Plasma ω-3 and ω-6 fatty acids in thyroid diseases

XIANG LI[1], HUI LI[2], JING ZHAO[3], QI DAI[1], CHAORAN HUANG[4], LANGPING JIN[5], FAN YANG[5], FUXUE CHEN[1], OUCHEN WANG[5] and YING GAO[3]

[1]School of Life Sciences, Shanghai University, Shanghai 200444; [2]Department of Nutrition and Food Hygiene, School of Public Health, Peking University, Beijing 100191; [3]Chinese Academy of Sciences Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031; [4]Shanghai University of Traditional Chinese Medicine, Shanghai 201203; [5]Department of Surgical Oncology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang 325000, P.R. China

Received April 7, 2017; Accepted February 19, 2018

DOI: 10.3892/ol.2018.9288

Abstract. The incidences of nodular goiter (NG), thyroid adenoma (TA), and thyroid cancer (TC) are increasing rapidly; however, the etiologies of these diseases remain unclear. The present study aimed to evaluate the differences in plasma fatty acids among these three thyroid diseases to facilitate etiological research. Four ω-3 and seven ω-6 polyunsaturated fatty acids were measured from 97 TC, 14 TA and 11 NG patient plasma samples with gas chromatography-flame ionization detector. Fatty acids levels were expressed as the percentage of each fatty acid out of the total fatty acids evaluated. The present study identified that the level of 22:6n-3 [median, interquartile range (IQR)] was significantly increased in TA (5.2%, 4.3-6.4%) compared with NG (3.6%, 3.1-4.6%) and TC patients (4.2%, 3.2-4.8%). Though not statistically significant, the levels of 20:5n-3 and 22:5n-3 demonstrated a similar pattern. The level of 22:4n-6 expressed (median, IQR) was significantly increased in NG patients (0.21%, 0.18-0.26%) compared with TA (0.16%, 0.15-0.18%) and TC (0.17%, 0.14-0.22%) patients. Furthermore the fatty acids 18:3n-6, 20:2n-6, 20:3n-6, 20:4:6, and 22:5n-6 demonstrated a similar but statistically insignificant pattern. This suggests that different fatty acids exhibit various etiological roles in NG, TA and TC and warrant further study.

Correspondence to: Professor Ying Gao, Chinese Academy of Sciences Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, P.R. China
E-mail: yinggao@sibs.ac.cn

Professor Ouchen Wang, Department of Surgical Oncology, The First Affiliated Hospital of Wenzhou Medical University, 2 Fuxuexiang Road, Wenzhou, Zhejiang 325000, P.R. China
E-mail: woc099@sina.com

Abbreviations: GC-FID, gas chromatography flame ionization detector; IQR, interquartile range; SEER, Surveillance, Epidemiology, and End Results; NCCR, National Central Cancer Registry; BMI, body mass index; FAME, fatty acid methyl esters; ANOVA, analysis of variance

Key words: fatty acids, thyroid adenoma, thyroid cancer, nodular goiter

Introduction

Nodular goiter (NG), thyroid adenoma (TA) and thyroid cancer (TC) are common thyroid diseases, and their incidence rates are rapidly increasing worldwide (1-3), and in China (4).

NG is a benign thyroid disease and, like hyperplasia, stems from recurrent attacks of simple goiter. It is diagnosed following thyroid ultrasonography and fine-needle aspiration (FNA); if the results suggest malignancy or malignant behavior, the patient is referred for a thyroidectomy (5).

TA is the most prevalent benign thyroid tumor; it primarily originates from thyroid follicular cells, and has a favorable prognosis following surgical resection (6).

TC is the most common malignant tumor among all endocrine system and head and neck neoplasms, and has a low but rapidly increasing incidence rate worldwide (7). According to the Surveillance, Epidemiology, and End Results (SEER) database (http://seer.cancer.gov/statfacts/html/ld/thyro.html), the number of new cases of TC has increased from 4.8 (per 100,000) in 1975 to 6.2 (per 100,000) in 1995, and 15.1 (per 100,000) in 2013. In China, it was estimated that the new cases and mortalities increased to 90,000 and 6,800, respectively, in 2015, according to the National Central Cancer Registry (NCCR) data of the average incidence rates from 2009-2011 in 72 population-based cancer registries (4). Differentiated TC originates from follicular thyroid cells, and includes papillary TC (PTC) and follicular TC, which account for 80-85 and 10-15% of TC cases, respectively (8).

Despite being the most commonly used diagnostic tool, the ultrasonography-guided FNA biopsy is not useful to distinguish between benign nodules, follicular adenoma and follicular carcinoma in cytology (3,9-11). Therefore, it would be beneficial in clinical practice to identify biomarkers that may distinguish NG, TA and TC in a convenient and noninvasive manner.

Fatty acids are important nutrients and bioactive molecules that are involved in energy storage, signal pathways and key biochemical activities (12,13). There are a number of studies demonstrating that fatty acids, in particular ω-3 and ω-6 fatty acids, are closely associated with the risk of certain diseases, including cancer, diabetes, and cardiovascular diseases (14,15). The thyroid is an important metabolic organ, which synthesizes thyroid hormones and controls energetic metabolism, that is closely associated with fatty acids (16,17). Schneider and Chen (3) hypothesized that an increasing body mass index (BMI), which is closely associated with fatty acids, is a possible explanation for the increasing incidence of TC. Furthermore, additional studies have reported differences in fatty acids in the serum (18-20), urine (21), and thyroid tissue samples (22,23) between TC patients and healthy controls.

