Adenosine-Induced Coronary Flow Reserve in Watanabe Heritable Hyperlipidemic Rabbits

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The Watanabe heritable hyperlipidemic (WHHL) rabbit develops coronary atherosclerosis and hypercholesterolemia because of a genetic deficiency of low-density lipoprotein receptors and is therefore a good animal model for studying the relationships of coronary atherosclerosis, hypercholesterolemia and coronary flow reserve. The aim of the present study was to assess myocardial perfusion at baseline and during adenosine infusion ($0.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in 8 WHHL rabbits (13.8±0.5 months) with ^{13}N -ammonia, small-animal positron emission tomography (PET) and colored microspheres. Results were compared with those from 6 age-matched Japanese white rabbits. Plaque distribution was also examined in the extramural coronary arteries. All 8 WHHL rabbits had coronary plaques, with 6 showing multiple plaques. Mean global myocardial blood flow ($\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$) did not differ significantly between control and WHHL groups both at baseline (3.67 ± 0.72 vs $4.26 \pm 1.12 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$, $p=\text{NS}$) and with adenosine (7.92 ± 2.00 vs $9.27 \pm 2.91 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$, $p=\text{NS}$), nor did coronary flow reserve (2.16 ± 0.37 vs 2.18 ± 0.41 , $p=\text{NS}$). None showed evidence of regional perfusion abnormalities by visual and semiquantitative analyses of PET images. It was concluded that WHHL rabbits preserve adenosine-induced coronary flow reserve despite coronary atherosclerosis and hypercholesterolemia, suggesting that a compensatory mechanism develops in this animal model. (*Jpn Circ J* 2000; 64: 971–976)

Key Words: Coronary artery stenosis; Coronary flow reserve; Positron emission tomography; WHHL rabbit

Studies in animals have shown that the coronary flow reserve (CFR), defined as the ratio of maximal to basal coronary blood flow, can be used as a functional index of the severity of coronary artery stenosis.^{1,2} This relation forms the basis for the diagnosis of coronary artery disease using nuclear perfusion imaging, which uses pharmacologically induced increases in myocardial perfusion, most commonly with intravenous dipyridamole or adenosine.³ However, recent studies have shown that myocardial perfusion reflects the integrated effects of single or multiple stenosis, diffuse atherosclerosis and vasomotor dysfunction, and in particular endothelial dysfunction induced by hypercholesterolemia.⁴ Thus, the relation between the percent diameter stenosis detected by coronary angiography and the CFR is relatively poor in patients,⁵ compared with animal studies in which there is a mechanical constrictor on the coronary arteries.¹ Recent intravascular ultrasound findings and postmortem studies have also shown that many coronary artery atherosclerotic plaques do not encroach on the lumen because of the compensatory enlargement of the arteries,^{6,7} which therefore makes them angiographically invisible.⁸

In addition, several investigations using positron emission tomography (PET) have revealed that adenosine or dipyridamole-induced CFR was decreased in hypercholesterolemic patients.^{9–13} On the other hand, interestingly, Pitkanen et al recently reported that dipyridamole-induced CFR in young men with phenotype IIA familial combined hyperlipidemia (FCHL) was preserved, although serum total cholesterol concentrations were significantly higher than those in control subjects.¹⁴

The Watanabe heritable hyperlipidemic (WHHL) rabbit, an animal model of familial hypercholesterolemia, develops atherosclerotic plaques in the coronary artery, thus providing an opportunity to investigate the relation between coronary atherosclerosis, hypercholesterolemia and CFR.¹⁵ We recently developed a high-resolution cardiac PET system for imaging and quantitation of myocardial perfusion in rabbits.¹⁶ For the present study, we chose 13-month-old WHHL rabbits, because the cellular composition of their coronary atherosclerotic lesions resemble those in humans,¹⁵ and we investigated the relation between coronary plaque distribution and myocardial blood flow (MBF) at both baseline and during adenosine infusion using the small-animal PET system and the microsphere technique. The results were compared with those in normal age-matched Japanese white rabbits.

