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The effect of ezetimibe on serum lipids and lipoproteins in patients with homozygous familial hypercholesterolemia undergoing LDL-apheresis therapy

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Abstract

LDL-apheresis is now commonly used as the only practical treatment for homozygous familial hypercholestreolemia (homozygous FH). However, even when applying apheresis therapy, the use of a drug or drugs is recommended to suppress the rapid rebound of cholesterol, which usually takes place after each apheresis procedure, and keep the LDL-cholesterol level within or near the optimal range for as long as possible. In this study, the usefulness of ezetimibe, a novel cholesterol-lowering drug, in enhancing the efficacy of apheresis therapy was evaluated in six Japanese patients with homozygous FH undergoing LDL-apheresis in combination with atorvastatin or simvastatin. With the exception of one patient, significant decreases in LDL-cholesterol at 2 weeks after each apheresis procedure were obtained during the period from 4 to 12 weeks of treatment, with an average reduction rate of 9.0% and a range of 4.3–12.6%. This corresponds to a suppression of rebound by approximately 36 mg/dl, from 391 to 355 mg/dl on average, in LDL-cholesterol values. Although the effect is not very strong, ezetimibe nevertheless appears to be a useful drug in combination with statins for those with homozygous FH undergoing LDL-apheresis.

Keywords: LDL-apheresis; Homozygous familial hypercholesterolemia; Ezetimibe; Treatment of hyperlipidemia; Combination therapy of familial hypercholesterolemia

1. Introduction

Most cases of heterozygous familial hypercholesterolemia (heterozygous FH) can now be adequately treated with a combination drug therapy including hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) as the first choice drug [1]. In contrast, homozygous FH still remains an intractable disease due to its resistance to drug therapy [2], and LDL-apheresis is currently the only practical way of treating such patients [3–5]. However, as the severe rebound of cholesterol that occurs after each apheresis procedure diminishes the effect of this therapy [5], there is a need for suitable agents that can suppress such an acute rebound. Recently a novel cholesterol-lowering drug with a particular mechanism of blocking the cholesterol absorption at the brush border of the intestine has been developed [6,7]. Because the functional site of ezetimibe is different from LDL-receptors, it was expected that the drug would be effective in lowering cholesterol in homozygous FH. A

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report from a clinical trial carried out in America, Europe and South Africa showed that the drug lowered LDL-cholesterol almost to the same extent as in patients with ordinary primary hypercholesterolemia; a 20.5% additional reduction in patients under treatment with atorvastatin or simvastatin at a dose of 80 mg/day with a consistent reduction in LDL-cholesterol being obtained regardless of LDL-apheresis [8]. However, the patients in the above trial were not stratified by LDL-apheresis. Furthermore, there is a wide rage of geneotype in FH. Previous studies have shown that there is a large variation in the efficacy of atorvastatin among patients with homozygous FH undergoing LDL-apheresis [9,10]. In this study, we therefore tried to see if ezetimibe would be useful in suppressing the rebound of cholesterol in patients with homozygous FH of the receptor-negative type under LDL-apheresis treatment combined with statin therapy.

2. Subjects and methods

2.1. Patients

Six homozygous FH patients undergoing LDL-apheresis treatment every 2 weeks were enrolled in the study (Table 1). All patients had receptor-negative phenotype almost completely lacking the LDL affinity binding to fibroblasts or mononuclear cells. Among the six patients, Cases 3–1 and 4–1 were sisters and Cases 2–1 and 2–2 were brother and sister. The original total cholesterol level at the first visit to hospitals or lipid clinics was 695 ± 115 mg/dl, and the average total cholesterol level before each apheresis procedure was 373 ± 59 mg/dl. Atorvastatin was used at a dose of 10–40 mg/day (10 mg dose in Case 2–1, 20 mg dose in Cases 3–1, 3–2 and 4–1, 40 mg dose in Case 2–2), while simvastatin was prescribed in Case 5–1 at a dose of 10 mg/day. Probucol was used in combination in 4 cases at a dose of 500 or 1000 mg/day (500 mg/day in Cases 3–2 and 5–1, 1000 mg/day in Cases 3–1 and 4–1). Ezetimibe was given at a dose of 10 mg/day after a meal once every day for 12 weeks.

2.2. LDL-apheresis

LDL-apheresis was carried out using the affinity chromatography technique with a dextran sulfate–cellulose column (Liposorber System MA-01; Kaneka Corporation; Osaka) (two patients) or the double membrane filtration technique (KM 8500 or 8800 equipped with EVAL-4A new type, Kuraray Co., Osaka, or Plasauto 1000 equipped with Plasmaflo, Asahi Medicals, Tokyo) (three patients) [11]. A consistent volume of blood plasma (3000–6000 ml depending on the individual patient) was treated at each procedure. Heparin was regularly used as an anticoagulant.

2.3. Therapy schedule

For the first 4 weeks of prechallenge observation, each patient remained on their conventional apheresis regimen including any drug(s) prescribed. They were asked to keep a regular life style during the course of prechallenge observa-

Table 1
Patient demographies

Patient no.	Sex, age	LDL receptor activity[a] (%)	TC[b] at first visit	Complications	Regular anti-lipidemic agents	Other cardiovascular drug(s)	LDL-C[c] at the start of the study period
2–1	F, 26	Negative (0)	806	IHD	Atorvastatin, 10 mg	–	423
2–2	M, 28	Negative (0)	822	Hypertension, IHD, aortic stenosis	Atorvastatin, 40 mg	Ca-channel blocker ARB β-blocker	421
3–1	F, 63	Negative (0)	748	Hypertension, stable angina, aortic steno-regurgitation, carotid artery stenosis, aortic aneurysm, cervical spondylosis	Atorvastatin, 20 mg, probucol, 1000 mg, eicosapentaemic acid (EPA), 1200 mg	Ca-channel blocker nitrate aspirin	418
3–2	F, 23	Negative (0)	672	–	Atorvastatin, 20 mg, probucol, 500 mg	–	282
4–1	F, 66	Negative (9)	661	Angina pectoris, cataract, carotid artery stenosis	Atorvastatin, 20 mg, probucol, 1000 mg, EPA, 1800 mg	Nicorandil β-blocker aspirin	325
5–1	M, 24	Negative (0)	700	Hypertension, stable angina, arteriosclerotic retinopathy, aortic steno-regurgitation	Simvastatin, 10 mg, probucol, 500 mg	Nitrate β-blocker aspirin	370

[a] Activity on lymphocytes: % of the average value of normal individuals.
[b] Total serum cholesterol (mg/dl).
[c] Low density lipoprotein cholesterol (mg/dl).

tion and the treatment (ezetimibe) period. After the prechallenge observation period, patients were given ezetimibe at a constant dose of 10 mg/day for 12 weeks. Compliance with drug administration was checked by the attending physician each time patients visited the clinic. This was almost 100%, except for one patient (Patient 5–1), who forgot to take the drug occasionally (5 days in total) during the period from the 7th to the 11th week. The compliance rate in this patient over the whole study period was 93.5%.

