[Environmental Health and Preventive Medicine 9, 13–21, January 2004]

Original Article

Serum Nutritional Status of Tocopherol and Retinol Normalized to Lipids of Persons Living in the Southern Rural Terai Region in Nepal

Kazuko HIRAI[1], Yoshimi OHNO[2], Mayumi JINDAI[1], Yoko AOKI[1], Eriko HAYASHI[1], Hisa HIGUCHI[3], Seiko MIZUNO[4], Kumiko NAGATA[5], Toshihide TAMURA[5], Shiva K. RAI[6] and Mathura P. SHRESTHA[7]

[1]*Department of Health and Nutrition, Graduate School of Human Life Science, Osaka City University, Osaka, Japan*
[2]*Department of Food Science and Nutrition, School of Human Environmental Sciences, Mukogawa Women's University, Hyogo, Japan*
[3]*Osaka Joshigakuen Junior College, Osaka, Japan*
[4]*Soai College, Osaka, Japan*
[5]*Department of Bacteriology, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan*
[6]*Department of Microbiology, Nepal Medical College, Kathmandu, Nepal*
[7]*Department of Community Medicine and Public Health, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal*

Abstract

Objective: The present study examined the levels of serum α-Tocopherol (Toc), retinol (Ret), cholesterol (Chol) and triglycerides (TG), and their correlations in the sera of people in Nepal.

Methods: The survey was conducted on the general populace in the agricultural Terai region in southern Nepal. The study population consisted of 93 males and 83 females aged 10–68 years. Serum Toc and Ret were measured by high-performance liquid chromatography.

Results: No significant differences were observed between the genders for the average of total Chol (T-Chol) (140 and 145 mg/100 ml, respectively), HDL-C (45 and 47 mg/100 ml), LDL-C (94 and 97 mg/100 ml), and TG (106 and 110 mg/100 ml), and the ratio of LDL/HDL (2.16). The levels of mean Toc (4.32 and 4.27 μg/ml) were about the same for both genders, while the mean Ret levels were significantly higher for males (624 ng/ml) than for females (535 ng/ml) ($p<0.001$). A direct relationship was found between the levels of Toc and Ret ($r=0.46$, $p<0.001$ and $r=0.28$, $p<0.05$ for males and females, respectively). Serum levels of Toc and Ret were positively related to the levels of Chol ($r=0.48$ and $r=0.58$, $p<0.001$ for males and $r=0.49$, $p<0.001$ and $r=0.33$, $p<0.01$ for females, respectively) and TG ($r=0.23$ and $r=0.28$, $p<0.05$ for males and $r=0.29$, $p<0.01$ and $r=0.28$, $p<0.05$ for females, respectively). The ratio of Toc/TG normalized to serum TG was directly correlated to the ratio of Ret/TG ($r=0.79$ for males, and $r=0.72$ for females, $p<0.001$, respectively) and the ratios of Toc/TG and Ret/TG were negatively related to the LDL/HDL levels ($r=-0.49$ and $r=-0.43$, for males, and $r=-0.46$ and $r=-0.57$ for females, $p<0.001$, respectively).

Conclusion: The levels of Toc and Ret were low in the sera of people living in the southern rural Terai region in Nepal, and it was found that lower levels of Toc and Ret normalized to TG increased the ratio of LDL/HDL. These results suggest that greater intake of foods rich in Toc and Ret should be encouraged to reduce the risk of coronary heart disease.

Key words: tocopherol, retinol, LDL/HDL, serum, Nepalese

Introduction

Nepal is a small impoverished country of very low human development indicators (1). Infectious diseases are prevalent and account for over 70% of health problems in Nepal (1), being aggravated by coexistent malnutrition (2). Malnutrition is a major public health problem in developing countries (3–5) and promotes parasite establishment (6). Infection decreases the concentrations of serum vitamin A (7). Severe vitamin A, or, retinol (Ret), deficiency results in night blindness, which occurs in 16% of the population in Nepal (8). Epidemiological studies have revealed a link between vitamin A deficiency and increased morbidity and mortality (9). Thus, vitamin A deficiency is a

Received Sep. 8 2003/Accepted Nov. 20 2003
Reprint requests to: Kazuko HIRAI
Department of Health and Nutrition, Graduate School of Human Life Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan
TEL & FAX: +81(06)6605-2811

major public health problem in developing countries (10, 11).

Another important nutrition indicator is vitamin E, or, tocopherol (Toc), which enhances immune responses (12) and is effective against bacterial infections (13). Toc as an antioxidant and a free radical scavenger (14) inhibits oxidation of LDL (15) and is thus effective for prevention of cancer (16). An inverse correlation between plasma α-Toc and mortality from ischemic heart diseases (17), and a role of γ-Toc as a marker of atherosclerosis (18), have been reported. In hyperlipidemia, elevated vitamin E concentration is found in the serum (19), and the ratio of Toc to lipid in serum has been proposed as an index of vitamin E status (20–22).

Malnutrition in developing countries also aggravates problems with child survival (23). In order to improve the nutritional status, biochemical measurements of nutrients can be important for nutritional assessment and monitoring. Previously, we have reported the findings of a nutritional survey conducted in the industrial Itahari district of the Terai region in southeastern Nepal where we found low levels of Toc and Ret in serum (24). As shown above, such a poor nutritional status of Toc and Ret adversely affects public health in Nepal. In this paper, we report the findings of a study on the serum biochemical parameters and the levels of Toc and Ret for inhabitants aged 10-68 years in the southern rural Terai region in Nepal. We also assessed the nutritional status of Toc and Ret normalized to serum lipids for these subjects.