Although there is a possibility that benign diseases, including NG and TA, may become malignant, it is unconfirmed whether these diseases are the precursors of TC and whether they share any common etiology, including body fat percentage and fat intake. Therefore studying the association between fatty acids and NG, TA and TC simultaneously may provide insights that would be beneficial in clinical practice. Consequently, the present study utilized a gas chromatography-flame ionization detector (GC-FID) method to measure the percentages of polyunsaturated fatty acids (PUFAs) in 122 plasma samples from patients with thyroid diseases.

Materials and methods

Participants. A total of 122 patients with thyroid diseases were recruited at 2 time points from Wenzhou Medical School Subsidiary Hospital (Wenzhou, China), including 97 patients with thyroid carcinoma (female, n=77), 11 patients with NG (female, n=7), and 14 patients with TA (female, n=9). All blood samples were collected following overnight fasting. Following centrifugation at 3,000 x g at room temperature for 15 min, plasma samples were removed and stored at -80˚C until measurement. Clinical parameters, including age, sex, weight, height, fasting blood glucose, systolic/diastolic blood pressure, thyroid hormone (Thy), triiodothyronine (T3), thyroid-stimulating hormone (TSH), free tetraiodothyronine (FT4), free triiodothyronine (FT3), thyroglobulin antibody (TGA), anti-thyroperoxidase antibody (TPOA), thyroglobulin (HTG), and parathormone (PTH), were obtained from the hospital researchers. The study was approved by Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical University and informed consent was obtained from all individual participants included in the study.

Chemicals and reagents. The fatty acid methyl esters (FAMEs; including 38 FAMEs) internal standard (IS) C21:0 (purity >99%), used as the calibration standard solution, was purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA). High-performance liquid chromatography (HPLC)-grade methanol, dichloromethane, n-hexane, deionized H_2O, and iso-octane were purchased from Honeywell (Morris Plains, NJ, USA). NaCl (purity >99.5%) was purchased from Jiangsu Hengrui Medicine Co., Ltd. (Lianyungang, China). Na_2SO_4 (purity >99%) and H_2SO_4 (purity >95%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Profile of fatty acids in plasma. Fatty acids were extracted from the plasma sample using methanol/dichloromethane (V/V=1:1), then dried with nitrogen, trans-methylated with methanol/concentrated sulfuric acid (V/V=25:1) and bathed in 80˚C water for 1 h. FAMEs were extracted by n-hexane, then dried by nitrogen, and finally dissolved in iso-octane for GC-FID (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a 100-m HP-88 NEFA phase column (100 m x 0.25 mm x 0.2 μm; Agilent Technologies, Inc.).

Four ω-3 and seven ω-6 fatty acids evaluated in the present study. The percentage of each individual fatty acid was calculated according to a response value of the standards using C21:0 as the IS. The standards were used to adjust the measurement deviation, and the IS was used to adjust the extraction procedure deviation. A uniform quality control (QC) sample was inserted into every 12 samples. The coefficient of variation (CV) of QC was <12% for all 11 fatty acids.

Statistics analysis. The clinical parameters were compared among three groups by non-parametric Kruskal-Wallis test for continuous variables, as the data did not follow a normal distribution. A χ^2 test was used for categorical variables. The percentages or ratios of fatty acids were compared between TC, NG and TA by the non-parametric Kruskal-Wallis test. Each group comparison was performed by the rank-based ANOVA among three groups. All tests were two-sided. $P<0.05$ was considered to indicate a statistically significant difference. Statistical analyses were performed using SAS 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

Baseline clinical characteristics. The baseline clinical characteristics are summarized shown in Table I. In thyroid-related hormones, only the TSH was significantly different among the three groups; and the level in the TC group was the highest. However, the other hormones evaluated, including Thy, T3, FT4, FT3, TGA, TPOA, and HTG, did not show any statistically significant differences (Table I). No significant difference among the three groups was observed for PTH, and the majority of thyroid-related hormone indexes in the experimental population were in the normal range according to 'China Thyroid Disease Diagnosis and Treatment Guidelines' (24), except TGA and TPOA. In total, 33 out of 95 patients exhibited higher TGA, and 24 out of 93 patients exhibited higher TPOA. Out of 110 patients, 40 were in the normal range according to 'China Thyroid Disease Diagnosis and Treatment Guidelines' (24) for all 9 thyroid-related hormone parameters (data not shown).

PUFAs among three groups. The plasma levels of 22:6n-3, ω-3 PUFAs, 22:4n-6, ω-6/ω-3 fatty acids, and total PUFAs were significantly different among the three groups (Fig. 1A-D). Total PUFA level (Fig. 1E) was significantly increased in the TA group compared with the TC and NG groups. The levels of 22:6n-3 (Fig. 1A) and total ω-3 PUFAs (Fig. 1B) were significantly increased in the TA group compared with the TC and NG groups. However, 20:5n-3 and 22:5n-3 did not demonstrate a statistically significant difference among the three groups. In addition, among ω-6 fatty acids, 22:4n-6 (Fig. 1H) was

Table I. Baseline characteristics and thyroid-related hormone levels in the TC, TA and NG groups.