2.4. Lipoprotein and lipid analysis

Serum or plasma lipid values (total cholesterol:TC, HDL-cholesterol:HDL-C, triglycerides:TG and LDL-cholesterol:LDL-C) were measured at the Hachioji Laboratory of SRL Teijin Laboratories (Tokyo), where the measurement of the major lipid components was controlled indirectly by CDC through the Lipid Reference Laboratory, Osaka Medical Center for Health Science and Promotion [12]. TC and TG were measured enzymatically. HDL-cholesterol and LDL-cholesterol were measured by a newly developed homogeneous method applying an enzymatic–colorimetric assay in the presence of specific detergents, Cholestest HDL [13] and Cholestest LDL [14]. Apolipoproteins were measured turbidimetrically using corresponding antibodies [15].

Serum lipid levels were measured before the start and at the end of each apheresis procedure. Values obtained before each apheresis procedure (2 weeks after the previous treatment) were used to evaluate the effect of ezetimibe.

Defects in LDL-receptors in our patients were estimated by measuring the receptor activity on blood monocytes by flow-cytometric assay [16].

2.5. Monitoring adverse effects

Adverse events were monitored during the whole trial period. Clinical signs, symptoms and laboratory data (biochemical measurements of plasma components, hematology and urinalysis) were checked at least every 4 weeks.

2.6. Statistical analysis

The average, standard deviation and the confidence interval (CI) for the percent change (%) from before administration of ezetimibe to the measurement times at 2, 4, 6, 8, 10 and 12 weeks after initiation of the treatment period were calculated. Over-time changes in the measurements at the above time points were analyzed according to the mixed effect model of repeated measurements, where the effect measured by time was defined as fixed effect and the effect in individual subjects as variate effect. If the by-time effect was significant, comparison between before administration and the effect at subsequent time points was tested by the Dunnett's method.

3. Results

3.1. Validity of samples

In one of the patients (Patient 3–2), no data were judged as satisfying the validation criteria due to the irregularity of the apheresis procedure (intervals and treated blood volume) and also inconsistencies in dietary habits during the summer season. Accordingly, the data from this patient were excluded in the analysis of the efficacy, but were included in the analysis of adverse effects. Treatment conditions were also inconsistent in another patient (Patient 5–1) and only data at 6 weeks were judged to be valid. In Patient 3–1, data at 12 weeks were excluded due to the same reason.

3.2. Changes in serum lipid levels

Pretreatment levels of serum lipids and changes in TC, LDL–C, HDL–C and TG in five patients are summarized in Table 2.

LDL-C levels, the primary endpoint of this study, showed significant decreases in all of the five patients for whom efficacy data were available. They were analyzed by use of a mixed effect model of repeated measurements, and the point of measurement was found to have significant effect. Following this finding, the baseline measurement was compared with subsequent measurements by the Dunnett's method. Only one patient (Patient 3–1) showed a remarkable reduction in LDL-C at 2 weeks of the ezetimibe treatment, while in the other four patients, the reduction rates obtained at 2 weeks were remarkably smaller than the values obtained from the later weeks of the treatment; the difference was statistically significant by Dunnett's procedure. The average reduction rates in the period from 4 to 12 weeks of treatment in individual patients were 4.3–12.6% with an average of 9.0% for all patients (Table 3).

Table 2
Changes in LDL-cholesterol, total cholesterol, triglycerides and HDL-cholesterol following treatment with ezetimibe

	LDL-cholesterol	Total cholesterol	Triglycerides	HDL-cholesterol
Pretreatment level (mg/dl)	391.5 ± 43.4	474.5 ± 67.4	107.3 ± 52.8	30.7 ± 8.6
On completion of treatment (mg/dl)	354.6 ± 47.4	432.5 ± 79.2	113.6 ± 61.4	28.3 ± 7.5
Percent change (%)	−9.57	−9.07	18.78	−7.58
95% CI	−14.11 ~ −5.03	−17.43 ~ −0.72	−42.51 ~ 80.06	−18.98 ~ 3.82

Average ± standard deviation (five patients).

Table 3
Percent changes in LDL-cholesterol in individual patients during treatment with ezetimibe

Treatment period	Patient no.				
	2–1	2–2	3–1	4–1	5–1
2 weeks	7.48	−3.80	−15.62	−2.67	–
4 weeks	−5.51	−4.99	−9.88	−10.06	–
6 weeks	−3.15	−7.13	−8.21	−8.52	−10.0
8 weeks	−12.36	−9.74	−10.60	−14.68	–
10 weeks	−3.15	−1.43	−21.83	−10.37	–
12 weeks	2.76	−12.59		−11.29	–
Average during the period from 4 to 12 weeks	−4.28	−7.18	−12.63	−10.98	−10.0

The level of LDL-C in five patients before the start of the ezetimibe treatment was 391 ± 43 mg/dl on average, while the value at the end was 355 ± 48 mg/dl, the rate of change being −9.6% with a 95% confidence interval ranging between −14.1 and −5.03%. The rebound of LDL-C after an apheresis procedure (increase in LDL-C 2 weeks after completing an apheresis procedure) was calculated in three of the five patients and the values were −17, −29 and −57 mg/dl. One of our patients (Case 2–2) was administered atorvastatin 40 mg/day (maximum dose approved for Japanese). However, the effect of ezetimibe (7.2% reduction in LDL-cholesterol) was no more remarkable than the other patients who were given a smaller dose of atorvastatin or simvastatin).

TC values also showed significant decreases, with a pretreatment average value of 475 ± 68 mg/dl and an average value at the end of the treatment in individual patients of 433 ± 79 mg/dl (−9.1%). TG values did not show any remarkable changes. HDL-C showed a tendency to decrease, although the difference from the pretreatment level was not statistically significant; 7.6% reduction with a 95% confidence interval ranging between −19.0 and +3.8% (Table 2). The change in HDL-C showed the same pattern as LDL-C, with the reduction rate at 2 weeks being much less than that in later weeks and this difference was shown to be statistically significant by the Dunnett's procedure.