Methods

Animal Preparation

Experiments were performed on 8 male WHHL rabbits and 6 male Japanese white rabbits. Both groups of animals were fed a standard rabbit chow (LRC-4, Oriental Yeast Co,

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Japan) and water ad libitum. Mean age, body weight and lipid profiles for each group are shown in Table 1. There were no differences in age, body weight or high density lipoprotein cholesterol levels between the 2 groups. Total serum cholesterol and triglyceride levels in the WHHL rabbits were significantly higher than in the control rabbits.

Animals were anesthetized with pentobarbital (35 mg/kg) intravenously via the right marginal ear vein. Anesthesia was maintained by a constant intravenous infusion of pentobarbital at $6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ commenced 1 h after induction. Surgical sites were surface-anesthetized with 1% lidocaine. The rabbits were ventilated by tracheotomy using a small-animal ventilator (SN-480-5; Shinano, Tokyo, Japan). The settings of this ventilator and the oxygen content of inspired air were adjusted to maintain the blood gases in the physiological range throughout the experimental period. Blood gases were analyzed using a Ciba-Corning pH/Blood Gas Analyzer (Model No.238; Chiron Diagnostics, Emeryville, CA, USA). The rabbits were paralyzed with pancuronium bromide (0.3 mg/kg iv), followed by 0.15 mg/kg iv every 40–50 min. Rectal body temperature was maintained at $38\text{--}39^\circ\text{C}$ using a heat pad. The right femoral artery was cannulated for collection of the microsphere reference sample and for arterial input function, the left femoral artery for the measurement of blood pressure (BP), the right jugular vein for the infusion of adenosine and normal saline, and the left marginal ear vein for the injection of ^{13}N -ammonia. The right carotid artery was cannulated for the injection of $15\text{-}\mu\text{m}$ colored polystyrene microspheres (E-Z Trac, Los Angeles, CA, USA) via a polyethylene catheter (ID: 0.87 mm ; OD: 1.27 mm ; Natume, Tokyo, Japan) advanced into the left ventricle. The animals were heparinized (400 U/kg) after the cannulations. Arterial pressures and heart rate (HR) were monitored with a multichannel polygraph (Omniace RT3200N; NEC San-ei, Tokyo, Japan). The rabbits were then placed in a supine position within the animal PET device.

The experiment was approved by the Animal Welfare Committee of the Institute, and was performed in compliance with the guidelines for the care and use of laboratory animals as described by the National Institutes of Health.

Positron Emission Tomography

PET images were obtained with a small-animal PET (SHR-2000, Hamamatsu Photonics KK, Hamamatsu, Japan) that provided 7 transaxial slices simultaneously.¹⁷ The slices had a transaxial resolution of 3.0 mm FWHM and were separated by 6.5 mm . Axial resolution was 4.8 and 4.1 mm FWHM in the direct and cross planes, respectively.

Initially, blank and transmission scans were acquired using a Ge-68/Ga-68 source for the correction of detection efficiency and photon attenuation. Subsequently, 111 MBq of ^{13}N -ammonia was injected intravenously into the marginal ear vein as a 20-s slow bolus. Three-minute PET imaging was begun 2 min after the start of tracer injection.

The reconstructed images were obtained using a parallel processing system (NuSprint, YARC Systems Corp, Newbury Park, CA, USA) on a personal computer (Macintosh Quadra950, Cupertino, CA, USA), and transferred to a graphics work station (Indigo 2, Silicon Graphics, Mountain View, CA, USA) for further processing. Each image was displayed as 180×180 pixels with a pixel size of $1.0 \times 1.0 \text{ mm}$. Count losses at the high count rates were corrected. All reconstructions were performed without ECG gate and corrected for physical decay of the tracer.

Table 1 Animal Characteristics

	Control (n=6)	WHHL (n=8)
Age (months)	13.8 ± 1.9	13.8 ± 0.5
Body weight (kg)	3.4 ± 0.3	3.3 ± 0.3
Total cholesterol (mg/dl)	19 ± 7	$486 \pm 72^*$
Triglycerides (mg/dl)	26 ± 11	$163 \pm 36^*$
HDL cholesterol (mg/dl)	13 ± 4	16 ± 8

WHHL, Watanabe heritable hyperlipidemic rabbits; HDL, high-density lipoprotein. * $p < 0.01$ vs control.