Characteristic	Value			P-value
	TC	TA	NG	
Age, years	46 (38-55)	43 (38-55)	56 (41-62)	0.25
Sex, n				
Female	77	9	8	0.55
Male	20	5	3	
Height, cm (range)	160 (158-165)	160 (157-170)	158 (151-168)	0.34
Weight, kg (range)	60 (53.5-67)	63 (49.5-79)	57 (55-63)	0.69
BMI, kg/m^2 (range)	23 (21-26)	24 (20-28)	23 (20-25)	0.91
Glu, mmol/l (range)	5.6 (4.9-6.5)	5.7 (5.5-6.1)	5.95 (5.2-7.3)	0.44
SBP, mmHg (range)	124 (117-140)	125.5 (114-134)	129 (118-135)	0.91
DBP, mmHg (range)	80 (74-87)	80.5 (70-90)	83 (79-88)	0.77
HT, %	32/97 (34)	2/11 (18)	2/10 (20)	0.56
Thy, nmol/l (range)	106 (95-117)	90 (85-111)	108 (92-130)	0.18
T3, nmol/l (range)	1.6 (1.4-1.8)	1.6 (1.3-1.7)	1.75 (1.4-1.8)	0.49
TSH, mIU/l (range)	1.4 (0.88-1.91)	1.13 (0.62-1.46)	0.67 (0.48-1.6)	0.03
FT4, pmol/l (range)	11 (9-12)	11 (10-12)	12 (9.5-13)	0.61
FT3, pmol/l (range)	4.4 (4-4.8)	4.1 (3.8-4.4)	4.4 (4.1-4.71)	0.16
TGA, IU/ml (range)	0.9 (0.9-12.8)	0.9 (0.9-0.9)	0.9 (0.9-0.9)	0.29
TPOA, IU/ml (range)	106 (95-117)	90 (85-110)	108 (92-130)	0.37
PTH, pg/ml (range)	37 (28-43)	31 (27-33)	42 (32-47)	0.20

Data are presented as the median (interquartile range), unless otherwise indicated. Comparisons between three groups were performed with the Kruskal-Wallis test, except for HT and sex, which were analyzed with the Chi-squared test. $P<0.05$ was considered to indicate a statistically significant difference. Glu, fasting blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; HT, hypertension; Thy, thyroid hormone; T3, three iodine threonine; TSH, thyroid stimulating hormone; FT4, free tetraiodothyronine; FT3, free triiodothyronine; TGA, thyroglobulin antibody; TPOA, anti-thyroperoxidase antibody; HTG, thyroglobulin; PTH, parathormone; TC, thyroid cancer; TA, thyroid adenoma; NG, nodular goiter.

significantly increased in the NG group compared with the TC and TA groups. A number of other ω-6 fatty acids, including 18:3n-6 (Fig. 1I), 20:2n-6 (Fig. 1J), 20:3n-6 (Fig. 1K), 20:4n-6 (Fig. 1L), and 22:5n-6 (Fig. 1M), demonstrated a similar pattern. The ω-6/ω-3 (Fig. 1D) ratio was significantly decreased in the TA group compared with the TC group.

PUFAs among three groups in females. In females (Table II), the present study did not identify a statistically significant difference in any ω-3 fatty acids among the three groups examined. While the ω-6 fatty acids 20:4n-6 and 22:4n-6 were significantly different among three groups in females. In females, the levels of 18:3n-6, 20:2n-6, 20:3n-6 and 22:5n-6 in the NG group were higher than those of the other groups, and their pattern that was similar to that of the whole study population (Fig. 1).

PUFAs among three groups with normal level of thyroid-related hormones. The percentage of fatty acids in patients with a normal level of thyroid-related hormones were compared by Kruskal-Wallis test among three groups (Table III). The results identified that 22:6n-3, total ω-3 fatty acids, 22:4n-6, and total PUFA levels were significantly different among three groups, which were consistent with the whole study population.

Cut-off of total ω3 fatty acids and 22:4n6 to distinguish TA and NG groups. When the 75th percentile cutoff of the ω-3 fatty acids and 22:4n-6 in the group from the first time point was applied to the group from the second time point, the patients with TA were 100% correctly classified (Tables IV and V). Consistent with the significant difference (Table II), it demonstrates that ω-3 PUFAs and 22:4n-6 are helpful to distinguish TA and NG among three diseases although sample size is limited.

Discussion

The present study identified that the plasma levels of 22:6n-3, total ω-3 fatty acids, and PUFAs were significantly increased in the TA group compared with those of the TC and NG groups and that 22:4n-6 level was significantly increased in NG group compared with that of TC and TA groups.

