Correlation of functional and structural alterations of the coronary arterioles during development of type II diabetes mellitus in rats

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Abstract

Objective: Cardiac complications in diabetes mellitus (DM) are frequently ascribed to microangiopathy. Therefore, we sought to directly correlate the serial changes in coronary arterial function with the extent of coronary arteriolar remodeling in a model spontaneously developing type II DM.

Methods: Otsuka Long-Evans Tokushima Fatty (OLETF) rats and control Long-Evans Tokushima Otsuka (LETO) rats were used. At 5, 15 and 30 weeks of age, ten rats in each group were subjected to systemic and coronary hemodynamic measurements using the colored microsphere technique before and during maximal coronary hyperemia and histological assessment with Azan-Mallory stain of the coronary arterioles.

Results: As early as 15 weeks of age, at which time fasting plasma glucose concentration remained normal, OLETF rats exhibited a lower coronary flow reserve and a greater coronary vascular resistance during hyperemia than did LETO rats. On histomorphometry, OLETF rats exhibited a greater wall-to-lumen ratio and a greater degree of perivascular fibrosis of arterioles at 15 weeks of age and thereafter, both of which exhibited a significant correlation with the minimal coronary vascular resistance.

Conclusions: The degree of functional deterioration in coronary circulation was directly correlated with the severity of coronary arteriolar structural remodeling during the development of microangiopathy in early stage of DM.

Keywords: Blood flow; Coronary circulation; Diabetes; Fibrosis; Histo(patho)logy; Remodeling

1. Introduction

Cardiovascular complications are the leading causes of morbidity and mortality in patients with type II diabetes mellitus (DM) [1,2]. Despite angiographically normal epicardial coronary arteries, the coronary vasodilator reserve is frequently impaired in DM patients [3–5]. It has been suggested that structural alterations of the coronary arterioles may contribute to the increased minimal coronary resistance and the diminished coronary flow reserve (CFR) [3,6]. However, no studies have directly compared functional and structural alterations in coronary circulation during the development of type II DM either in humans or in animal models.

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Otsuka Long-Evans Tokushima Fatty (OLETF) rat is an established model of human type II DM. The model is characterized by hyperinsulinemia from 8 weeks of age; insulin resistance of the peripheral tissues from 12 weeks of age; late onset of hyperglycemia after 20 weeks of age and diagnosable DM by oral glucose tolerance test (OGTT) from 25 week of age; reduction in insulin level from 60 weeks of age; inheritance by males and the contribution of sex hormones to the onset of DM [7,8].

We have recently reported that left ventricular diastolic filling is impaired and aortic wall stiffness is increased in the prediabetic stage in this model [9,10]. In the present study, we investigated the serial changes in coronary small arterial function by measuring myocardial blood flow (MBF) to correlate this with the structural remodeling of coronary arterioles in the myocardium during the development of DM in OLETF rats.

Time for primary review 22 days.
2. Methods

2.1. Subjects

Thirty male OLETF rats were used as the experimental subjects. The recessive genes responsible for type II DM in OLETF rats are located on chromosome 14 and X, and OLETF rats develop the diabetic syndrome in nearly 100% of male rats at 25 weeks of age [11,12]. Thirty male Long-Evans Tokushima Otsuka (LETO) rats, which were developed from the same colony by selective mating but do not develop DM, were used as control animals. All animals were maintained at the Kagawa Medical University animal experiment center from 5 weeks of age, and housed in a specific pathogen-free facility under controlled temperature (23 °C) and humidity (55%) with a 12-h artificial light/dark cycle. Animals were given free access to standard laboratory rat chow (MF, Oriental Yeast Corp., Tokyo, Japan) and tap water. The present investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

2.2. Animal preparation

At the age of 5, 15 and 30 weeks, 10 rats of each strain were anesthetized with 50 mg/kg sodium pentobarbital, and were instrumented for determination of systemic and coronary hemodynamics using the reference standard colored microsphere method [13,14]. Rats were intubated and ventilated with room air using a ventilator (Model 680, Harvard Apparatus, Holliston, MA, USA). The right femoral vein and artery were cannulated with polyethylene catheters (PE-50) for infusion of dipyridamole and reference sample withdrawal, respectively. Another polyethylene catheter (PE-50) was inserted into the left carotid artery to record arterial pressure and heart rate (HR). Then a thoracotomy via the third left intercostal space was performed, and a polyethylene catheter (PE-10) was placed into the left atrium through the left atrial appendage for microsphere injection. The proximal portion of the catheter was passed out of the thorax before the chest was closed under compression. The carotid catheter was connected to a pressure transducer of a multiple channel physiograph (RM-6000, Nihon Kohden, Tokyo, Japan) and mean arterial pressure (MAP) and HR were derived. Rats were then placed in nonrestrictive polyethylene cages and allowed to recover fully for 3–5 h.