Materials and Methods

Subjects and methods

The present survey was conducted in a medical camp for Japanese encephalitis vaccinations on the general populace in the Khargual village of the Chitwan district, in the agricultural Terai region in southern Nepal, in December 1989. The subjects studied were a random sampling from among those who voluntarily came to the camp and consisted of over 50% of the population of the village. The study population consisted of 93 males and 83 females of a mean age of 27.2±14.0 and 26.4±13.6 years, respectively, with a range of 10–68 years (Table 1). Two persons with hypertriglyceremia (460 mg/100 ml) and with high γ-GPT (62 IU/l) were excluded from the study. The majority of the residents in this district were peasants engaged in agriculture. Most of them were Hindu believers.

Body mass index (BMI), (wt/ht^2 in kg/m^2) was calculated, and blood pressure, systolic (SBP) and diastolic (DBP), were measured using a mercury sphygmomanometer. After informed consent had been obtained from each individual included in the study, fasting venous blood samples were collected using disposable syringes. Serum was separated by centrifugation, kept in ice, and stored under −30°C until analysis. A food intake survey was simultaneously conducted (25).

Analyses of all biochemical parameters in serum were examined with an autoanalyzer (Hitachi-736, Hitachi, Tokyo) using commercial reagent kits. The aspartate aminotransferase (AST, GOT), alanine aminotransferase (ALT, GPT), γ-glutamyltransferase (γ-GTP), lactate dehydrogenase (LDH), urea nitrogen (UN) and uric acid (UA) were measured by enzymatic methods (AST, ALT, LDH, and UA: Shino Test Co., Tokyo; γ-GTP: Kanto Chem. Co., Tokyo; and UN: Wako Pure Chem. Ltd., Osaka), and creatinine (Cr) was measured by colorimetric method (Daiichi Pure Chem. Co. Ltd., Tokyo).

Serum lipid, total cholesterol (T-Chol), high-density lipoprotein cholesterol (HDL-Chol), triglycerides (TG), phospholipid (PL), and nonesterified free fatty acid (NEFA) were measured by enzymatic methods (Chol and TG: Kyowa Medex. Co., Ltd., Tokyo; PL: Daiichi Pure Chem. Co. Ltd., Tokyo; NEFA: Kainos Lab. Inc., Tokyo). LDL-Chol and atherogenic index (AI) were calculated using the following equations: LDL-Chol=T-Chol–HDL-Chol, and AI (LDL/HDL)=LDL-Chol/HDL-Chol.

Serum vitamin E (α- and γ-tocopherol (α-Toc and γ-Toc)), vitamin A (retinol (Ret) and esterified retinol (retinyl palmitate, Ret-pal)) were extracted by hexane and separated by high-performance liquid chromatography on a Diasil 5NH$_2$ column (4 mm i.d.×250 mm) with isopropanol in hexane (0.8:100) as the mobile phase, as described previously (26). The amounts of Toc and Ret were measured fluorometrically (excitation, 298 nm and 340 nm; emission, 325 nm and 480 nm, respectively) and calculated from the recovery of the added internal standard of β-tocopherol for Toc and benzo(a)pyrene for Ret. To measure the nutritional status of vitamin E and A, Toc and Ret concentrations were expressed as the concentration divided by the concentration of Chol and TG in serum, as suggested by us (24), as well as Thurnham et al. (21) and Horwitt et al. (20).

Statistical analysis

Statistical analyses were carried out using Stat View software Version 5.0. (SAS Institute Inc., NC, USA). Data were analyzed by two-way analysis of variance (ANOVA) for between age and gender. Pearson's correlation coefficients and partial correlation coefficients were used to examine the relationship between the variables.

Results

Physical constitution and distribution of serum lipid levels

The physical status statistics of the 176 subjects are given in Table 1. The age-specific mean values for height increased regularly from 139 and 135 cm in the 10–14-year age group for males and females, respectively, to 158 and 152 cm in the 15–19-year age group. The mean height showed a continuous slow increase to the 20–29-year group in males but remained about the same in higher aged females ($p<0.001$ for age). Therefore, the increase in mean height for the males was higher than that for the females, with the mean height of males being greater (158 cm) than that of females (149 cm) ($p<0.001$).

Weight means were approximately 29 kg for males in the 10–14-year age group, increased to 51 kg by 20–29 years and then declined to 49 kg in the age group above 50 years. In females, the weight mean was 28 kg in the 10–14-year age group and increased to 45 kg by 20–29 years and then declined to 41 kg in the age group above 50 years ($p<0.001$ for age). The mean weight of males (45 kg) was heavier than that of females (40 kg) ($p<0.001$) and an interaction between age and gender was found ($p<0.05$).

The body mass index (BMI) rose from 14.7 (males) and

15.3 (females) for the 10–14-year age group to 18.9 for the 20–29-year male group and 19.0 for the 15–19-year female group, and then remained the same for higher aged groups ($p<0.001$ for age). Overall, the males were taller and heavier than the females, BMI values were related to gender ($p<0.05$) and an interaction between age and gender was found ($p<0.05$).

The mean systolic blood pressure (SBP) was 111 in males and females in the 10–14-year age group, and then increased to 148 and 139, respectively, in those over 50 years old. The mean diastolic blood pressure (DBP) was 71 and 69 in males and females in the 10–14-year age group and increased to 85 and 84 in those over 50 years old, respectively. The overall mean levels of SBP and DBP were significantly higher in males than in females ($p<0.05$ and $p<0.01$, respectively). The levels of SBP and DBP were related to age ($p<0.001$, respectively).