Thyroid diseases are common endocrine diseases, although some investigators hypothesize that thyroid diseases, especially TC, are over-diagnosed (1,25,26). Conversely, other researchers postulate that the incidence rate of TC is increasing worldwide (2,4); however, this increasing trend may be associated with environmental changes and the development of detection technology (3). Regardless, the clear etiological

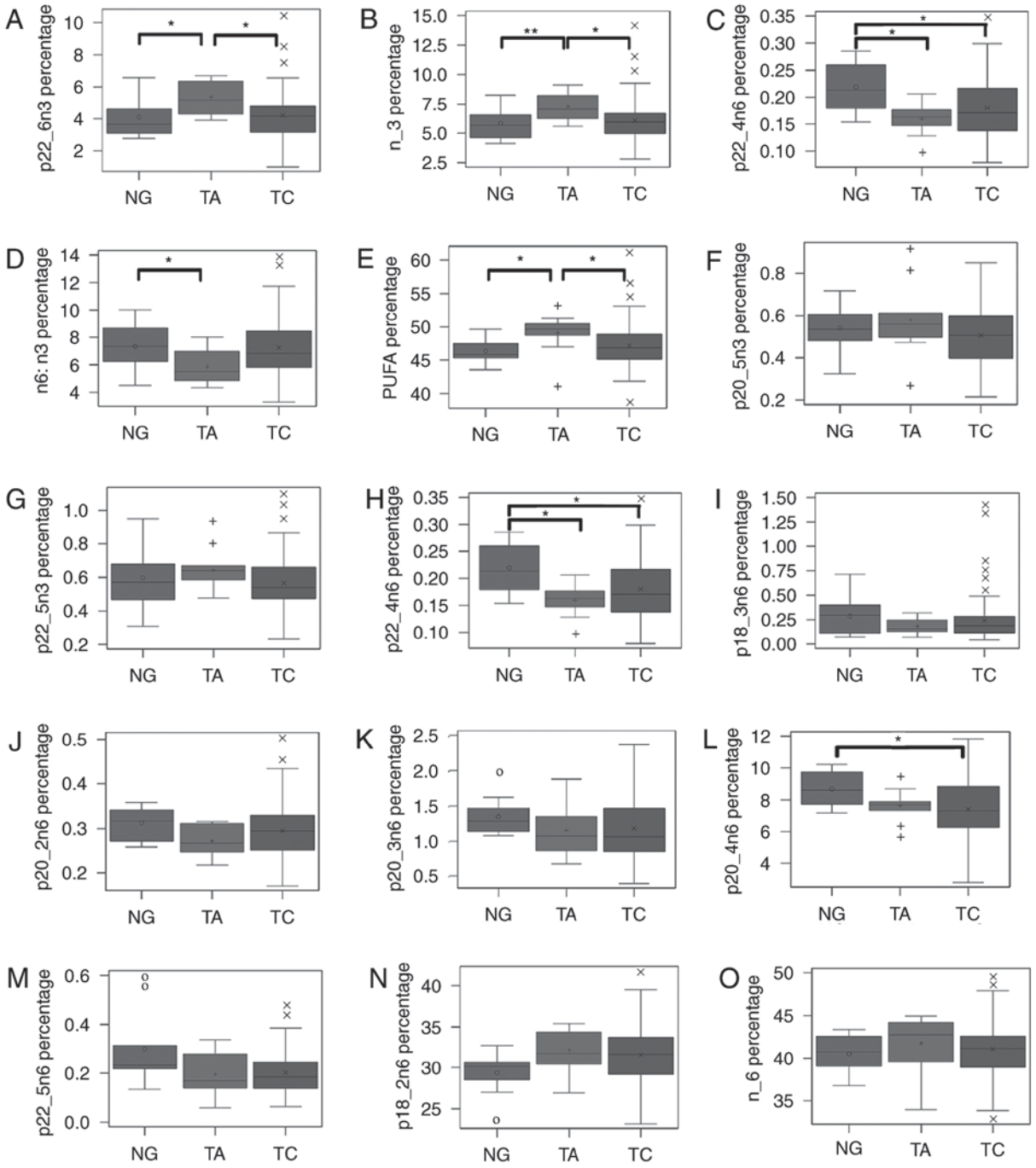

Figure 1. Percentage and the ratio of fatty acids and different kinds of fatty acids are compared among three diseases. (A) Demonstrates the percentage of total PUFA; (B) exhibits the ratio of total ω-6 to total ω-3; (C-J) were the percentage of total ω-6, 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, and 22:5n-6; (K-O) were the percentage of total ω-3, 18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3. Fatty acids are compared by Kruskal-Wallis test among three groups, and two groups are compared using an analysis of based on rank adjusted by Bonferroni Correction. *P<0.05. 0, +, and x denote median values of NG, TA and TC, respectively, inside the interquartile range, and outliers outside the interquartile range.

mechanism of this substantial rise requires examination. It is difficult to distinguish the different thyroid diseases of follicular based on clinical symptoms and cytology (3,9-11). Therefore, it is important to develop biomarkers and easier-to-use technologies to facilitate clinical practice.

The estimated numbers of new cases of TC in 2015 were 40,200 in east, 14,300 in central, 10,700 in northeast and <10,000 in other regions of China (4). A number of studies reported that the fish and seafood-rich dietary intake was associated with the increased risk of TC (27,28), in particular dried or salted fish that specifically occur among Asian seasonings (29). Wenzhou is an eastern coastal city where people consume an increased amount of seafood compared with the national average (30), and seafood is rich in ω-3 fatty acids. Therefore, evaluating the link between thyroid disease and fatty acids may provide beneficial insights; hence the patient sample was obtained from a Wenzhou-based hospital.

Activation of *de novo* lipogenesis may result in TC tumorigenesis through fatty acid synthase (FASN) catalyzing

Table II. The percentage and the ratio of fatty acids and different kinds of fatty acids in female are compared among three diseases.

