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各種高脂血症治療薬の糖尿病性心血管病進展予防効果の総合的検討

（臨床研究チームの整備）

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主任研究者　横手　幸太郎

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（臨床研究実施チームの整備）

主任研究者　横手　幸太郎　千葉大学助手

研究協力者　小林　一貴　千葉大学大学院生

同　前澤　善朗　千葉大学大学院生

研究要旨

本研究の目的は、各種高脂血症治療薬が日本人糖尿病・耐糖能異常患者における心血管イベントの発症、生命予後、生活の質に及ぼす影響を評価することにあり、その達成のため千葉大学医学部附属病院において臨床研究実施チームを編成した。さらに本学臨床治験管理センターを中心とした千葉臨床試験ネットワーク(Chiba University Clinical Research Network)と連携し、内分泌･代謝医としての専門性を活かしつつ、調査・研究をより効果的に遂行する体制を確立した。その結果、276 症例の糖尿病・耐糖能異常患者について初年度登録を完了した。加えて本チームを活用し、HMG-CoA 還元酵素阻害剤（スタチン）を用いた介入試験（CHIBA スタディ）を開始、次年度以降の研究につなげる基盤を得た。

A. 採択された研究事業での研究概要

食習慣の西洋化と社会の高齢化に伴い、わが国における糖尿病・耐糖能障害患者の急増が社会問題となっている。糖尿病患者には動脈硬化に基づく心血管疾患の発症頻度が世界的に高いことが知られ、その生命予後のみならず生活の質（Quality of Life:QOL)、ひいては医療経済にもにも重大な影響を及ぼす。糖尿病患者における心血管疾患の発生は、血糖コントロールの改善だけでは十分に抑制することができず、むしろ合併する高脂血症に対する治療がイベント抑制に有効であるとの成績が海外から報告されている。しかし、わが国における日本人を対象とした臨床研究成績は未だ乏しい。

本研究は、各種高脂血症治療薬が日本人糖尿病・耐糖能異常患者における心血管イベントの発症、生命予後、生活の質に及ぼす影響を評価することを目的として開始された。初年度である平成１６年度は、千葉大学医学部附属病院において３名の医師によって内分泌・代謝・老年病専門医師によって構成される臨床研究実施チームを編成した。そして、本学臨床治験管理センターを中心に準備を進めている千葉臨床試験ネットワーク(Chiba University Clinical Research Network)と連携し、専門性を活かしつつ、調査・研究をより効果的に遂行する体制を確立した。その結果、本チームの当初目標であった２００症例を上回る２７６症例の糖尿病・耐糖能

異常患者について登録を完了した。さらに、個別の高脂血症治療薬の有効性をより直接的に解析するため、本チームを活用し、千葉大学医学部倫理委員会の審査を経て、HMG-CoA 還元酵素阻害剤（スタチン）を用いた介入試験を開始した（CHIBA スタディ）。このように、次年度以降に成果が期待できる体制の準備が整えることができた。

B. 採択された研究事業での研究実績

本研究は、動脈硬化にもとづく心血管疾患の合併率が高い糖尿病ならびに耐糖能異常患者を対象として、高脂血症の治療（特に脂質値と高脂血症薬の種類）が心血管イベントの発症ならびに生命予後、QOL、高齢者においては日常生活活動度（activity of daily livig: ADL）にどのような影響を及ぼすか、日本人におけるエビデンスを構築することを目的として開始した。研究計画を実施するにあたり、本研究費に基づき千葉大学医学部附属病院において、若手医師、臨床研究協力者ならびにその指導者からなる臨床研究実施チームを編成した。さらに、大規模な調査をより効果的に実施するため、本学齋藤康病院長指導のもと臨床治験管理センターを中心に準備を進めている千葉臨床試験ネットワークと連携し、被検者のリクルートならびに調査遂行のノウハウを習得してきた。この組織は、専門性に基づいた地域ネットワークによる臨床試験の推進体制の充実・強化をはかり、臨床試験に参加するすべての者（医師、被験者、依頼者、規制当局）を対象とした安全性情報の共有化と教育活動を通して最終的に新しい治療方法の確立を目指すものである。その第一歩として我々のチームとの連携により代謝疾患群を対象としたネットワークを構築を試みた結果、当チームの当初目標であった２００症例を上回る２７６症例の糖尿病・耐糖能異常患者について登録を行うことができた。初年度登録者２７６名の内訳は、男性１４４名、女性１３２名、平均年齢は６４歳であった。２７６名の７７％にあたる２１３名に、日本動脈硬化学会の診断基準による「高脂血症」の合併を認めた。一方、同学会の診療ガイドラインによる治療目標値である血清総コレステロール値２００mg/dl 未満または LDL コレステロール値１８０mg/dl 未満を達成していた症例は全体の４８％にあたる１３２名に過ぎないことがわかった。

本研究チームでは、特に、糖尿病・高脂血症合併患者における脳血管疾患ならびに認知機能障害の評価と追跡を充実させたいと考えている。そこで、Minimental State Examination（MMSE）や Geriatric Depression Scale-15（GDS-15）を含む総合機能評価（Comprehensive geriatric assessment: CGA）に加え、登録症例の 3 分の 1 強にあたる 80 症例において、頭部 MRI 撮影および脳血流シンチを実施した。頭部 MRI では、T1 強調・T2 強調・フレア画像を撮像し、確立した脳梗塞のみならず、脳梗塞に

至らない皮質下虚血病変（leukoaraiosis）の定量的評価を試みている。さらに、臨床パラメーターとして調査に必要となる各種バイオマーカーの測定系を実験室レベルで確立し、2年目以降の研究に活用できる土台を築いた。今後、ベースラインデータの充実をはかり、次年度以降の追跡調査を遺漏なく進める所存である。

（倫理面への配慮）

本研究の実施にあたっては、臨床研究に関する倫理指針を遵守し、千葉大学医学部倫理委員会の承認を得たのち、各被験者よりインフォームドコンセントを取得している。また2005年4月1日以降、特に個人情報保護に細心の注意を払っている。