Serum levels of biochemical parameters

Table 2 shows that the mean serum levels of biomarkers of liver function in males and females, AST (GOT) (36.1 and 28.8 IU/l), ALT (GPT) (17.5 and 12.9 IU/l), γ-GTP (18.6 and

Table 1 Physical constitution of the Nepalese in the southern rural Terai regions

Gender (age)	Subjects (n)	Height (cm)	Weight (kg)	Body mass index (kg/m^2)	Blood pressure (mmHg)	
					SBP	DBP
Male						
10–14	18	139±9	28.7±5.1	14.7±1.0	111±7	71±7
15–19	15	158±7	42.8±5.8	17.0±1.3	123±12	81±10
20–29	29	165±7	51.3±5.3	18.9±1.4	129±16	82±8
30–39	12	163±6	49.3±5.5	18.5±1.4	129±12	86±7
40–49	9	160±8	48.0±3.6	18.7±1.7	124±13	80±8
50–68	10	161±6	49.1±5.3	18.9±1.6	148±21	85±8
All	93	158±12***	44.7±9.8***	17.7±2.1	127±17*	81±9**
Female						
10–14	16	135±8	28.4±6.2	15.3±1.7	111±7	69±6
15–19	16	152±6	43.8±4.6	19.0±1.8	120±6	74±7
20–29	23	154±5	44.6±4.9	18.8±1.5	124±13	78±9
30–39	12	151±5	39.9±4.5	17.4±1.5	121±13	81±10
40–49	6	146±4	39.0±2.4	18.4±1.3	123±10	78±8
50–60	10	151±7	41.4±8.7	18.0±3.2	139±15	84±5
All	83	149±9***	39.8±8.0***	17.9±2.3	123±13*	77±9**
ANOVA	Age	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
	Gender	ns	ns	p<0.05	ns	p<0.05
	Age x Gender	ns	p<0.05	p<0.05	ns	ns

between genders (Fisher's PLSD): * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 2 Serum levels of biomarkers of the liver and kidney for the Nepalese in the southern rural Terai regions

Gender (age)	AST (GOT) (IU/l)	ALT (GPT) (IU/l)	γ-GTP (IU/l)	LDH (IU/l)	Cr (mg/100 ml)	UN (mg/100 ml)	UA (mg/100 ml)
Male							
10–14	33.3±11.8	12.1±6.1	7.50±2.2	370±73	0.661±0.085	9.39±3.84	3.99±1.04
15–19	38.7±12.3	19.8±7.6	16.2±10.4	375±116	0.820±0.121	8.93±1.94	5.41±0.640
20–29	36.8±10.2	20.5±10.3	25.9±21.0	307±78	0.893±0.0700	8.45±2.20	5.30±0.75
30–39	37.5±11.4	19.3±6.7	19.4±12.9	286±59	0.900±0.0850	9.67±2.61	5.13±1.61
40–49	32.8±10.2	18.2±8.0	12.8±7.8	287±34	0.911±0.117	9.33±3.32	5.52±0.720
50–68	36.1±15.1	12.6±2.3	25.3±18.2	317±84	0.920±0.162	10.3±3.37	5.14±0.830
All	36.1±11.5*	17.5±8.5*	18.6±16.1*	326±86	0.842±0.136*	9.15±2.83	5.05±1.07*
Female							
10–14	32.6±7.7	14.9±7.0	7.44±1.36	366±61	0.650±0.082	8.13±1.75	4.03±0.70
15–19	25.7±5.2	13.4±4.5	7.38±2.85	346±75	0.713±0.0960	8.69±3.22	4.38±0.640
20–29	28.8±10.0	13.5±5.9	6.91±2.45	337±92	0.691±0.0670	8.83±2.57	4.09±0.67
30–39	30.0±6.3	12.7±3.5	7.42±3.18	312±62	0.700±0.148	8.83±2.62	3.61±1.06
40–49	23.2±2.8	8.33±3.1	6.50±1.05	272±83	0.733±0.0820	10.5±1.97	3.57±0.430
50–60	29.4±8.9	10.4±3.6	7.00±1.25	372±99	0.820±0.103	10.3±2.11	3.90±0.850
All	28.8±8.0*	12.9±5.4*	7.16±2.26*	340±82	0.707±0.105*	8.96±2.53	4.00±0.77*
ANOVA							
Age	ns	ns	ns	p<0.05	p<0.001	p<0.01	ns
Gender	ns	ns	ns	ns	p<0.05	ns	ns
Age x Gender	ns	ns	ns	ns	ns	ns	p<0.01

between genders (Fisher's PLSD): * $p<0.001$.

Table 3 Serum levels of lipids for the Nepalese in the southern rural Terai regions

Gender (age)	T-Chol (mg/100 ml)	HDL-C (mg/100 ml)	LDL-C (mg/100 ml)	LDL/HDL	HDL/T-Chol (%)	TG (mg/100 ml)	PL (mg/100 ml)	NEFA (mg/100 ml)
Male								
10–14	123±24	41±7	82±22	2.03±0.62	34.3±7.3	90±36	158±21	459±296
15–19	134±14	43±7	92±11	2.19±0.44	31.9±4.6	105±30	172±16	506±251
20–29	142±22	46±10	97±21	2.22±0.67	32.4±7.3	109±35	182±28	415±219
30–39	167±31	51±11	116±29	2.38±0.750	31.0±6.90	134±65	209±31	432±228
40–49	131±23	41±6	90±19	2.17±0.32	31.9±3.1	106±64	164±23	353±220
50–68	146±31	53±14	84±19	1.93±1.18	37.5±10.4	95±47	200±32	479±266
All	140±27	45±10	94±23	2.16±0.68	33.0±7.0	106±45	179±30	441±244
Female								
10–14	136±16	41±6	94±19	2.36±0.76	31.1±6.8	123±49	170±20	379±202
15–19	142±26	41±9	101±20	2.51±0.53	29.1±4.1	126±41	175±47	487±257
20–29	146±24	51±11	95±24	1.93±0.64	35.8±7.6	98±54	188±33	495±276
30–39	149±36	56±14	93±31	1.75±0.690	38.1±8.10	79±26	196±45	538±345
40–49	151±34	54±10	97±27	1.80±0.41	36.4±6.3	94±27	194±50	675±176
50–60	152±35	44±10	108±31	2.53±0.76	29.6±6.7	136±63	196±27	455±268
All	145±27	47±11	97±24	2.16±0.71	33.2±7.4	110±50	185±37	486±266
ANOVA								
Age	$p<0.01$	$p<0.001$	ns	ns	ns	ns	$p<0.001$	ns
Gender	ns	ns	ns	ns	ns	ns	ns	ns
Age x Gender	ns	ns	ns	ns	ns	ns	ns	ns

