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Age-related distributions of nine fasting plasma free fatty acids in a population of Chinese adults

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ABSTRACT

Background: Free fatty acids (FFAs) play important roles in health and disease. We investigated the distributions of nine plasma FFAs in a population of Chinese adults.
Method: Three hundred and ninety-nine healthy individuals aged 18–104 years were divided into 4 groups: 18–39 years; 40–59 years; 60–79 years; and 80–104 years. Nine plasma FFAs, including C12:0, C14:0, C16:0, C16:1, C18:0, C18:1 C18:2, C20:4 and C20:5 were determined using a validated HPLC method.
Results: There were significant differences among the 4 age groups in the plasma total FFA (TFA), saturated fatty acid (SFA), unsaturated fatty acid (UFA), and eight specific FFA concentrations, and the ratios of SFA to UFA, SFA to TFA, and UFA to TFA as well (all $P<0.05$), except for FFA C16:1. However, no significant difference was found between males and females. The 4 most abundant FFAs, C16:0, C18:0, C18:1 and C18:2 account for >90% of plasma total FFA. Reference intervals for individual FFAs are set at the 10th–90th percentile.
Conclusions: Significant differences in eight specific plasma FFAs among various age groups were found in a population of Chinese adults. C16:0, C18:0, C18:1 and C18:2 are the most abundant FFAs in the fasting plasma. Reference intervals are established for the local Chinese community.

1. Introduction

Free fatty acids (FFAs) play important roles in providing energy for tissues, particularly in fasting conditions [1,2]. Numerous studies have been performed on FFA metabolism in human and animal models. Relationships between FFAs and glucose and lipid metabolism were firstly demonstrated by Randle et al. [3]. Increasing evidence suggests that elevated FFA concentrations are related to insulin resistance, and individuals with insulin resistance are at higher risk for disorders in lipoprotein metabolism, cardiovascular diseases, type 2 diabetes, central obesity, and primary hypertriglyceridemia [2].

Plasma FFAs play critical regulatory roles in the lipolytic activity by modulating the secretion of hormones, such as insulin, catecholamine, and growth hormone. The higher levels of FFAs not only enhance glucose-stimulated insulin secretion [4], but also suppress growth hormone secretion [5], which can feed back to reduce lipolysis. On the other hand, FFAs may themselves directly inhibit adipose tissue fatty acid release [6]. Thus, normal plasma FFA concentration is vital for human health. Different types of FFAs exert various effects on the development of insulin resistance, type 2 diabetes and atherosclerosis [2,7,8]. Saturated long-chain fatty acids (C16 or greater) exert a much more powerful early toxicity than shorter chain saturated FFAs [9,10], palmitic acid (C16:0) and stearic acid (C18:0) were demonstrated to be potent inducers of insulin resistance [2,7]; Polyunsaturated FFAs, particularly n-3 fatty acids such as eicosapentaenoic acid (C20:5) and docosahexanoic acid (C22:5), have been shown to have beneficial effects on inflammation [11], cancer [12,13], atherosclerosis in patients with dyslipoproteinaemia, insulin resistance and type 2 diabetes; and C20:5 could improve retina, brain and nerves system development in infants [10]. Certain monounsaturated FFAs, such as palmitoleic acid (C16:1) and oleic acid (C18:1), actively promote cell proliferation and viability [14–16]. Natural C18:1 and linoleic acid (C18:2) affect total serum cholesterol equally [15–17]. Furthermore, elevated C18:2 FFA level could promote platelet aggregation and thrombus formation, and be positively related to inflammation that expedites the growth and transformation of tumor cells [18]. Therefore, it is more significant to analyze specific FFAs than the total FFA level because specific FFA abnormalities may contribute to disease despite normal total FFA level.

Plasma FFA is a very useful companion test for evaluation of the metabolic status of individuals with endocrinopathies; detection

Abbreviations: FFA, free fatty acid; UFA, unsaturated fatty acid; SFA, saturated fatty acid; FPG, fasting plasma glucose.

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Table 1
Clinical characteristics of subjects.

	18–39 years	40–59 years	60–79 years	80–104 years	18–104 years
n (male/female)	108 (45/63)	142 (83/59)	55 (31/24)	94 (41/53)	399 (200/199)
Age (y)	29.9±6.6	47.8±5.8	70.6±5.7	85.5±4.2	54.7±21.5
BMI (kg/m^2)	21.2±3.2	22.7±2.7	22.6±2.9	21.2±3.3	21.9±3.1
FPG (mmol/l)	5.3±0.4	5.3±0.4	5.4±0.6	4.8±0.7*	5.2±0.6
HDL-C (mmol/l)	1.2±0.2	1.3±0.3	1.4±0.3	1.5±0.3#	1.3±0.3
LDL-C (mmol/l)	2.3±0.5	2.4±0.5	2.4±0.6	2.0±0.5*	2.3±0.6
TC (mmol/l)	4.1±0.6▲	4.5±0.7	4.6±0.7	4.7±0.9	4.4±0.7
TG (mmol/l)	1.0±0.3	1.1±0.4	1.1±0.3	1.0±0.3	1.0±0.3

* 80–104 years age group vs. the other 3 groups, $P<0.05$.
\# 80–104 years age group vs. 18–39 years age group, $P<0.05$.
▲ 18–39 years age group vs. the other 3 groups, $P<0.05$.

of pheochromocytoma and of glucagon-, thyrotropin-, and adrenocorticotropin-secreting tumors; as well as in the assessment of glycemic control in diabetes mellitus.

