厚生労働科学研究費補助金（長寿科学総合研究事業）
分担研究報告書

虚血性脳血管障害と血中レムナントリポ蛋白

分担研究者　米原敏郎　済生会熊本病院脳卒中センター　副部長

研究要旨
【目的】虚血性脳血管障害患者における血中レムナントリポ蛋白（RLP）値を明らかにするとともに、脳梗塞の臨床病型間における血中レムナントリポ蛋白値の違いの有無について検証する。【方法】2004年2月23日から2005年2月26日までの期間中、済生会熊本病院脳卒中センターに入院した虚血性脳血管障害患者42例を対象に、臨床病型、血中RLP値を登録し、全体の血中RLPの平均値を算出した後、臨床病型毎に同値を比較した。【結果】虚血性脳血管障害患者における血中RLP値（平均3.9±2.0mg/dl）は高値の傾向にあり、特にアテローム血栓性脳梗塞症で高かった。

A. 研究目的

高脂血症は脳梗塞の主要危険因子のひとつとして広く認知されている。このうち高コレステロール血症に対してはスタチン製剤の投与が有用であることが既に確立し，脳卒中においても発症予防のエビデンスが出現してきた。一方で高トリグリセライド血症に関しても、レムナントリポ蛋白（RLP）がその病態に重要な役割を有していることが明らかになりつつある。

今回は新たな動脈硬化惹起性リポ蛋白として注目されてきたこのRLPと虚血性脳血管障害の関連に焦点をあて、虚血性脳血管障害患者における血中RLP値を明らかにするとともに、脳梗塞の臨床病型間における違いの有無について検証した。

B. 研究方法

2004年2月23日から2005年2月26日までの期間中、当院の脳卒中センターに入院した虚血性脳血管障害患者42例（男性24例、女性18例、平均76.2±10.9歳）を対象とした。臨床病型、血中RLP値の他、高血圧、糖尿病、喫煙、肥満、心房細動、冠動脈疾患、末梢動脈疾患の有無について調査した。臨床病型は、臨床症候、頭部X線CT、MRI、神経超音波検査、心電図（12誘導、24時間）、心エコー（経胸壁、経食道）などにより、米国National Institute of Neurological Disorders and Strokeの脳血管疾患分類第Ⅲ版に準じ、アテローム血栓性脳梗塞、ラクナ梗塞、心原性脳塞栓症、その他の脳梗塞、一過性脳虚血発作に分類した。対象患者全体の血中RLPの平均値を算出した後、臨床病型毎に同値を比較した。統計解析には、Kruskal-Wallis検定を用いた。

（倫理面への配慮）

本研究の過程において個人名は特定されないよう配慮した。

C. 研究結果

臨床病型の内訳は、アテローム血栓性脳梗塞10例、ラクナ梗塞12例、心原性脳塞栓症14例、その他の脳梗塞2例、一過性脳虚血発作4例であった。その他の項目については、高血圧26例(62%)、糖尿病11例(26%)、喫煙16例(38%)、肥満5例(12%)、心房細動10例(24%)、冠動脈疾患3例(7%)の頻度であった。対象患者全体の血中RLPの平均値は3.9±2.0mg/dl、中央値は3.5mg/dlであった。臨床病型別の血中RLPの平均値(中央値)は各々、アテローム血栓性脳梗塞4.7±3.1(3.5)mg/dl、ラクナ梗塞3.6±1.4(3.5)mg/dl、心原性脳塞栓症3.7±1.4(3.4)mg/dl、その他の脳梗塞3.3±0.9(3.3)mg/dl、一過性脳虚血発作4.1±2.4(3.7)mg/dlであったが、統計学的に有意差を認めなかった。

D. 考察

対象患者全体の血中RLPの平均値は、高い傾向にあり、血中RLPの虚血性脳血管障害への関連性の存在の可能性がある。臨床病型別の検討で、高脂血症は脳梗塞の臨床病型の中でも動脈硬化に起因するアテローム血栓性脳梗塞の発症に強く関与していることから、血中RLP値も他の病型に比してアテローム血栓性脳梗塞で高値を示すことが予想された。正常上限値を超える高値を示した２例はいずれもアテローム血栓性脳梗塞であり、症例数の増加によりこれらの間に関連が認められてくる可能性もありうる。

E. 結論

虚血性脳血管障害患者における血中RLP値は高値の傾向にあり、特にアテローム血栓性脳梗塞症で高かった。RLPが虚血性脳血管障害の危険因子である可能性が示唆された。脳梗塞の臨床病型によっては高値の症例が存在した。今後の更なる症例の蓄積、あるいは大規模な前向き調査に期待する。

F. 健康危険情報

特記すべきことなし。

G. 研究発表

1. 論文発表
   なし。
2. 学会発表
   なし。

H. 知的財産権の出願・登録状況(予定を含む。)

1. 特許取得

なし。

2. 実用新案登録

なし。

3. その他

特記すべきことなし。

厚生労働科学研究費補助金（長寿科学総合研究事業）
分担研究報告書

ネットワークを利用したデータ収集を行う臨床試験における データ品質管理に関する考察

分担研究者　比江島欣慎　山梨大学総合分析実験センター　助教授

研究要旨
【目的】ネットワークを利用したデータ収集を伴う臨床試験におけるデータ品質管理の検討。【方法】医薬品の開発に関連する臨床試験（治験）におけるデータ管理の方法を調査・検討し，ネットワークを利用したデータ収集を行う臨床研究におけるデータの品質管理を治験のそれと比較し，問題点の抽出と解決策の導出を試みる。【結果】ネットワークを利用したデータ収集を行う臨床試験におけるデータ品質管理に関して，品質確保のための方策を複数考案した。

A. 研究目的

近年，医学研究をはじめ，新薬開発，トランスレーショナルスタディなどで多くの臨床試験が実施されている。また，いくつかの臨床試験では，複数の研究参加施設で多くの患者をフォローアップすることができるよう，ネットワークを利用したデータ収集が採用されている。こうした試験では，ネットワークにインターネットを利用するため，収集されるデータが外部に漏れないこと，外部からの改竄を受けないことなどのセキュリティー確保が重要な問題となっており，色々なIT技術を利用した対策が採られている。

一方，臨床研究において収集されるデータの品質を確保するために様々な議論が行われてきた。特に，医薬品の開発に関連する臨床試験(治験)においては医薬品の許認可に関わるため，当該データが精確に収集され分析されるように，十分すぎる運営・管理・監査体制を用意している。近年では，治験を実施する各施設にクリニカル・リサーチ・コーディネイター(CRC)を配置するなど，その体制の整備が進んでいる。

本研究では，ネットワークを利用したデータ収集を行う臨床研究におけるデータの品質管理について検討を行う。

B. 研究方法

治験におけるデータ管理の方法を調査・検討し，ネットワークを利用したデータ収集を行う臨床研究におけるデータの品質管理を，治験のそれと比較しながら，問題点の抽出と解決策の導出を試みる。

C. 研究結果

治験におけるデータ管理体制

治験では基本的に被験者と担当医師の間でデータのやりとりが行われる。通常の診療と同様にその内容はカルテに記載(入力)される。治験において必要とされる被験者からのデータは，別途ケース・レポート・フォーム(CRF)にカルテから転記するという形で，CRCによって記録される。CRFの内容がカルテと矛盾していないか確認するため適切な時期に監査が入る。CRFに記録された情報は分析のために担当者によってデジタル化される。このデジタル化の作業においても，CRFの内容が正しくデジタルされているか確認するために監査が入る。

こうした一連の業務において，データの品質を確保するために，担当医師，CRC，入力担当者，監査人の4者で責任を分担している。担当医師は日常の診療と同様にカルテに記載(入力)される情報について，CRCはCRFに記載される情報について，入力担当者はデジタル化されたデータについて，監査人はカルテ→CRF，CRF→デジタルデータにおいての正確性についての責任をそれぞれ負っている。