7.16 IU/l) and LDH (326 and 340 IU/l)) were within the normal range for Japanese people. The levels of AST, ALT, and γ-GPT were higher in males than in females ($p<0.001$, respectively).

The levels of kidney function biomarkers of males and females, Cr (0.842 and 0.707 mg/100 ml), UN (9.15 and 8.96 mg/100 ml) and UA (5.05 and 4.00 mg/100 ml) were within the normal range (Table 2). The overall mean levels of Cr and UA were significantly higher in males than in females ($p<0.001$, respectively). The levels of Cr and UN increased with age ($p<0.001$ and $p<0.01$, respectively).

As shown in Table 3, the mean serum level of T-Chol in males was 123 mg/100 ml in the 10–14-year age group, increased to 167 mg/100 ml in the 30–39-year age group, and decreased to 146 mg/100 ml at above 50 years of age. In females, the T-Chol increased from 136 mg/100 ml in the 10–14-year age group to 152 mg/100 ml at above 50 years of age. The T-Chol levels were within the normal ranges and were related to age ($p<0.01$), and no significant differences were observed between genders.

The serum levels of HDL-Chol for males and females ranged from 41 mg/100 ml in the 10–14-year age group to 53 and 44 mg/100 ml in those above 50 years of age, respectively. The serum HDL-Chol levels were related to age ($p<0.001$), and were almost the same in both genders. The percentage of HDL-Chol to T-Chol in males and females ranged from 34% and 31% in the 10–14-year age group to 38% and 30% in those above 50 years of age, respectively. The serum HDL-Chol percentage was about the same in both genders.

The serum LDL-Chol levels for males and females ranged from 82 and 94 mg/100 ml in the 10–14-year age group to 84 and 108 mg/100 ml in those above 50 years old, respectively. The average of the overall values of LDL-Chol, 94 and 97 mg/100 ml, were within the normal range and no differences were observed in these levels in all age groups of both genders. The atherogenic index (AI), the ratio of LDL-Chol to HDL-Chol (LDL/HDL) for males and females, ranged from 2.03 and 2.36 in the 10–14-year age group to 1.93 and 2.53 in those above 50 years of age. The overall average ratio was the same, 2.16, for both genders.

The mean levels of TG for males was 90 mg/100 ml in ages 10–14 and increased to 105 mg/100 ml in ages 15–19. In the 30–39-year group, there was a sharp increase to 134 mg/100 ml followed by a decline to 95 mg/100 ml in the above 50-year group. The TG level in females was 123–126 mg/100 ml in ages 10–19 and decreased to 79 mg/100 ml in ages 30–39, but reached 136 mg/100 ml in the above 50-year group. The TG level seemed to be higher in females at 10–14 years and in males at 30–39 years of age. However, no significant differences were observed in the overall average values of TG, 106 and 110 mg/100 ml, in males and females, respectively.

The serum PL levels in males and females increased with age, from 158 mg/100 ml to 200 mg/100 ml and from 170 to 196 mg/100 ml, respectively ($p<0.001$). The serum levels of NEFA varied between 459–479 and 379–455 mg/100 ml in males and females, respectively, over the age groups. The levels of PL and NEFA showed no differences between genders.

Serum levels of tocopherol and retinol and the ratio to lipids

The serum α-Toc levels for males were 3.93 μg/ml in ages 10–14, then increased to 5.14 μg/ml in ages 30–39 years, reaching 4.53 μg/ml in the 50–68-year group ($p<0.001$ for age) (Table 4). In females, the level of α-Toc varied between 3.90–4.07 μg/ml through age 29, then slowly increased to 4.94 μg/ml in those above 50 years ($p<0.001$ for age). More than 5-fold variation in serum α-Toc values was evident among the participants, from 1.35 to 6.99 μg/ml. The overall average levels of α-Toc were the almost same for both genders (4.32 and 4.27 μg/ml in males and females, respectively).

Table 4 Serum levels of tocopherol and retinol for the Nepalese in the southern rural Terai regions