C. 考察

本年度整備を行った臨床研究実施チームは3名の臨床医により構成され、実施する研究にふさわしい被験者の選択、倫理審査結果に基づくインフォームド・コンセントの取得、臨床検査成績の収集等に大きな力を発揮した。さらに、本学医学部附属病院臨床治験管理センターを基盤とする千葉臨床試験ネットワークと連携し、研究をより効果的に遂行する体制を確立することができた。次年度以降は、本計画の主研究である「各種高脂血症治療薬の糖尿病性心血管病進展予防効果の総合的検討」に関して各種評価項目のフォローアップの重要性が増し、被験者からの血液採取や血中バイオマーカーのアッセイ等が必要とされるため、より効率的な研究遂行を意図して医師ではなく、臨床検査技師を臨床研究協力者にあてたいと考えるに至った。また、“E. その他実施した臨床研究・治験の概要及び実績”においても触れるが、この臨床研究実施体制を基盤に、千葉県下の糖尿病・代謝・内分泌専門病院と連携するネットワークを構築、本年度すでにスタチンを用いた無作為割付による介入試験を開始している。この研究は登録症例200程度の臨床研究であるが、今後、この体制を活用していく上での試金石的役割を果たすものと考えている。得られた成果をもとに改良を加え、次年度以降は千葉大学、千葉県下においてより信頼性・応用性の高いより大規模な臨床研究を実施できる体制を充実させていきたい。

D. 健康危険情報

特にありません。

E. その他実施した臨床研究・治験の概要及び実績

当初の計画にはなかったことであるが、本チームを活用し、千葉臨床試験ネットワークと共同で、高脂血症治療薬のスタンダード的存在であるスタチンを用いた介入試験を開始している。これは千葉大学医学部倫理委員会の審査を経て開始した多施設研究であり、世界第一位のシェアを占めるアトルバスタチンと国産のストロングスタチンであるピタバスタチンの臨床効果、耐糖能等に及ぼす影響を比

較検討する無作為割付試験である。現在、糖尿病患者を含む１８０症例のエントリーを終え、介入を開始している。

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研究成果の刊行に関する一覧表レイアウト

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
横手幸太郎	食後高脂血症	代田浩之,野出孝一	循環器医が治療する糖尿病と大血管障害.	メディカルレビュー社	東京	2004	296-297
横手幸太郎,村野俊一,齋藤康	肥満症	大内尉義監修,井藤英喜担当編集	高齢者の生活習慣病	メディカルレビュー社	東京	2004	82-91
前澤善朗,横手幸太郎,山田研一	ACE阻害薬	阿部好文,西川哲男	臨床に直結する内分泌・代謝疾患治療のエビデンス:ベッドサイドですぐに役立つリファレンスブック	文光堂	東京	2004	167-169

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Yokote K, Honjo K, Kobayashi K, Fujimoto M, Kawamura H, Mori S, Saito Y.	Metabolic improvement and abdominal fat redistribution in Werner syndrome by pioglitazone.	J Am Geriat Soc	52	52:1582-1583	2004
Kawamura H, Yokote K, Asaumi S, Kobayashi K, Fujimoto M, Maezawa Y, Saito Y, Mori S.	High glucose-induced upregulation of osteopontin is mediated via a Rho/Rho Kinase pathway in cultured rat aortic smooth muscle cells.	Arteriosc Thromb Vasc Biol	24	276-281	2004

Our report is, to the best of our knowledge, the first one describing the effects of obesity surgery in type 1 diabetes. In our opinion, gastric bypass surgery, which is being performed increasingly often (~100,000 operations in the U.S. annually [10]) in obese individuals, also with type 2 diabetes (4–8), is a feasible, safe, and effective method of weight reduction in young type 1 diabetic patients with severe obesity and comorbidities leading to metabolic syndrome (e.g., hypertension, hyperlipidemia) (11). In our patients, surgery-induced weight loss was also associated with a decrease in insulin requirement per kilogram of body weight (0.60 to 0.53 IU/kg in the first patient and from 0.95 to 0.83 IU/kg in the second patient). This observation may suggest the presence of clinically significant insulin resistance in severely obese type 1 diabetic subjects (12), which was subsequently reduced once weight loss occurred. Importantly, neither of the patients had any significant hypoglycemic episodes after the surgery, despite considerable reduction in HbA_{1c} level and apparent increase in insulin sensitivity.

In conclusion, gastric bypass surgery not only leads to a significant and maintained weight loss in type 1 diabetic patients, but also results in remarkable improvement in metabolic control (absolute reduction in HbA_{1c} of 3–4%) and concomitant disorders. Interestingly, the need for constant intensive insulin therapy in these patients had no detrimental influence on weight loss as an effect of obesity surgery. Both patients lost 50–60% of their excessive body weight during the follow-up period, which is also the rate reported in nondiabetic subjects (4,5,7).

LESZEK CZUPRYNIAK, MD, PHD[1]
JANUSZ STRZELCZYK, MD, PHD[2]
KATARZYNA CYPRYK, MD, PHD[3]
MACIEJ PAWLOWSKI, MD[1]
DARIUSZ SZYMANSKI, MD, PHD[2]
ANDRZEJ LEWINSKI, MD, PHD[3]
JERZY LOBA, MD, PHD[1]

From the [1]Department of Diabetology and Metabolic Diseases, Medical University of Lodz, Lodz, Poland; the [2]Department of General and Transplant Surgery, Medical University of Lodz, Lodz, Poland; and the [3]Department of Endocrinology and Isotope Therapy, Polish Mother's Memorial Hospital Research Institute, Medical University of Lodz, Lodz, Poland.

Address correspondence to Leszek Czupryniak, MD, PHD, Department of Diabetology and Metabolic Diseases, Barlicki University Hospital, No. 1, Ul, Kopcinskiego 22, 90-153 Lodz, Poland. E-mail: bigosik@poczta.onet.pl.

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Dysadipocytokinemia in Werner Syndrome and Its Recovery by Treatment With Pioglitazone

Werner syndrome (WS) (Mendelian Inheritance in Man no. 277700) is an autosomal recessive disorder known for progeroid phenotypes including graying and loss of hair, juvenile cataracts, insulin-resistant diabetes, skin atrophy, premature atherosclerosis, and cancer (1). Mutations in *WRN*, a RECQ family DNA/RNA helicase gene, have been identified to cause this disease. The mechanism for insulin resistance in WS remains to be elucidated.

Adipocytes secrete a number of hormones (or adipocytokines), such as tumor necrosis factor-α (TNF-α), leptin, adiponectin, and resistin, thereby regulating insulin sensitivity (2). WS patients typically show the lipoatrophic skinny extremities with an obese trunk (1). The accumulated intra-abdominal visceral fat (3) suggests an altered production of adipocytokines.

To investigate the role of adipocytokines in the pathophysiology of WS, we examined the serum levels of TNF-α and adiponectin in WS. Sera sampled from 24 WS patients (14 men and 10 women; 16 with and 8 without diabetes) proven to be homozygous for *WRN* mutations, and 40 age- and sex-matched normoglycemic healthy volunteers were assayed after informed consent was obtained. Age (43 ± 8.1 vs. 41.6 ± 7.5 years) and BMI (19.4 ± 1.9 vs. 18.8 ± 2.0 kg/m^2) were similar for diabetic and nondiabetic WS patients.

