district of Sri Lanka. *Ann Nutrit Metab* 1995; 39 : 9-15.

Hellerstein MK, Wu K, McGrath M, *et al.* Effects of dietary n-3 fatty acid supplementation in men with weight loss associated with the acqired immune deficiency syndrome: Relation to indices of cytokine production. *J Acquired Imm Defic Syndr Human Retrovirol* 1996; 11 : 257-70.

Kromhout D, Menotti A, Bloembery B, *et al.* Dietery saturalted and trans fatty acids and cholesterol and 25-year mortality from coronary heart disease: the Seven Countries Study. *Prevent Med* 1995; 24 : 308-15.

McElhaney RN, Souza KA. The relationship between environmental temperature, cell growth and the fluidity and physical state of the membrane lipids in Bacillus slearothermophilus. *Biochim Biophys Acta* 1976; 443 : 348-59.

Pawlowshi ZS, Schard GA, Stott GJ. Hookworm infection and anaemia. World Health Organization 1991.

Umemura U, Iso H, Koike K, *et al.* Dietary habits and serum fatty acid compositions of women college students--changes in serum lipids and fatty acids by dietary education. *Jpn J Public Health* 1993; 40 : 1139-54 [Japanese].

Prevention and control of intestinal parasitic infections. Report of a WHO Expert Committee. Geneva, World Health Organization. *WHO Techn Rep Ser* No. 749, 1987; 57-9.

Yu SH, Shen WX. Hookworm infection and disease in China. In: Schad GA, Warren KS eds. Hookworm disease: current status and new directions. London: Taylor and Francis. 1990; 44-54.