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Relationship Between Angiopoietin-Like Protein 8 and Fasting Serum Triglyceride Level

Hodaka Yamada[a, c], Ikuyo Kusaka[a], Rika Saikawa[a], Kazuo Hara[a],
Masafumi Kakei[a], San-e Ishikawa[b]

Abstract

Background: The aim of the study was to evaluate the correlation between angiopoietin-like protein 8 (ANGPTL8) and metabolic parameters in non-diabetic healthy humans.

Methods: We enrolled 30 healthy Japanese adults (25 men and five women). After 9 h of fasting, we collected blood samples and analyzed the ANGPTL8, lipoprotein lipase (LPL), plasma lipid and glucose metabolic parameters. In addition, we performed 75-g oral glucose tolerance test (OGTT) and measured adipokines (tumor necrosis factor-α, leptin and adiponectin).

Results: Median serum ANGPTL8 level was 224 (167 - 437) pg/mL, and serum ANGPTL8 level positively correlated with serum triglyceride level (r = 0.42, P = 0.021) and negatively correlated with LPL level (r = -0.44, P = 0.015). ANGPTL8 level showed no correlation with body mass index (BMI), waist-hip ratio, and homeostasis model assessment of insulin resistance (HOMA-IR) or with adipose tissue-derived adiponectin and leptin levels. Further, ANGPTL8 showed no association with glucose and insulin levels after 75-g OGTT.

Conclusion: Serum ANGPTL8 level negatively correlated with LPL levels in healthy Japanese adults. Regulation of ANGPTL8 could be a promising therapeutic target for hypertriglyceridemia.

Keywords: Angiopoietin-like protein 8; Hypertriglyceridemia; Lipoprotein lipase; Insulin resistance

Introduction

Angiopoietin-like protein 8 (ANGPTL8) is a lipid metabolic

[a]First Department of Comprehensive Medicine, Division of Endocrinology and Metabolism, Jichi Medical University Saitama Medical Center, 1-847 Amanuma-cho, Omiya-ku, Saitama 330-8503, Japan
[b]Division of Endocrinology and Metabolism, International University of Health and Welfare Hospital, 537-3 Iguchi, Nasushiobara, Tochigi 329-2763, Japan
[c]Corresponding Author: Hodaka Yamada, Department of Medicine, Jichi Medical University Saitama Medical Center, 1-847 Amanuma-cho, Omiya-ku, Saitama 330-8503, Japan. Email: hyamada0510@jichi.ac.jp

regulator derived from adipose tissues and liver [1]. ANGPTL8 (as betatrophin) is believed to control beta cell proliferation [2]. However, this finding could not be confirmed in subsequent replication studies [3, 4]. Phenotypical *Angptl8⁻/⁻* mice exhibit low serum triglyceride (TG) levels [5], and it is thought that ANGPTL8/betatrophin is a crucial factor for TG metabolism [6]. Zhang et al originally revealed that male mice that overexpressed ANGPTL8 (with the use of an adenovirus vector) exhibited hypertriglyceridemia compared with controls overexpressing green fluorescent protein. In addition, they identified that recombinant ANGPTL8 inhibited lipoprotein lipase (LPL) activity [7]. In both mice and humans, some studies (including our previous report) showed that serum ANGPTL8 is elevated in individuals with diabetes and was positively correlated with TG levels [8, 9]. However, the correlation between ANGPTL8 and metabolic parameters (i.e., adipokines and LPL) in non-diabetic healthy humans is unknown.

Methods

We enrolled 30 healthy Japanese adult volunteers (25 men and five women) who were not under any medications and had no significant past medical history. After 9 h of fasting, blood samples were collected into tubes from all subjects. The blood was centrifuged at 3,000 rpm and 4 °C for 15 min to obtain serum, which was frozen at -80 °C until the time of assay.

We analyzed the following metabolic parameters and serum adipokine concentrations using commercially available ELISA kits: ANGPTL8 ELISA kit (Catalog No. E11644h; Wuhan Eiaab Science, Wuhan, China), LPL (Sekisui Medical Co. Ltd, Tokyo, Japan), C-reactive protein (CRP; EIA kit American Research Products, Belmont, MA, USA), tumor necrosis factor-α (TNF-α; Invitrogen Corporation, CA, USA), leptin (R&D Systems, Minneapolis, USA), and adiponectin (Fujirebio Inc., Tokyo, Japan). Other parameters were measured in the central laboratory section of the Jichi Medical University Saitama Medical Center using standard techniques. We performed a 75-g oral glucose tolerance test (OGTT) during morning fasting state and measured fasting plasma glucose (FPG) and fasting immunoreactive insulin (FIRI); 60 and 120 min later, we measured plasma glucose (PG) and immunoreactive insulin (IRI) levels, respectively. The homeostasis model assessment of insulin resistance (HOMA-IR, an index of insulin resistance) and HOMA-β (an index of the capacity of insulin secretion in a fasting state) were calculated using the following equations: HOMA-IR = (FPG × FIRI)/405 and

