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Urine lipids in patients with a history of filariasis

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Abstract The presence of lipids in postprandial urine was assessed in 116 patients with a history of filariasis and 70 normal individuals using a biochemical autoanalyzer. Urinary triglycerides (TGs) ranging from 10 to 1955 mg/dl were detected in 13 individuals with a history of chyluria, including 3 with TG levels ranging from 233 to 1955 mg/dl and cholesterol levels of 6–35 mg/dl. Three patients who had a filarial history but without chyluria were also found to have urinary TGs (13–15 mg/dl) without detectable cholesterol. Neither TGs nor cholesterol were detected in the urine of normal individuals. Fluctuations in postprandial urine lipid contents were measured by time course determinations of urinary TG and cholesterol in 17 patients with filariasis and a history of chyluria, 16 patients with filariasis and hydrocele and 16 normal individuals. The level of urine lipid excretion was found to increase within 1–4 h postprandially, with urinary TG levels ranging between 7.8 and 1284 mg/h in eight patients and urinary cholesterol levels between 1.2 and 138 mg/h in seven patients with a history of chyluria. To evaluate the origin of the urine lipids in hematochyluria, fish oil containing 360 mg eicosapentaenoic acid (EPA) and 240 mg docosahexaenoic acid (DHA) was prescribed to a patient with hematochyluria. The excretion of EPA and DHA in urine was increased postprandially in the patient, similar to the elevation of urinary TG and cholesterol. The profile of fatty acids from urine samples showed it was of dietary origin. Our results suggest that postprandial urine lipids, especially TG, might be used as markers for the clinical evaluation of chyluria.

Key words Filariasis · Chyluria · Triglyceride · Cholesterol

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Introduction

Chyluria is defined as the presence of chyle in the urine [17, 18]. The etiology of chyluria has been classified as either parasitic or nonparasitic [11, 16–18]. In Asia, most of the chyluria results from *Wuchereria bancrofti* infection [7, 14, 18], and the frequency of chyluria in the filarial endemic area ranges from 0.7% to 10% [7, 14]. *Wuchereria bancrofti* organisms have been shown in the lymphatics of the testes by ultrasonography [2]. It is believed that chyluria occurs by the retroperitoneal lymph nodes receiving lymph flow from the intestinal lymphatic vessels, which have become obstructed [4]. Since there are communications between intestinal and renal lymphatic vessels prior to emptying into the lymphatic trunks, the lymph node obstruction causes dilatation of the intestinal lymphatic vessels and the backflow of chyle in the renal lymph vessels into the renal collection system [11]. There are various methods for chyluria evaluation: by questionnaire [7, 14], gross examination of urine specimens [4], microscopic examination of fat droplets [1], intravenous pyelography, cystoscopy with retrograde urography [6, 17], lymphangiography [11, 17, 18] and lymphoscintigraphy [15]. However, clinical evaluation of asymptomatic chyluria is still uncommon. An easily quantitative method may be needed for the differential diagnosis of chyluria in asymptomatic patients. This report presents results from determinations of the urine lipids in patients with a

filarial history using a biochemical autoanalyzer. The findings suggest the examination of postprandial urine lipids might be very useful for chyluria evaluation.

Materials and methods

Postprandial urine samples were collected within 2–4 h after meals from 116 subjects with a previous history of filariasis who had been treated with diethylcarbamazine (DEC) for 5–15 years [7]. Of these, 53 subjects had a history of chyluria, while the other 63 subjects were patients without a history of chyluria. Postprandial urine samples from 70 age-matched normal individuals were also collected as a control group. All the urine samples were collected on the condition that no drug had been taken by the individuals for at least 1 week before collection. By gross examination of the total of 186 urine samples, most (178) had a clear appearance except one which was milky, with red cell clumps (hematochyluria) and six which had a cloudy appearance. None of the 53 patients with a history of chyluria had received surgical intervention. Urine triglyceride (TG) and cholesterol levels were measured using a biochemical autoanalyzer (Synchron CX-5 system, Beckman Instruments, Brea, CA) at least 3 times. All the data presented are the mean values of the repeated measurements. In order to evaluate the reproducibility of the autoanalyzer in measuring the urine samples, we repeatedly measured a milky urine sample by twofold dilution with normal saline and calculated the correlation coefficient.

In order to assess the timing variation of the urine lipids after a meal, the overnight and postprandial urine samples were collected from the 33 subjects with a history of filariasis including 17 subjects with a history of chyluria and 16 subjects with hydrocele. Urine samples from 16 normal individuals were also collected for the control group. The postprandial urine samples were collected hourly after the meal. The urine triglyceride and cholesterol levels were determined by biochemical autoanalyzer. For the high-lipid-content samples, appropriate dilutions were made by normal saline and the total amount of urinary excretion per hour was calculated.