Gender (age)	α-Toc (μg/ml)	γ-Toc (μg/ml)	γ-Toc/α-Toc	Toc/Chol (μg/mg)	Toc/TG (μg/mg)	Ret (ng/ml)	Ret-pal (ng/ml)	Ret-pal/Ret	Ret/Chol (ng/mg)	Ret/TG (ng/mg)
Male										
10–14	3.93±0.71	0.42±0.28	0.104±0.063	3.24±0.58	5.28±2.81	456±124	15.7±6.8	0.035±0.015	369±74	590±294
15–19	4.00±0.93	0.35±0.20	0.086±0.038	3.00±0.70	4.01±1.12	579±126	22.2±11.4	0.041±0.024	437±107	593±200
20–29	4.42±0.98	0.27±0.11	0.061±0.022	3.18±0.85	4.48±1.84	696±142	27.2±14.1	0.039±0.017	497±110	696±264
30–39	5.14±0.93	0.37±0.13	0.070±0.018	3.10±0.52	4.73±2.19	759±220	21.6±9.2	0.029±0.012	453±98	706±388
40–49	4.02±0.70	0.35±0.14	0.086±0.028	3.16±0.83	4.67±2.12	636±143	19.3±9.1	0.031±0.015	492±128	731±342
50–68	4.53±1.04	0.37±0.20	0.080±0.035	3.13±0.55	5.44±1.99	611±140	33.7±20.5	0.056±0.035	423±78	751±302
All	4.32±0.96	0.34±0.19	0.079±0.040	3.15±0.70	4.71±2.06	624±175**	23.4±13.3	0.038±0.021	448±109**	669±290*
Female										
10–14	4.06±0.63	0.45±0.20	0.108±0.037	3.02±0.46	3.67±1.20	459±88	23.2±11.8	0.050±0.023	341±71	408±119
15–19	3.90±1.00	0.32±0.15	0.080±0.029	2.82±0.82	3.29±0.96	538±104	21.2±12.7	0.040±0.022	390±101	469±174
20–29	4.07±0.96	0.45±0.33	0.107±0.066	2.81±0.62	4.92±0.46	553±128	22.1±10.7	0.042±0.022	386±95	648±204
30–39	4.69±1.59	0.36±0.21	0.082±0.052	3.10±0.44	6.14±1.85	531±154	21.1±15.6	0.039±0.026	362±88	721±258
40–49	4.62±1.26	0.32±0.16	0.068±0.026	3.19±1.17	5.12±1.28	540±116	18.4±6.6	0.035±0.015	366±72	605±152
50–60	4.94±0.84	0.38±0.24	0.077±0.051	3.35±0.81	4.23±1.76	606±78	19.6±10.5	0.033±0.020	414±90	508±154
All	4.27±1.07	0.39±0.24	0.092±0.050	2.99±0.69	4.47±0.21	535±120**	21.4±11.6	0.041±0.022	377±89**	558±212*
ANOVA										
Age	p<0.001	ns	p<0.05	ns	p<0.05	p<0.01	ns	ns	ns	p<0.05
Gender	ns	ns	ns	ns	ns	ns	ns	p<0.05	ns	ns
Age x Gender	ns	ns	ns	ns	ns	ns	p<0.05	p<0.05	ns	ns

between genders (Fisher's PLSD): * p<0.01, ** p<0.001.

The serum γ-Toc levels were 0.42 and 0.45 μg/ml in males and females at ages 10–14 years, respectively. At 20–29 years, the serum γ-Toc levels decreased to 0.27 μg/ml in males, and were lower than those of 0.45 μg/ml in females. The level of γ-Toc varied between 0.35–0.37 μg/ml and 0.32–0.38 μg/ml at above 30 years in males and females, respectively. The ratio of γ-Toc/α-Toc decreased from 0.104 in the 10–14 year age group to 0.080 in males in the above 50-year group, and from 0.108 to 0.077 in females, respectively ($p<0.05$ for age).

The ratios of α-Toc to T-Chol, Toc/Chol in μg/mg, in males was 3.24 μg/mg at ages 10–14, decreased to 3.00 μg/mg at 15–19 years, and then reached 3.13 μg/mg for the above 50-year group (Table 4). In females, the level of Toc/Chol was at 3.02 μg/mg in ages 10–14, then decreased to 2.81 μg/mg at 20–29 years, then slowly increased to 3.35 μg/mg in the above 50-year group. No differences were observed in the mean levels of Toc/Chol at all ages in both genders (3.15 and 2.99 μg/mg in males and females, respectively).

The ratio of α-Toc to TG, Toc/TG in μg/mg, in males decreased from 5.28 μg/mg in the 10–19-year age group to 4.01 μg/mg in the 15–19-year group and increased to 5.44 μg/mg at above 50 years ($p<0.05$ for age). In females, the ratio of Toc/TG was 3.67 μg/mg in ages 10–14, increased to 6.14 μg/mg in the 30–39-year group, then decreased steadily to 4.23 μg/mg above 50 years of age ($p<0.05$ for age). The average ratios of Toc/TG at all ages were about the same in both genders (4.71 and 4.47 μg/mg in males and females, respectively).

The serum Ret levels for males increased steadily from 456 ng/ml in ages 10–14 to 759 ng/ml in ages 30–39 and decreased to 611 ng/ml above 50 years of age (Table 4). In females, the levels of Ret were 459 ng/ml at 10–14 years and increased to 553 ng/ml in the 20–29 year group. The Ret levels decreased to 531 ng/ml in the 30–39-year group, increased to 540 ng/ml at the age of 40–49 years, and then reached 606 ng/ml for the above 50-year group. The Ret levels were related to age ($p<0.01$) and the average of the Ret levels in all the subjects was higher in males (624 ng/ml) than in females (535 ng/ml) ($p<0.001$).

For the 10–14-year group, the serum Ret-pal levels were 15.7 ng/ml in males, which seemed to be lower than those in females, 23.2 ng/ml. The level of Ret-pal in males varied between 22.2–19.3 ng/ml in the 15–49-year groups, and increased to 33.7 ng/ml at above 50 years. In females, the level of Ret-pal varied between 21.2–19.6 ng/ml at above 15 years. The average levels of Ret-pal in all ages were about the same in both genders (23.4 and 21.4 in males and females, respectively) and an interaction between age and gender was found ($p<0.05$).