Table 1. Basic Clinical Parameters and Correlation Analysis With ANGPTL8

Parameters	Mean ± SD or median	r	P-value
Age (years)	51 (44 - 56)	0.295	0.114
BMI (kg/m^2)	23.2 ± 2.7	0.291	0.118
Waist-hip ratio	0.87 ± 0.05	0.185	0.329
FPG (mg/dL)	97 ± 6	-0.23	0.221
FIRI (µU/mL)	8.9 (3.9 - 13.9)	0.063	0.739
PG60 (mg/dL)	121 ± 29	-0.284	0.128
IRI60 (µU/mL)	51 (29 - 67)	-0.294	0.184
PG120 (mg/dL)	108 ± 21	0.092	0.630
IRI120 (µU/mL)	39 (24 - 53)	0.095	0.617
HbA1c (%)	5.1 ± 0.2	0.009	0.963
TC (mg/dL)	194 (173 - 208)	0.144	0.448
TG (mg/dL)	94 (71 - 167)	0.42	0.021
HDL-C (mg/dL)	52 ± 10	-0.279	0.136
LPL (ng/mL)	1.65 ± 0.45	-0.438	0.015
HOMA-β	103 (47 - 133)	0.079	0.677
HOMA-IR	2.12 (0.92 - 3.46)	0.052	0.784
CRP (mg/dL)	0.04 (0.03 - 0.07)	0.154	0.416
TNF-α (pg/mL)	0.53 (0.48 - 0.72)	-0.186	0.325
Leptin (pg/mL)	26 (12 - 35)	-0.023	0.903
Adiponectin (ng/mL)	9.5 (5.6 - 16.2)	-0.127	0.503

BMI: body mass index; FPG: fasting plasma glucose; FIRI: fasting immunoreactive insulin; HbA1c: glycated hemoglobin; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LPL: lipoprotein lipase; HOMA-β: homeostasis model assessment of β; HOMA-IR: homeostasis model assessment of insulin resistance; TNF-α: tumor necrosis factor-α; CRP: C-reactive protein.

HOMA-β = (360 × FIRI)/(FPG - 63).

Data are expressed as the mean ± standard deviation (SD) or median with interquartile range. A Spearman correlation coefficient analysis was used to calculate correlations between serum ANGPTL8 and all dependent variables. All analyses were performed using EZR (Saitama Medical Center, Jichi Medical University), a graphical user interface for R (The R Foundation for Statistical Computing, ver. 2.13.0) and a modified version of the R Commander (ver. 1.6-3), which was designed to add frequently used biostatistical functions [10]. A threshold of P < 0.05 was considered significant. The study was approved by the Ethics Committee at Jichi Medical University Saitama Medical Center and performed in compliance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Results

Median participant age was 51 years and body mass index (BMI) was 23.2 ± 2.7 kg/m^2. No subject exhibited abnormal glucose tolerance at baseline or following 75-g OGTT. Other clinical characteristics of the 30 adult participants are presented in Table 1. Median serum ANGPTL8 level was 224 (167 - 437) pg/mL, and there was no difference in serum ANGPTL8 between men and women (men 237 (164 - 442), women 210 (168 - 447), P = 0.867), though only five women were included in a total of 30 participants. Serum ANGPTL8 level positively correlated with serum TG level (r = 0.42, P = 0.021) and negatively correlated with LPL level (r = -0.44, P = 0.015). ANGPTL8 level showed no correlation with BMI, waist-hip ratio, and HOMA-IR or with adipose tissue-derived adiponectin and leptin levels. In addition, ANGPTL8 showed no association with 75-g OGTT or with FPG and FIRI levels.

Discussion

We performed this study to clarify the relationship between circulating ANGPTL8 and lipid metabolic parameters, including LPL and adipokines. Our results demonstrated that fasting serum ANGPTL8 levels were closely associated with LPL in healthy adults. In an *Angptl8$^{-/-}$* mouse model, LPL activity increased and the uptake of very low-density lipoprotein-TG decreased in adipose tissue [5]. We do not assert a causal relationship between ANGPTL8 and LPL activity in this cross-sectional study. A prospective cohort study revealed that fasting ANGPTL8 level positively correlated with fasting TG levels in 240 Korean children [11]. Our results were consistent with previous animal and human studies [5, 11]. In contrast, a study reported that serum ANGPTL8 levels decreased in subjects with dyslipidemia [12] and negatively correlated with serum TG levels [13]. There is no clear evidence to explain these discrepancies. Fu et al reported that variability in ANGPTL8 measurement kits could affect results, namely the difference in N-terminus-recognizing antibodies (full-length ANGPTL8) or C-terminus-recognizing antibodies (total ANGPTL8) [14]. We used the Eiaab N-terminus-recognizing ELISA kit in the present study. Previous studies have shown a positive relationship between circulating ANGPTL8 and TG levels using ELISA kits [8, 9, 11]. An ANGPTL8 assay system is needed to clarify whether full-length ANGPTL8 or fragmented ANGPTL8 truly reflects its bioactivity [8]. In humans, one study exhibited variable associations between ANGPTL8 (single nucleotide polymorphism, variant) and the serum lipid profile [6]. Peloso et al revealed that loss-of-function mutations of ANGPTL8 had low TG levels [15]. In the future, ANGPTL8 regulation might be a novel therapeutic option for targeting hypertriglyceridemia [8].

There were limitations to the present study. We used a cross-sectional design and examined a small cohort of participants. In addition, this study included deflection of the sex of the participant. Consequently, we cannot determine if elevated ANGPTL8 level is a causative factor. Large sample size study is also necessary. We did not analyze postprandial ANGPTL8 and lipid profiles. A previous study demonstrated elevated postprandial (2 h after a meal) ANGPTL8 levels [16]. In a fasting state, ANGPTL8 inhibited LPL of white adipose tissue and muscles, resulting in hypertriglyceridemia [6]. Further study is required to determine the postprandial lipid profile, including

LPL and ANGPTL8.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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