ネットワークを利用したデータ収集

さて，ネットワークを利用したデータ収集について考えてみよう。治験と同様に，被験者と担当医師の間でデータのやりとりが行われ，その内容はカルテに記載(入力)される。それと同時に，担当医師(もしくは入力担当者)は端末を使って研究に必要なデータを別途入力する。分析に必要なデジタルデータはこの時点で作成される。治験のそれと比べるととても簡素化されているが，その反面，治験において4者に分担されていた責任を担当医師(と入力担当者)がすべて抱えることになっている。責任の集中は業務の集中を意味しているが，日常の診察業務も並行せねばならない医師に各業務を行わせるのは，データの品質低下につながるおそれがある。収集されるデータのセキュリティー確保のためのシステム構築も重要な問題であるが，品質確保のための体制作りもそれと同じくらい重要である。

D. 考察

ネットワークを利用したデータ収集を行う臨床試験において，その品質を確保する方法としてはいくつか考えられる。1つは，デジタルデータの内容が参加施設の被験者カルテの内容と食い違ってないかを第三者によって確認・監査することである。全症例について行うのが理想であるが，難しい場合は抜き取りにて行うことになろう。実施に当たっては，参加施設に事前に

承諾をとる必要があるが，承諾がとれるかどうかが問題となるかもしれない。

別の方法としては，被験者カルテの内容を端末から入力する業務を行うCRCを各参加施設に配置し，治験とほぼ同様の体制を準備することである。この場合，臨床試験ごとにCRCを準備するとすると人件費などのコストがかかってしまうが，参加施設にこうした業務を専門に行う部門があれば，いくらかのコスト削減が可能である。治験を担当するCRC部門を利用するのも手である。

もし，電子カルテが広く普及しているなら，カルテのデータから必要な情報のみを研究データベースに転送する方法がある。参加施設の医療情報部の協力が必要となるが，もっとも精確にやりとりができる方法かもしれない。

E. 結論

ネットワークを利用したデータ収集を行う臨床試験におけるデータ品質管理に関して，品質確保のための方策を複数考案した。

F. 健康危険情報

なし。

G. 研究発表

論文発表

なし。

学会発表

1. 比江島欣慎，寺田信幸 ： ネットワークを使った臨床研究におけるデータ管理．「科学的根拠を支える生物統計学」研究会，2005，3月．

H. 知的財産権の出願・登録

1. 特許取得

なし。

2. 実用新案登録

なし。

3. その他

特記すべきことなし。

厚生労働科学研究費補助金（長寿科学総合研究事業）
分担研究報告書

生活習慣病ナレッジデータベースの構築

分担研究者　寺田信幸　山梨大学総合分析実験センター　助教授

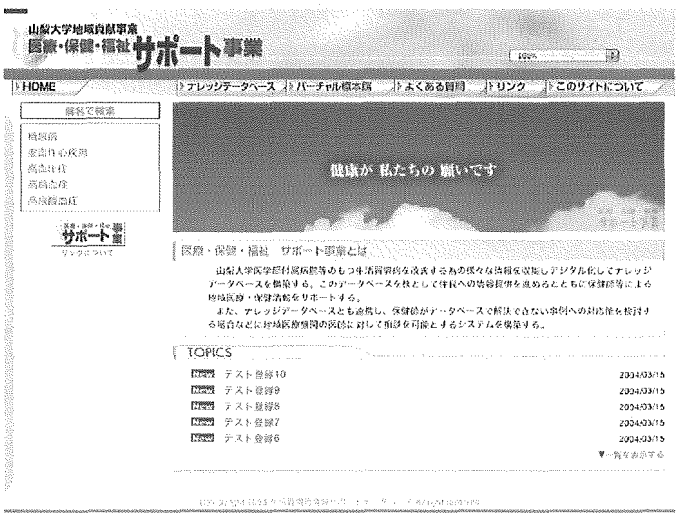
研究要旨
【目的】山梨大学医学部附属病院等のもつ生活習慣病を改善する為の様々な情報を収集、デジタル化してナレッジデータベースを構築し、広く一般に情報提供を行う。これにより患者の理解を深め、本試験研究を側面から支援する。【方法】生活習慣病データベースを構築し、その情報を自治体側の「生活習慣病ナレッジデータベース」に提供することにより、地域住民への情報サービスとしてのインターネットサービスを構築した。【結果】主に循環器疾患と糖尿病に関する情報を中心に、情報の配信を行っている。

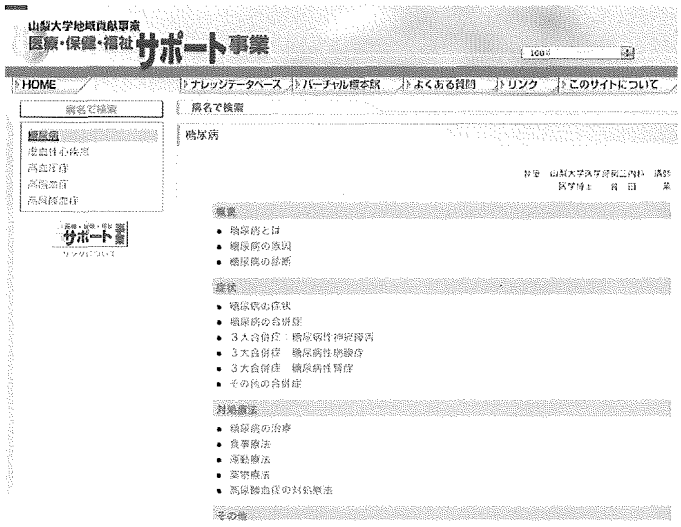
A. 研究目的

高レムナントリポ蛋白血症がIII型高脂血症などの稀な疾患のみならず虚血性心疾患例の20～30%、糖尿病例の30～50%に伴う高頻度の高脂血症であることが明らかとなっている。そこで、山梨大学医学部附属病院等のもつ生活習慣病を改善する為の様々な情報を収集、デジタル化してナレッジデータベースを構築し、広く一般に情報提供を行う。これにより患者の理解を深め、本試験研究を側面から支援する。

B. 研究方法

「医療・保健・福祉サポート事業」と連携して生活習慣病データベースを構築し、その情報を自治体側の「生活習慣病ナレッジデータベース」に提供することにより、地域住民への情報サービスとしてのインターネットサービスを構築した。内容については、主に循環器疾患と糖尿病に関する情報を中心に情報配信を行っている。





C. 研究結果および考察

平成16年2月1日より配信を行い、当初より1日平均約3000件のアクセスがあり、生活習慣病への関心が高いことが伺える。現在掲載している疾病に関してはほぼまんべんなくアクセスされている状況で、どの疾病も関心が高いといえる。また、閲覧者がYes/No形式で答えることにより簡易的に疾病の可能性を調べる健康チェックはアクセスが多く、生活習慣病の改善効果が期待できる。現在のところは糖尿病のみの配信だが今後、他疾病に関してもチェックシステムを構築し、配信していく必要がある。

また、本ホームページ内の全文検索でも収録している生活習慣病以外のキーワードでデータベースを検索しているケースが見られることから、今後、様々な疾病に対応できるよう各診療科と協力し内容の充実を図り、より一層地域住民への生活習慣病予防の啓発ができる情報内容にすることが今後の課題である。

D. 結論

生活習慣病を改善する為の様々な情報を収集、デジタル化してナレッジデータベースを構築し、広く一般に情報提供を行った。これにより患者の理解を深め、本試験研究を側面から支援する環境を構築した。

E. 健康危険情報

なし。

F. 研究発表

論文発表

なし。

学会発表

1. 比江島欣慎, 寺田信幸 ： ネットワークを使った臨床研究におけるデータ管理. 「科学的根拠を支える生物統計学」研究会, 2005, 3月.