2.3. Experimental protocol

The baseline measurements of systemic and coronary hemodynamics in nonrestrained rats were obtained after they had fully recovered from anesthesia. The first set of colored microspheres (E-Z Trac, Los Angeles, CA, USA) was injected into the left atrial appendage for cardiac output (CO) and MBF measurement, while the reference blood was withdrawn from the femoral artery. After these basal measurements had been obtained, maximal coronary vasodilatation was induced by intravenous infusion of dipyridamole (4 mg/kg/min) for 10 min using an infusion/withdrawal pump (Harvard Apparatus, Holliston, MA, USA). The hemodynamic studies were then repeated using the second set of colored microspheres. The dipyridamole infusion was maintained for 1–2 min after the injection of microspheres. At the end of the study, blood was sampled in heparinized tubes to measure blood glucose concentrations by a blood glucose test meter (MIWA Chemical Laboratory, Nagoya, Japan). The rats were then sacrificed by an overdose of pentobarbital and the heart was removed immediately.

2.4. Measurements of cardiac output and coronary flow by microsphere method

CO and MBF were measured using the reference sample microsphere method [13,14]. Approximately 1×10⁶ colored microspheres 15±0.43 μm in diameter were injected into the left atrium over a period of 20 s and the tubing was flushed immediately with 0.3 ml of saline for 30 s. In order to ensure uniform mixing and even distribution, the microsphere suspension was mixed thoroughly with a vortex mixer before infusion and the plastic tube, containing the microsphere suspension, was constantly tapped during the infusion. The reference blood sample was withdrawn from the right femoral catheter beginning 10 s before the injection and continued for 60 s at a constant rate of 1.00 ml/min with another pump (Model 2400, Harvard Apparatus, Holliston, MA, USA). Then all blood samples were placed in plastic tubes for later analyses.

For MBF measurement, the heart was removed on completion of data collection. After the left ventricle was isolated, cleaned, and weighed, a small transmural piece of the left ventricular free wall was obtained and weighed. Then the microspheres in each blood and tissue sample were recovered as described previously [13]. The total number of microspheres was counted under a microscope at ×200 magnification using a Fuchs–Rosenthal hemocytometer (Erma Inc., Tokyo, Japan). CO (ml/min) was calculated as \( CO = \frac{(C_i \times Q_i)}{C_i} \), while MBF (ml/min/g) was calculated as \( MBF = \frac{(C_m \times Q_i)}{C_i} \), where \( C_i \) was the number of microspheres injected, \( Q_i \) was the withdrawal rate of the reference blood sample (ml/min), \( C_i \) was microsphere count in the reference blood sample, and \( C_m \) was microsphere count per gram of myocardial tissue. CO was normalized by body weight to derive the cardiac index (CI: ml/min/kg). The total peripheral resistance index (mmHg/ml/min/kg) was calculated by dividing MAP with CI. CFR was calculated as the ratio of MBF after dipyridamole to the flow at baseline. Coronary vascular resistance (CVR: mmHg/ml/min/g) was calculated by dividing the MAP by the MBF at baseline.
Minimal CVR was defined as CVR determined during dipyridamole infusion.

2.5. Histopathological examination

At 5, 15 and 30 weeks of age, after a portion of tissue was removed from the left ventricular free wall for microspheres recovery, the remainder of the left ventricle was fixed with formalin solution. The specimens were embedded in paraffin and cut into sections 4 μm thick for Azan-Mallory staining.

Thickening of the coronary arterial wall and the degree of perivascular fibrosis were assessed according to the method of Takemoto et al. [15]. All Azan-Mallory stained sections were carefully scanned with a light microscope (VM-30, Olympus, Tokyo, Japan) connected to a computer using the NIH image-analysis system for histomorphometry at a magnification of ×100. The trans-sectional images of the small arterioles with diameters <100 μm were examined. The area encircled by the outer border of the media (total vascular area) and that of the vascular lumen (lumen area) were measured. The total vessel wall area was calculated as the difference between these two areas. Then, the thickness of the wall of the coronary arteriole was determined as the wall-to-lumen ratio (the total vessel wall area divided by the lumen area). The area of fibrosis immediately surrounding the arteriole was measured and corrected for the total vascular area. In each heart, no less than seven arterioles were examined and the average values were used for analysis.