The serum level of TNF-α, a mediator of insulin resistance, was significantly elevated in WS regardless of having diabetes (21.8 ± 8.7 pg/ml, $P < 0.0001$ by Mann-Whitney test) or not having diabetes (14.0 ± 3.2 pg/ml, $P = 0.002$) compared with the healthy control group (6.05 ± 3.0 pg/ml). Adiponectin levels in diabetic WS patients (3.1 ± 2.9 μg/ml) was significantly lower than in nondiabetic WS patients (11.6 ± 9.2 μg/ml, $P = 0.006$) or control subjects (14.4 ± 8.8 μg/ml, $P < 0.0001$). The growing evidence indicates insulin sensitizing as well as antiatherogenic actions of adiponectin and the association of decreased serum adiponectin with insulin resistance, obe-

sity, and type 2 diabetes (2,4). Although WS patients are usually not obese by the definition of BMI, the visceral fat specifically accumulated by an unknown mechanism (3) might cause high TNF-α and low adiponectin levels, characteristics similar to morbid obesity.

We recently reported the successful improvement of glycemic control and insulin sensitivity by pioglitazone in diabetic WS patients (5). Therefore, we next assessed adipocytokines before and after 16 weeks on pioglitazone (15 mg/day) in three diabetic WS patients. The treatment significantly elevated adiponectin levels from 2.57 ± 1.36 to 7.07 ± 2.48 μg/ml ($P = 0.03$ by paired t test). TNF-α and HbA_{1c} levels showed a tendency to decline from 16.1 ± 4.75 to 3.53 ± 0.58 pg/ml ($P = 0.052$) and from 7.7 ± 0.6 to 6.4 ± 0.5% ($P = 0.17$), respectively.

To our knowledge, this is the first study to examine serum adipocytokine levels in WS patients. Reduced insulin sensitivity with increased visceral adiposity is the hallmark of both WS and normal aging. Because pioglitazone achieved improvement of glycemic control as well as correction of adiponectin and TNF-α levels, these cytokines are likely to be at least in part responsible for insulin resistance in WS. Adipocyte function may be a key element linking *WRN* mutation and the metabolic abnormalities observed in WS. It is also of our interest to know whether pioglitazone and other thiazolidinediones can prevent or delay the onset of diabetes in WS by modulating adipocytokines. Our present findings raise a possibility that pioglitazone could extend the lifespan of WS patients by improving metabolism and preventing early cardiovascular death.

KOUTARO YOKOTE, MD[1,2]
KAZUO HARA, MD[3]
SEIJIRO MORI, MD[1,2]
TAKASHI KADOWAKI, MD[3]
YASUSHI SAITO, MD[1,2]
MAKOTO GOTO, MD[4,5]

From the [1]Division of Endocrinology and Metabolism, Department of Internal Medicine, Chiba University Hospital, Chiba City, Japan; the [2]Department of Clinical Cell Biology, Chiba University Graduate School of Medicine, Chiba City, Japan; the [3]Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; the [4]Department of Rheumatology, Tokyo Metropolitan Otsuka Hospital, Tokyo, Japan; and the [5]Institute of Bioengineering, Toin Yokohama University, Yokohama, Japan.

Address correspondence to Koutaro Yokote, MD, Division of Endocrinology and Metabolism, Department of Internal Medicine, Chiba University Hospital, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan. E-mail: kyokote-cib@umin.ac.jp.

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Effect of α-Linolenic Acid-Containing Linseed Oil on Coagulation in Type 2 Diabetes

Blood coagulation in diabetes is known to be increased (1,2). Because levels of n-3 and n-6 polyunsaturated fatty acids (PUFAs) influence the parameters of blood coagulation, the aim of this study was to determine the effects of n-3 PUFA supplementation on coagulation and fibrinolytic factors in type 2 diabetic subjects. While it is not clear what the appropriate intake ratio of n-6 to n-3 PUFAs should be for diabetic subjects, it is known that the dietary intake ratio of n-6 to n-3 PUFAs is roughly 4:1 in Japanese subjects (3).

Ten subjects (six women and four men, average age 59.6 years) with type 2 diabetes participated in this study as inpatients. Their average BMI and HbA_{1c} values were 20.9 ± 3.8 kg/m^2 and 10.8 ± 1.1%, respectively. Their daily energy intake during the course of the study was 1,490 ± 166 kcal. After 2 weeks on the control diet, our subjects were placed on a diet in which 5 g linseed oil was added (in salads, miso soup, etc., without heating) in exchange for 5 g cooking oil. The ratio of PUFAs to saturated fatty acids in the subjects' prestudy and study diets were 1.2 and 1.6, respectively, while the ratios of n-6 to n-3 PUFAs in their prestudy and study diets were 3.6 and 1.5, respectively. Blood samples were collected before and 14 days after initiation of the study. Plasmin α2-plasmin inhibitor complex (PPI) level and plasminogen activator inhibitor-1 (PAI-1) activity in plasma was measured using a latex photometric immunoassay, while thrombin antithrombin III complex (TAT) level was measured using an enzyme-linked immunoassay. Differences in these parameters obtained at the start and end of the study were analyzed using a paired t test; values were considered to be significant if the P value was <0.05. Values are expressed as the mean ± SD.

After 2 weeks on a linseed oil–supplemented diet, PPI level, PAI-1 activity, and TAT level fell significantly (0.72 ± 0.19 vs. 0.47 ± 0.14 μg/ml, $P = 0.0009$; 73.3 ± 37.5 vs. 51.6 ± 25.0 ng/ml, $P = 0.02$; and 9.6 ± 9.1 vs. 2.5 ± 1.1 ng/ml, $P = 0.04$; respectively).

Boberg et al. (4) reported that PAI-1 activity was increased in type 2 diabetic subjects after supplementation of their diet with 10 g eicosapentaenoic acid. Kelly et al. (5) reported that a diet containing flaxseed oil (60% α-linolenic acid) did not alter indexes of blood coagulation, i.e., bleeding time, prothrombin time, and partial prothrombin time. Chan et al. (6) showed that altering the dietary n-6–to–n-3 PUFA ratio had no effect on

High Glucose-Induced Upregulation of Osteopontin Is Mediated via Rho/Rho Kinase Pathway in Cultured Rat Aortic Smooth Muscle Cells

Harukiyo Kawamura, Koutaro Yokote, Sunao Asaumi, Kazuki Kobayashi, Masaki Fujimoto, Yoshiro Maezawa, Yasushi Saito, Seijiro Mori

Objective—Osteopontin is upregulated in the diabetic vascular wall and in vascular smooth muscle cells cultured under high glucose concentration. In the present study, we analyzed the mechanism of high glucose-induced upregulation of osteopontin in cultured rat aortic smooth muscle cells.