To verify the origin of urine lipids of hematochyluria, an 80-year-old female patient with gross hematochyluria voluntarily ate fish oil, which contained 360 mg eicosapentaenoic acid (EPA) and 240 mg docosahexaenoic acid (DHA) approximately 30% by weight, as lipid markers with breakfast before urine collection. Urine samples were then collected every hour after breakfast and extracted by the Folch method to recover the total lipids [8]. After saponification in 10% KOH solution, the released fatty acids were derivatized with 2-bromo-2′-acetonaphthone (ANT-Br) in the presence of 18-crown-6 and excess potassium carbonate. The n-3 fatty acid derivatives were determined by reverse-phase high-performance liquid chromatography (RP-HPLC) with an isocratic elution. The results were expressed as the peak areas of corresponding fatty acid derivatives with UV detection (247 nm). Simultaneous blood sample collection was refused by the patient.

Results

Using the biochemical autoanalyzer, we found that of the 116 collected urine samples from patients with a history of filariasis, 16 (14%) were detected to contain TGs ranging from 10 to 1955 mg/dl (Table 1). Of the 53 cases who had a history of chyluria, 13 (24.5%) urine samples were urine TG positive. In addition, although the urine cholesterol level was too low to be detected in almost all the samples collected, 3 of the 13 samples were found to contain cholesterol levels ranging from 6 to 35 mg/dl. Very high TG amounts were found in two milky urine samples with (936 mg/dl) or without gross red cell clumps (1955 mg/dl) and one cloudy urine sample (233 mg/dl). In the 63 patients with filariasis without a history of chyluria, most of the urine samples were TG negative except for three subjects (4.8%), in whom a low amount of urine TG was unexpectedly detected (13–15 mg/dl). Neither urine TG nor cholesterol was detectable in the 70 urine samples from normal individuals.

To discern the reproducibility of the urine lipid detection by biochemical autoanalyzer, a milky urine sample was analyzed by twofold dilution and repeatedly measured by biochemical autoanalyzer. Figure 1 shows the linear regression of the titration experimental results with a correlation coefficient (r) of 0.997. The coefficients of variation (CV) in each dilution were 5.5%, 1.6%, 1.2%, 2.7%, 3.95% and 4.6% in the original and 2-fold,

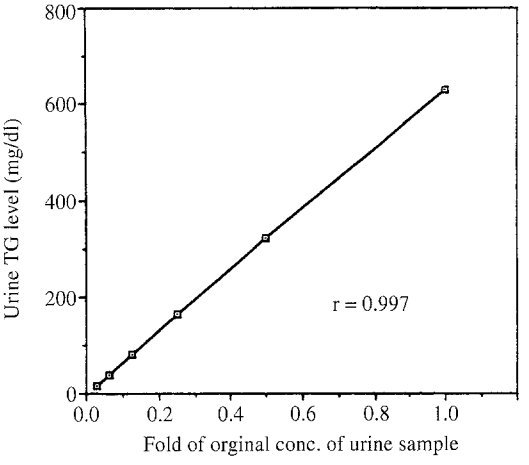

Fig. 1 Titrational urine triglyceride (TG) level of a milky urine sample. The urine was diluted twofold with normal saline. Each diluted sample was detected repeatedly 20 times. Mean values are shown, and the coefficients of variation (CV) were 5.5% for undiluted samples, 1.6% for twofold diluted samples, 1.2% for 4-fold, 2.7% for 8-fold, 3.95% for 16-fold and 4.6% for 32-fold dilution samples. Linear regression of the curve produced a correlation coefficient (r) of 0.997

Table 1 Spot urine analysis for urine triglycerides (TGs)

	Case number	Positive for urine TGs	Range (mg/dl)
With a history of chyluria	53	13 (24.5%)	10–1955
Without a history of chyluria	63	3 (4.8%)	13–15
Total	116	16 (14%)	

Table 2 Postprandial urine lipid (TG/cholesterol) excretion in eight individuals (total amount mg/h) (– undetectable, ~* no sample available)

Case			Overnight	After breakfast						After lunch		
No.	Sex	Age (years)		1 h	2 h	3 h	4 h	5 h	6 h	1 h	2 h	3 h
1	F	80	41.3/-	78.3/9	9.5/1.5	39/4.7	302.7/5	2348/0.8	246/–			
2	M	33	–/–	–/–	164/1.2	447.2/42.4	324.3/27.7			205/4.5	172.7/8.8	187/22.5
3	M	37	109/–	9/–	183.6/–	582.4/66.4	1284/138			653/50.4	350/7	442.4/60.2
4	F	42	–/–	–/–	7.8/–	28.1/–	17.3/–			229/9.5	248.5/–	28.3/–
5	F	39	~*	463/41	208.5/2	69.4/3	55.7/4.7			233.7/33.4	391/6.3	732.5/105
6	M	53	17.2/–	–/–	–/–	–/–	–/–			33/–	126/–	121/17.9
7	M	59	–/–	–/–	19.8/–	80.6/–	265.4/30.9			225/–	90/–	50/–
8	M	43	–/–	–/–	–/–	–/–	–/–			–/–	203/–	25/–

4-fold, 8-fold, 16-fold and 32-fold dilutions of the urine sample, respectively.