None of the other lipid parameters, VLDL-C, RLP-C, PL, FFA, apolipoproteins A-I, A-II, B, C-II, C-III, E and Lp(a), showed remarkable changes.

3.3. Adverse effects

Adverse effects occurring in more than one of the six patients following treatment with ezetimibe included nausea (three patients), and fatigability, cough and albuminuria (two patients). Adverse effects, whose relation to the drug could not be denied, were found in three patients; nausea 1, fatigability 2, cough 1, anorexia 1, increase in liver enzymes (AST and ALT) 1 and albuminuria 1. All the adverse effects were slight to mild. There were no remarkable changes in vital signs.

4. Discussion

Ezetimibe is a unique cholesterol-lowering drug, that exerts its function by inhibiting the absorption of cholesterol at the brush border of the intestine with Niemann-Pick C1 like 1 protein as a target of its action [17]. Clinical studies have shown that the drug is effective in reducing LDL-cholesterol by nearly 20% with monotherapy [6,7] and useful in achieving the goal of NCEP guidelines in combination with statins [18,19].

Homozygous FH is extremely resistant to drug therapy. According to Raal et al., atorvastatin or simvastatin at a large dose succeeded in reducing LDL-cholesterol from 15 mmol/l (about 600 mg/dl) to 10.9 mmol/l (about 440 mg/dl). However, increasing the dose above 80 mg/day did not result in any further reduction, which suggests a plateau effect in their patients [20]. Although the effect of ezetimibe in lowering LDL-cholesterol may be limited due to the induction of cholesterol synthesis in the liver [21] in a somewhat similar way to the effect of bile acid sequestering agents [22,23], it is worth testing its effect in homozygous FH as no antilipidemic drugs other than probucol [24] can exert their cholesterol-lowering effect in patients with homozygous FH. Atorvastatin has been shown to exert its effect at a relatively high dose and to be useful in assisting the efficacy of LDL-apheresis [9]. However, the effect is virtually limited to the receptor-defective type with a remnant LDL-receptor activity, and only a very small number of patients with the receptor-negative type do respond to treatment with atorvastatin due to the complete lack of LDL-receptor activity [10].

Our present study showed that ezetimibe suppressed the rebound of LDL-cholesterol after apheresis to some extent, with an average 9% reduction in LDL-cholesterol levels after 4 weeks. A report from clinical trials carried out in America, Europe and South Africa showed that ezetimibe lowered LDL-cholesterol almost to the same extent as in patients with ordinary primary hypercholesterolemia; a 20.5% additional reduction in patients under treatment with atorvastatin at a dose of 80 mg/day [8]. In our study, patients with homozygous FH of the receptor-negative type undergoing LDL-apheresis were registered. In the study in America, Europe and South Africa [8], a variety of mutations in LDL-receptors

with different residual receptor activity were included and patients were not stratified by LDL-apheresis. It has been shown that an acute lowering of cholesterol by apheresis beyond a certain level results in an increase in cholesterol synthesis and there is a wide range of individual variation in the increase in cholesterol synthesis among FH patients [25,26]. The influence of dietary habits should also be considered regarding the low efficacy of ezetimibe in our study.

The dose of atorvastatin or simvastatin in our study was smaller than that used in previous studies conducted by Raal et al. [20] and Gagne et al. [8]. The possiblility still exists that the efficacy of ezetimibe in our study could be improved by increasing the dose of atorvastatin up to 40 or 80 mg/day. Such a study should be carried out in the future through careful observation, case by case, avoiding the appearance of serious side effects, resulting from possible genetic differences in drug tolerance.

A marked rebound of LDL-cholesterol after each apheresis procedure shortens the period in the post-apheresis stage, during which LDL-cholesterol is kept within the range of serum concentration in which the progression of atherosclerosis is stopped [5,27]. Therefore, even a 10% reduction in such a rebound can be expected to lengthen this effective period and save lives. This 10% reduction is equivalent to increasing the dose two to four times in statin monotherapy [9].

Bile acid sequestering agents have been shown to be not only ineffective but also to increase LDL-cholesterol by strongly enhancing cholesterol synthesis [1,22,23]. An enhancement of cholesterol synthesis has also been reported for ezetimibe [21]. However, it has been shown that ezetimibe is effective in lowering cholesterol even in cases of homozygous FH [8,28,29] and the present study supports this by showing that the drug is effective even in a state where the synthesis of cholesterol is enhanced after apheresis procedure. Bile acid sequestering agents often result in an increase in TG, to a great extent in some cases [30]. There were no changes in TG levels in our patients. This shows that the mechanism of regulation of cholesterol synthesis by cholesterol and bile aids is different and is probably related to why ezetimibe is effective in patients with homozygous FH, while bile acid sequestering agents are not.

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実験技術

食後高トリグリセリド血症家兎(PHT)の開発経緯と生活習慣病モデルとしての有用性

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要約：血管病対策は21世紀の医療の重要な課題であり，血管病の原因は動脈硬化性変化である．動脈硬化の危険因子としては高コレステロール血症のほうが臨床的に重大である．しかし，虚血性心疾患を発症した全ての患者に高コレステロール血症を認めるわけではなく，むしろ他の危険因子（高トリグリセリド（TG）血症，耐糖能異常，肥満，高血圧等）を同一人が複数併せ持つ方が，虚血性心疾患を発症する頻度が高いことが報告されており，特に高トリグリセリド血症と虚血性心疾患との関係が注目されている．さらに，動脈硬化症を主要所見とする臨床例の中に，食後高脂血症を示す症例が知られており，血管病進展の重篤な危険因子と考えられている．著者らは，動脈硬化に対する血中のコレステロール高値の要因を排除するために，通常（絶食状態）は血中のコレステロール（CHO）とトリグリセリド（TG）が低値であり，食後にTGのみが異常高値を示す家兎（PHT: Postprandial Hyper Triglyceridemia，食後高トリグリセリド血症家兎）の分離に成功した．PHTはCHO代謝異常に依存しない新しい脂質代謝異常のモデル動物である．PHTの食後高トリグリセリド血症は，絶食時に100 mg/dl以下を示し，食後12～24時間で1,000 mg/dl以上のTG値を示す．制限食給餌（120 g/day）下でも食後TG値は1,000 mg/dlを超える．PHTの食後高トリグリセリド血症の発症時期は6ヵ月齢前後から顕著となる．また，PHTの雄性でより食後高トリグリセリド血症が顕著である．PHTの食後の血漿をリポタンパク分析した結果，VLDLの増加が顕著であった．PHTに糖負荷試験を行ったところ，耐糖能異常並びにインスリン抵抗性を認めた．PHTは野生型家兎（日本白色家兎）に比較して体型がやや小型だが，腹腔内脂肪の沈着が顕著である．