Methods and Results—We found that an inhibitor of Rho-associated protein kinase, Y-27632, suppressed osteopontin mRNA expression under high glucose concentration. Transfection of cells with a constitutive active Rho mutant, pSRα-myc-RhoDA, enhanced osteopontin mRNA expression. Furthermore, incubation of cells under high glucose concentration activated Rho, indicating that Rho/Rho kinase pathway mediates high-glucose–stimulated osteopontin expression. Treatment of cells with an inhibitor of protein kinase C, GF109203X, and azaserine, an inhibitor of the hexosamine pathway, suppressed high glucose-induced Rho activation. Glucosamine treatment was shown to activate Rho. Treatment of cells with an inhibitor of MEK1, PD98059, suppressed osteopontin mRNA expression under high glucose concentration. Incubation of cells under high glucose concentration activated ERK. Finally, transfection of cells with pSRα-myc-RhoDA also activated ERK.

Conclusions—In conclusion, our present findings support a notion that Rho/Rho kinase pathway functions downstream of protein kinase C and the hexosamine pathways and upstream of ERK in mediating high-glucose–induced upregulation of osteopontin expression. (*Arterioscler Thromb Vasc Biol.* 2004;24:276-281.)

Key Words: osteopontin ■ Rho ■ glucose ■ atherosclerosis ■ smooth muscle cells

Osteopontin (OPN)[1] is a multifunctional phosphoprotein secreted by many cell types such as osteoclasts, lymphocytes, macrophages, epithelial cells, and vascular smooth muscle cells (SMC).[1,2] Overexpression of OPN has been found in several physiological and pathological conditions, including immunologic disorders,[3] neoplastic transformation,[4] progression of metastasis,[5] formation of urinary stones,[6] and wound healing.[7]

It was reported that OPN protein and mRNA were expressed in the neointima and in calcified atheromatous plaque.[8] A neutralizing antibody against OPN was found to inhibit rat carotid neointimal formation after endothelial denudation.[9] These results have suggested that OPN promotes the development of atherosclerosis. Recently, we found upregulation of OPN expression in diabetic human and rat vascular walls.[10] It was also noted that high glucose concentrations stimulated OPN expression via a protein kinase C (PKC)-dependent pathway and the hexosamine pathway in cultured rat aortic SMC.[11] Furthermore, OPN was found to stimulate migration and enhance platelet-derived growth factor-mediated DNA synthesis of cultured rat aortic SMC.[10] Based on these data, we suggest that OPN plays a role in accelerated atherogenesis in diabetes mellitus.

In the present study, we further analyzed the mechanism of high glucose-induced upregulation of OPN in cultured rat aortic SMC. We show that Rho/Rho kinase pathway functions downstream of PKC and the hexosamine pathways and upstream of ERK in mediating high glucose-stimulated OPN expression.

Methods

Reagents

GGTI-298, an inhibitor of geranylgeranyltransferase I, FTI-277, an inhibitor of farnesyltransferase, Y-27632, an inhibitor of Rho-associated protein kinase, GF109203X, an inhibitor of PKC, PD98059, an inhibitor of MEK1, SB203580, an inhibitor of p38 mitogen-activated protein (MAP) kinase, and SP600125, an inhibitor of c-Jun N-terminal kinase (JNK), were purchased from Calbiochem (La Jolla, CA). Azaserine, an inhibitor of glutamine:fructose-6-phosphate amidotransferase (GFAT) was from Sigma (St. Louis, MO). The p44/42 MAP kinase assay kit, p38 MAP kinase assay kit, and SAPK/JNK assay kit were from Cell Signaling Technology (Beverly, MA). Rho activation assay kit was from UBI (Lake Placid,

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From the Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, Inohana, Chiba, Japan

Correspondence to Koutaro Yokote Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chiba 260-8670, Japan. E-mail kyokote-cib@umin.ac.jp

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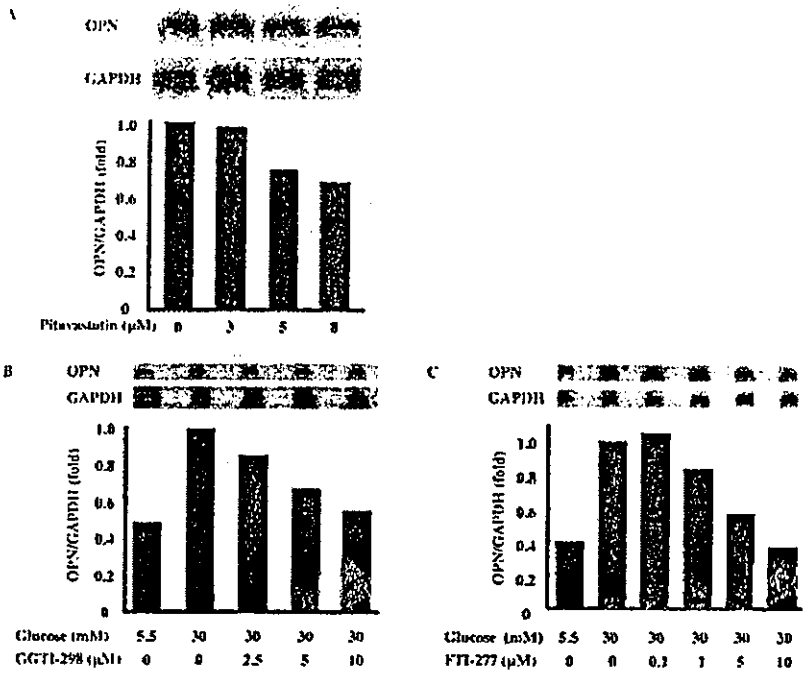

Figure 1. Effects of inhibitors for HMG-CoA reductase, geranylgeranyltransferase, and farnesyltransferase on OPN expression in cultured rat aortic SMC. After serum-starvation for 24 hours, cells were incubated with the indicated concentrations of Pitavastatin (A), GGTI-298 (B), or FTI-277 (C) in serum-free medium containing either 5.5 mmol/L or 30 mmol/L glucose for 48 hours. After incubation, cells were processed for Northern blotting with ^{32}P-labeled rat OPN and GAPDH cDNA probes. The level of OPN mRNA expression was estimated by the ratio of OPN signal to GAPDH signal. Data are expressed as fold increase relative to the value obtained in 30 mmol/L glucose without inhibitors. Data shown in this figure are representative of at least 2 independent experiments providing essentially similar results.