G. 知的財産権の出願・登録

1. 特許取得

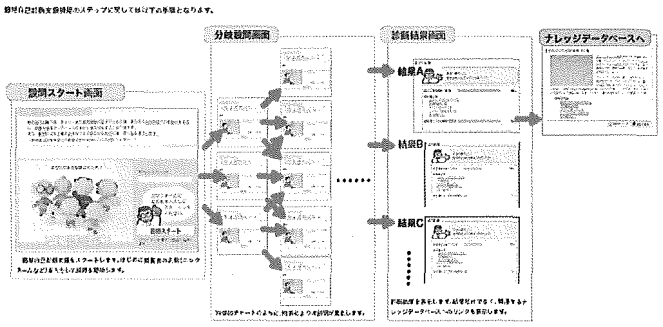
なし。

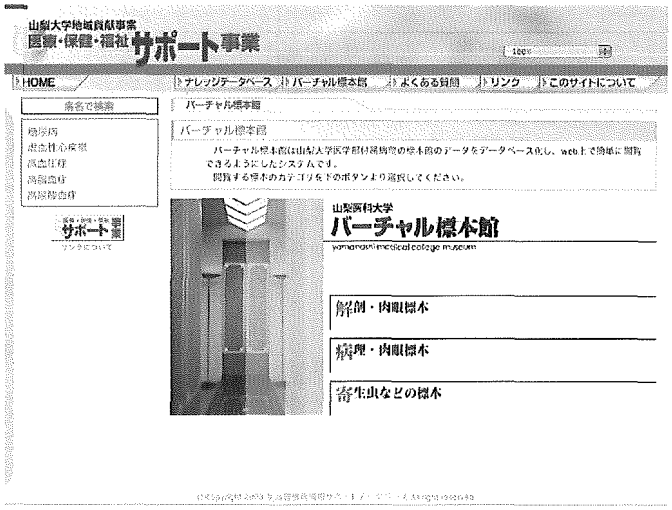
2. 実用新案登録

なし。

3. その他

特記すべきことなし。





研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Takashi Iida, et al	Blunted reduction of pulse pressure during nighttime is associated with leftventricular hypertrophy in elderly hypertensive patients.	Hypertens Res	27	573 - 579	2004
Norio Tada, et al	Effects of diacylglycerol ingestion on postprandial hyperlipidemia in diabetes	Clinica Chimica Acta	353	87-94	2005
Takamitsu Nakamura, et al	Remnant lipoproteinemia is a risk factor for endothelial vasomotor dysfunction and coronary artery disease in metabolic syndrome.	Atherosclerosis	In press	In press	2005
Yoshihide Ichigi, et al.	Increased ambulatory pulse pressure is a strong risk factor for coronary endothelial vasomotor dysfunction	J Am Coll Cardiol	In press	In press	2005

Original Article

Blunted Reduction of Pulse Pressure during Nighttime Is Associated with Left Ventricular Hypertrophy in Elderly Hypertensive Patients

Takashi IIDA, Isao KOHNO, Daisuke FUJIOKA, Yoshihide ICHIGI, Ken-ichi KAWABATA, Jun-ei OBATA, Mitsuru OSADA, Hajime TAKANO, Ken UMETANI, and Kiyotaka KUGIYAMA

Increased pulse pressure (PP) is recognized as a risk factor for cardiovascular disease, especially in elderly patients. However, blood pressure (BP) is known to have a circadian variation. Therefore, this study asked whether or not PP has a circadian variation and, if so, whether a circadian variation of PP has clinical importance. Ambulatory BP monitoring (every 30 min for 48 h) was performed in 255 patients with untreated essential hypertension (24 to 82 years old; mean: 52±12 years). Left ventricular mass index (LVMI) was estimated from M-mode echocardiography. PP was decreased during nighttime (10±11% reduction from daytime PP). Multivariate linear regression analysis showed that, among four variables—the degree of nighttime PP reduction, daytime PP, 48-h systolic BP, and nondipper hypertension—the degree of nighttime PP reduction had the strongest (inverse) correlation with LVMI in a subgroup of elderly patients (≥60 years old, n=67) (standardized regression coefficient=−0.32, p=0.02), whereas this association was not significant in the whole patient population unclassified by age. Furthermore, a blunted reduction of nighttime PP in combination with nondipper hypertension was an incremental risk for increase in LVMI in the elderly patients. In conclusion, PP is reduced during nighttime, but the degree of reduction varies among patients. The blunted reduction of nighttime PP is a risk for left ventricular hypertrophy, an established predictor of hypertension-induced cardiovascular events, and it may thus play a role in cardiovascular complications, especially in elderly patients with nondipper hypertension. (*Hypertens Res* 2004; 27: 573–579)

Key Words: **blood pressure, hypertension, hypertrophy**

Introduction

Hypertension is the most prevalent cardiovascular disease and causes various cardiovascular complications. Recently, increased pulse pressure (PP) was shown to be a risk factor for cardiovascular disease, especially in elderly patients (*1*). The association between PP and cardiovascular diseases is thought to be independent of systolic and diastolic blood pressure (SBP and DBP, respectively).

Mean ambulatory PP correlates with organ damage more closely than does office PP (*2*). It is known that blood pressure (BP) rises during the day and decreases during nighttime (*3*), although the magnitude of fluctuation varies among

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Table 1. Clinical Characteristics of Study Patients

	<60 years (n=188)	≥60 years (n=67)
Sex (% men)	55	52
Age (years)	47±9	68±6##
BMI (kg/m²)	25±3	23±3##
Smoker (%)	44	35
Diabetes mellitus (%)	10	3
Total cholesterol (mg/dl)	208±41	208±41
HDL cholesterol (mg/dl)	56±16	57±15
Triglyceride (mg/dl)	152±95	124±63#
Serum creatinine (mg/dl)	0.68±0.19	0.74±0.21#
Hemoglobine A1c (%)	5.3±0.7	5.4±0.4
LVMI (g/m²)	121±33	132±35#
SV (ml)	71±16	68±19
Office pulse rate (bpm)	71±9	71±9
Office BP (mmHg)		
Systolic	160±18	160±16
Diastolic	100±10	92±9##
Pulse pressure	59±14	68±15##

Values represent % of total or mean±SD. #$p<0.05$, ##$p<0.01$ compared with <60 years old patients. BMI, body mass index; HDL, high-density lipoprotein; LVMI, left ventricular mass index; SV, stroke volume; BP, blood pressure.

individual cases. Previous studies (*4–6*) have demonstrated that hypertensive target organ damage—including left ventricular hypertrophy (LVH)—is frequent in nondipper hypertension, a decreased fall in nighttime BP. It is well-known that LVH is causally related to high BP and represents hypertensive target organ damage (*7*). This study, therefore, investigated a possible circadian change in PP and its relation to LVH in 255 patients with untreated essential hypertension.

Methods

Study Subjects

The study population consisted of 255 consecutive patients with essential hypertension. All of these patients were untreated for hypertension and visited Yamanashi University Hospital. Hypertension was defined according to the Sixth Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-VI) criteria (*7*). All patients fulfilled the following inclusion criteria: the averaged values of two or more BP measurements obtained on at least two separate occasions were ≥140 mmHg SBP or ≥90 mmHg DBP, with waking ambulatory BP measurements ≥135/85 mmHg or sleeping ambulatory BP measurements ≥120/75 mmHg. None of the patients had secondary hypertension, congestive heart failure, previous myocardial infarction, cardiomyopathy, valvular heart diseases, previous stroke, or serum creatinine concentration >1.5 mg/dl. Diabetes mellitus was diagnosed when the random glucose level was ≥200 mg/dl, or when the hemoglobin A1c was ≥6.5%, or when the patient was treated by dietary restrictions, oral hypoglycemics, or insulin. Smoking was defined as >10 cigarettes per day for >1 year. Patients' characteristics are shown in Table 1. Written informed consent was obtained from all patients prior to commencement of the study. The study protocol was approved by the Ethics Committee of the University of Yamanashi.

Ambulatory BP Measurements

SBP, DBP, PP, and heart rate (HR) during daily activities were measured every 30 min for 48 h by the oscillometric method, using a noninvasive ambulatory BP monitoring system (TM-2425; A&D, Tokyo, Japan) (*8*). The daytime and nighttime mean values of SBP, DBP, PP, and HR during the 48-h period were analyzed by reviewing the patients' diaries. We defined daytime as the period from the time the patients awoke to the time they went to sleep, and nighttime as the period during which they were sleeping. The daytime and nighttime SBP, DBP, PP, and HR were the averages of the respective values over the 2 days of monitoring. Dipper hypertension was defined by the presence of a fall (≥10%) in the mean nighttime SBP and DBP from the respective daytime values. Nondipper hypertension was defined by the absence of the fall (≥10%) in the mean nighttime SBP, and/or in the mean nighttime DBP. The percent reduction of nighttime PP from daytime PP was calculated as (daytime mean PP−nighttime mean PP)×100 / (daytime mean PP).

Echocardiography

We performed echocardiography using a 2.5 MHz transducer with a Sonos-5500 echocardiographic unit (Hewlett Packard, Andover, USA). Left ventricular mass (LVM) and stroke volume (SV) were estimated from M-mode echocardiography (*8*). The LVM index (LVMI) was defined as LVM divided by body surface area (BSA). All echocardiographic studies were performed by physicians unaware of the patients' clinical data.