The ratio of the Ret-pal to Ret (Ret-pal/Ret) in males (0.035) seemed to be lower than those in females (0.050) in the 10–14-year group. The ratio of Ret-pal/Ret in males varied between 0.041 and 0.031 in the 15–49-year groups and increased to 0.056 in the above 50-year group. In females, the ratio of Ret-pal/Ret decreased from 0.040 to 0.033 at above 50 years. The average of the ratio of Ret-pal/Ret was about the same in both genders (0.038 in males and 0.041 in females) and was related to gender ($p<0.05$). An interaction between age and gender was found for the ratio of Ret-pal/Ret ($p<005$).

The ratios of Ret to T-Chol, Ret/Chol in ng/mg, in males was 369 ng/mg at ages 10–14, increased to 497 at 20–29 years, and then reached 423 ng/mg for the above 50-year group (Table 4). In females, the level of Ret/Chol was at 341 ng/mg in ages 10–14, then increased to 390 ng/mg at 15–19 years, then slowly increased to 414 ng/mg above 50 years of age. The average ratios of Ret/Chol of males were higher than those of females (448 and 377 ng/mg in males and females, respectively) ($p<0.001$).

Table 5 Correlation between parameters

Parameter	T-Chol	HDL-Chol	LDL-Chol	LDL/HDL	TG	Toc	Toc/Chol	Toc/TG	Ret	Ret/Chol	Ret/TG
T-Chol		0.41***	0.93***	0.49***	0.30**	0.48***	–0.41***	–0.07	0.58***	–0.10	0.13
HDL-Chol	0.44***		0.09	–0.57***	–0.32**	0.36***	–0.03	0.47***	0.45***	0.17	0.61***
LDL-Chol	0.91***	0.03		0.73***	0.43***	0.36***	–0.44***	–0.25*	0.50***	–0.12	–0.06
LDL/HDL	0.34**	–0.67***	0.69***		0.57***	0.10	–0.32**	–0.49***	0.11	–0.22*	–0.43***
TG	0.26*	–0.29**	0.42***	0.51***		0.23*	–0.03	–0.74***	0.28*	0.15	–0.67***
Toc	0.49***	0.31**	0.40***	0.07	0.29**		0.59***	0.29**	0.46***	0.20	0.14
Toc/Chol	–0.28*	–0.05	–0.28*	–0.17	0.15	0.69***		0.36***	–0.04	0.31**	0.02
Toc/TG	0.10	0.54***	0.15	–0.46***	–0.69***	0.39***	0.31**		–0.02	–0.01	0.79***
Ret	0.33**	0.28*	0.23*	–0.02	0.28*	0.28*	0.04	–0.10		0.74***	0.43***
Ret/Chol	–0.44***	–0.10	–0.45***	–0.25*	0.08	–0.09	0.27*	–0.16	0.68***		0.37***
Ret/TG	–0.07	0.52***	–0.32**	–0.57***	–0.75***	–0.07	–0.06	0.72***	0.25*	0.29**	

Upper right, male; lower left, female (Pearson's correlation coefficient): * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 6 Correlation between parameters adjusting for age, gender and BMI

Parameter	T-Chol	HDL-Chol	LDL-Chol	LDL/HDL	TG	Toc	Toc/Chol	Toc/TG	Ret	Ret/Chol	Ret/TG
T-Chol	1.00										
HDL-Chol	0.40***										
LDL-Chol	0.92***	0.04									
LDL/HDL	0.43***	–0.63***	0.71***								
TG	0.26***	–0.33***	0.41***	0.53***							
Toc	0.46***	0.29***	0.38***	0.10	0.27***						
Toc/Chol	–0.37***	–0.07	–0.37***	–0.23**	0.08	0.64***					
Toc/TG	–0.01	0.49***	–0.20**	–0.46***	–0.72***	0.32***	0.32***				
Ret	0.43***	0.34***	0.34***	0.04	0.24**	0.35***	–0.01	–0.05			
Ret/Chol	–0.33***	0.01	–0.34***	–0.26**	0.08	0.03	0.31***	–0.06	0.69***		
Ret/TG	0.00	0.54***	–0.20*	–0.49***	–0.73***	0.00	–0.02	0.77***	0.34***	0.32***	1.00

Adjusting for age, gender and BMI (partial correlation coefficient): * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

The ratio of Ret to TG, Ret/TG in ng/mg, in males increased from 590 ng/mg in the 10–14-year age group to 751 ng/mg at above 50 years ($p<0.05$ for age). In females, the ratio of Ret/TG was 408 ng/mg in ages 10–14, increased to 721 ng/mg in the 30–39-year group, then decreased steadily to 508 ng/mg in the above 50-year group ($p<0.05$ for age). The average ratios of Ret/TG ng/mg of males were higher than those of females (669 and 558 ng/mg in males and females, respectively) ($p<0.01$).

Correlation among serum levels of tocopherol, retinol, and lipids

The Peason's correlation coefficients between the levels of Chol, TG, Toc and Ret in males and females, respectively (Table 5), and the partial correlation coefficients adjusting for age, gender and BMI (Table 6) were examined. Those p values of the partial correlation coefficients were almost the same as or higher than those of the Peason's correlation coefficients.

The levels of Chol were positively related to the levels of HDL ($r=0.41$ and $r=0.44$, $p<0.001$ for males and females, respectively), LDL ($r=0.93$ and $r=0.91$, $p<0.001$), and TG ($r=0.30$, $p<0.01$ and $r=0.26$, $p<0.05$), and the ratio of LDL/HDL ($r=0.49$, $p<0.001$ and $r=0.34$, $p<0.01$).

Comparison of the serum levels of Toc with lipids showed the levels of Toc to be positively related to the levels of Chol ($r=0.48$ and $r=0.49$, $p<0.001$ for males and females, respectively) and TG ($r=0.23$, $p<0.05$ and $r=0.29$, $p<0.01$). The Toc/Chol ratios were negatively related to the levels of Chol ($r=-0.41$, $p<0.001$, and $r=-0.28$, $p<0.05$), and Toc/TG ratios were negatively related to the levels of TG ($r=-0.74$ and $r=-0.69$, $p<0.001$).