Rho/Rho Kinase Pathway Mediates High Glucose-Induced Upregulation of OPN Expression

It is well known that geranylgeranylation is prerequisite for Rho, a small GTP-binding protein, to exert its cellular function. Therefore, Rho seemed to be a possible candidate involved in mediating a positive signal for OPN expression. To evaluate a role of Rho, we first examined effect of an inhibitor of Rho-associated protein kinase, Y-27632, on high glucose-induced upregulation of OPN expression in cultured rat aortic SMC. As shown in Figure 2A, Y-27632 dose-dependently decreased OPN mRNA level under high glucose concentration, suggesting a critical role of Rho kinase activity in OPN expression.

Next, we examined effect of transient transfection of a constitutive active Rho mutant, pSRα-myc-RhoDA, on OPN expression in cultured rat aortic SMC. As shown in Figure 2B, transfection of pSRα-myc-RhoDA enhanced OPN mRNA expression in proportion to the efficiency of its transfection, confirming that Rho mediates a positive signal for OPN expression.

Finally, we examined effect of high glucose on Rho activation in cultured rat aortic SMC. As shown in Figure 2C, the amount of GTP-Rho in cells cultured in 30 mmol/L glucose was found to be much higher than that in 5.5 mmol/L glucose. No difference was found in total Rho protein levels between 5.5 mmol/L glucose and 30 mmol/L

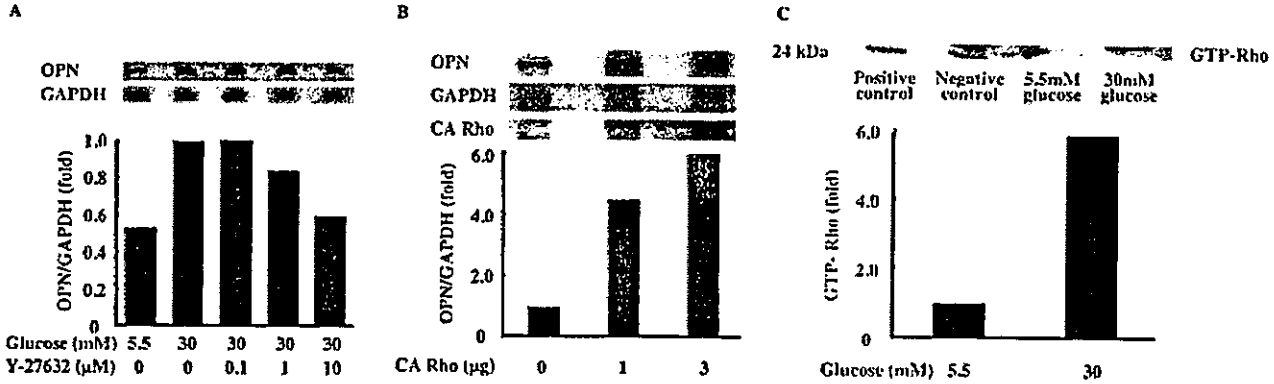

Figure 2. A, Effect of a Rho kinase inhibitor on OPN expression in cultured rat aortic SMC. After serum-starvation for 24 hours, cells were incubated with the indicated concentrations of Y-27632 in serum-free medium containing either 5.5 mmol/L or 30 mmol/L glucose for 48 hours. After incubation, cells were processed for Northern blotting as described in the legend to Figure 1. B, effect of transient transfection of a constitutive active Rho mutant (CA Rho) on OPN expression in cultured rat aortic SMC. At 50% confluency, cells were transfected with 1 to 3 μg of pSRα-myc-RhoDA and incubated for 48 hours, as described in Methods. After incubation, cells were processed for Northern blotting. The blots were re-proved with ^{32}P-labeled Rho cDNA probe to estimate the efficiency of transfection. Data are expressed as fold increase relative to the value obtained in the absence of CA Rho. C, High glucose-induced Rho activation in cultured rat aortic SMC. After serum-starvation for 24 hours, cells were incubated in serum-free medium containing either 5.5 mmol/L or 30 mmol/L glucose for 24 hours. After incubation, GTP-Rho in cell lysates was adsorbed to GST-Rhotekin Rho-binding domain and subjected to Western blotting with an anti-Rho antibody. Data are expressed as fold increase relative to the value obtained in 5.5 mmol/L glucose. Data shown in this figure are representative of at least 2 independent experiments providing essentially similar results.