1. はじめに

生活習慣病の死因は癌（悪性新生物），心臓病（虚血性心疾患），脳血管障害であり，心臓病と脳血管障害を合わせると癌死を越える．したがって，血管病対策は21世紀の医療の重要な課題である．

血管病の原因は動脈硬化性変化である．地域住民を対象とした疫学調査によって動脈硬化症の発症と重症度を規定する危険因子が明らかとなり，その代表的なものは高脂血症，高血圧，糖尿病，喫煙，性（男性，閉経後の女性）である．高脂血症には高コレステロール血症と高トリグリセリド（中性脂肪）血症，それらの合併したものとがある．動脈硬化の危険因子としては高コレステロール血症のほうが臨床的に重大である．

しかしながら，欧米や日本の先進諸国では食事内容として年々高栄養価のものが摂取される傾向にあり，高トリグリセリド血症が問題視されるようになってきている．米国のFramingham研究によると，虚血性心疾患患者の35%は血清コレステロール（CHO）が200 mg/dl以下であったと報告している(1)．また，泰江らは心筋梗塞患者1,032例を対象として行ったJapanese Antiplatelets Myocardial Infarction Study（JAMIS）の結果において，220 mg/dl以上の高コレステロール血症を有する患者の割合はたった27.1%であったと報告している(2)．このように虚血性心疾患を発症した全ての患者に高コレステロール血症を認めるわけではなく，むしろ他の危険因子（高トリグリセリド（TG）血症，耐糖能異常，肥満，高血圧等）

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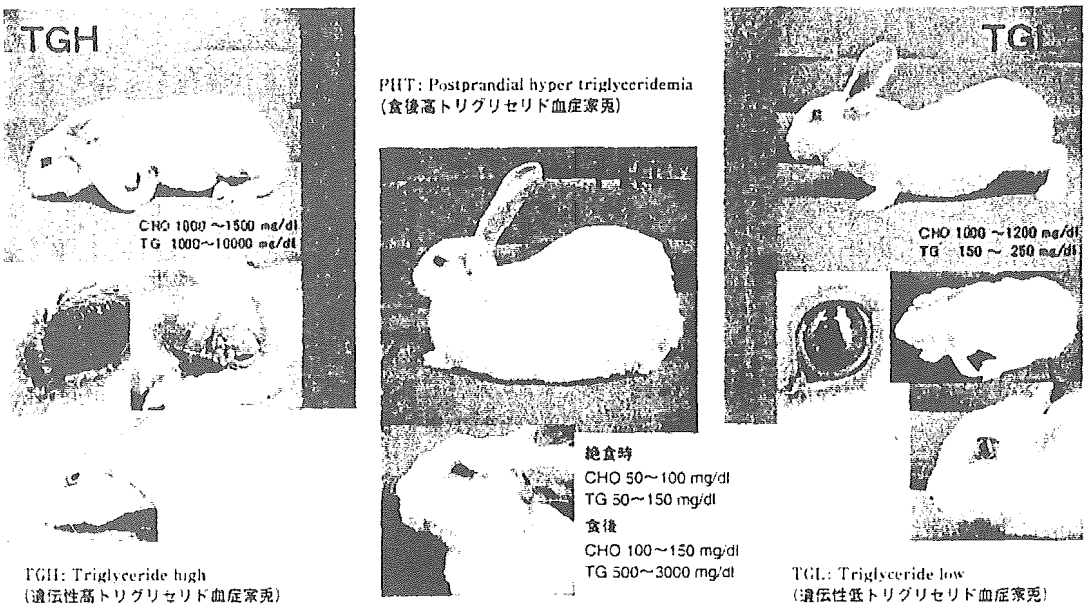

図1 山形大学で開発および維持されているウサギの系統

を同一人が複数併せ持つ方が，虚血性心疾患を発症する頻度が高いことが示され，特に高トリグリセリド血症と虚血性心疾患との関係が注目されている．さらに，動脈硬化症を主要所見とする臨床例の中に，食後高脂血症を示す症例が知られており，血管病進展の重篤な危険因子と考えられている．

しかし臨床例において，遺伝性の有無，脂質代謝の特徴は系統的に解析されていない．なぜ，高トリグリセリド血症が発生するのか，医学的な解明は進んでいない．その理由は，食事と高トリグリセリド血症の関係が未解決な事による．そのために，食後に高トリグリセリド血症を示す動物モデルの開発は，この問題の解明に必須と考えられる．

2. 遺伝性高トリグリセリド血症家兎(TGH)と遺伝性低トリグリセリド血症家兎(TGL)の系統確立

著者らはWatanabe heritable hyperlipidemic rabbit (WHHL) の分与を受け(3-5)，日本白色家兎 (JW) と戻し交配を行った後，ホモ接合体の確立を試みた．血清コレステロールと血清トリグリセリドを酵素法で測定したところ，WHHLのトリグリセリド値（中性脂肪）は200～900 mg dl と幅広い分布を示す事が分かった．一方，野生型である日本白色家兎では血清トリグリセリド値は多くの場合100 mg dl 以下を示す．そこで，血清トリグリセリド値が高い個体の掛け合わせを行った結果，世代を追う毎に血清トリグリセリド値が500 mg dl を超すWHHLの発現が増加した．即ち，遺伝性高トリグリセリド血症を示す家兎の発現頻度が上昇することが観察された．1995年の第4世代では90%，第5世代以降では100%の進達率で発現したことから，遺伝性高トリグリセリド血症家兎 (TGH) を系統として確立できた．同様に血清トリグリセリド値が低い個体の掛け合わせを行い，遺伝性低トリグリセリド血症家兎 (TGL) を系統として確立した．著者らはこれらの家兎を遺伝性高トリグリセリド血症家兎 (TGH, CHO = 1000～1500 mg dl, TG = 1000～10000 mg dl)，遺伝性低トリグリセリド血症家兎 (TGL, CHO = 1000 mg dl, TG = 150～200 mg dl) と命名した (図1)．