The serum Ret levels were also positively related to the levels of Chol ($r=0.58$, $p<0.001$, and $r=0.33$, $p<0.01$ for males and females, respectively) and TG ($r=0.28$, $p<0.05$ for males and females, respectively). The Ret/Chol ratios were negatively related to the levels of Chol ($r=-0.44$, $p<0.001$ for females) and Ret/TG ratios were negatively related to the levels of TG ($r=-0.67$ and $r=-0.75$, $p<0.001$ for males and females, respectively).

The levels of Ret were directly correlated with the levels of Toc ($r=0.46$, $p<0.001$ and $r=0.28$, $p<0.05$ for males and females, respectively). The ratios of Toc/Chol were positively related to the ratios of Ret/Chol ($r=0.31$, $p<0.01$, and $r=0.27$, $p<0.05$). The ratios of Toc/TG were directly correlated with the ratio of Ret/TG ($r=0.79$ and $r=0.72$, $p<0.001$).

The ratios of LDL/HDL (AI) were negatively related to the ratios of Toc/Chol ($r=-0.32$, $p<0.01$ for males) and Ret/Chol ($r=-0.22$ and $r=-0.25$, $p<0.05$ for males and females, respectively). Also, the ratios of LDL/HDL (AI) were negatively related to the ratios of Toc/TG ($r=-0.49$ and $r=-0.46$, $p<0.001$) and Ret/TG ($r=-0.43$ and $r=-0.57$, $p<0.001$).

Discussion

Data from the current study were compared with previous findings (Table 7). The heights of subjects in this study were similar to those of subjects aged 10–72 years in the Itahari district of the Terai region in southeastern Nepal (131–163 cm

Table 7 Comparison of the serum levels of lipid, tocopherol and retinol

Country (Reference)		T-Chol (mg/100 ml)	HDL-Chol (mg/100 ml)	LDL-Chol (mg/100 ml)	TG (mg/100 ml)	Toc (μg/ml)	Toc/Chol (μg/mg)	Toc/TG (μg/mg)	Ret (ng/ml)	Ret/Chol (ng/mg)	Ret/TG (ng/mg)
Nepal											
Present data (10–68 y):	Male	140	45	94	106	4.32	3.15	4.71	624	448	669
	Female	145	47	97	110	4.27	2.99	4.45	535	377	558
Itahari (10–72 y) (24):	Male	133			132	3.76	2.83	3.36	534	409	473
	Female	128			102	3.37	2.67	3.66	420	328	462
Japan											
National Survey (27):	Male	198	53	145	162						
	Female	205	61	144	129						
University Students (34):	Male	170	54	116	74	5.16	3.11	7.88			
	Female	181	62	118	59	6.15	3.46	11.48			
Adult (50–74 y) (35):	Male	183			160	8.7	4.75	5.43	731	399	457
	Female	201			160	10.1	5.03	6.32	632	314	395
Tunisia (32–85 y) (29):	Mixed gender	176	46	130	112	9.34	5.31	8.34			
Tanzania (52±4 y) (31):	Male	131	39	77	186						
	Female	170	39	116	195						
Dutch (43±15 y) (30):	Mixed gender	217	62	149	100						
British (45–64 y) (32):	Mixed gender	232	58	143	124	10.2	4.64	8.22	602	259	485
USA (41±15 y) (36):	Mixed gender					11.2			530		

The values were transformed from moles to grams to allow comparison.

and 140–152 cm for males and females, respectively) (24). The weights of the subjects seemed lower in this study than those of the Itahari districts (25–59 kg and 28–53 kg for males and females, respectively) (24). Therefore, the BMI values tended to be lower than those of the Itahari districts (14.1–22.2 and 14.2–23.5 for males and females, respectively) (24). The reason for the variation in findings for weight and BMI between the two studies was not clear, but could have been related to lower income or regional differences. The Itahari district is an industrial region (24), while the Chitwan district of this study is an agricultural region where the residents are self-supporting but live on a low income. The corresponding BMI values of the Nepalese appear to be lower than the values of the Japanese (20.7–24.2 and 20.4–23.6 for males and females, respectively, 15–69 years of age (27).

Epidemiological studies indicate that serum Chol levels increased throughout adulthood, reaching a plateau at approximately the sixth decade of life (28). Differences were observed in our present survey for Chol levels, related to age ($p<0.01$). The highest Chol levels was found at 30–39 years for males, which may be due to the custom of the father eating first in the family and therefore tending to eat more. The Chol levels in this study seemed higher than those for the subjects in the Itahari district (133 and 128 mg/100 ml, in males and females, respectively) (24). The levels of Chol found in Nepal were lower than those of the Japanese (198 and 205 mg/100 ml for males and females, respectively, above 20 years of age) (27), of Tunisians (176 mg/100 ml as the average in males and females) (29), and of the Dutch (217 mg/100 ml as the average in males and females) (30).

HDL-Chol levels were similar to those of Tunisians (46 mg/100 ml as the average in males and females) (29), and tended to be lower than those of the Japanese (53 and 61 mg/100 ml, in males and females, respectively) (27) and of the Dutch (62 mg/100 ml as the average in males and females) (30), and higher than those of Tanzanians (39 mg/100 ml, in males and females, respectively) (31).

The LDL-Chol levels were lower than those of the Japanese (145 and 144 mg/100 ml, in males and females, respectively) (27), of the Dutch (149 mg/100 ml as the average in males and females) (30) and of the British (143 mg/100 ml as the average in males and females) (32), and appear to be higher than those of males in Tanzania (77 mg/100 ml) (31).