Experimental Protocols

The rabbits were allowed to stabilize for 20–30 min after completion of the procedures. Microsphere and PET studies were performed at baseline and during the continuous infusion of adenosine. An interval of approximately 50 min was used between control and adenosine studies to allow for physical decay of the isotopes. In each study, approximately 1.0×10^6 colored microspheres were dispersed with a mechanical mixer immediately before injection and injected into the left ventricle. A blood sample was withdrawn from the femoral artery at a constant rate of 1.5 ml/min for 2 min. Blood withdrawal began 15 s before microsphere injection. Immediately after the injection of microspheres, ^{13}N -ammonia was administered as a 20-s slow bolus. Three-minute PET imaging was begun 2 min after the start of tracer injection. Low-molecular dextran was infused into the ear vein at a rate of 1.5 ml/min , concurrent with the withdrawal of the blood sample, to prevent a significant decrease in BP during withdrawal. Adenosine (Wako Pure Chemical Industries Ltd, Osaka, Japan) at a concentration of $0.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ was infused for 8 min. At 3 min after the start of adenosine infusion, microspheres and ^{13}N -ammonia were administered. At the end of the studies, the animals were killed during deep anesthesia with KCl solution; the hearts were removed and the left ventricular myocardium was dissected. The upper part of the left ventricular myocardium was used for histological examination of the extramural coronary arteries and the remaining left ventricular myocardium was weighed and used for microsphere measurements.

The extraction of microspheres from the blood and tissue samples was performed as described by Hale et al.¹⁸ Regional MBF was calculated from the formula: regional MBF = $\text{Cm} \times \text{Qr} / \text{Cr}$, where Cm represents the total number of microspheres/g of myocardial tissue, Qr the withdrawal rate of the reference blood sample (ml/min), and Cr the total number of microspheres in the reference blood sample.

Coronary flow reserve was defined as the ratio of hyperemic to resting MBF. To relate the hyperemic blood flow to one of its major determinants (the coronary driving pressure), minimal coronary vascular resistance (CVR) was also calculated as the ratio of mean arterial blood pressure (mmHg) to myocardial blood flow ($\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$).

A separate group of 8 rabbits weighing $3.3 \pm 0.5 \text{ kg}$ and aged about 6 months were used to determine the dose of adenosine infusion rate for the maximally vasodilated myocardial vasculature under the same experimental settings. Adenosine at concentrations of $0.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($n=4$) and $0.4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($n=4$) was infused after the baseline measurement. MBF at baseline (3.23 ± 1.09 vs $2.32 \pm 0.54 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$), during adenosine infusion (6.08 ± 1.41 vs $5.35 \pm 1.50 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$), CFR (1.96 ± 0.41 vs 2.32 ± 0.65), minimal CVR (10.5 ± 2.52 vs $9.52 \pm 1.64 \text{ mmHg} \cdot \text{ml}^{-1} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$), mean BP at baseline (89 ± 12 vs $92 \pm 9 \text{ mmHg}$) and during adenosine

Table 2 Baseline Hemodynamic Values

	Control (n=6)	WHHL (n=8)
HR (beats/min)	320±35	322±42
Systolic BP (mmHg)	124±12	131±15
Diastolic BP (mmHg)	107±12	105±15
Mean BP (mmHg)	112±12	115±15
RPP (beats/min × mmHg)	39601±5900	42361±7660
MBF (ml·min ⁻¹ ·g ⁻¹)	3.67±0.72	4.26±1.12

WHHL, Watanabe heritable hyperlipidemic rabbits; HR, heart rate; BP, blood pressure; RPP, rate pressure product; MBF, myocardial blood flow.

infusion (63±16 vs 49±10 mmHg) did not differ significantly between the 2 groups. However, mean BP during adenosine infusion at a rate of 0.4 mg·kg⁻¹·min⁻¹ tended to be lower than that of 0.2 mg·kg⁻¹·min⁻¹, suggesting that it may be outside the range of the autoregulation of coronary circulation. Thus, we chose the adenosine infusion rate of 0.2 mg·kg⁻¹·min⁻¹ for this study.

PET Image Analysis

Five to 8 regions of interest (ROIs) with an area of 11 pixels each were drawn on the left ventricular myocardium at the midventricular level and divided into 3 segments (septal, anterior, lateral). Segmental tracer activities were determined by averaging ROI values in each segment and normalized by dividing by the maximum segmental value in each image. PET images were also visually interpreted independently by 2 experienced PET observers blinded to experimental data and scored as normal=0, probably normal=1, equivocal=2, mild defect=3, moderate defect=4, severe defect=5. When there was disagreement between the observers, the images were reviewed together to obtain consensus.