WHHLの高コレステロール血症はLDL (low density lipoprotein：低比重リポタンパク) 受容体の異常であることが明らかにされているが，遺伝性高トリグリセリド血症家兎 (TGH) および遺伝性低トリグリセリド血症家兎 (TGL) はWHHL家兎亜系より選抜した家兎であり，WHHLの特徴であるコレステロール高値に加えトリグリセリドの異常高値および低値を特徴とする．

3. 食後高トリグリセリド血症家兎(PHT)の発見

著者らは，TGHの遺伝様式を検索する目的で，TGHと日本白色家兎 (JW) の交配により雑種第1世代を作出した．さらに雑種第1世代 (MHF1) の中での交

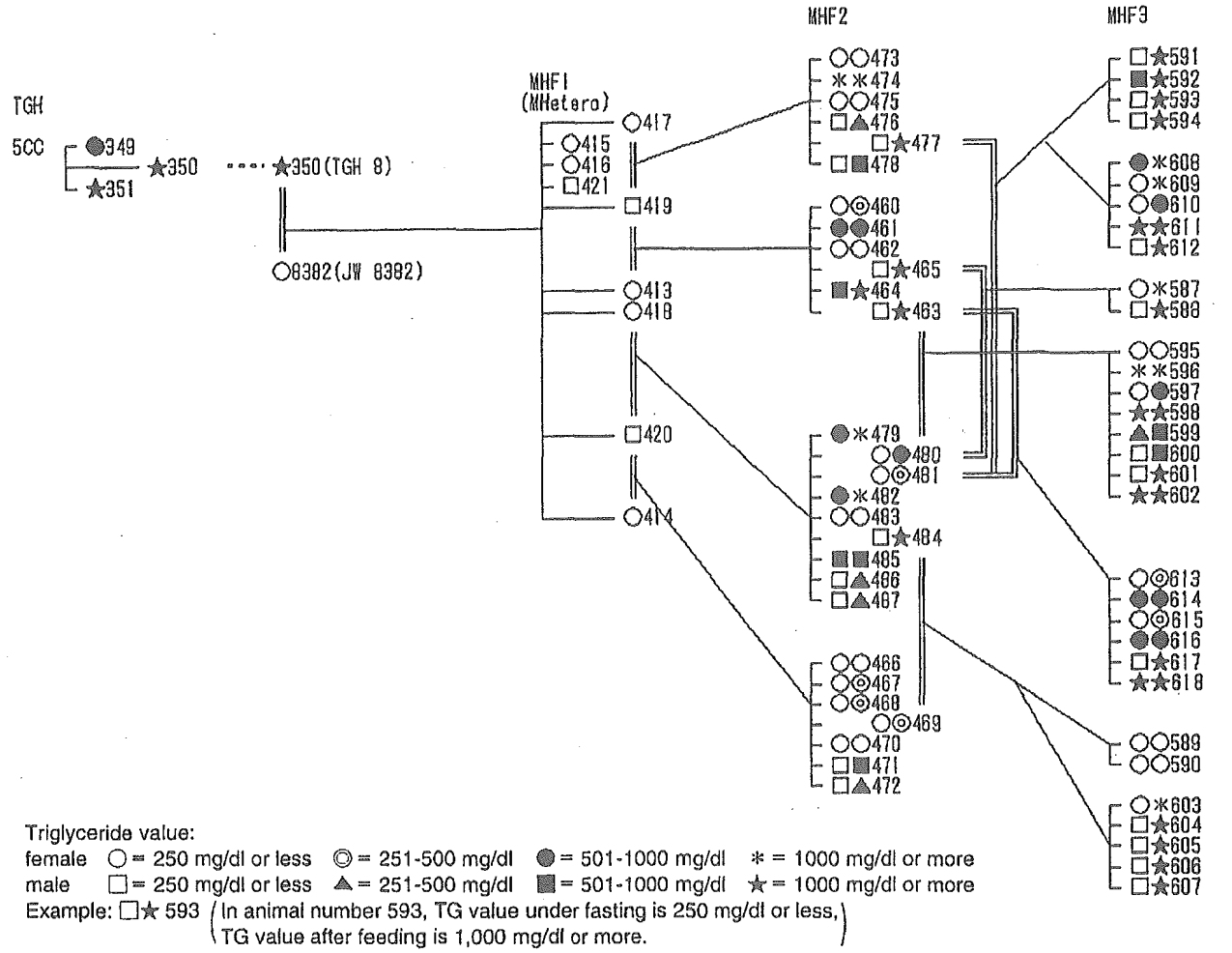

図2　PHT ウサギの家系図

配（兄妹交配／ヘテロ接合体どうしの交配なので，メンデルの法則から 1/4 はホモ接合体，1/4 は野生型，2/4 はヘテロ接合体が産まれる）により第二世代（MHF2）を作出し，遺伝形質の解析を行ったところ，ホモ接合体以外の個体に食後高トリグリセリド血症家兎を見出した．すなわち，食餌摂取と血清脂質レベルの関係を検討したところ，空腹時には脂質レベルは正常であり，食後 12 時間以降に血清トリグリセリド値が 1000 mg/dl 以上になる特異な家兎の存在に気づいた．遺伝形質を調べたところ，食後高トリグリセリド血症は常染色体優性遺伝の形質を示唆することが分かった．図 2 に遺伝性食後高トリグリセリド血症家兎を作出した際の家系図を示した．WHHL と日本白色家兎との戻し交配により，第一世代（MHF1）および第二世代（MHF2）を作出した．MHF2 においては TGH 7 匹，正常の脂質レベルを示す個体 26 匹を得た．その 26 匹の中で，食後に高トリグリセリド血症を示す 7 匹（雄 4 匹，雌 3 匹）を種動物として掛け合わせを行った結果，第三世代（MHF3）を，32 匹（雄 20 匹，雌 12 匹）得た．その中で，食餌に関係なく血清高トリグリセリド血症を示す TGH の性質を有する個体は 9 匹，空腹時の脂質レベルは正常で食後に高トリグリセリド血症を示す個体は 23 匹であった．従って，TGH は約 25% を占め，常染色体性劣性であることが確認された．食後に高トリグリセリド血症を示す個体は 23 匹だったことから，本形質は TGH と別の遺伝子支配による可能性が高く示唆された．そこで，本形質を示す家兎をモデル動物系として確立し，食後高トリグリセリド血症家兎（PHT: Postprandial Hyper Triglyceridemia）と命名し（図 1），日本および米国の特許を取得した(6,7)．食後にのみ高トリグリセリド血症を示すという遺伝形質を有するモデル動物はこれまでに例がない．PHT はコレステロール代謝異常に依存しない新しいヒト虚血性心疾患のモデル動物とし