In order to determine the factor of timing in the sample collection, overnight and the continuous time course postprandial urine samples were collected from 17 individuals with a history of chyluria. The results showed that eight individuals were positive for urine lipids (Table 2). The level of urine lipid excretion was increased within 1–5 h after breakfast with urine TG levels ranging between 7.8 and 1284 mg/h and urine cholesterol levels of between 1.2 and 138 mg/h. The positivity of postprandial urine TG and cholesterol at the 4th h were 75% (6/8) and 62.5% (5/8), respectively. Although the urine lipids were not detectable in two post-breakfast urine samples, they appeared in the urine collected after lunch.

In order to understand the origin of the urine lipids in the 80-year-old female with hematochyluria, fish oil which contained EPA and DHA as lipid markers was taken voluntarily with breakfast by the patient and the urine collected hourly and analyzed. Elevation of EPA (C20:5) and DHA (C22:6) could be found gradually from 1 to 3 h and dramatically increased in the 4th h after breakfast and then continuously sustained in the urine until the 6th h (Fig. 2). The time period when the lipid markers were elevated was similar to the elevation period of the urine TG and cholesterol in this case (Table 2, patient 1). Figure 3 shows the fatty acid profiles of fasting urine and the 4th h of fish oil ingestion. The fatty acid profile of fasting urine shows a peak for linolenic acid (C18:3), which is the marker of soybean oil which may result from a normal diet. The fatty acid profile of lipoprotein in urine samples is distinct from

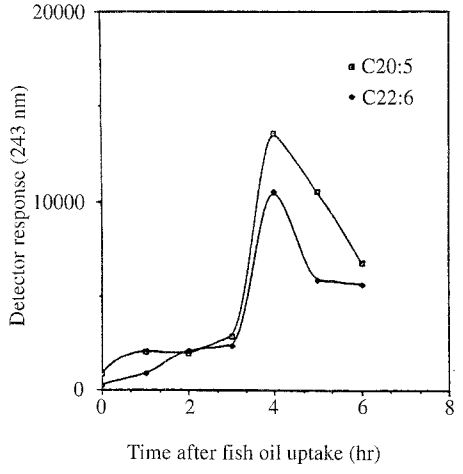

Fig. 2 Responses of C20:5 (eicosapentaenoic acid, *squares*) and C22:6 (docosahexaenoic acid, *diamonds*) in urine after fish oil uptake in the patient with hematochyluria detected by reverse-phase high-performance liquid chromatography

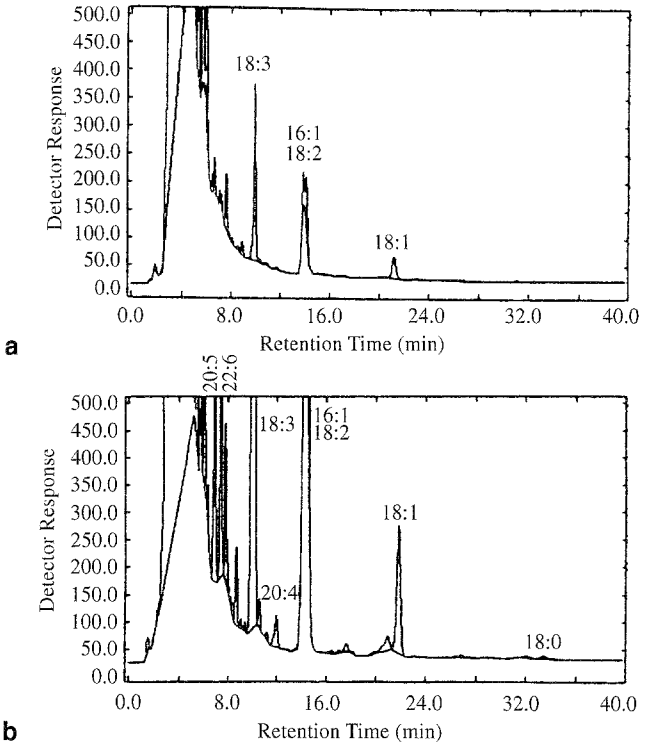

Fig. 3 Reverse-phase liquid chromatograms illustrating the increase in n-3 polyunsaturated fatty acids in urine lipids collected from a patient with hematochyluria after fish oil uptake. *Chromatogram* **a** fasting urine, **b** 4 h after fish oil uptake. Increasing urinary C20:5 and C22:6 during the 4th h after fish oil ingestion and absence of prominent palmitic acid and oleic acid were found. However, the results show urine lipids for the patient with hematochyluria is of intestinal origin. HPLC: column, Cosmosil 5C18-AR (4.6 × 250 mm, 5 mm; Nacalai Tesque, Kyoto, Japan); mobile phase, 100% acetonitrile; flow rate, 1.0 ml/min; Detection, UV at 247 nm

that of human serum because no prominent palmitic acid (C16:0) and oleic acid (C18:1) portions, which are the markers for serum lipoproteins, were found. These results strongly suggest that the urine lipid excretion in this patient with hematochyluria was of intestinal origin.