Statistical Analysis

Results are expressed as the mean±SD. The mean values and frequencies of continuous variables were compared between the 2 groups using the unpaired t-test and χ^2 analysis, respectively. Comparison between more than 3 groups was performed using one-way ANOVA. Analyses for the associations were performed using a linear regression technique. Nondipper hypertension, 48-h SBP, and daytime PP, which have traditionally been considered to be risk factors for LVH, were included as independent covariables in a multivariate linear regression analysis for the association between

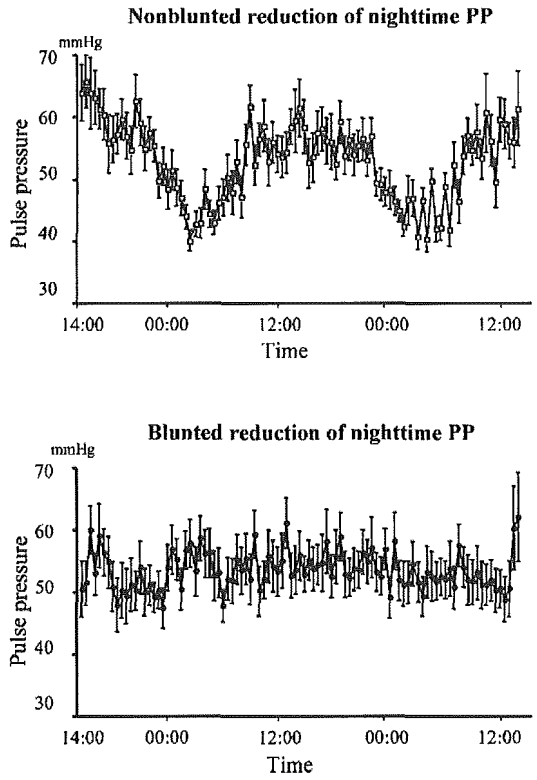


Fig. 1. *Circadian changes in pulse pressure during 48-h ambulatory blood pressure monitoring. The upper panel shows the circadian changes in pulse pressure from 20 representative patients with nonblunted reduction of nighttime pulse pressure. The lower panel shows the data from 20 representative patients with blunted reduction of nighttime pulse pressure. Data are shown as the mean±SD.*

LVMI as a dependent variable and percent reduction of nighttime PP as an independent variable. The circadian variation of BP was coded by the following dummy variables: 0 for dipper hypertension; or 1 for nondipper hypertension. A confidence level of $p<0.05$ was considered statistically significant. Analyses were partially assessed using StatView 5.0 (SAS Institute, Cary, USA).

Results

Circadian Changes of PP

In all patients, PP was not constant throughout either day or night, but, in a majority of patients, there was a circadian fluctuation of PP (*i.e.*, a reduction during nighttime), as shown in Fig. 1. The percent reduction of nighttime PP from daytime PP reached up to 40% with a mean of 10±11% of the daytime PP. The percent reduction of nighttime PP from daytime PP had a significant and inverse relation with 48-h SBP, nighttime SBP, nighttime DBP, 48-h PP, and nighttime PP, as shown in Table 2. It was significantly lower in those with nondipper hypertension than those with dipper hypertension (% reduction from daytime PP: 3±9% in nondippers [$n=114$] *vs.* 16±8% in dippers [$n=141$], respectively, $p<0.01$). The percent reduction of nighttime PP had no significant relation with serum levels of total cholesterol or high-density lipoprotein cholesterol, as shown in Table 2. The percent reduction of nighttime PP was comparable between patients with and without diabetes (data not shown).

Table 2. Relation of % Reduction of Nighttime Pulse Pressure to Clinical Parameters in the Whole Patient Population Unclassified by Age

		r	*p*
Age		−0.11	0.09
Body mass index		−0.04	0.52
Total cholesterol		−0.02	0.82
HDL cholesterol		0.06	0.72
Left ventricular mass index		−0.11	0.07
Stroke volume		−0.25	0.48
Office BP			
Systolic		−0.07	0.22
Diastolic		0.05	0.48
Pulse pressure		−0.12	0.049
Ambulatory BP			
Systolic	48 h mean	−0.18	0.003
	Daytime	0.05	0.44
	Nighttime	−0.55	<0.001
Diastolic	48 h mean	−0.03	0.57
	Daytime	0.07	0.28
	Nighttime	−0.24	<0.001
Pulse pressure	48 h mean	−0.25	<0.001
	Daytime	−0.04	0.93
	Nighttime	−0.63	<0.001

HDL, high-density lipoprotein; BP, blood pressure.

Association of Nighttime PP Reduction with LVMI

The percent reduction of nighttime PP from daytime PP had a significant and inverse relation to LVMI in a subgroup of elderly hypertensive patients (≧60 years), as shown in Fig. 2 and in Table 3, whereas this relation was not significant in either the whole patient population unclassified by age or a subgroup of non-elderly hypertensive patients (<60 years), as shown in Tables 2 and 3. Daytime PP and 48-h SBP also had a significant and positive relation with LVMI in the elderly patients ($r=0.33$, $p=0.004$ and $r=0.31$, $p=0.007$, respectively) in univariate linear regression analyses. Further, nondippers had a greater LVMI than dippers (139±36 g/m^2 *vs.* 121±31 g/m^2, respectively, $p=0.04$) in the elderly hypertensive patients. However, multivariate regression analy-

Table 3. Comparisons of Relation of % Reduction of Nighttime Pulse Pressure to Clinical Parameters between the Elderly Patients (≥ 60 Years) and the Non-Elderly Patients (<60 Years)

		<60 years		≥60 years	
		r	*p*	*r*	*p*
Age		−0.01	0.87	−0.16	0.20
Body mass index		−0.06	0.41	−0.13	0.31
Total cholesterol		−0.04	0.58	0.08	0.52
HDL cholesterol		0.05	0.61	0.16	0.31
Left ventricular mass index		−0.01	0.86	−0.39	0.009
Stroke volume		−0.04	0.59	−0.10	0.40
Office BP					
Systolic		−0.04	0.61	−0.23	0.06
Diastolic		0.03	0.67	−0.16	0.43
Pulse pressure		−0.07	0.33	−0.20	0.11
Ambulatory BP					
Systolic	48 h mean	−0.15	0.04	−0.34	0.004
	Daytime	0.09	0.22	−0.16	0.20
	Nighttime	−0.54	<0.001	−0.58	<0.001
Diastolic	48 h mean	0.04	0.38	−0.14	0.25
	Daytime	−0.06	0.38	−0.06	0.61
	Nighttime	−0.25	<0.001	−0.26	0.03
Pulse pressure	48 h mean	−0.20	0.007	−0.36	0.003
	Daytime	0.07	0.37	−0.17	0.18
	Nighttime	−0.62	<0.001	−0.65	<0.001
Heart rate	48 h mean	0.09	0.22	0.08	0.51
	Daytime	0.14	0.06	0.09	0.47
	Nighttime	−0.03	0.64	0.12	0.92

HDL, high-density lipoprotein; BP, blood pressure.

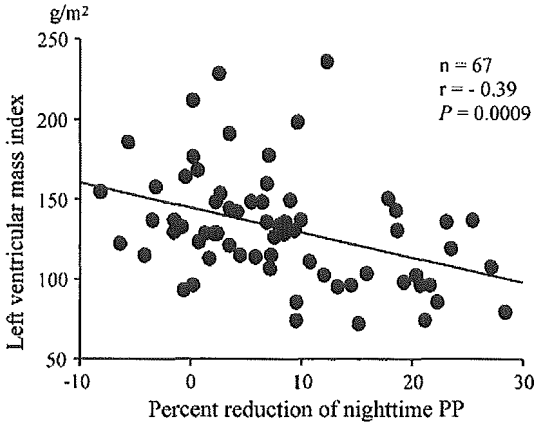


Fig. 2. *Correlation between the percent reduction of nighttime pulse pressure (PP) and left ventricular mass index in elderly hypertensive patients.*

sis showed that, among four variables—the percent reduction of nighttime PP, daytime PP, 48-h SBP, and nondipper hypertension—only the percent reduction of nighttime PP and daytime PP remained significantly correlated with LVMI in the elderly patients, as shown in Table 4. And the percent reduction of nighttime PP had the strongest (inverse) association with LVMI.