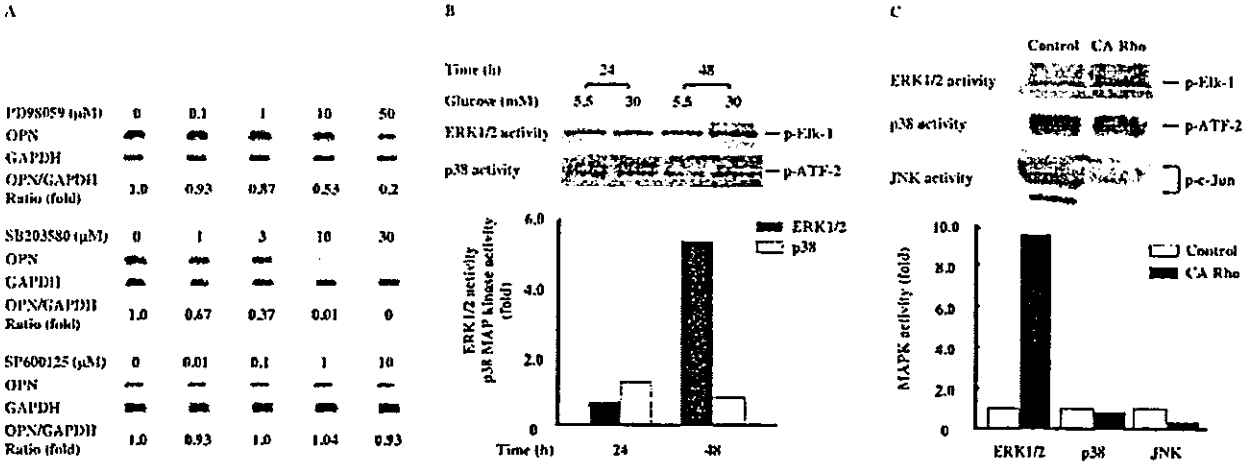

Figure 4. A, Effects of MAP kinase inhibitors on OPN expression in cultured rat aortic SMC. After serum-starvation for 24 hours, cells were incubated with the indicated concentrations of PD98059, SB203580, or SP600125 in serum-free medium containing 30 mmol/L glucose for 48 hours. After incubation, cells were processed for Northern blotting as described in the legend to Figure 1. B, High glucose-induced ERK activation in cultured rat aortic SMC. After serum-starvation for 24 hours, cells were incubated in serum-free medium containing either 5.5 mmol/L or 30 mmol/L glucose for 24 to 48 hours. After incubation, activities of ERK1/2 and p38 MAP kinase in cell lysates were measured by immune complex kinase assay with an immobilized phospho p44/42 MAP kinase antibody and Elk-1 protein as substrate, or with an immobilized phospho p38 MAP kinase antibody and ATF-2 protein as substrate, respectively. After phosphorylation reactions, samples were processed for Western blotting with phospho Elk-1 antibody or phospho ATF-2 antibody. Data are expressed as fold increase relative to the value obtained in 5.5 mmol/L glucose at the indicated times. C, Effect of transient transfection of a constitutive active Rho mutant (CA Rho) on activation of MAP kinases in cultured rat aortic SMC. Cells were transfected with 3 μg of pSRα-myc-RhoDA and incubated for 48 hours as described in the legend to Figure 2. After incubation, MAP kinase activities in cell lysates were determined. Data are expressed as fold increase relative to the value obtained in the absence of CA Rho. Double bands in the JNK activity assay correspond to 37- and 35-kilodalton forms of phosphorylated c-Jun fusion proteins. Data shown in this figure are representative of at least 2 independent experiments providing essentially similar results.

UTP-induced OPN increase and migration, demonstrating the central role of OPN in this process. The finding, together with our present observation, underscores the importance of Rho in OPN expression.

Our present finding that high glucose induces Rho activation sheds new light on the mechanism of the accelerated atherogenesis in diabetes mellitus, because involvement of Rho/Rho kinase pathway has been implicated in a wide variety of atherosclerotic processes, including neointimal formation,[15] vasospastic response,[16,17] proliferation,[18,19] migration,[19,20] and anti-apoptosis[20,21] of vascular SMC, and vascular gene expression of monocyte chemoattractant protein-1,[22] transforming growth factor-β1,[22] and inducible nitric oxide synthase.[23] Besides our present study using rat aortic SMC, high glucose-induced Rho activation was also observed in cultured rat mesangial cells[24] and in basilar artery derived from streptozotocin-induced diabetic rats.[25] It is thus conceivable that high glucose promotes diabetic vascular complications not only by upregulation of OPN but also by more diverse effects resulting from Rho activation.

It was reported that transfection of vascular SMC with the c-Ha-rasEJ oncogene induced overexpression of OPN.[26] It is well known that farnesylation is prerequisite for Ras to exert its cellular effect; therefore, our present finding that the inhibitor of farnesyltransferase, FTI-277, suppressed OPN expression might be ascribed to the inhibition of Ras function by the drug. In our previous study, however, the inhibitory effect of Pitavastatin on OPN expression in cultured rat aortic SMC was almost completely reversed by the addition of mevalonate or geranylgeranylpyrophosphate but not by farnesylpyrophosphate.[13] Studies using other types of cells, fibroblasts,[27] or keratinocytes[28] showed that transfection of dominant-negative Rho or dominant-negative Rac suppressed Ras-induced activation of Raf-MEK-ERK pathway, indicating that Ras requires either Rho or Rac function in activation of Raf-MEK-ERK pathway. Based on these findings, it is speculated that the inability of farnesylpyrophosphate to rescue the cells from the inhibition of OPN expression by Pitavastatin might be caused by suppression of Rho family function in Pitavastatin-treated cells. Further study is necessary to prove this possibility.

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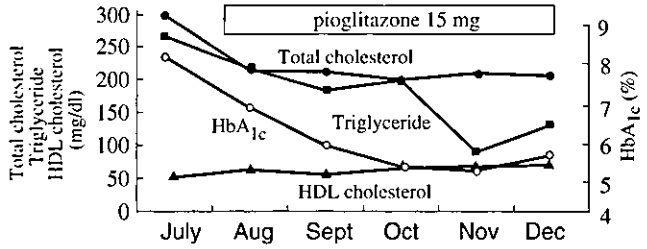

Fasting serum insulin (μU/ml)	45.5	13.0
Insulin sensitivity index	19.5	24.9
Visceral fat area (cm^2)	111.6	104.3
Subcutaneous fat area (cm^2)	162.5	218.5

Fig. 1 Metabolic parameters and abdominal fat areas before and during pioglitazone treatment. HDL = high-density lipoprotein; Hb = hemoglobin.

METABOLIC IMPROVEMENT AND ABDOMINAL FAT REDISTRIBUTION IN WERNER SYNDROME BY PIOGLITAZONE

To the Editor: Werner syndrome is a rare autosomal recessive disorder known for its premature aging phenotype including loss of hair, cataracts, atrophy of peripheral soft tissue, diabetes mellitus, and atherosclerosis. Mutations in the deoxyribonucleic acid (DNA) helicase gene have been identified as the cause of this disease.[1] One common feature of Werner syndrome is insulin resistance, but the mechanism by which insulin resistance occurs in this syndrome is unknown. We have previously described that visceral fat accumulation is strongly associated with insulin resistance in Werner syndrome.[2] We report a case of Werner syndrome in which administration of pioglitazone, a thiazolidinedione derivative, improved insulin sensitivity, glucose tolerance, lipid metabolism, and abdominal fat distribution.

A 46-year old woman with Werner syndrome came to our hospital for glycemic control. After obtaining written informed consent, we analyzed genomic DNA from peripheral leukocytes, which revealed that the patient was homozygote for type 4 mutation in the Werner helicase gene.[3] She was thin (body mass index = 16.5 kg/m^2) but had accumulated visceral fat in excess, as determined using a computed tomography scan at the umbilical level (visceral fat area = 111.6 cm^2, normal range for Japanese women <90).[4] She also had type IIb hyperlipidemia according to World Health Organization classification. She had significant insulin resistance, as determined using an insulin sensitivity index calculated from the value of steady state plasma glucose (19.4, normal range 55–162).[5] After 1 week of treatment with diet, pioglitazone 15 mg daily was initiated. After 16 weeks of pioglitazone treatment, the patient's fasting plasma glucose had decreased from 198 mg/dL to 115 mg/dL, glycated hemoglobin A1c from 8.4% to 5.9% (normal = 5.9% or less), serum total cholesterol from 270 mg/dL to 209 mg/dL (normal = 130–220 mg/dL), serum triglyceride from 301 mg/dL to 90 mg/dL (normal = 80–150 mg/dL), and serum high-density lipoprotein-cholesterol increased from 52 mg/dL to 64 mg/dL (normal $\geq$40 mg/dL). Fasting serum insulin decreased from 45.5 μU/mL to 13.0 μU/mL (normal = 6–26 μU/mL), and insulin sensitivity index had improved to 24.9 (Figure 1, July to November). Although the patient gained weight, from 35.9 kg to 39.0 kg, during the period, her visceral fat area (V) decreased to 104.3 cm^2. In contrast, abdominal subcutaneous fat area (S) increased from 162.5 cm^2 to 218.5 cm^2. As a result, her V/S ratio decreased from 0.69 to 0.48 (normal range for Japanese

<0.4).[4] Liver function monitored using serum transaminase level did not show abnormality throughout the period.