2.6. Statistical analysis

All values were expressed as mean±S.D. All statistical analyses were performed using Statview software (Abacus Concepts Inc., Cary, NC, USA). Comparison of values between the age-matched OLETF and LETO rats was performed using the Mann–Whitney U-test. Serial changes were examined by repeated measure of one-way analysis of variance (ANOVA). A probability value of <0.05 was considered statistically significant.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>LETO (g)</th>
<th>OLETF (g)</th>
<th>LETO (g)</th>
<th>OLETF (g)</th>
<th>LETO (g)</th>
<th>OLETF (g)</th>
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<tbody>
<tr>
<td>Body weight</td>
<td>95±7</td>
<td>93±5</td>
<td>350±18</td>
<td>457±32*</td>
<td>468±18</td>
<td>581±14*</td>
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<tr>
<td>LVW (g)</td>
<td>0.37±0.04</td>
<td>0.38±0.03</td>
<td>0.79±0.07</td>
<td>0.93±0.06*</td>
<td>0.94±0.06</td>
<td>1.13±0.09*</td>
</tr>
<tr>
<td>LVI (mg/g)</td>
<td>3.84±0.31</td>
<td>4.08±0.43</td>
<td>2.27±0.22</td>
<td>2.05±0.24</td>
<td>2.01±0.11</td>
<td>1.94±0.14*</td>
</tr>
<tr>
<td>PGC (mg/dl)</td>
<td>78±13</td>
<td>79±7</td>
<td>101±5</td>
<td>116±9*</td>
<td>90±5</td>
<td>112±12*</td>
</tr>
</tbody>
</table>

Data are presented as mean±S.D.; n = 10 for each group. LVW, left ventricular weight; LVI, left ventricular weight index; PGC, plasma glucose concentration.

* P<0.05 versus LETO rats of the same age.

3. Results

3.1. Body weight, left ventricular weight, and plasma glucose concentration

Table 1 summarizes the changes in body weight, left ventricular weight, and plasma glucose concentration in both groups. The body weight and left ventricular weight of all rats increased with age; the increases were significantly greater in OLETF than in LETO rats from 15 weeks of age. No significant difference in left ventricular weight index was observed between OLETF and LETO rats at any weeks of age. Plasma glucose concentrations in both groups remained within the normal range, although OLETF rats showed a higher value at 15 weeks of age.

3.2. Systemic hemodynamics

Serial changes in systemic hemodynamics under basal conditions and during dipyridamole infusion are summarized in Table 2. Under basal conditions, HR remained unchanged with age in both groups. MAP in OLETF rats increased gradually and was significantly higher than that in LETO rats at 30 weeks of age. CI decreased with age in both groups, and the decrease was greater in OLETF than in LETO rats. Consequently, the increase in total vascular resistance index was significantly greater in OLETF than in LETO rats. There was no significant difference in HR or MAP between OLETF and LETO rats under basal condition at any weeks of age. None of the changes in HR and MAP in response to dipyridamole infusion reached statistical significance in either group at any weeks of age. CI tended to be increased by dipyridamole, while TPRI was significantly decreased to the similar extent in the two groups.

3.3. Coronary hemodynamics

Serial changes in coronary hemodynamic parameters are presented in Fig. 1. There were no significant differences in baseline MBF (Fig. 1A) or baseline CVR (Fig. 1B) between OLETF and LETO rats at any weeks of age.
Table 2
Systemic hemodynamic parameters before and during dipyridamole infusion in LETO and OLETF rats at 5, 15 and 30 weeks of age

<table>
<thead>
<tr>
<th></th>
<th>5 weeks LETO</th>
<th>5 weeks OLETF</th>
<th>15 weeks LETO</th>
<th>15 weeks OLETF</th>
<th>30 weeks LETO</th>
<th>30 weeks OLETF</th>
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<tr>
<td><strong>HR (beats/min)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>357±6</td>
<td>348±25</td>
<td>351±25</td>
<td>348±20</td>
<td>340±31</td>
<td>349±32</td>
</tr>
<tr>
<td>Dipyramrole</td>
<td>361±37</td>
<td>355±32</td>
<td>366±34</td>
<td>361±6</td>
<td>352±26</td>
<td>360±30</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>99±18</td>
<td>98±21</td>
<td>108±21</td>
<td>98±11</td>
<td>119±15</td>
<td>103±14</td>
</tr>
<tr>
<td>Dipyramrole</td>
<td>88±16</td>
<td>86±12</td>
<td>91±12</td>
<td>88±18</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CI (ml/min/kg)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>821±47</td>
<td>810±6</td>
<td>292±28</td>
<td>275±22</td>
<td>246±24</td>
<td></td>
</tr>
<tr>
<td>Dipyramrole</td>
<td>846±31</td>
<td>837±62</td>
<td>282±39</td>
<td>257±30</td>
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<td></td>
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<tr>
<td><strong>TPRI (mmHg/ml/min/kg)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.13±0.02</td>
<td>0.12±0.03</td>
<td>0.42±0.06</td>
<td>0.41±0.08</td>
<td>0.55±0.09</td>
<td></td>
</tr>
<tr>
<td>Dipyramrole</td>
<td>0.10±0.02†</td>
<td>0.10±0.01</td>
<td>0.27±0.06†</td>
<td>0.32±0.07†</td>
<td>0.42±0.08†</td>
<td></td>
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</tbody>
</table>