Table 4. Multivariate Linear Regression Analysis for Association of Left Ventricular Mass Index with Parameters Related to Blood Pressure in the Elderly Patients

	Standardized regression coefficients	*p*
% reduction of nighttime PP	−0.32	0.02
Daytime PP	0.39	0.03
48 h mean SBP	0.14	0.46
Nondipper hypertension	0.16	0.38

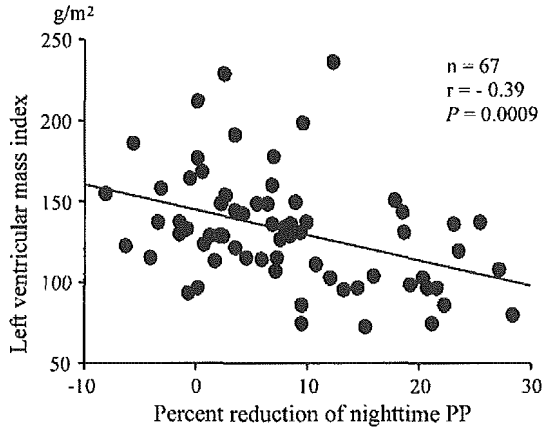
PP, pulse pressure; SBP, systolic blood pressure.

Blunted reduction of nighttime PP (<7% reduction of nighttime PP from daytime PP, with 7.0% corresponding to the median value of the % reduction of nighttime PP in the elderly patients) in combination with nondipper hypertension or higher daytime PP (>60 mmHg, corresponding to the median value of the daytime PP in the elderly patients) conferred an incremental risk of increase in LVMI in the elderly

Table 3. Comparisons of Relation of % Reduction of Nighttime Pulse Pressure to Clinical Parameters between the Elderly Patients (≥ 60 Years) and the Non-Elderly Patients (<60 Years)

		<60 years		≥60 years	
		r	*p*	*r*	*p*
Age		−0.01	0.87	−0.16	0.20
Body mass index		−0.06	0.41	−0.13	0.31
Total cholesterol		−0.04	0.58	0.08	0.52
HDL cholesterol		0.05	0.61	0.16	0.31
Left ventricular mass index		−0.01	0.86	−0.39	0.009
Stroke volume		−0.04	0.59	−0.10	0.40
Office BP					
Systolic		−0.04	0.61	−0.23	0.06
Diastolic		0.03	0.67	−0.16	0.43
Pulse pressure		−0.07	0.33	−0.20	0.11
Ambulatory BP					
Systolic	48 h mean	−0.15	0.04	−0.34	0.004
	Daytime	0.09	0.22	−0.16	0.20
	Nighttime	−0.54	<0.001	−0.58	<0.001
Diastolic	48 h mean	0.04	0.38	−0.14	0.25
	Daytime	−0.06	0.38	−0.06	0.61
	Nighttime	−0.25	<0.001	−0.26	0.03
Pulse pressure	48 h mean	−0.20	0.007	−0.36	0.003
	Daytime	0.07	0.37	−0.17	0.18
	Nighttime	−0.62	<0.001	−0.65	<0.001
Heart rate	48 h mean	0.09	0.22	0.08	0.51
	Daytime	0.14	0.06	0.09	0.47
	Nighttime	−0.03	0.64	0.12	0.92

HDL, high-density lipoprotein; BP, blood pressure.



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PP, pulse pressure; SBP, systolic blood pressure.

Blunted reduction of nighttime PP (<7% reduction of nighttime PP from daytime PP, with 7.0% corresponding to the median value of the % reduction of nighttime PP in the elderly patients) in combination with nondipper hypertension or higher daytime PP (>60 mmHg, corresponding to the median value of the daytime PP in the elderly patients) conferred an incremental risk of increase in LVMI in the elderly

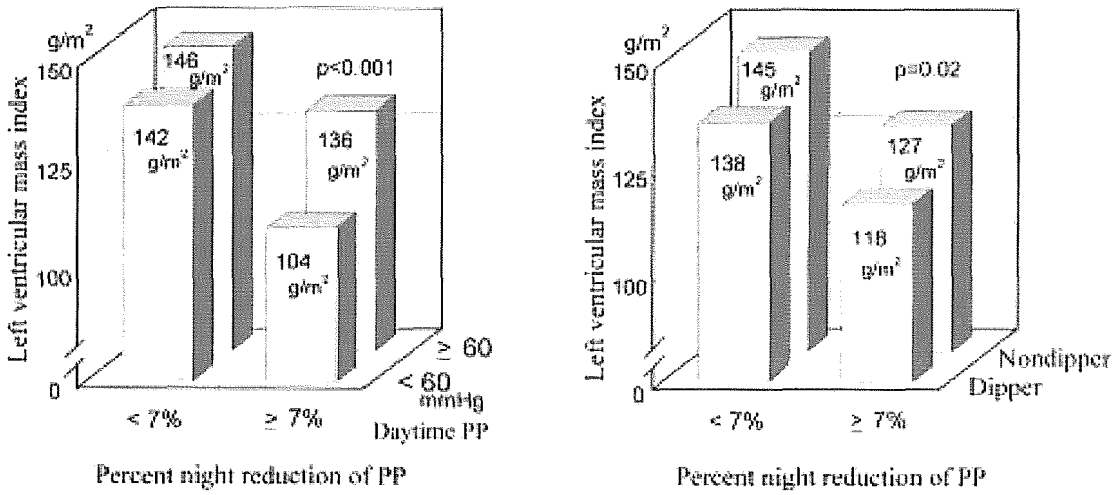

Fig. 3. *Incremental effects on left ventricular mass index with the combination of blunted reduction of nighttime pulse pressure (PP) and nondipper hypertension (right panel) or higher daytime PP (left panel). Blunted reduction of nighttime PP was defined as less than 7.0% reduction in nighttime PP with respect to daytime PP, with 7.0% corresponding to the median value in the elderly patients. Higher PP during daytime was defined as daytime PP exceeding 60 mmHg, which corresponds to the median value in the elderly patients. Statistical analyses were performed with ANOVA.*

Table 5. Comparisons of Variables of Amburatoly BP Measurments between Eldery Patients with and without Blunted Reduction of Nighttime PP

	% reduction of nighttime PP	
	≥7% (n=33)	<7% (n=34)
Systolic BP		
48 h mean	140±10	147±13#
Daytime	148±10	150±13
Nighttime	127±12	140±16##
Diastolic BP		
48 h mean	83±6	84±9
Daytime	86±6	88±8
Nighttime	75±7	78±10
PP		
48 h mean	58±9	62±8#
Daytime	61±9	63±8
Nighttime	52±9	62±9##

Values are mean±SD. #p<0.05, ##p<0.01 compared with ≥7% reduction of nighttime PP. BP, blood pressure; PP, pulse pressure.

patients, as shown in Fig. 3.

Comparisons of Ambulatory BP Measurements between Elderly Patients with and without Blunted Reduction of Nighttime PP

The elderly patients with <7% reduction of nighttime PP had higher 48-h SBP, nighttime SBP, 48-h PP, and nighttime PP, and similar levels of DBP parameters as compared with those with ≥7% reduction of nighttime PP, as shown in Table 5. The extent of decrease in SBP was greater than that of DBP, leading to a greater reduction in nighttime PP in the elderly patients with ≥7% reduction of nighttime PP. On the other hand, the extent of decrease in SBP was comparable to that of DBP, leading to a blunted reduction in nighttime PP in the elderly patients with <7% reduction of nighttime PP, as shown in Fig. 4. The elderly patients with <7% reduction of nighttime PP had a higher frequency of smoking, but not

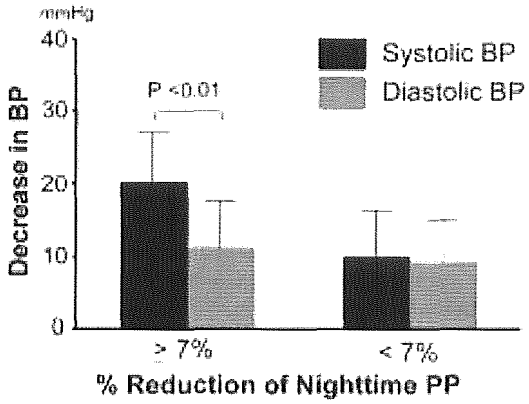

Fig. 4. *Comparisons of decreases in systolic and diastolic BP from daytime to nighttime in elderly patients.*