Histological Examination

The presence of coronary atherosclerosis was investigated in the main trunk of the left coronary artery (LMT), left anterior descending artery (LAD) and left circumflex artery (LCX), because all rabbits showed left-side dominance of coronary artery distribution and the right coronary artery was small and supplied only the right ventricular wall. The upper part of the heart was immersed in methanol–Carnoy's fixative. After immersion fixation, the left coronary arteries were sliced at 2-mm distal from each ostium (LMT, LAD, LCX). All slices were processed routinely by embedding in paraffin, sectioning at 5-μm thickness, and staining with Weigert's elastica van Gieson stain.

Morphometric Analysis

The degree of coronary atherosclerosis was quantified using a computerized morphometry system, MacSCOPE Ver 2.2 (Mitani Corporation). The lumen, the internal elastic lamina (IEL), and the outer limit of the media in plaque-carrying sections were traced and the actual cross-sectional area of the lumen (lumen area), the lesion area (the cross-sectional area occupied by plaque), and the IEL area were determined from these contours. According to the method reported by Pesonen et al.¹⁹ the IEL area in the collapsed or contracted arteries was corrected to the area of the idealized circle using the length of the IEL.²⁰ The % plaque area was expressed as lesion area/corrected IEL area×100.

Statistical Analysis

Experimental animal characteristics and hemodynamic

Table 3 Plaque Distribution in WHHL Rabbits

Exp. animal no.	% Plaque area of coronary artery		
	LMT	LAD	LCX
1	77	59	56
2	65	0	x
3	65	63	62
4	51	44	x
5	0	22	22
6	50	x	63
7	0	40	6
8	0	61	x

WHHL, Watanabe heritable hyperlipidemic; LMT, left main trunk; LAD, left anterior descending artery; LCX, left circumflex artery.

Fig 1. A cross-section of a coronary atherosclerotic lesion of a WHHL rabbit (×152, Weigert's elastica van Gieson).

data were expressed as mean±SD. Differences between mean were compared by Student's 2 tailed t test for unpaired data. A value of p<0.05 was considered significant.

Results

Baseline Hemodynamic Data and MBF

There were no statistical differences in baseline HR, arterial pressure (systolic, diastolic and mean), rate-pressure product and MBF between the 2 groups (Table 2).

Coronary Atherosclerosis in WHHL Rabbits

Atherosclerotic lesions were found in all 8 WHHL rabbits (Table 3, Fig 1); 6 of them had advanced atherosclerosis that reduced the luminal area by more than 50%, 5 rabbits had atherosclerotic plaque in the left main trunk and, moreover, 6 rabbits had multiple lesions. In contrast, control rabbits were free of lesions.

Response to Adenosine

Both the control (n=6) and WHHL (n=8) groups showed a similar decrease in mean arterial pressure in response to adenosine (28.1±13.8 vs 24.9±13.5 mmHg, p=NS) (Fig 2A), and the differences from baseline values were statistically significant (both groups, p<0.01). In WHHL rabbits, HR significantly decreased from 322±42 to 267±53 (beats/min) with adenosine infusion (p<0.05), but did not decrease in the control group. Changes were statistically different between the 2 groups (Fig 2B).

Fig 2. Differences in (A) mean arterial pressure (dMAP) (-28.1 ± 13.8 vs -24.9 ± 13.5 mmHg) and (B) heart rate (dHR) between baseline and adenosine infusion (3.3 ± 22.0 vs -54.6 ± 57.8 beats/min) in control (n=6) and WHHL (n=8) rabbits.

Fig 3. (A) Coronary flow reserve (CFR) (2.16 ± 0.37 vs 2.18 ± 0.41) and (B) minimal coronary vascular resistance (CVR) (11.0 ± 1.7 vs 10.1 ± 2.0 mmHg·ml⁻¹·min⁻¹·g⁻¹) in control (n=6) and WHHL (n=8) rabbits.