	Fatty acid level, median (Q1, Q3)			
Fatty acid	TC (n=78)	TA (n=9)	NG (n=8)	P-value
PUFA	47 (45,49)	50 (49,51)	46 (45,48)	0.01
n-3	6.2 (5.2,6.9)	7.4 (6.5,8.1)	6.0 (4.6,7.9)	0.08
18:3n-3	0.71 (0.59,1.0)	0.53 (0.49,0.65)	0.61 (0.49,0.84)	0.13
20:5n-3	0.49 (0.4,0.61)	0.57 (0.52,0.61)	0.51 (0.45,0.55)	0.24
22:5n-3	0.54 (0.48,0.67)	0.6 (0.53,0.66)	0.64 (0.45,0.86)	0.66
22:6n-3	4.3 (3.3,5.1)	5.7 (4.5,6.3)	3.7 (3.0,6.0)	0.08
n-6	41 (39,43)	43 (42,44)	41 (37,42)	0.15
18:2n-6	32 (29,34)	33 (31,35)	30 (27,31)	0.05
18:3n-6	0.2 (0.12,0.29)	0.16 (0.13,0.23)	0.28 (0.11,0.4)	0.70
20:2n-6	0.29 (0.26,0.33)	0.27 (0.25,0.31)	0.32 (0.3,0.34)	0.18
20:3n-6	1.1 (0.87,1.5)	1.2 (0.91,1.4)	1.2 (1.1,1.6)	0.38
20:4n-6	7.3 (6.3,8.8)	7.8 (7.6,8.3)	9.1 (8.1,9.9)	0.04
22:4n-6	0.17 (0.14,0.21)	0.16 (0.14,0.18)	0.22 (0.18,0.28)	0.02
22:5n-6	0.18 (0.13,0.25)	0.2 (0.14,0.31)	0.24 (0.22,0.56)	0.06
n-6:n-3	6.7 (5.7,8.1)	5.8 (5.2,6.6)	7.2 (4.6,9.3)	0.30

All variables were compared with the Kruskal-Wallis test. The data are presented as the median with the lower quartile (Q1) and the upper quartile (Q3). P<0.05 was considered to indicate a statistically significant difference. TC, thyroid cancer; TA, thyroid adenoma; NG, nodular goiter.

Table III. Percentage and ratio of fatty acids compared among three groups of patients with five types of normal level thyroid-related hormone.

	Fatty acid level, median (Q1, Q3)			
Fatty acid	TC (N=56)	TA (N=8)	NG (N=6)	P-value
PUFA	46 (44,49)	50 (49,51)	46 (45,48)	0.03
n-3	5.9 (4.9,6.7)	7.4 (6.8,8.0)	5.0 (4.5,6.0)	0.02
18:3n-3	0.68 (0.58,1.1)	0.53 (0.51,0.7)	0.52 (0.43,0.63)	0.12
20:5n-3	0.51 (0.4,0.61)	0.57 (0.54,0.61)	0.53 (0.51,0.55)	0.24
22:5n-3	0.54 (0.43,0.68)	0.6 (0.59,0.66)	0.57 (0.47,0.68)	0.65
22:6n-3	4.2 (3.1,4.8)	5.7 (5.0,6.3)	3.4 (3.0,3.7)	0.04
n-6	40 (38,43)	43 (42,43)	41 (39,43)	0.21
18:2n-6	31 (28,33)	33 (31,34)	30 (29,31)	0.20
18:3n-6	0.2 (0.12,0.29)	0.15 (0.13,0.22)	0.12 (0.11,0.32)	0.64
20:2n-6	0.3 (0.25,0.33)	0.27 (0.25,0.31)	0.33 (0.27,0.35)	0.24
20:3n-6	1.1 (0.87,1.6)	1 (0.86,1.3)	1.3 (1.2,1.4)	0.50
20:4n-6	7.2 (6.3,8.3)	7.8 (7.5,8.7)	8.4 (7.7,9.1)	0.15
22:4n-6	0.18 (0.14,0.22)	0.15 (0.13,0.17)	0.22 (0.21,0.26)	0.03
22:5n-6	0.2 (0.14,0.26)	0.16 (0.14,0.23)	0.26 (0.22,0.31)	0.06
n-6:n-3	7.0 (5.7,8.6)	5.8 (5.4,6.2)	8.1 (7.2,9.3)	0.11

The hormones Thy, T3, TSH, FT4, and FT3 of all 70 cases are normal. The variables were tested with the Kruskal-Wallis test. The data are presented as the median with the lower quartile (Q1) and the upper quartile (Q3). P<0.05 was considered to indicate a statistically significant difference.

the synthesis of 16-carbon saturated fatty acids and AKT pathway signaling (31). However, consistent with the results of previous studies (18,21,23), the present study identified that the ω-3 and ω-6 fatty acids with chains longer than 16 carbons

Table IV. The 75% threshold value for total ω-3 fatty acids was 6.550 (75th percentile), which was used to distinguish TA from TC and NG.

	Batch 1	Batch 2
Disease (%)	(Proportion ≥Q3)	(Proportion ≥Q3)
TC	14/67=21	17/30=57
TA	6/9=67	2/2=100
NG	2/7=29	1/3=33

TC, thyroid cancer; TA, thyroid adenoma; NG, nodular goiter.

Table V. The 75% threshold value for 22:4n-6 was 0.209 (75th percentile), which was used to distinguish NG from TC and TA.