Table 6. Comparisons of Clinical Characteristics between Eldery Patients with and without Blunted Reduction of Nighttime PP

	% reduction of nighttime PP	
	≥7% (n=33)	<7% (n=34)
Sex (% men)	48	56
Age (years)	67±6	66±7
BMI (kg/m^2)	22±3	23±3
Smoker (%)	21	44$^{\#}$
Diabetes mellitus (%)	5	9
Total cholesterol (mg/dl)	209±32	207±48
HDL cholesterol (mg/dl)	60±17	55±14
Serum creatinine (mg/dl)	0.7±0.2	0.8±0.3
LVMI (g/m^2)	119±34	146±30$^{\#\#}$
SV (ml)	65±18	70±21

Values are mean±SD. $^{\#}p<0.05$, $^{\#\#}p<0.01$ compared with ≥7% reduction of nighttime PP. PP, pulse pressure; BMI, body mass index; HDL, high-density lipoprotein; LVMI, left ventricular mass index; SV, stroke volume.

of diabetes or dyslipidemia, as compared with those with ≥7% reduction of nighttime PP, as shown in Table 6.

Discussion

This study demonstrated that PP has a circadian variation, decreasing during nighttime in a majority of patients. However, the extent of nighttime reduction of PP varies among individuals. The present study further showed that the blunted reduction of nighttime PP is significantly associated with LVH in elderly hypertensive patients independently of daytime PP, 48-h SBP, and nondipper hypertension, and that the association of the blunted reduction of nighttime PP with LVH is the strongest among the covariates. Therefore, the blunted reduction of PP during nighttime might play a role in the pathogenesis of hypertension-induced cardiac damage in elderly patients. The present study also showed that nondipper hypertension is associated with a smaller reduction of nighttime PP. This was expected, because patients with nondipper hypertension are known to show a minimal decrease in BP during nightitme, which would lead to a blunted reduction of nighttime PP. In fact, this study also showed that a smaller reduction of nighttime SBP resulted in a blunted reduction of nighttime PP. Thus, the blunted reduction of nighttime PP may be intimately related to the pathogenesis of cardiovascular complications in nondipper hypertension. Furthermore, the present study demonstrated that the blunted reduction of nighttime PP confers an additional risk—in patients with nondipper hypertension—for LVH. Thus, the combination of a blunted reduction of nighttime PP and nondipper hypertension is a strong risk factor for LVH in elderly hypertensive patients.

Recently, increased PP was shown to be a risk factor for cardiovascular mortality in elderly patients (*1*). Stiffening of the central elastic arteries, which reflects biological aging of the arterial system, tends to raise SBP and lower DBP. The former, which causes a disproportionate increase in end-systolic stress, promotes the development of cardiac hypertrophy and requires a greater coronary blood flow. The latter reduces the pressure on which coronary flow is dependent, and together they increase the vulnerability of the heart to ischemia. This explains why an increase in PP is a major predictor of cardiovascular risk in elderly hypertensive patients. The blunted reduction of PP from day to night, which persistently increases PP, might cause further progression of hypertension-induced organ damage, thereby resulting in a higher incidence of cardiovascular events. In fact, the present study showed that, in patients with higher daytime PP, the blunted reduction of nighttime PP conferred an additional risk for LVH. Taken together, these results indicate that the combination of an increase in PP and the blunted reduction of nighttime PP could be a risk for cardiovascular complications in elderly hypertensive patients. It is, however, necessary to confirm the blunted reduction of nighttime PP as a new risk for cardiovascular disease in a prospective study with a large number of the patients. Previous studies (*9*, *10*) have shown that diabetes and dyslipidemia are associated with an increase in PP. The present study, however, showed that the elderly patients with a blunted reduction of nighttime PP had higher frequency of smoking, but not higher frequency of diabetes or dyslipidemia. Although the mechanism for the positive association between smoking and the blunted reduction of nighttime PP remains to be determined, this association may play a possible role in the pathogenesis of smoking-related cardiovascular diseases.

SBP increases with age, while DBP rises only until 50 years of age, after which it either becomes constant or even decreases slightly. In the Framingham Heart Study (*1*), increasing age entailed a shift from DBP to SBP and then to PP as the major predictor of cardiovascular risk. Thus, PP became superior to SBP as a predictor of cardiovascular diseases in elderly hypertensive patients (*1*). These features in elderly hypertension may explain why, in the present multivariate analysis, the percent reduction of nighttime PP, but not 48-h SBP, remained significantly associated with LVMI in elderly patients, and why the significant association was observed in the elderly hypertensive patients but not in the younger patients or the total population.

In conclusion, PP is decreased during nighttime in elderly hypertensive patients, but the extent of the decrease varies case-by-case. The blunted reduction of PP during nighttime is a risk factor for LVH and may play a role in cardiovascular complications in elderly patients.

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Effects of diacylglycerol ingestion on postprandial hyperlipidemia in diabetes

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Abstract

Background: We previously reported that diacylglycerol (DAG) as compared with triacylglycerol (TAG) suppressed increases in postprandial lipids in healthy volunteers. This study was to investigate the effects of DAG on postprandial lipids, particularly remnant lipoproteins in diabetics.

Methods: Emulsified DAG oil or TAG oil with a fatty acid composition similar to DAG oil was orally administered (30 g fat/m^2 of body surface) to moderately controlled six diabetics, with hemoglobin A1c (HbA1c) below 8%, after fasting for at least 12 h in a randomized crossover manner. Serum cholesterol and TAG, lipids in remnant-like particles (RLP), and other lipid parameters including serum ketone bodies were measured prior to and 2, 4, and 6 h after fat loading.

Results: DAG loading significantly suppressed increases in postprandial serum TAG and lipids in RLP as compared with TAG loading. The incremental area under the curve (IAUC) for serum TAG and that for lipids in RLP with DAG loading were also significantly smaller than those with TAG loading. However, changes in serum levels of insulin, free fatty acids, and ketone bodies during fat loading were essentially the same for DAG and TAG.

Conclusions: This pilot study suggests that substituting DAG intake for TAG may be beneficial to moderately controlled diabetics due to its effect in reducing postprandial hyperlipidemia.

Keywords: Diacylglycerol; Remnant lipoprotein; Remnant-like particles; Postprandial hyperlipidemia; Diabetes

1. Introduction

A greatly enhanced risk for coronary heart disease (CHD) has been reported in diabetes [1,2]. Lipid abnormalities in diabetes, including elevated plasma tri-

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acylglycerol (TAG), low cholesterol in high-density lipoproteins (HDL), an increase in small, dense low-density lipoproteins (LDL), and postprandial hyperlipidemia contribute to this increased risk [3–6]. Among them, postprandial increase in remnant lipoproteins (remnants) has been recognized as a powerful CHD risk not only for diabetic but also for nondiabetic subjects [7–12].

Remnants are the metabolites of TAG-rich lipoproteins, such as chylomicrons (CM) and very-low-density lipoproteins (VLDL), and are formed in the circulation by the effect of lipoprotein lipase. These remnants are readily incorporated into endothelial macrophages, leading to the accumulation of cholesterol in these cells, consequently forming a premature atherosclerotic lesion [9,12–14].

Therapeutic approaches to reduce remnant levels in the postprandial phase are believed to be important for the management of patients with diabetes and also with metabolic syndrome [5]. We have previously reported [15] that the substitution of diacylglycerol (DAG) oil intake for TAG oil significantly suppressed postprandial increases in serum TAG and lipids in remnants measured by the method of Nakajima et al. [16] in healthy male volunteers. Therefore, we had an interest whether this favorable effect of DAG intake on postprandial hyperlipidemia can be applied to diabetic subjects without any serious adverse phenomenon.

Diacylglycerol is a natural component of various edible oils and consists mainly of the 1,3-species. The intake of DAG has been reported to reduce fasting serum TAG concentration and hemoglobin A1c (HbA1c) levels in type 2 diabetics and to prevent the accumulation of body fat in experimental animals and in humans [17,18]. Decreased activities of enzymes of fatty acid synthesis and increased activities of enzymes involved in the β-oxidation pathway by DAG ingestion have also been reported [18].