These results suggest that pioglitazone was effective in ameliorating impaired insulin sensitivity, glycemic control, and hyperlipidemia in the patient. Human and animal studies have shown that a possible mechanism for thiazolidinedione to improve insulin sensitivity is through the specific promotion of subcutaneous adipocyte differentiation through the activation of peroxisome proliferator-activated receptor-γ.[6] It has also been reported that troglitazone-treatment of type 2 diabetic patients resulted in subcutaneous fat increase in accordance with improvement of glucose tolerance.[7] It was also proven experimentally that, in lipoatrophic diabetes mellitus, lack of fat is directly associated with insulin resistance and hyperglycemia.[8] Marked atrophy of soft tissues in the extremities, a characteristic feature of Werner syndrome, may at least in part account for the insulin resistance. Leptin administration was recently reported to ameliorate severe insulin resistance in leptin-deficient lipodystrophic patients,[9] but in our patient, serum leptin levels were in the normal range before and during the pioglitazone treatment (data not shown). Therefore, in this case, induction of subcutaneous fat using pioglitazone would have accompanied production of another mediator than leptin to improve insulin sensitivity.

Recently, accumulating evidence suggests that thiazolidinedione has direct antiatherosclerotic effects on vascular cells.[10] Because atherosclerotic vascular disease is a leading cause of middle-age mortality in Werner syndrome, pioglitazone may provide an ideal choice for the treatment of metabolic disorders to improve prognosis of this syndrome.

Koutaro Yokote, MD
Satoshi Honjo, MD
Kazuki Kobayashi, MD
Masaki Fujimoto, MD
Harukiyo Kawamura, MD
Seijiro Mori, MD
Yasushi Saito, MD
Second Department of Internal Medicine
Chiba University Hospital
Chiba, Japan

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耐糖能障害

—基礎・臨床研究の最新情報—

V. 臨床的事項

耐糖能異常(IGT)・境界型で発症・進展する病態・疾患

動脈硬化

横手幸太郎　　齋藤　康

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耐糖能異常(IGT)・境界型で発症・進展する病態・疾患

動脈硬化

Atherosclerosis

横手幸太郎[1]　齋藤　康[2]

Key words：食後高血糖，メタボリック症候群，インスリン抵抗性，心血管イベント，IMT

1. 概　　念

糖尿病が動脈硬化の独立した危険因子であることは，広く認知された事実である．糖尿病患者が冠動脈イベントを発症するリスクは，非糖尿病者に比べて男性で約2倍，女性では4倍高くなると報告されている[1]．すなわち動脈硬化は糖尿病の重要な合併症の一つであり，腎症・網膜症・神経障害などのいわゆる'細小血管症'に対して'大血管症'とも呼称される．

一方，'前糖尿病状態'に相当する'耐糖能異常'群においても，正常耐糖能の人々に比べると動脈硬化の進みやすいことが明らかとなり，近年，この範疇に該当する人口が世界的に急増していることもあって注目されている．本稿では糖尿病には至らない耐糖能障害(主としてIGT)を便宜上，耐糖能異常と定義し，耐糖能異常と動脈硬化，特に冠動脈性心疾患(coronary heart disease: CHD)との関連について，これまでに得られている主な臨床的知見を解説する．

2. 分　　類

a. 耐糖能異常と動脈硬化

Coutinhoらは，非糖尿病者の血糖値と心血管リスクとの関連に着目して，1966-96年の30年間に発表された20の臨床研究の成績をメタ解析している[2]．その結果によると，空腹時血糖値が110 mg/dlの場合は75 mg/dlの人に比べて心血管リスクが約1.3倍，経口ブドウ糖負荷後2時間の血糖値が140 mg/dlでは75 mg/dlに比べて約1.6倍それぞれ上昇しており，糖尿病と診断されない人々の中でも血糖の上昇が心血管イベント発症のリスクとなることが示された．我が国でも，久山町研究において同様の結論が導かれている．すなわち，住民2,427人を対象に75 g経口ブドウ糖負荷試験(75 gOGTT)を施行の後5年間の追跡調査を行ったところ，正常耐糖能群に比べて糖尿病群ではCHDと脳卒中を合わせた心血管イベントの発症リスクが約3倍，耐糖能異常群においても約1.9倍有意に上昇していた[3]．つまり，耐糖能異常は人種差を越えて動脈硬化の進展要因となることをこれら疫学研究の成績は示している．

b. 食後高血糖と動脈硬化

アメリカ糖尿病学会および世界保健機関(WHO)により提唱された糖尿病の診断基準では，耐糖能異常を①75 gOGTTの2時間血糖値が140-199 mg/dlで食後血糖値や随時血糖値が正常と糖尿病の境界域に属する'impaired glucose tolerance(IGT)'と，②早朝空腹時血糖値110-125 mg/dlだが2時間血糖値は正常範囲にある'impaired fasting glucose(IFG)'とに分類し

[1]Koutaro Yokote: Division of Endocrinology and Metabolism, Department of Internal Medicine, Chiba University Hospital　千葉大学医学部附属病院糖尿病・代謝・内分泌内科　[2]Yasushi Saito: Department of Clinical Cell Biology, Chiba University Graduate School of Medicine　千葉大学大学院医学研究院細胞治療学

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表1 高血糖とCHD/予後の関係について報告した主な前向き研究のまとめ(発表年順に記載)