	Batch 1	Batch 2
Disease (%)	(Proportion ≥Q3)	(Proportion ≥Q3)
TC	17/67=25	12/30=40
TA	0/9=0	0/2=0
NG	4/7=57	2/3=67

TC, thyroid cancer; TA, thyroid adenoma; NG, nodular goiter.

were not increased in patients with cancer. We hypothesize that this is due to long chain ω-3 and ω-6 fatty acids being primarily from diet, and produced through other enzymatic reactions.

ω-3 and ω-6 fatty acids, including 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3 and 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6, are involved in two different metabolic pathways in KEGG. It is reported that ω-3 fatty acids are protective in the majority of diseases, while ω-6 fatty acids do not exhibit this activity type (32). The present study identified that ω-3 and ω-6 fatty acid profiles were exhibited differently among three diseases: ω-3 fatty acids, including 20:5n-3, 22:5n-3, and 22:6n-3, were highest in the TA group; and ω-6 fatty acids, including 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, and 22:5n-6, were highest in the NG group. We hypothesize that ω-3 and ω-6 fatty acids may have different metabolic pathways in TA and NG compared with TC, which may result in the varying etiological characteristics of those diseases.

The incidence of nodules generally increases with age (33). The present study corroborated this, as NG patients of the study sample were older than in the other two groups. TGA and TPOA are related to autoimmune thyroid disease and thyroiditis (34,35). TSH level, which is a risk factor for TC (3,36), was highest in the TC group followed by the TA group in our study.

Total PUFA levels were significantly increased in the TA group compared with the TC and NG groups in the present study. Berg *et al* (18) reported that the sum of arachidonic acid (20:4n-6) and docosahexaenoic acid (DHA) concentrations and the sum of arachidonic acid, EPA, and DHA were significant decreased in patients with cancer compared with healthy controls. Guo *et al* (23) reported C20:4, C22:4 and C22:5 were significantly decreased in six types of cancer tissues (breast, lung, colorectal, esophageal and gastric cancer and TC); however, C22:4 and C22:5 were increased in TC tissue compared with normal tissue. Furthermore, urinary PUFAs are increased in TC compared with healthy controls (21). To date, the results of previous studies on total PUFA in cancer have been inconclusive, suggesting that total PUFA may not serve as an appropriate indicator for thyroid disease assessment.

The present study demonstrated that DHA and total ω-3 fatty acids were significantly increased in TA compared with TC and NG. If confirmed through further study, this exhibits potential to aid in distinguishing TA from other thyroid diseases. Although statistically significant differences were not identified for other ω-3 fatty acids, they exhibited similar trends. Therefore a study with a larger cohort would be required to examine this. Consistent with our results, Xu *et al* (37) reported that DHA levels were increased in TA compared with TC tissue. Yao *et al* (20) reported serum DHA levels were decreased in PTC and NG compared with healthy controls; however, they did not identify a difference between PTC and NG. Zhang *et al* (19) reported that the serum DHA and 18:3n-3 were significantly increased in TC patients compared with those with benign thyroid diseases, and the difference may be due to the different types of fatty acids. Zhang *et al* (19) measured the free fatty acids (FFAs) while Yao *et al* (20) measured the total fatty acids, including FFA and esterified fatty acids. Furthermore, Zhang *et al* (19) used concentration (μM), whereas the present study merely obtained percentages of fatty acids, which may be responsible for the differences observed.

In the present study, 22:4n-6 was significantly decreased in the TC and TA groups compared with NG group; a number of other ω-6 fatty acids, including 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, and 22:5n-6, exhibited similar, though statistically insignificant trends. ω-6 fatty acids also can be helpful to differentiate NG from other thyroid diseases. In a study by Kim *et al* (21), the urinary concentrations of ω-6 fatty acids, including 18:2n-6 and 20:4n-6, were decreased in TC compared with healthy controls. However, Yao *et al* (20) reported that serum 18:2n-6, 20:4n-6 and 22:4n-6 were increased in PTC compared with NG. Furthermore, Zhang *et al* (19) reported the serum 20:4n-6 was significantly increased in TC patients compared with those with benign thyroid diseases. However, the differences may be related to the types of diseases examined and the units used.

When the analysis was restricted to female subjects, the DHA and total ω-3 fatty acids did not demonstrate a significant difference. This may be due to the smaller sample size. The 20:4n-6 was significantly decreased in the TC and TA groups compared with the NG group in female subjects, which may suggest that more 20:4n-6 would be transferred into the downstream product. This is due to arachidonic acid being the substrate of prostaglandin E2, which may inhibit the anti-tumor reaction of the immune system (38). Therefore, 20:4n-6 may be associated with a high incidence of TC in female subjects.

To the best of our knowledge, the present study is amongst the first to explore the plasma fatty acid profiles among three common thyroid diseases. TA was identified to be different from TC and NG in ω-3 fatty acids, while NG was different from TC and TA in ω-6 fatty acids. FNA is the most common tool for thyroid disease diagnosis; however, its sampling accuracy is limited. While the mutational analyses of BRAF, RAS, RET/PTC, and PAX8-PPARγ rearrangement contribute to distinguishing follicular lesions (39,40), the tissue damage resulting from surgery is not negligible. Furthermore, this method also has uncertainty due to the dependency of accurate sampling on the equipment and the operator. However, if validated, fatty acids have the potential to serve as diagnostic biomarkers, which may facilitate the diagnosis of thyroid diseases.