The objectives of this study were to investigate the effects of oral DAG loading on postprandial changes in serum lipids, related parameters including ketone bodies, and changes in remnants in moderately controlled diabetics.

2. Materials and methods

2.1. Subjects

The subjects were six patients with type 2 diabetes mellitus (five females and one male; aged 46–70 years) who had moderately controlled HbA1c levels that were <8%. The study was performed in accordance with the principle of the Helsinki Declaration. The subjects were fully informed concerning the study and gave their informed consent. The clinical characteristics of subjects are shown in Table 1. All of them were not receiving insulin therapy, but they were medicated as follows: one was taking both a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor and a hypotensive drug; five subjects were taking oral antidiabetic drugs (sulfonylurea, biguanides, and α-glucosidase inhibitors), and two of these five were also taking HMG-CoA reductase inhibitors, and the others were taking hypotensive drugs concomitantly.

Table 1
Characterics of subjects

No.	Age (years)	Height (m)	Weight (kg)	FPG (mmol/l)	HbA1c (%)	Serum TAG (mmol/l)	Serum T-Chol (mmol/l)	HDL-C (mmol/l)
1	59	1.47	59	7.3	6.5	1.75	4.85	2.12
2	46	1.50	65	7.5	7.8	0.89	5.35	1.27
3	70	1.48	54	6.1	6.5	0.69	5.53	1.32
4	67	1.64	57	11.5	7.6	1.34	5.06	1.11
5	59	1.47	47	9.3	7.6	0.88	5.47	2.24
6	68	1.57	56	9.6	6.8	0.59	5.64	1.86
Mean±S.E.	62±4	1.52±0.03	56±2	8.6±0.8	7.1±0.2	1.03±0.18	5.32±0.12	1.65±0.20

TAG: triacylglycerol; BMI: body mass index; FPG: fasting plasma glucose; T-Chol: total cholesterol.

2.2. Experimental oils

The DAG oil was prepared by esterifying glycerol with fatty acids from soybean and rapeseed oil according to the method of Huge-Jensen et al. [19]. The product contained 1,3-DAG and 1,2-DAG isomers in a ratio of 7:3 with a total DAG content of approximately ≥80%. Triacylglycerol oil was prepared by mixing rapeseed, safflower, and perilla oils to give a final fatty acid composition that was similar to that of the DAG oil. The fatty acid compositions of the DAG and TAG oils are shown in Table 2. No appreciable differences in combustion energy were detected between the DAG and TAG oils [20].

2.3. Study design

The study was designed in a double-blind crossover style with a 2-week interval. The medications were not changed during the period of interval. The protocol was substantially the same as that as previously reported [15]. Briefly, the patients ingested emulsified test oil (TAG or DAG oil) at a dose of 30 g fat/m^2 of body surface area in the morning after fasting for approximately 12 h. The test oil emulsions contained 35% oil, 1% casein sodium, 3% skim milk, 0.5% fatty acid sucrose polyester, 0.36% soybean lecithin, and 60.14% water. During the study, patients were asked to remain seated with minimum physical activity. Patients were also requested not to take any drugs or food except for water from the morning of the test day to the final blood sample collection.

Blood samples were collected before fat loading for baseline measurements (initial value) and at 2, 4, and 6 h after fat loading. Two weeks later, the same patients received the opposite test oil, and blood samples were collected at the same time points.

Table 2
Fatty acid composition of test oils (wt.%)

	DAG	TAG
C16	3.1	5.4
C18	1.1	2.1
C18:1	38.3	34.3
C18:2	47.7	49.2
C18:3	9.0	7.8
C20	0.3	0.5
C20:1	0.2	0.3

DAG: diacylglycerol; TAG: triacylglycerol.

2.4. Sample analysis

Serum lipids, lipids in lipoproteins, HDL-cholesterol (HDL-C), and serum apolipoprotein concentrations were measured as described elsewhere [15]. The concentrations of serum total ketone bodies [21] and plasma glucose [22] were measured by enzymatic methods. Serum insulin [23] and plasma plasminogen-activator inhibitor 1 (PAI-1) [24] were measured by enzyme immunoassay methods. Plasma preheparin lipoprotein lipase (LPL) protein mass was measured by the method of Kobayashi et al. [25]. Serum leptin was measured by radioimmunoassay [26]. The remnant-like particle (RLP) fraction was isolated from the serum of each sample by means of an immunoaffinity-mixed gel conjugated with anti-apoB-100 and anti-apoAI monoclonal antibodies [16].

Lipoprotein fractions were obtained from the serum sample by sequential preparative ultracentrifugation [27] using a Hitachi RP65T rotor. Chylomicrons (Svedberg floatation >400 lipoproteins) were isolated at 20,000 rpm for 30 min at 15 °C, and very-low-density lipoproteins (VLDL; d<1.006 g/ml) were isolated further at 40,000 rpm for 16 h at 4 °C. Then, serum density was adjusted by adding potassium bromide. LDL (1.006<d<1.063 g/ml) and HDL (1.063<d<1.21 g/ml) fractions were isolated sequentialy at 40,000 rpm for 20 and 40 h, respectively, at 4 °C. Separated LDL and HDL were dialyzed against 0.15 mol/l NaCl. TAG concentrations of the isolated lipoprotein fractions were determined by enzymatic methods.

2.5. Statistical analysis

Measured values and changes (Δ) from the initial values are presented as mean±standard error (S.E.). Statistical analyses were performed using SPSS (version 11.0: SPSS, Chicago IL). Differences between the groups in the time course changes from the initial values were analyzed using repeated-measures two-way ANOVA. The difference between the measured values during DAG and TAG loading test at each time point was assessed by a paired t-test. The incremental area under the curve (IAUC) of blood variables during 6 h after fat loading was calculated,

Table 3
Changes in serum concentrations of lipids

		Time after fat loading (h)				p value of ANOVA
		0	2	4	6	
T-Chol (mmol/l)	DAG	5.25±0.16 (0.00)	5.35±0.13 (0.08±0.08)	5.22±0.10 (−0.05±0.08)	5.28±0.13 (0.03±0.08)	p=0.330
	TAG	5.38±0.16 (0.00)	5.35±0.13 (−0.03±0.05)	5.33±0.13 (−0.05±0.05)	5.28±0.13 (−0.10±0.05)	
LDL-C (mmol/l)	DAG	3.28±0.23 (0.00)	3.34±0.26 (0.08±0.08)	3.23±0.23 (−0.05±0.05)	3.18±0.23 (−0.10±0.08)	p=0.617
	TAG	3.26±0.28 (0.00)	3.26±0.26 (0.00±0.05)	3.18±0.26 (−0.08±0.05)	3.18±0.28 (−0.08±0.05)	
HDL-C (mmol/l)	DAG	1.66±0.21 (0.00)	1.63±0.18 (−0.03±0.03)	1.60±0.18 (−0.08±0.05)	1.63±0.21 (0.00±0.03)	p=0.567
	TAG	1.63±0.18 (0.00)	1.66±0.18 (0.00±0.05)	1.58±0.18 (−0.08±0.03)	1.60±0.18 (−0.05±0.00)	
TAG (mmol/l)	DAG	0.94±0.19 (0.00)	1.46±0.33 (0.52±0.23)	1.59±0.42 (0.64±0.23)	1.41±0.26 (0.14±0.11)	p=0.005
	TAG	1.11±0.20 (0.00)	1.77±0.32 (0.68±0.27)	1.80±0.34 (0.69±0.18)	1.41±0.36 (0.32±0.17)	
FFA (mmol/l)	DAG	0.51±0.06 (0.00)	0.63±0.07 (0.11±0.04)	0.87±0.09 (0.36±0.07)	0.82±0.11 (0.31±0.09)	p=0.567
	TAG	0.59±0.08 (0.00)	0.75±0.11 (0.16±0.16)	0.79±0.06 (0.21±0.11)	0.85±0.13 (0.27±0.13)	
RLP-C (mmol/l)	DAG	0.11±0.01 (0.00)	0.17±0.03 (0.06±0.03)	0.17±0.04 (0.06±0.02)	0.13±0.02 (0.03±0.01)	p=0.021
	TAG	0.11±0.02 (0.00)	0.21±0.02 (0.10±0.02)	0.20±0.03 (0.09±0.02)	0.15±0.03 (0.05±0.02)	
RLP-TAG (mmol/l)	DAG	0.14±0.04 (0.00)	0.48±0.15 (0.34±0.14)	0.52±0.19 (0.38±0.14)	0.29±0.08 (0.15±0.05)	p=0.004
	TAG	0.15±0.04 (0.00)	0.67±0.19 (0.52±0.18)	0.59±0.13 (0.44±0.10)	0.41±0.13 (0.26±0.09)	

Values are Mean±S.E. Mean±S.E. changes from baseline are shown in parentheses (Δ). *Significantly different from TAG ingesition at the same time points by paired t-test: $p<0.05$. P values are calculated by repeated-measures two-way ANOVA (Δ). DAG: diacylglycerol; TAG: triacylglycerol; T-Chol: total cholesterol; FFA: free fatty acids.

and the differences between the treatment groups were assessed by a paired t-test. P values <0.05 were considered to be significant for all analyses.