Recent investigations on plasma FFA concentrations have focused on the detection of total FFA, and most of them have adopted enzymatic colorimetric methods [19,20]. Although the enzymatic procedure is much simpler and less time consuming, but it fails to provide information about the content of individual fatty acids. Several studies involved in the measurement of specific FFAs using high performance liquid chromatography (HPLC) were carried out with relatively small sample sizes (n<43) or FFAs that are different from those analyzed in this study [21–24] and, to the best of our knowledge, none in different age groups with representative samples in the Chinese populations. In the present study, we measured nine plasma FFAs, including lauric acid (C12:0), myristate acid (C14:0), C16:0, C16:1, C18:0, C18:1, C18:2, arachidonic acid (C20:4) and C20:5, in 399 Chinese healthy adults aged 18–104 years using HPLC method with margaric acid (C17:0) as the internal standard, and in an attempt to establish the reference intervals of plasma FFAs for the local Chinese community.

2. Research design and methods

2.1. Subjects

A total of 399 healthy Chinese adults (54.7±21.5 years; male:female, 200:199) attending the physical examinations in Zhongnan Hospital of Wuhan University participated in the study. The subjects were divided into 4 groups according to their age: 18–39 years (n=108); 40–59 years (n=142); 60–79 years (n=55); and 80–104 years (n=94). Informed consent was obtained from each participant, and the study was approved by the ethics committee of Zhongnan Hospital, Wuhan University, China. Only subjects with no history of coronary heart disease, hypertension, diabetes and other lipid-related diseases were recruited; and their liver, kidney and thyroid function tests were within the normal range. All participants were asked to maintain their usual diet and stop fish-oil supplement for at least 1 week prior to their assessment.

Table 2
Standard curves, RSD and LOD of different fatty acids.

FFA	Calibration equation	Regression coefficient (R^2)	Range of detection (μmol/l)	CV (%)		LOD (μmol/l)
				Inter-day	Intra-day	
C12:0	y=0.4741 X+0.0033	0.9986	0.10–6.40	4.4	3.5	0.08
C14:0	y=0.9045 X+0.0004	0.9996	0.85–54.44	2.5	1.9	0.32
C16:0	y=0.8319 X−0.0619	0.9990	3.81–243.83	1.5	0.9	0.35
C18:0	y=0.9427 X+0.0105	0.9985	0.87–44.01	2.8	1.7	0.02
C16:1	y=5.9485 X+0.0672	0.9993	0.76–49.11	3.1	2.6	0.33
C18:1	y=0.8427 X+0.0920	0.9992	3.46–221.21	2.2	1.4	0.30
C18:2	y=0.7750 X+0.1040	0.9993	3.48–222.72	1.9	1.9	0.30
C20:4	y=0.5561 X+0.0072	0.9987	0.13–8.23	2.9	2.8	0.36
C20:5	y=0.6540 X+0.0023	0.9994	0.12–8.25	3.2	2.9	0.05

X: the content of fatty acids; Y: relative peak area = peak area of fatty acid / peak area of internal standard.

Table 3
Accuracy and precision of different FFA concentrations(n=8).

FFA	Concentration (μmol/l)	HPLC method (μmol/l)	Accuracy (% of expected value)	Precision (C.V., %)
C12:0	5.00	5.11	102.2	4.4
	1.25	1.22	97.6	1.9
	0.325	0.34	104.8	3.4
C14:0	43.78	42.95	98.1	2.5
	10.95	10.86	99.2	2.2
	2.74	2.84	103.6	1.5
C16:0	195.00	188.18	96.5	4.5
	48.75	48.26	99.0	4.0
	12.19	12.10	99.3	3.7
C18:0	35.20	36.36	103.3	1.6
	8.80	8.62	98.0	3.3
	2.20	2.26	102.7	3.1
C16:1	39.31	40.96	104.2	1.6
	9.83	9.61	97.8	2.1
	2.46	2.52	102.4	2.8
C18:1	177.02	183.22	103.5	3.9
	44.26	44.75	101.1	1.8
	11.06	10.86	98.2	2.6
C18:2	178.29	174.37	97.8	3.8
	44.57	44.17	99.1	2.0
	11.14	10.98	98.6	2.2
C20:4	6.57	6.73	102.4	2.3
	1.64	1.61	98.2	3.2
	0.41	0.40	97.6	4.4
C20:5	6.61	6.80	102.9	2.4
	1.65	1.59	96.4	2.7
	0.41	0.42	102.4	3.9

2.2. Specimens

An amount of 4 ml EDTA blood was obtained from each subject after an overnight fast. Fresh plasma was stored at −80 °C immediately after separated from the whole blood until assay without repeated freeze–thawing cycles. FFA concentrations were measured using HPLC method within 7 days.

2.3. Baseline subject characteristics

In all subjects, height and weight were measured, and body mass index (BMI) was calculated as weight (kg)/height (m^2). Fasting plasma glucose (FPG), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were assayed by using an automated chemistry analyzer (Abbott-AEROSET, Abbott Diagnostics, Abbott Park, IL).

2.4. Extraction and derivatization of plasma FFAs

The extraction and derivatization procedures were modified from the method described by Mehta et al. [22]. Margaric acid (C17:0) was used as an internal standard in the extraction step. For derivatization, 50 μl each of α-bromoacetophenone (20 mg/ml) and triethylamine (25 mg/ml) were added to the dried extract. The vials were incubated