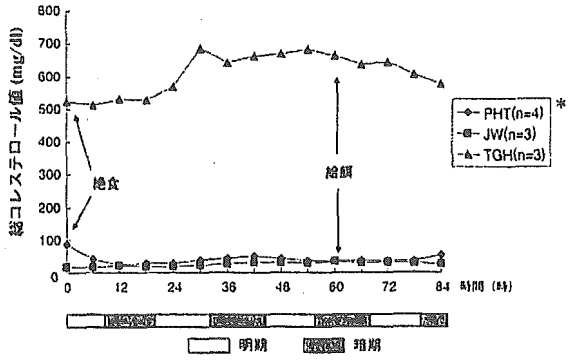

図3 食餌によるコレステロールの変動
＊PHT：食後高トリグリセリド血症家兎，JW：日本白色家兎，TGH：遺伝性高トリグリセリド血症家兎

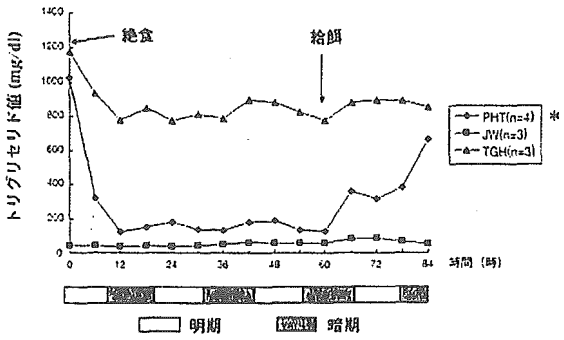

図4 食餌によるトリグリセリドの変動
＊PHT：食後高トリグリセリド血症家兎，JW：日本白色家兎，TGH：遺伝性高トリグリセリド血症家兎

て有用と考えられる．また，ヒトのマルチプルリスクファクター症候群や食後高脂血症の診断や解析に重要と考えられる．以下，PHTの特徴について述べる．

4. PHTにおける食後高トリグリセリド血症の特徴

PHTは，絶食時には10 mg dl以上200 mg dl以下の血清トリグリセリド値を示し，かつ食後12時間以後48時間以内に500 mg dl以上3000 mg dl以下の血清トリグリセリド値を示す事を特徴とする．

著者らが規定する高トリグリセリド血症とは500 mg dl以上のトリグリセリド値を示すことを意味し，1000～2000 mg dlをPHTの育種選抜目標としている．同様に食後とは食事開始後12～24時間後のことである．

各種血液脂質成分値を比較検討するためには，家兎の飼育条件，繁殖条件を一定にする必要がある．山形大学において作出維持されている家兎は温度22±2℃，湿度40-60%に管理された飼育室の中で，固型飼料（Labo R Grower，日本農産工業，東京）120 gを毎日一定量給餌されている．水は自由摂取である．飼育室の明暗は午前6時から午後6時までを照明時間としている．家兎の繁殖は自家で行い，娩出された家兎は生後30日で離乳された後に個別飼育された．生後1カ月齢から2カ月齢までの幼若家兎には80 gの固型飼料を毎日定量摂取させ，2ヵ月齢以後は120 gの制限給餌としている．

血液脂質の測定は，家兎の耳動脈から1 mlを採血し，1分間3000回転で15分間（4℃）の冷却遠心を行って血清または血漿を分離後，Vision Analyzer (Dinabot社，日本）を用いてコレステロールとトリグリセリドの測定を行った．著者らはPHTのスクリーニングとして，制限食を給餌した家兎に24～48時間の絶食を施した後に飽食給餌し，給餌開始24時間後の血漿トリグリセリド値を測定して評価している．

5. 野生型家兎（日本白色家兎，JW: Japanese White）との比較

PHTは，空腹時には血清トリグリセリド値は正常であるが，食後に高トリグリセリド血症を示すという特徴を有することから，ヒトの食後高脂血症のモデル動物として有用である．日本白色家兎（JW）では，食後のトリグリセリド増加は認められない．PHT, JWおよびTGHの絶食と食後の影響について血清コレステロールと血清トリグリセリドを指標に比較検討した．コレステロールは3系統とも特に変化を認めなかった（図3）．しかし，トリグリセリドは，JWとTGHでは絶食や食餌の影響を受けなかったのに対し，PHTでは，絶食前に約1000 mg/dlあったトリグリセリド値が，絶食開始後急激に低下し，12時間で約100 mg/dlを示した．絶食期間中は100～200 mg/dlで推移したが，その後食餌を与えるとトリグリセリド値は急激に上昇し，食後24時間で600 mg/dlを示した（図4）．このように，PHTは絶食と食餌に対してトリグリセリドの鋭敏な反応を示す(8)．

6. PHTの平時におけるトリグリセリド値

平時（制限食条件下で絶食等の前処置をしていない）におけるPHTの血清トリグリセリド値の経時変化を詳細に測定した（図5）．通常の給餌時間である昼の12時を食前（0時）とし，以後3時間おきに翌日の昼の12時まで24時間（0, 3, 6, 9, 12, 15, 18, 21, 24時）の血漿脂質成分値を測定した．すると食後6時間を過ぎる頃から血清トリグリセリド値は急速に上昇し，