Data are presented as mean±S.D.; n=10 for each group. HR, heart rate; MAP, mean arterial pressure; CI, cardiac index; TPRI, total peripheral resistance index.

† P<0.05 versus rats aged 5 weeks of the same strain.

* P<0.05 versus LETO rats of the same age.

§ P<0.05 versus baseline.

![Fig. 1.](image)

Fig. 1. Coronary hemodynamics for LETO and OLETF rats at 5, 15, and 30 weeks of age. Values are expressed as mean±S.D.; n=10 for each group. Baseline MBF, baseline myocardial blood flow; baseline CVR, baseline coronary vascular resistance; CFR, coronary flow reserve; minimal CVR, minimal coronary vascular resistance. † P<0.05 versus rats aged 5 weeks of the same strain; * P<0.05 versus LETO rats of the same age.
These parameters at rest remained unchanged with age in both groups. In contrast, maximal hyperemia induced by dipyridamole revealed deterioration of coronary dilator reserve in OLETF rats. Although both CFR (Fig. 1C) and minimal CVR (Fig. 1D) remained unchanged in LETO rats, CFR was significantly decreased and minimal CVR was increased in OLETF rats at 15 weeks of age and thereafter.

3.4. Histopathological findings

Fig. 2 shows representative sections of coronary arterioles from LETO (top) and OLETF (bottom) rats at 5, 15, and 30 weeks of age. The wall of the arteriole was thickened, which was accompanied by significant perivascular fibrosis in OLETF rats as early as 15 weeks of age. Such morphological alterations were not seen in LETO rats.

Fig. 3 compares the serial changes in histopathological parameters between the two groups. Both the wall-to-lumen ratios (Fig. 3A) and the degrees of perivascular fibrosis (Fig. 3B) in coronary arterioles were increased in OLETF rats at 15 weeks of age and thereafter, although both parameters remained unchanged in LETO rats.

In histomorphometry, the number of arterioles examined in each heart ranged from seven to 12 (8.6±1.3/animal). The average values for individual animals were used for comparison. The S.D. values (coefficient of variation) of the measurements in individual animals ranged from 0.03 (15.6%) to 0.08 (16.3%) for the wall-to-lumen ratios and from 0.02 (9.1%) to 0.09 (13.4%) for the degrees of perivascular fibrosis. Thus, intra-animal variations of the histopathological parameters were small.

Furthermore, regression analysis showed that both the wall-to-lumen ratios ($r=0.49, P<0.01$) and the degrees of perivascular fibrosis ($r=0.65, P<0.01$) were significantly correlated with minimal CVR in OLETF rats (Fig. 4).

4. Discussion

Impaired CFR in patients with type II DM with angiographically normal coronary arteries has been demonstrated in studies using coronary Doppler flow wire [16], positron emission tomography [17], and argon-gas chromatography [18]. The majority of these studies ascribed the reduced CFR to microangiopathy. In fact, numerous studies demonstrated remodeling of small coronary arteries in DM [3,19–21]. However, no studies have directly compared their functional and structural alterations in DM.

In the present study, using OLETF rats as a model of type II DM, we found that CFR could be impaired even at the prediabetic stage, which was evidenced by reduced vasodilator reserve to dipyridamole as early as 15 weeks of age. The alteration in coronary arterial function was associated with morphological changes of the coronary

![Fig. 2. Light micrographs of intramyocardial small arteries from LETO and OLETF rats at 5, 15, and 30 weeks of age. Compared with LETO rats, increases in arterial wall thickness and degree of perivascular fibrosis are seen in OLETF rats from 15 weeks of age. Scale bar=50 μm.](image-url)
arterioles during the development of DM. When all data from OLETF rats at 5, 15 and 30 weeks of age were combined, the minimal CVR exhibited significant correlations with both the wall-to-lumen ratio and the degree of perivascular fibrosis. Thus, the remodeling of coronary arterioles has been shown to directly correlate to functional deterioration of coronary circulation in the process of DM development. To the best of our knowledge, this is the first study that provides serial data on both functional and morphological changes of intramyocardial small arterioles to demonstrate their close association in the course of the development of microangiopathy in type II DM.