The present study is not without limitations. It had a small sample size, which limited the ability to detect moderate differences. Second, fatty acids in the plasma only reflect short-term dietary exposure, and the absence of analysis of PUFAs in a healthy population is a limitation. However, the present study focused on comparing three thyroid diseases, as opposed to a case-control study with healthy controls, in order to provide insight on the etiological evolution of the diseases. This could aid in the understanding of whether NG or TA are precancerous forms of TC from the aspect of fatty acid metabolism. The results obtained may also provide data for the prevention/treatment of TC; however, further studies with somatic tissues are required to investigate the long-term effects of the exposure to ω-3 and ω-6 PUFAs.

In summary, the present study demonstrated the differences in clinical parameters and plasma ω-3 and ω-6 PUFA profiles among three different common thyroid diseases, namely NG, TA and TC. The results of the present study suggest that ω-3 fatty acids, especially 22:6n-3, are advantageous for distinguishing TA from NG and TC; and ω-6 fatty acids, especially 22:4n-6, are effective for distinguishing NG from TA and TC. However, further study is required with a larger cohort and prospective design, including used of somatic tissues.

Acknowledgements

Not applicable.

Funding

This research was supported by funds from the Key Laboratory of Nutrition and Metabolism (awarded to OW and YG) and the 100 Talented Plan of Chinese Academy of Sciences (awarded to YG).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YG and FC designed the current study. OW, LJ, and FY obtained biological samples. XL, JZ, QD and CH performed the experiments. XL and HL analyzed the data. All authors were involved in the interpretation of the results. XL, FC and YG wrote the manuscript. All authors read, gave comments, and approved the final version of the manuscript. All authors had taken responsibility for the integrity of the data and the accuracy of the data analysis.

Ethics approval and consent to participate

Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical University approved the study and informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Davies L, Ouellette M, Hunter M and Welch HG: The increasing incidence of small thyroid cancers: Where are the cases coming from? Laryngoscope 120: 2446-2451, 2010.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 65: 87-108, 2015.
3. Schneider DF and Chen H: New developments in the diagnosis and treatment of thyroid cancer. CA Cancer J Clin 63: 374-394, 2013.
4. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J: Cancer statistics in China, 2015. CA Cancer J Clin 66: 115-132, 2016.
5. Burman KD and Wartofsky L: Clinical Practice. Thyroid nodules. N Engl J Med 373: 2347-2356, 2015.
6. Hedinger C, Williams ED and Sobin LH: The WHO histological classification of thyroid tumors: A commentary on the second edition. Cancer 63: 908-911, 1989.
7. La Vecchia C, Malvezzi M, Bosetti C, Garavello W, Bertuccio P, Levi F and Negri E: Thyroid cancer mortality and incidence: A global overview. Int J Cancer 136: 2187-2195, 2015.
8. Xing M: Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer 13: 184-199, 2013.
9. American Thyroid Association Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, *et al*: Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 19: 1167-1214, 2009.
10. Crippa S, Mazzucchelli L, Cibas ES and Ali SZ: The Bethesda System for reporting thyroid fine-needle aspiration specimens. Am J Clin Pathol 134: 343-345, 2010.
11. Zablotska LB, Nadyrov EA, Polyanskaya ON, McConnell RJ, O'Kane P, Lubin J, Hatch M, Little MP, Brenner AV, Veyalkin IV, *et al*: Risk of thyroid follicular adenoma among children and adolescents in Belarus exposed to iodine-131 after the Chornobyl accident. Am J Epidemiol 182: 781-790, 2015.
12. Dutta-Roy AK: Cellular uptake of long-chain fatty acids: Role of membrane-associated fatty-acid-binding/transport proteins. Cell Mol Life Sci 57: 1360-1372, 2000.
13. Nickerson JG, Alkhateeb H, Benton CR, Lally J, Nickerson J, Han XX, Wilson MH, Jain SS, Snook LA, Glatz JF, *et al*: Greater transport efficiencies of the membrane fatty acid transporters FAT/CD36 and FATP4 compared with FABPpm and FATP1 and differential effects on fatty acid esterification and oxidation in rat skeletal muscle. J Biol Chem 284: 16522-16530, 2009.
14. Santos CR and Schulze A: Lipid metabolism in cancer. FEBS J 279: 2610-2623, 2012.