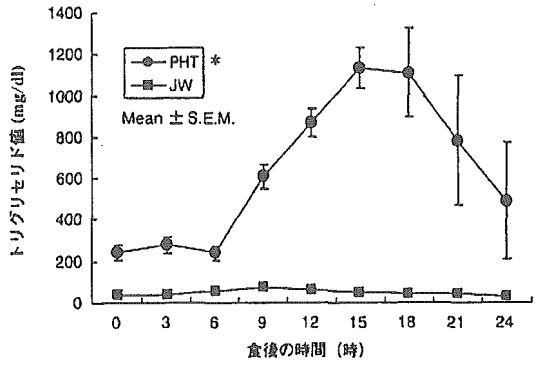

図5　PHTウサギの平時トリグリセリド値
＊PHT：食後高トリグリセリド血症家兎，JW：日本白色家兎

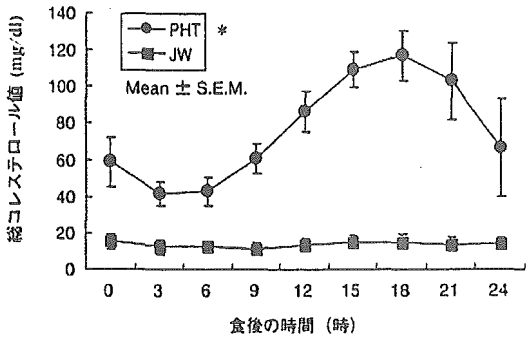

図6　PHTウサギの平時コレステロール値
＊PHT：食後高トリグリセリド血症家兎，JW：日本白色家兎

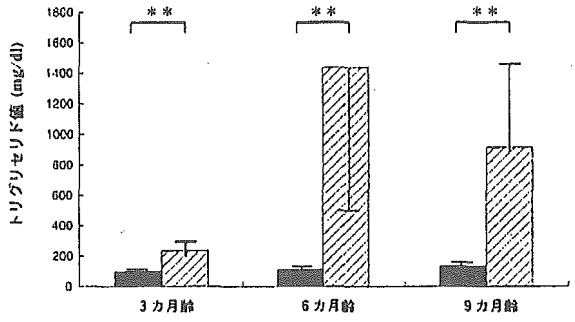

図7　PHTウサギの食後TG値における月齢差
■絶食，▨食後，平均値±標準偏差，n=23（雄15，雌8），$^{**}P<0.01$

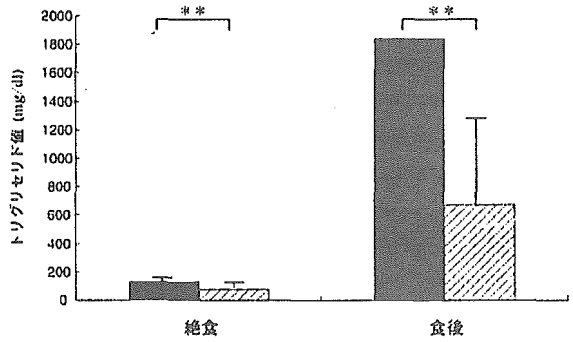

図8　PHTウサギの食後TG値における性差
■雄，▨雌，平均値±標準偏差，n=23（雄15，雌8），$^{**}P<0.01$

食後18時間で血清トリグリセリド値は1000 mg/dl以上という最高値に達した．野生型であるJWは，食事開始後6～9時間後にわずかなトリグリセリドの上昇（グラフからは判別つかないが）を認めるが，PHTのようなトリグリセリド値の極端な上昇は認められなかった．一方，PHTの平時における血清コレステロール値は，トリグリセリド値と同様の変化パターンを示したが，JWでは認められない（図6）．

7. PHTの食後高トリグリセリド血症の発症時期と性差

PHTの同一個体を用いて3，6，9カ月齢の食餌と血清脂質との関係を調べた．食餌の摂取量と食後のトリグリセリドレベルの間には有意の相関は認められなかった．従って，食後高トリグリセリド血症は食物の摂取と関連するが，食餌の摂取量は血液中の濃度を規定する因子ではないことになる．絶食時と食後の血清総コレステロール値の比較において，3カ月齢では有意の差を認めないが，6カ月齢では平均44 mg/dlから118 mg/dlへ，9カ月齢では41 mg/dlから80 mg/dlへと食餌により有意に増加した．血清トリグリセリド値を絶食時と食後で比較したところ，3カ月齢で103 mg/dlから236 mg/dlへ，6カ月齢で113 mg/dlから1437 mg/dlへ，9カ月齢では131 mg/dlから915 mg/dlへと，食餌により極端に増加した（図7）．上記6ヵ月齢の動物に対し，トリグリセリド値の性差を検討した結果，空腹時の血清トリグリセリド値には性差を認めないが，食後のレベルは雄性が1844 mg/dl，雌性が675 mg/dlであり，雄性の方が顕著に高いという結果を得た（図8）．

8. PHTの食後のリポタンパク分画

PHTの食後のリポタンパク分画についてアガロースゲル電気泳動法を用いて検討した結果，他のリポタンパクに対して食後のVLDLコレステロールとVLDLトリグリセリドの増加が顕著であった．VLDL（Very Low Density Lipoprotein：超低比重リポタンパク）はカイロミクロンにつぐ大きさを持つ粒子で，アガロース電気泳動ではpreβ位に分画される．VLDL

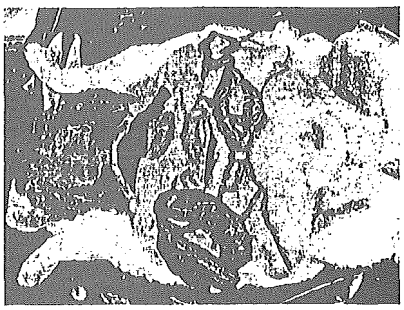

図9 PHTウサギ（雌2.5年齢）の腹腔内脂肪

の役割は肝臓で生合成されるトリグリセリドやコレステロールを，血中を介して全身の組織に運搬することにあるとされている．PHTの血中におけるVLDLの増加は，肝臓からのVLDL分泌亢進なのか，血中のVLDLの代謝が阻害されているかは不明である．また，食後に小腸上皮細胞で生成されるカイロミクロンおよびカイロミクロンレムナントの動態についても未検討である．

9. PHTの耐糖能異常と内臓脂肪

PHTにヒトの経口糖負荷試験に準じて経口糖負荷試験（1.5 g/kg OGTT）を行った結果，日本白色家兎では，負荷後2時間値が負荷前の血糖値（140 mg/dl）に戻ったのに対し，PHTでは200 mg/dl以上を示した．各血糖測定値の和である血糖和も統計学的有意な高値を示した．この結果からPHTは耐糖能異常を示すことが分かった．耐糖能異常の原因については不明である．また，同様の試験において，血中のインスリン濃度について検討した結果，PHTはインスリン抵抗性を示した．PHTの耐糖能異常ならびにインスリン抵抗性は，静脈内糖負荷試験（0.6 g/kg IVGTT）においても確認された．PHTはJWに比較すると腸管膜や腎周囲などの腹腔内に多量の脂肪組織が存在する．図9に2.5年齢雌のPHTの内臓脂肪を示した．

