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# Proposed cut-off value of the intrahepatic lipid content for metabolically normal persons assessed by proton magnetic resonance spectroscopy in a Japanese population

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ABSTRACT

*Aims:* To determine the threshold intrahepatic lipid (IHL) content separating metabolically normal from abnormal in a Japanese population based on proton magnetic resonance spectroscopy ($^1$H-MRS).

*Methods:* A total of 305 Japanese subjects aged 20–69 years were investigated. The subjects underwent general examination, blood tests, and $^1$H-MRS of the liver after an overnight fast. They completed a questionnaire about daily drinking habits and their daily alcohol intake was calculated.

*Results:* The median IHL content was 4.7% in men and 1.7% in women, and it increased along with the number of features of metabolic syndrome (MetS). The optimum IHL cut-off value for separating normal subjects from those with at least one feature of MetS was 6.5% in men (AUC of ROC: 0.727, 95%-CI: 0.649–0.804) and 1.8% in women (0.765, 0.685–0.844). Alcohol intake was not correlated with the IHL content according to multiple logistic regression analysis.

*Conclusion:* This study demonstrated a close association of IHL with features of MetS and identified IHL content cut-off values for metabolic normality in Japanese subjects.



## 1. Introduction

Metabolic syndrome (MetS) is a cluster of risk factors (visceral obesity, hyperglycemia, dyslipidemia, and hypertension) that is considered to promote the development of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) [1]. It has been reported that persons with MetS have a fivefold and threefold increased risk of developing T2DM and CVD, respectively [2]. In addition, Klein et al. estimated that persons with four or more features of MetS are approximately 35 times more likely to develop T2DM than persons without any features [3]. The features of MetS include visceral fat

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accumulation, hyperglycemia, dyslipidemia, and hypertension. Among them, visceral fat accumulation is fundamental to MetS and precedes the others, because excess visceral fat with enlarged adipocytes and infiltrating macrophages causes insulin resistance that induces other features of MetS by disrupting the balance of adipokines and chemokines [4,5].

Recently, excessive accumulation of intrahepatic lipid (IHL), generally known as fatty liver, has also been reported to cause insulin resistance [6–10], although it is not included among the features of MetS. In fact, some reports have suggested that the association of excess IHL with indices of insulin resistance may be stronger than that of excess visceral fat [11,12]. We previously reported that the IHL content measured by proton magnetic resonance spectroscopy ($^1$H-MRS), but not the visceral fat volume measured by whole-abdominal computed tomography (CT) scanning (700–800 slices), was significantly correlated with two indices of insulin resistance, which were Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and the Matsuda Index [13]. While the mechanism by which excess IHL causes insulin resistance has not been fully elucidated, it has been suggested that some intracellular metabolites of triglycerides, such as ceramide or diacylglycerol, may interfere with intracellular insulin signaling in the liver [14,15]. Additionally, there have been reports that the hepatokine fetuin-A, production of which is increased in fatty liver, induces insulin resistance and subclinical inflammation in mice and humans [16–18]. These previous reports have indicated that measurement of IHL is important for assessing MetS.

$^1$H-MRS focusing on segment 6 of the liver has been used as a noninvasive method for quantitative measurement of the IHL content. Recent $^1$H-MRS studies have also demonstrated that the 95th percentile of IHL is 5.6% and 3.0% in a low-risk mixed population (Hispanic, Caucasian, and African American) [19] and a lean Caucasian population [20], respectively. The median IHL content has also been reported for several ethnic groups other than Japanese [19,21,22]. However, there have been no reports about IHL values in metabolically normal persons. Accordingly, the cut-off value for a Japanese population that separates metabolically normal persons without any features of MetS from persons with at least one feature of MetS has not been determined. Thus, the aim of this study was to identify the IHL thresholds separating metabolically normal persons from metabolically abnormal persons in a Japanese population by using $^1$H-MRS for measurement of hepatic lipid.

## 2. Materials and methods

### 2.1. Subjects

A total of 318 consecutive Japanese subjects aged 20–69 years undergoing general health screening at the International University of Health and Welfare Hospital (IUHW) were recruited for this study during the period from April 2014 to March 2015. We excluded persons with a history of treatment with medications for diabetes, dyslipidemia, or hypertension, alcohol abuse (alcohol intake >60 g/day) [23], liver disease (hepatitis B or C, autoimmune hepatitis, etc.), atherosclerotic disease (stroke, coronary heart disease, or peripheral vascular disease), or chronic kidney disease, as well as pregnant or breast-feeding women. After measurement of the height, body weight, waist circumference, and blood pressure, blood tests and $^1$H-MRS of the liver were performed in the morning after an overnight fast. Drinking habits were assessed using a questionnaire (Table 1), and daily alcohol intake was calculated as follows: alcohol intake at one session (g) × frequency of drinking alcohol (days per week or month)/7 (days) or 28 (days). We divided the subjects into three groups, which were non-drinkers, light drinkers (<20 g/day for men and <10 g/day for women), and moderate drinkers (≧20 g/day for men and ≧10 g/day for women) [24]. The present study was conducted according to the principles of the Declaration of Helsinki and was approved by the institutional ethics committee of IUHW (registration number: 13-B-48). Informed consent was obtained from all of the subjects.

### 2.2. Measurements

Height and body weight were measured by an automated device (BF-220, Tanita Co. Ltd., Japan). Waist circumference was measured at the umbilicus in a standing position during quiet breathing by using a nonstretchable tape measure. In subjects with marked periumbilical fat accumulation, measurement was done at a point midway between the lower margin of the ribs and the anterior superior iliac spine. Blood pressure was measured in the sitting position by using one of two automatic devices (UA-786, A&D Co. Ltd., Japan, Kentaro HBP-9020, Omron Co. Ltd., Japan).

### 2.3. Laboratory tests

A blood sample was collected in the morning while fasting. Free fatty acids (FFA) and insulin were measured by an enzymatic method (LSI Medience Co. Ltd., Tokyo, Japan) and a chemiluminescence immunoassay (LSI Medience Co. Ltd.), respectively. Glycohemoglobin (HbA1c) was measured by the latex agglutination method, and other blood tests were performed by standard methods (SRL Co. Ltd., Tokyo, Japan). The homeostasis model assessment of insulin resistance (HOMA-R) and HOMA of β-cell function (HOMA-β) were calculated as reported previously [25].

### 2.4. Evaluation of MetS

To evaluate MetS, the following five criteria recommended for Asian populations by the Joint Scientific Statement [26] were used: (1) waist circumference (WC) ⩾90 cm in men and ⩾80 cm in women, (2) fasting serum triglyceride (TG) level ⩾150 mg/dL, (3) fasting serum HDL-cholesterol (HDL-C) level <40 mg/dL in men and <50 mg/dL in women, (4) systolic blood pressure (SBP) ⩾130 mmHg and/or diastolic blood pressure (DBP) ⩾85 mmHg, and (5) fasting plasma glucose (FPG) level ⩾100 mg/dL. Subjects with none of these criteria were defined as metabolically normal, while those with three or more of the above criteria were classified as having MetS.