15. de Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, Kishibe T, Uleryk E, Budylowski P, Schünemann H, Beyene J and Anand SS: Intake of saturated and trans unsaturated fatty acids and risk of all-cause mortality, cardiovascular disease, and type 2 diabetes: Systematic review and meta-analysis of observational studies. BMJ 351: h3978, 2015.
16. Lagrou A, Dierick W, Christophe A and Verdonk G: Lipid composition of normal and hypertrophic bovine thyroids. Lipids 9: 870-875, 1974.
17. Butte NF, Puyau MR, Vohra FA, Adolph AL, Mehta NR and Zakeri I: Body size, body composition, and metabolic profile explain higher energy expenditure in overweight children. J Nutr 137: 2660-2667, 2007.
18. Berg JP, Glattre E, Haldorsen T, Høstmark AT, Bay IG, Johansen AF and Jellum E: Longchain serum fatty acids and risk of thyroid cancer: A population-based case-control study in Norway. Cancer Causes Control 5: 433-439, 1994.
19. Zhang Y, Qiu L, He C, Wang Y, Liu Y, Zhang D and Li Z: Serum unsaturated free fatty acids: A potential biomarker panel for differentiating benign thyroid diseases from thyroid cancer. J Cancer 6: 1276-1281, 2015.
20. Yao Z, Yin P, Su D, Peng Z, Zhou L, Ma L, Guo W, Ma L, Xu G, Shi J and Jiao B: Serum metabolic profiling and features of papillary thyroid carcinoma and nodular goiter. Mol Biosyst 7: 2608-2614, 2011.
21. Kim KM, Jung BH, Lho DS, Chung WY, Paeng KJ and Chung BC: Alteration of urinary profiles of endogenous steroids and polyunsaturated fatty acids in thyroid cancer. Cancer Lett 202: 173-179, 2003.
22. Leng J, Guan Q, Sun T, Wu Y, Cao Y and Guo Y: Application of isotope-based carboxy group derivatization in LC-MS/MS analysis of tissue free-fatty acids for thyroid carcinoma. J Pharm Biomed Anal 84: 256-262, 2013.
23. Guo S, Wang Y, Zhou D and Li Z: Significantly increased monounsaturated lipids relative to polyunsaturated lipids in six types of cancer microenvironment are observed by mass spectrometry imaging. Sci Rep 4: 5959, 2014.
24. Chinese Society of Endocrinology, Chinese Medical Association: China Thyroid Disease Diagnosis and Treatment Guidelines. Zhonghua Nei Ke Za Zhi 47: 867-868, 2008 (In Chinese).
25. Davies L and Welch HG: Current thyroid cancer trends in the United States. JAMA Otolaryngol Head Neck Surg 140: 317-322, 2014.
26. Hoang JK, Nguyen XV and Davies L: Overdiagnosis of thyroid cancer: Answers to five key questions. Acad Radiol 22: 1024-1029, 2015.
27. Kolonel LN, Hankin JH, Wilkens LR, Fukunaga FH and Hinds MW: An epidemiologic study of thyroid cancer in Hawaii. Cancer Causes Control 1: 223-234, 1990.
28. Glattre E, Haldorsen T, Berg JP, Stensvold I and Solvoll K: Norwegian case-control study testing the hypothesis that seafood increases the risk of thyroid cancer. Cancer Causes Control 4: 11-16, 1993.
29. Horn-Ross PL, Morris JS, Lee M, West DW, Whittemore AS, McDougall IR, Nowels K, Stewart SL, Spate VL, Shiau AC and Krone MR: Iodine and thyroid cancer risk among women in a multiethnic population: the Bay Area Thyroid Cancer Study. Cancer Epidemiol Biomarkers Prev 10: 979-985, 2001.
30. Wang Yilong: Study of Wenzhou food culture. Zhejiang Ocean University, 2014 (In Chinese).
31. Uddin S, Siraj AK, Al-Rasheed M, Ahmed M, Bu R, Myers JN, Al-Nuaim A, Al-Sobhi S, Al-Dayel F, Bavi P, *et al*: Fatty acid synthase and AKT pathway signaling in a subset of papillary thyroid cancers. J Clin Endocrinol Metab 93: 4088-4097, 2008.
32. Calder PC: Polyunsaturated fatty acids, inflammation, and immunity. Lipids 36: 1007-1024, 2001.
33. Hegedus L: Clinical practice. The thyroid nodule. N Engl J Med 351: 1764-1771, 2004.
34. Bjoro T, Holmen J, Kruger O, Midthjell K, Hunstad K, Schreiner T, Sandnes L and Brochmann H: Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trondelag (HUNT). Eur J Endocrinol 143: 639-647, 2000.
35. Prummel MF and Wiersinga WM: Thyroid peroxidase autoantibodies in euthyroid subjects. Best Pract Res Clin Endocrinol Metab 19: 1-15, 2005.
36. Fiore E and Vitti P: Serum TSH and risk of papillary thyroid cancer in nodular thyroid disease. J Clin Endocrinol Metab 97: 1134-1145, 2012.
37. Xu Y, Zheng X, Qiu Y, Jia W, Wang J and Yin S: Distinct metabolomic profiles of papillary thyroid carcinoma and benign thyroid adenoma. J Proteome Res 14: 3315-3321, 2015.
38. Chen JH, Perry CJ, Tsui YC, Staron MM, Parish IA, Dominguez CX, Rosenberg DW and Kaech SM: Prostaglandin E2 and programmed cell death 1 signaling coordinately impair CTL function and survival during chronic viral infection. Nat Med 21: 327-334, 2015.
39. Nikiforova MN, Lynch RA, Biddinger PW, Alexander EK, Dorn GW III, Tallini G, Kroll TG and Nikiforov YE: RAS point mutations and PAX8-PPAR gamma rearrangement in thyroid tumors: Evidence for distinct molecular pathways in thyroid follicular carcinoma. J Clin Endocrinol Metab 88: 2318-2326, 2003.
40. Fugazzola L, Puxeddu E, Avenia N, Romei C, Cirello V, Cavaliere A, Faviana P, Mannavola D, Moretti S, Rossi S, *et al*: Correlation between B-RAFV600E mutation and clinico-pathologic parameters in papillary thyroid carcinoma: Data from a multicentric Italian study and review of the literature. Endocr Relat Cancer 13: 455-464, 2006.