Discussion

Parasitic chyluria is one of the late manifestation of filariasis. In endemic areas, most of epidemiological studies for chyluria have been carried out by questionnaire [7, 14]. However, the characteristics of spontaneous remission [13] and the intermittent pattern [18] of chyluria makes interpretation more difficult. In addition, some turbid urines, such as pyuria, phosphaturia and bacteriuria, could also be regarded as chyluria by the patients. Therefore, an easy and quantitative method is required for evaluation of the status of chyluria. In the studies of nonparasitic chyluria cases, the urine TG and cholesterol have been detected either by urinary electrophoresis [3, 12] or quantitatively by infrared spectrophotometry [5, 9]. Analysis of the urinary lipids by routine biochemical autoanalyzer serves as an alternative and easier way to assess chyluria. In this study, we showed that of the 53 patients with a history of chyluria, 13 individuals were urinary TG positive. However, three urinary TG-positive individuals were also found in 63 patients without chyluria (Table 1). Approximately half of the urinary TG-positive patients possessed clear urine at the examination except two in whom the urine was milky and four in whom the urine had a turbid appearance. Low levels of cholesterol were found in only three patients. Since 90% of the chylomicron lipid composition which comes from intestine is TG, the high amount of TG and low cholesterol content in these urine samples suggested that the source of the urine lipid was the intestinal chylomicron. This characteristic could be considered a criterion for differentiation of the chyluria from other lipiduria, e.g. lipiduria of blood origin. In view of the spontaneous remission, intermittent pattern of chyluria and long duration of the disease after treatment, the relatively low urine TG positive rate (24.5%) in our patients with a history of chyluria seems reasonable. The low TG contents in the postprandial urine of three patients without a history of chyluria indicated that the method might be sensitive enough to detect subclinical chyluria.

The time course results demonstrated that the level of urine TG excretion increased postprandially in comparison with the fasting urine. Although the period during which the excretion of urinary TG reached a peak was slightly different between individuals, e.g. 1–5 h post-breakfast or after lunch in this study, it increased postprandially in comparison with the fasting urine. This suggested that the postprandial period is the better time for urine collection for lipid detection.

In about 23% of the cases of the severe form of chyluria, simultaneous gross hematochyluria was present [17]. Therefore, the origin of the urine lipids is of interest. Although we did not have the high-performance liquid chromoatography (HPLC) data of the blood lipid profile for this case, the increasing urinary C20:5 and C22:6 composition at the 4th h after fish oil uptake and decreasing gradually thereafter suggested the fluctuation of postprandial urine lipids may have a dietary cause. The presence of linolenic acid and the absence of palmitic acid and oleic acid in urine samples suggested these urine lipids come from diet. However, we cannot rule out a trace amount of blood lipid leaking into the urine as the reason for hematuria.

There are several factors in the urine, for example, hemoglobin, bilirubin, ascorbic acid, and drugs, which could interfere with lipid measurement, usually negatively [10]. By keeping the sample fraction low, endogenously interfering substances were diluted by the detecting reagent. Since the ratio of sample to reagent was very low (1/100), the effect of the endogenous interference was of minor significance. Besides, dietary lipid content also influences urinary lipid excretion [12]. Thus, in clinically suspected cases of chyluria with urine negative TG, avoiding drug taking prior to examination and time course analyses after a fatty meal are suggested to reduce the effect of interference. On the other hand, similarly to hyperlipidemia, the lipid micelles formed on the top of the urine samples from severe chyluria patients with high lipid contents would also influence the accuracy of the quantitation by autoanalyzer. Dilution of urine samples before determination is necessary for high lipid measurements.

In conclusion, a routine biochemical method was used to evaluate the severity of chyluria and urine TG was found to be a better indicator than cholesterol for the detection of the presence of chyluria, and the high ratio of urine TG to cholesterol could be used as a diagnostic criterion. In addition, a close relationship between urine lipids and diet was verified. One case with hematochyluria which was studied also suggested the intestinal origin of urine lipids. The biochemical detection of urine lipids could be easily carried out in a filarial endemic area and may provide a positive contribution to the clinical evaluation of this disease.

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