研究名(論文)	対象人数	追跡期間	主な結果
Framingham Study[1]	男女3,476人, 45-84歳	10年	随時血糖の高値が女性ではCHDの発生予測に有効
Helsinki Policemen Study (Diabetes Care 2: 131-141, 1979)	男性4,326人, 30-59歳	4-5年	負荷後1および2時間血糖値がCHDの発生と単変量解析でのみ相関
Whitehall Study (Br Med J 287: 867-870, 1983)	男性18,000人, 40-64歳	10年	食後血糖値が高値を示す上位5%で心血管イベントの発生が増加
Paris Prospective Study (Horm Metab Res 15[Suppl]: 41-46, 1985)	男性約7,000人	10年	空腹，食後を問わず，血糖・インスリン高値の上位20%で心血管イベント増加
Chicago Heart Study (Am J Epidemiol 123: 504-516, 1986)	約18,000人	9年	ブドウ糖負荷後1時間血糖値が9年間の総死亡を予測
Honolulu Heart Study (Diabetes 36: 689-692, 1987)	日系男性8,006人, 45-70歳	20年以上	負荷後1時間血糖値が致死性・非致死性CHDの発生リスクと関連
British Regional Heart Study (J Epidemiol Community Health 48: 538-542, 1994)	男性7,735人, 40-59歳	9.5年	血糖高値上位20%でCHD発生が増加した
Hisayama Study[3]	日本人男女2,427人, 40-79歳	5年	正常耐糖能者に比べIGT者でCHDリスクが上昇
The Rancho Bernardo Study[4]	男女1,858人, 50-89歳	7年	負荷後2時間血糖の高値が女性において致死性心血管イベントの発生と相関
The Funagata Diabetes Study[5]	日本人男女2,016人, 58.8±10.6歳	7年	IGTおよび糖尿病患者で心血管死が増加したが，IFGはリスクとならなかった
DECODE Study[6]	30歳以上の男女 25,362人	7.3年	負荷後2時間血糖の高値が総死亡と相関したが，空腹時血糖との関連はなし

ている．近年，この差異に着目した前向き研究が国内外で相次いで行われ，その結果，約7年の追跡期間でIGTは総死亡および心血管死のリスクを1.5-2.9倍増加させるが，IFGにおけるリスクは正常耐糖能者と変わらないことが明らかとなった[4-6]．すなわち動脈硬化促進因子としては，'食後'の高血糖こそが重要であるとの考え方が確立しつつある．

もっともIFGも，将来IGTや2型糖尿病へと移行する可能性があるため，その経過を通じて動脈硬化の発症と関連することが予想される．したがって動脈硬化リスクとしてのIFGの臨床的意義の解明には，より長期間の観察に基づいた研究の集積が必要と考えられる．

高血糖とCHD/予後に関してこれまでに報告された主な前向き研究のまとめを**表1**に示す．

3. 病因と病態：食後高血糖かメタボリック症候群か？

耐糖能異常がなぜ動脈硬化の進展をもたらすのか，その機序はまだ十分には解明されていない．現時点では，①先にも触れた'食後高血糖'と②インスリン抵抗性を背景にリスクの重積を招く'メタボリック症候群'の役割が重要視されている．

a. 食後高血糖

食後の高血糖が心血管イベントの発症と関連することは既に述べたとおりである．また，動脈硬化の進展度をベッドサイドで客観的に評価する一手段として頸動脈壁の内膜・中膜厚(intima-media thickness: IMT)を測定する方法が普及しているが，75gOGTT 2時間時点でみら

表2 メタボリック症候群の定義

[WHOによる定義[11]]
1) IGTまたはインスリン抵抗性を示す糖尿病の存在
2) 以下の①から④のうち2項目を満たす：
① 血圧値>140/90 mmHgまたは降圧薬内服
② 血漿トリグリセリド値>1.7 mmol/l(>149 mg/dl)かつ/または男性でHDLコレステロール<0.9 mmol/l(<35 mg/dl)，女性でHDLコレステロール<1 mmol/l(<39 mg/dl)
③ 中心性肥満：ウエスト-ヒップ比が男性で>0.9，女性で>0.85，かつ/またはBMI*>20
④ 微量アルブミン尿：尿中アルブミン排泄≧20 μg/minまたは尿中アルブミン/クレアチニン比≧20

[ATP III (Adult Treatment Panel III)による定義[12]]
以下の①から⑤のうち3項目を満たす：
① 空腹時血糖≧6.1 mmol/l(110 mg/dl)
② 血圧値≧130/85 mmHgまたは降圧薬内服
③ 血漿トリグリセリド値>1.71 mmol/l(150 mg/dl)
④ HDLコレステロール値が男性で<1.0 mmol/l(40 mg/dl)，女性で<1.3 mmol/l(50 mg/dl)
⑤ 中心性肥満：腹囲が男性で>102 cm(49 in)，女性で>88 cm(35 in)**

*BMI: body mass index，体重(kg)/[身長(m)]^2
**参考：日本人の場合は男性で>85 cm，女性で>90 cmが内臓脂肪蓄積の指標とされる．

れる高血糖とIMT値の上昇との相関が，これまで横断的および前向き研究により明らかにされている[7,8]．食後高血糖が動脈硬化を促進するメカニズムについては，血糖の上昇に伴う酸化ストレスの増大がその中心的役割を担うと考えられている[9]．更にLDLの酸化促進，第VII凝固因子の活性化や易血栓形成などと相まって血管内皮細胞の機能障害をもたらし，動脈硬化の発症・進展を導くと考えられている．

b. メタボリック症候群

耐糖能異常を有する人は，高トリグリセリド血症や低HDLコレステロール血症などの脂質代謝異常，それに高血圧など他の危険因子をも合併することが多い．その背景要因として内臓型肥満とインスリン抵抗性が重要であること，そしてこれらが炎症機転や内皮機能障害を通じて動脈硬化の進展と深くかかわることが最近話題となっている．この病態は，Reavenがシンドローム X[10]として提唱して以来様々な言葉で語られてきたが，近年メタボリック症候群(metabolic syndrome)として統一された(表2)[11,12]．メタボリック症候群の発症には，加齢，肥満，運動不足，そして遺伝的素因が関与すると考えられており，脂肪細胞に由来してインスリン感受性の制御にかかわる様々なアディポサイトカインの分泌異常がその病態形成に重要と指摘されている．このように，耐糖能異常がメタボリック症候群の一表現型として現れる場合には，ほかに合併する複数の危険因子もまた動脈硬化の進展に寄与すると考えられる．

4. 診　　断

耐糖能異常における非侵襲的な動脈硬化検査法として，前述の頸動脈IMT測定がよく用いられる．これは7.5-10 MHzの端子を用い，総頸動脈壁の内膜と中膜の厚みの総和を計測するものである．Yamasakiらは，食後高血糖を認めるが糖尿病ではない人々112人を75 g OGTT 2時間血糖値が7.8 mmol/l(約140 mg/dl)以上の者(IGT群)と6.7 mmol/l(約120 mg/dl)以上，7.7 mmol/l以下の者(non-IGT群)とに分け，2型糖尿病患者211人ならびに健常コントロール55人とそれぞれ頸動脈IMTを比較した[7]．すると耐糖能異常の群ではnon-IGT，IGTにかかわらず，コントロールに比べ有意にIMTが高値を示し，糖尿病患者との間には有意差がみられなかったとしている．

またHanefeldらは，1年間に頸動脈IMTが増加する割合は，健常者で0.007-0.008 mm，2型糖尿病患者で0.02 mm，IGTでは0.013 mmであ