3. Results

3.1. Changes in serum lipids and apolipoproteins

Table 3 shows changes in serum lipids, LDL-C, HDL-C, and RLP lipids. Serum total cholesterol, LDL-C, and HDL-C did not change during TAG or DAG loading. However, serum TAG increased, peaking at 4 h after the loading with either oil and decreased at 6 h. Increases (Δ: shown in the parentheses in Table 3) in serum TAG from the initial value were significantly smaller during DAG loading than those observed during TAG loading (p=0.005), as determined by two-way ANOVA. No significant difference in serum TAG levels after fat loading was observed between the TAG and DAG study groups at any time point. Serum FFA was increased during TAG or DAG loading, and changes in these values were not different significantly between the two study groups.

The RLP lipids, RLP-C and RLP-TAG, were increased, peaking at 2 or 4 h and then decreased toward the initial value as a function of time after the loading of either oil. However, increases in RLP-C

Table 4
Changes TAG in CM, VLDL, LDL, and HDL

		Time after fat loading (h)				p value of ANOVA
		0	2	4	6	
CM (mmol/l)	DAG	0.01±0.01 (0.00)	0.27±0.13 (0.25±0.13)	0.30±0.12 (0.29±0.12)	0.12±0.05 (0.10±0.04)	p=0.141
	TAG	0.02±0.01 (0.00)	0.37±0.16 (0.35±0.16)	0.31±0.06 (0.30±0.05)	0.17±0.07 (0.15±0.07)	
VLDL (mmol/l)	DAG	0.38±0.16 (0.00)	0.55±0.18 (0.17±0.06)	0.59±0.22 (0.20±0.06)	0.36±0.16 (−0.02±0.05)	p=0.796
	TAG	0.52±0.16 (0.00)	0.70±0.17 (0.17±0.07)	0.72±0.22 (0.20±0.09)	0.53±0.21 (0.01±0.07)	
LDL (mmol/l)	DAG	0.34±0.04 (0.00)	0.33±0.04 (0.00±0.01)	0.33±0.04 (0.00±0.01)	0.32±0.05 (−0.01±0.02)	p=0.097
	TAG	0.34±0.04 (0.00)	0.34±0.04 (0.00±0.00)	0.35±0.04 (0.00±0.00)	0.35±0.04 (0.01±0.01)	
HDL (mmol/l)	DAG	0.14±0.02 (0.00)	0.16±0.02 (0.02±0.01)	0.18±0.03 (0.04±0.02)	0.16±0.03 (0.01±0.01)	p=0.585
	TAG	0.17±0.01 (0.00)	0.19±0.01 (0.02±0.01)	0.21±0.02 (0.03±0.02)	0.18±0.02 (0.01±0.01)	

Values are Mean±S.E. Mean±S.E. changes from baseline are shown in parentheses (Δ). P values are calculated by repeated-measures two-way ANOVA (Δ). DAG: diacylglycerol; TAG: triacylglycerol.

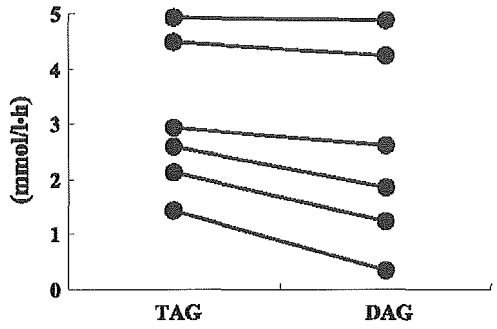

Fig. 1. Incremental area under the curve (IAUC) for serum TAG with either TAG or DAG loading (n=6). Increases in serum TAG were monitored during 6 h after a single loading of either TAG or DAG orally. This figure shows the difference of individual IAUC for serum TAG between TAG and DAG loading, and IAUC for serum TAG in the DAG group were significantly lower when compared with those in the TAG group (p<0.05). DAG: diacylglycerol; TAG: triacylglycerol.

and RLP-TAG from the initial values (Δ) after DAG loading were significantly smaller than those after TAG loading.

Changes in TAG level in each lipoprotein, separated by ultracentrifugation, with fat loading are shown in Table 4. No significant differences in terms of changes in these values were found between the TAG and DAG groups. However, the duration to the peak CM-TAG after fat loading was longer in the case of DAG loading compared with that in the TAG loading group, suggesting a slower production of CM during the DAG loading, although the difference between the groups was not significant. Apolipoproteins AI, AII, B, CII, CIII, and E were decreased during either fat loading, and changes in these parameters were not significantly different between TAG and DAG loading (data not shown).

3.2. Incremental area under the curve (IAUC) of serum TAG and RLP lipids

Differences of IAUC for serum TAG and RLP lipids between DAG and TAG ingestion, monitored up to 6 h after the loading with test oil, are shown in Figs. 1 and 2, respectively. The mean values of IAUC for serum TAG, RLP-C, and RLP-TAG in the DAG group were all significantly lower when compared with those in the TAG group (p<0.05).

3.3. Changes in serum insulin, plasma glucose, serum total ketone bodies, plasma LPL protein mass, serum leptin, and plasma PAI-1

As listed in Table 5, serum insulin was increased slightly at 2 h and then decreased at 4 and 6 h after either of the fat loading, and there were no significant differences between the values for TAG and DAG loading. Plasma glucose was gradually decreased with time after either of the fat loading. Decreases in plasma glucose from the initial value (Δ) at 2 and 4 h were significantly larger with TAG loading than those with DAG loading. Overall, decreases in plasma glucose during TAG loading were significantly greater

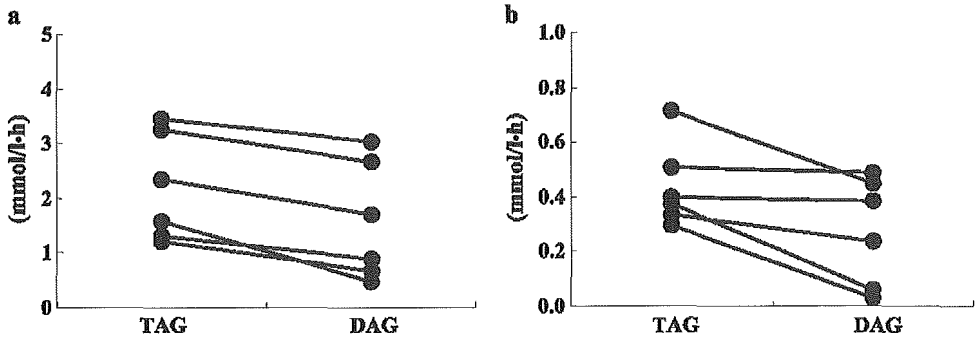

Fig. 2. Incremental area under the curve (IAUC) for RLP lipids with either TAG or DAG loading (n=6). Increases in RLP-TAG (a) and RLP-C (b) were monitored during 6 h after a single loading of either TAG or DAG orally. This figure shows the difference of individual IAUC for RLP-TAG (a) and RLP-C (b) between TAG and DAG loading. IAUC for both RLP-TAG (a) and RLP-C (b) in the DAG group were significantly lower when compared with those in the TAG group, respectively (each p<0.05). DAG: diacylglycerol; TAG: triacylglycerol; RLP: remnant-like particles.