10. 食後高トリグリセリド血症家兎の生殖と寿命

PHTの体型は日本白色家兎と同等か若干小型であり，寿命は野生型（対照）と比較して大差を認めなかった．また，正常の生殖活動が観察された．

11. 終わりに

これまで数多くの実験的研究により，虚血性心疾患等の動脈硬化性疾患に対する高コレステロール血症の影響が解明されてきた．また最近，高トリグリセリド血症を呈するモデルウサギが開発され(9-12)，多くの研究に用いられているが，高脂肪食の負荷が伴い，PHTと比較して血清脂質値が低い．著者らが開発したPHT家兎は，通常食環境下で動脈硬化性疾患に対する血中のコレステロール高値の要因を排除し，トリグリセリドのみの影響を研究する上で有用な動物モデルと考える．さらに，虚血性心疾患を取り巻く危険因子として，高トリグリセリド血症，肥満，高血圧，耐糖能異常などが挙げられ，これら危険因子の重複が虚血性心疾患のリスクを相乗的に増加させている．個々のリスクの重症度とは別に，リスクが重複することが重要とされ，その中心に高トリグリセリド血症がある．PHT家兎は食後高トリグリセリド血症，耐糖能異常，内臓脂肪の蓄積等が確認されることから，生活習慣病やそれらの重積によって引き起こされるmultiple risk factor syndromeと虚血性心疾患との関係を解明するモデル動物として期待している．PHTウサギを用いた実験をご計画の場合は，山形大学医学部附属動物実験施設のホームページ（http://www.id.yamagata-u.ac.jp/Animal/animal.htm）をご参照頂くか，メール（tito@med.id.yamagata-u.ac.jp）にてお問い合わせください．

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Diminution of angiotensin II-induced contraction of the abdominal aorta isolated from Watanabe heritable hyperlipidemic rabbits

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Abstract

The purpose of this study was to investigate the changes in vasocontractile responses in atherosclerosis, using abdominal aortic strips isolated from Watanabe heritable hyperlipidemic (WHHL) rabbits and Japanese White (control) rabbits. The aortic strips from WHHL rabbits showed a significantly lower contractile response to angiotensin II than that in strips from control rabbits. The contractile responses to phenylephrine and 5-hydroxytryptamine were not different in WHHL and control groups. The contractile response to angiotensin II was higher in endothelium-denuded aortic strips than in endothelium-intact strips, but to a greater extent in the control group than in the WHHL group. The contractile response to angiotensin II in the absence of the endothelium was also lower in the WHHL group than in the control group. Pretreatment with N^G-nitro-L-arginine significantly increased the contractile response to angiotensin II in the endothelium-intact aortic strips in both the WHHL and control groups, while pretreatment with diclofenac did not affects the aortic contractile response to angiotensin II. The contractile responses to angiotensin II in the presence of N^G-nitro-L-arginine and diclofenac were lower in the WHHL group than in the control group. The contractile response to angiotensin II in the presence of PD123319 was also lower in the WHHL group than in the control group. Endothelium-dependent relaxation by acetylcholine occurred to the some extent in the WHHL and control groups. These results suggest that the WHHL rabbit abdominal aorta displays attenuated angiotensin II-induced contraction, mainly due to an abnormality in the angiotensin II-specific contractile pathway of the medial smooth muscle.

Key words: hyperlipidemia, atherosclerosis, vasoconstriction, angiotensin II, endothelium

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Introduction

Both dyslipidemia and hypertension are major risk factors during the progress of atherosclerosis. As a positive correlation between blood pressure and serum cholesterol level is known from epidemiological studies (Kannel, 1988), it is of interest to investigate how dyslipidemia affects arterial tone. Abnormalities in vasocontractile responses have been reported in animals with atherosclerosis induced by hypercholesterolemia. 5-Hydroxytryptamine-induced contraction is known to be increased in arteries of Watanabe heritable hyperlipidemic (WHHL) rabbits (Yokoyama *et al.*, 1983) as well as in those of rabbits with diet-induced hyperlipidemia (Henry and Yokoyama, 1980; Merkel *et al.*, 1990; Chin *et al.*, 1990). However, the reported change in angiotensin II contraction in atherosclerotic vessels is not consistent (Merkel *et al.*, 1990; Dam *et al.*, 1997; Yang *et al.*, 1998). The rabbit thoracic aorta has often been used for the study of vasocontractility in experimental atherosclerosis. On the other hand, little is known about the vascular response of the abdominal aorta isolated from WHHL rabbits. We have recently reported that angiotensin II-induced contraction was attenuated in the thoracic aorta isolated from WHHL rabbits (Shishido *et al.*, 2004). However, the detailed mechanism of this attenuation of angiotensin II contraction still remains to be determined. Angiotensin II is a major vasoactive substance involved in the pathophysiology of hypertension and atherosclerosis. In addition to having a potent vasoconstrictive action, angiotensin II induces the release of vasodilatory substances such as nitric oxide (NO) and prostacyclin, mainly from the vascular endothelium (Vane and Botting, 1993). These suppress the contractile component of the vascular response to angiotensin II. Thus, the angiotensin II-induced vasocontractile response is increased in the absence of the endothelium (Yilmaz *et al.*, 1987; Zhang *et al.*, 1994). In the present study, we investigated whether angiotensin II-induced contraction was altered in the abdominal aorta of WHHL rabbits and was modulated by vasodilatory substances released from the vessels.

Materials and Methods

Animals

The study protocols regarding treatment of animals were in accordance with the Guidelines for Experiments Using Laboratory Animals in Yamagata University School of Medicine. Male WHHL rabbits and Japanese White (control) rabbits aged 3–4 months were used in the present study. Each rabbit was housed individually in a controlled environment with unlimited access to water and was fed standard rabbit chow (120 g/day, Labo R Grower, Nihon Nosan Kogyo, Ltd., Tokyo, Japan). The animals were anesthetized with an intravenous administration of 30 mg/kg sodium pentobarbital, and segments of the abdominal aorta were carefully removed and immediately immersed in ice-cold Krebs-Henseleit solution for isometric tension study.

Tissue preparation

Excess fat and connective tissue were carefully removed from the dissected abdominal aortae. The vessels were cut into 3 mm long rings which were then cut open. In some strips,