Fig 4. (Upper) Three contiguous cross-sectional myocardial images obtained at 3–6 min after administration of ¹³N-ammonia in a WHHL rabbit at baseline. (Lower) Three contiguous images corresponding to the upper row during adenosine infusion in the same rabbit. Myocardial uptake of the tracer is homogeneous at baseline and during adenosine infusion. A, anterior; S, interventricular septum; L, lateral; IP, inferoposterior.

MBF during adenosine infusion did not change significantly between the control and WHHL groups (7.92 ± 2.00 vs 9.27 ± 2.91 ml·min⁻¹·g⁻¹, $p = NS$). Consequently, there were no statistical differences in CFR and minimal CVR between the 2 groups (Fig 3).

Visual and Semiquantitative PET Image Analyses

Fig 4 shows the PET image at baseline and during adenosine infusion in a WHHL rabbit. As regards visual interpretation, the septal segment of case 5 during adenosine infusion and the anterior segment of case 8 at baseline and during adenosine infusion were given a score of 2 (equivocal).

Excepting these 2 segments, visual interpretation revealed normal or probably normal perfusion (scores 0 or 1) in the 2 groups. The relative distribution values of the myocardial ¹³N-ammonia uptake at baseline and during adenosine infusion were higher than 0.825 in the 2 groups.

Discussion

Adenosine-induced CFR in WHHL rabbits, measured with microspheres, was unexpectedly not decreased compared with that in age-matched control rabbits, despite the fact that all WHHL rabbits had coronary atherosclerotic

plaques and hypercholesterolemia. In addition, the visual findings on PET perfusion images indicated no obvious regional perfusion abnormalities at baseline or during adenosine infusion in the WHHL rabbits compared with control rabbits, except that 1 segment at baseline and 2 segments during adenosine infusion were given a score of 2 (equivocal). This is compatible with small differences in relative distribution values of the myocardial ^{13}N -ammonia uptake (0.825–1.0) at baseline and during adenosine infusion in both the WHHL and control rabbits. Compensatory enlargement of atherosclerotic coronary arteries and the relaxation response to adenosine in hypercholesterolemia are the 2 major factors involved in the basal mechanism of the preservation of CFR in WHHL rabbits.

Compensatory enlargement of atherosclerotic coronary arteries was first reported in human coronary arteries by Glagov et al⁶ and is the main reason for angiographic underestimation of coronary atherosclerosis⁸ which explains the poor correlation of relative angiographic measurements of stenosis severity with CFR in humans.⁵ Recently, Shiomi et al perfused fixed 4 WHHL rabbits at ages of 8–9 months and investigated the relation of coronary plaque and compensatory enlargement.²¹ They analyzed 20–27 cross-sections at 0.5 mm intervals in the LCX, revealing 11 plaques in total. The % plaque area ranged from 14 to 65%, but lumen size remained unaffected and outward bulges of coronary arteries were clearly seen. This probably explains, at least partly, the preservation of adenosine-induced CFR in the WHHL rabbits in the present study, despite the fact that all had coronary atherosclerotic plaques.

Hypercholesterolemia impairs the endothelium-dependent vasodilation induced by acetylcholine.^{22,23} However, the effect of hypercholesterolemia on vascular responses to purines (adenosine, ATP, etc) is complex and remains a matter of controversy, probably because subtypes of purinoceptors exist in both the endothelium and vascular smooth muscles²⁴ and they are differentially affected by progressive atherosclerosis.^{25,26} In any case, this may be involved in the preservation of adenosine-induced CFR in WHHL rabbits. Ragazzi et al found that the WHHL rabbit aorta maintained the relaxant ability induced by ATP and adenosine, despite serious impairment of endothelium-dependent relaxation mediated by acetylcholine.²⁷ They showed that adenosine relaxed the vessel by acting only on smooth muscle and hypothesized that this retained endothelial relaxant effect of ATP may involve activation of a remodeled purinergic receptor site.²⁸ On the other hand, Abebe et al reported that the coronary vasorelaxant effects of the adenosine agonists NECA and CGS-21680 were partially endothelium-dependent, and that the oxidation of low density lipoprotein caused impaired responses to these agonists in porcine coronary artery rings *in vitro*.²⁹