The TG levels in males in the present study tended to be lower than those in the Itahari district of the industrial region, but similar for females (132 and 102 mg/100 ml, in males and females, respectively) (24). The levels of TG tended to be lower than those of the Japanese (162 and 129 mg/100 ml, in males and females, respectively) (27) and of Tanzanians (186 and 195 mg/100 ml, in males and females, respectively) (31), and higher than those of the Dutch (100 mg/100 ml as the average in males and females) (30) and similar to those of Tunisians (112 mg/100 ml as the average in males and females) (29).

The mean serum Toc levels in this report on an agricultural region tended to be higher than those in the industrial Itahari district (3.76 and 3.37 μg/ml, in males and females, respectively) (24). Those values appear to be lower than the normal plasma concentration of vitamin E of 10 μg/ml with a range of 5–16 μg/ml (33). The Toc levels of the Nepalese were lower than those of the Japanese, at 5.16 and 6.15 μg/ml, in males and females, 21 years, respectively (34), and at 8.7 and 10.1 μg/ml, in males and females, 50–74 years, respectively (35). The levels of Toc were lower than those of Tunisians (9.34 μg/ml as the average in males and females) (29), of the British (10.2 μg/ml as the average in males and females) (32), and of Americans (11.2 μg/ml as the average in males and females) (36).

The average of the Ret levels in all the subjects was higher for males than females, and was the highest at 30–39 years in males. This may be due to the custom of the father eating first in the family, and therefore tending to eat more. The mean

serum Ret levels in this agricultural region tended to be higher than those in the industrial Itahari district (543 and 420 ng/ml, in males and females, respectively) (24). The Ret levels were lower than those of the Japanese (731 and 632 ng/ml, in males and females, respectively) (35). The levels of Ret tended to be similar to those of the British (602 ng/ml as the average in males and females) (32) but higher than those of Americans (530 ng/ml as the average in males and females) (36).

As shown in Table 5, the levels of Toc and Ret in serum are correlated with the serum levels of Chol and TG. These correlations concur with our previous findings for the Nepalese in the Itahari district (24), for university students (34) and elementary school children (26) in Japan, and with those of other reports (19–22). These results indicate that the changes in serum levels of Toc and Ret are closely related to the alterations in the Chol and TG levels. It is logical that when Chol and TG levels were high, Toc and Ret were high, and that the nutritional status of Toc and Ret should not be estimated only from the serum levels. These findings suggest that the serum levels of Toc and Ret normalized to serum lipids, the ratios of Toc/Chol and Ret/Chol and the ratios of Toc/TG and Ret/TG, is a more precise way of assessing the nutritional status of Toc and Ret than the serum Toc and Ret levels only (20, 22, 24, 26, 34).

The ratios of Toc/Chol and Toc/TG in this agricultural region tended to be higher than those in the industrial Itahari district (2.83 and 2.67 μg/mg of Toc/Chol, and 3.36 and 3.66 μg/mg of Toc/TG, for males and females, respectively) (24). These levels of Toc/Chol were lower than those found in the Japanese (4.75 and 5.03 μg/mg in males and females, respectively) (35), Tunisians (5.31 μg/mg as the average in males and females (29), and the British (4.64 μg/mg as the average in males and females) (32). The ratios of Toc/TG in Nepal appeared to be lower than those found in the Japanese (5.43 and 6.32 μg/mg for males and females, respectively) (35), Tunisians (8.34 μg/mg as the average for males and females) (29), and the British (8.22 μg/mg as the average for males and females) (32).

The ratio of the Ret/Chol levels was higher for males than for females. For males, the ratio of the Ret/Chol levels was the highest at 20–29 years, and was the higher than that of females. The reason for that may be due to the higher levels of serum Ret in males than in females in the 20–29-year group, and due to the lower levels of Chol in males than in females.

The ratios of Ret/Chol and Ret/TG in this report tended to be higher than those in the industrial Itahari district (409 and 328 ng/mg and 473 and 462 ng/mg, for males and females, respectively) (24). The levels of Ret/Chol of the Nepalese appeared to be higher than those of the Japanese (399 and 314 ng/mg, in males and females, respectively) (35) and the British (259 ng/mg as the average in males and females) (32). The ratios of Ret/TG in this study appeared to be higher than those of the Japanese (457 and 395 ng/mg for males and females, respectively) (35) and the British (485 ng/mg as the average for males and females) (32).

A positive correlation between the ratios of Toc/TG and Ret/TG concurs with our previous findings for people in the Itahari districts (24) and for elementary school children in Japan (26). From the report that the Ret store in the liver will be diminished by Toc deficiency and increased by its supplementation (37), it is reasonable that the nutritional status of Toc/TG in serum shows a positive correlation with the serum Ret/TG status, as shown in this work.

We found that the serum levels of Toc/TG and Ret/TG were negatively correlated with the ratio of LDL/HDL, which is a risk factor for the development of coronary heart disease. These correlations concur with our previous findings for elementary school children (26) and university students (34) in Japan. These results indicate that changes in serum levels of LDL/HDL are closely related to alterations in the serum levels of Toc/TG and Ret/TG. When Toc/TG and Ret/TG levels are low, the LDL/HDL ratio seems to be high. These results suggest that greater intake of foods rich in Toc and Ret, including carotenoids with provitamin A, should be encouraged to reduce the risk of coronary heart disease.

Acknowledgments

We thank the staff and students of Tribhuvan University Teaching Hospital and Ms Noriko Sato, Mr Masasi Ito and other members of the Japan Overseas Cooperation Volunteers (JOCV) in Nepal for their help.

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