The present study is the first to investigate *in vivo* the adenosine-induced CFR in hypercholesterolemic animals. The results are consistent with the *in vitro* data of the WHHL rabbit aorta discussed earlier^{27,28} but there is discrepancy between our data and some clinical data. In humans, several PET investigations have revealed that adenosine or dipyridamole-induced CFR was decreased in hypercholesterolemic patients.^{9–13} On the other hand, Pitkanen et al recently reported that, using PET and ^{15}O -water, the dipyridamole-induced CFR in young men with phenotype IIA FCHL was preserved, in contrast with that in phenotype IIB.¹⁴ They speculated that the genetic factors underlying FCHL may cause endothelial and/or smooth muscle dysfunction

in phenotype IIB, not phenotype IIA, by mechanisms unrelated to lipid metabolism. Thus it is possible that subtypes of hyperlipidemia differentially affect the coronary vasodilator response to adenosine, and, as a second possibility, this mechanism may be involved in the preservation of adenosine-induced coronary flow reserve in WHHL rabbits.

The present results seem to partly conflict with those of Clozel et al, who investigated carbochromen-induced CFR in WHHL rabbits at ages of 100 and 300 days and compared the findings with age-matched Burgundy rabbits.³⁰ These disparate results may relate to the methodological differences between the 2 studies. Clozel et al measured MBF in a conscious condition. In addition, the mechanism of vascular response to carbochromen is relatively unknown compared with adenosine, although it is recognized as a potent drug for estimation of coronary dilatory capacity.³¹ In their study, at 100 days, there was no difference in CFR between the 2 groups, despite the fact that the cholesterol level was already extremely high in the WHHL rabbits. However, at 300 days, the CFR in WHHL rabbits was lower than that in normal Burgundy rabbits. The baseline MBF in WHHL and Burgundy rabbits at the age of 300 days as reported by Clozel et al was almost the same as that in the WHHL and Japanese White rabbits at the age of 410 days in our study. Interestingly, however, WHHL rabbits achieved a higher blood flow with the present adenosine infusion compared with that by their carbochromen infusion. On the other hand, in their control rabbits, carbochromen induced a higher blood flow than adenosine.

Mean BP and HR values at baseline in the WHHL and control rabbits were slightly higher than those reported previously in open-chest anesthetized rabbits.^{32,33} It is possible that in the present experimental setting the rabbits had a high adrenergic tone at baseline, which could be why they appear to have a higher MBF at baseline and lower CFR compared with the open-chest anesthetized rabbits. On the other hand, the minimal CVR values in the WHHL and control rabbits were almost the same as those reported previously³³ which suggests that the adenosine infusion dose is enough to dilate coronary vessels.

There was a significant decrease in HR by adenosine infusion in the WHHL rabbits compared with the control rabbits. Morita et al reported that the baroreflex control of HR, stimulated or unloaded by raising or lowering of arterial pressure, was impaired in WHHL rabbits³⁴ and this probably explains our data. Morita et al also reported that the basal values of arterial pressure and HR in WHHL rabbits were significantly higher than those in normal rabbits in the conscious condition and may be related to the higher rate–pressure product and MBF at baseline in the WHHL rabbits, although no statistically significant increase was demonstrated.

Technical Limitations

We did not use a perfusion–fixation technique to evaluate coronary atherosclerosis because we needed to perform PET scans and microsphere injection simultaneously in our experimental protocol before the histological examination. Therefore, we corrected the IEL area in the collapsed or contracted arteries, assuming an idealized circle using the length of IEL.^{19,20}

We performed ^{13}N -ammonia PET scans to evaluate regional perfusion abnormalities. However, the rate of increase of ^{13}N -myocardial uptake tended to decrease at high flows because the extraction fraction of the tracer in the myocardium is inversely correlated with MBF. As a result, perfusion

abnormalities during adenosine infusion might be underestimated. We also did not evaluate intramural coronary arteries histologically in WHHL rabbits. Wu et al reported that in WHHL rabbits aged 6–12 months, stenosis of more than 50% due to intimal fibrous thickening was often seen in intramural coronary arteries less than 200 μ m in diameter.³⁵ Therefore, this abnormality may affect the coronary circulation in WHHL rabbits.

Conclusion

In summary, CFR and minimal CVR induced by adenosine-infusion in WHHL rabbits did not differ significantly compared with age-matched controls despite coronary atherosclerosis and hypercholesterolemia, suggesting that a compensatory mechanism develops in this animal model.

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