4.1. Stage of DM in OLETF rats

Our previous studies have shown that, from 10 to 20 weeks of age, OLETF rats presented postprandial hyperglycemia and relatively higher plasma insulin levels both at baseline and at 2 h of OGTT as compared with those in age-matched LETO rats. At 30 weeks of age, the OGTT resulted in a definite DM pattern [9,10]. In the present study, the plasma glucose concentration of OLETF rats was higher than that of LETO rats at 15 weeks of age but remained within the normal range. Although we did not perform OGTT in this study, according to our previous data [9,10] and those in other reports [7,8], it is likely that our subjects were prediabetic with insulin resistance at 10–20 weeks and diagnosable DM at 30 weeks.

4.2. Mechanism of remodeling of coronary arterioles

Vascular lesions of small coronary arteries have been described in diabetic patients and experimental animals. The morphological features represent thickening of the arterial wall [3] and capillary basement membrane, periodic acid–Schiff (PAS)-positive deposits in the vessel wall of small arteries [19], microaneurysms [20], perivascular and interstitial fibrosis, and fibrosis within the small coronary vessel wall [21,22]. In the present study, OLETF rats exhibited both significant wall thickening and substantial
perivascular fibrosis in coronary arterioles as early as at the stage of prediabetes and insulin resistance. We have previously shown that the receptor of tissue growth factor beta-1 that modulates cellular growth and differentiation and extracellular matrix deposition are significantly increased in the myocardial tissue in association with myocardial interstitial collagen deposition in the same stage in the same model [9]. We have recently reported that the wall thickness of intramyocardial small arteries significantly increases at 15 weeks in OLETF rats and that pretreatment with troglitazone, an insulin sensitizer, both improves metabolic abnormalities and prevents the development of remodeling of intramyocardial small arteries [23]. Zaman et al. [24] also found that genetically modified mice, which are rendered leptin-deficient and develop obesity, insulin resistance, hyperinsulinemia and NIDDM, exhibit increased perivascular fibrosis at an early stage of DM. They postulated that inhibition of fibrinolysis, evidenced as the increase in plasminogen activator inhibitor-type I concentration and augmented tissue factor expression in the heart, may contribute to the perivascular fibrosis [24]. Thus, it is conceivable that the key processes involved in coronary arteriolar remodeling, including both perivascular fibrosis and proliferation of arteriolar medial smooth muscle cells, are secondary to the serological alterations that we and other investigators have shown to precede manifest DM.

4.3. Functional alteration of coronary arterioles

In the present study, in contrast to the significant increase in the baseline total peripheral resistance index as the index of systemic vascular resistance, both baseline MBF and baseline CVR remained unchanged in OLETF rats that had significant remodeling of coronary arterioles. Correlation study revealed the lack of association between the magnitude of morphological alterations of coronary arterioles in OLETF rats and their baseline MBF or baseline CVR. This may be ascribed to the autoregulation mechanism of tissue perfusion specific to coronary circulation. Precapillary pressure is maintained by autoregulation that dilates arterioles to offset the increase in CVR due to epicardial conduit stenosis [25]. Since dipyridamole acts via an increase in adenosine concentration in the same level of precapillary arterioles [26], the reserve for further adenosine action may be reduced.

According to Folkow et al. [27], arterial medial hypertrophy reduces the lumen diameter even when vascular smooth muscle is fully relaxed. Therefore, the increase in wall-to-lumen ratio of arterioles observed on fixed pathological specimens should be reproducible during vasodilatation by dipyridamole in vivo and could elevate minimal CVR. In addition, the perivascular accumulation of collagen fibers could interfere with vasodilatation and thus affect both CFR and minimal CVR [28].

Recently, Fukui et al. [29] have demonstrated that oxidative stress is already increased in early stage type II DM in the same model as ours. Impairment of endothelial cell functions due to the increase in oxidative stress in DM was demonstrated both in vitro and in vivo [30,31]. Dipyridamole used in the present study increases the interstitial adenosine concentration in vascular smooth muscle, leading to relaxation of coronary resistance vessels [32]. It has been postulated that increased shear stress associated with an increase in blood flow induces the release of vasodilating substances from endothelial cells, and elicits more prominent vasodilatation in the vessels with preserved endothelial function [33]. Indeed, the coronary flow response to dipyridamole or adenosine has been found to relate to endothelium-dependent vasodilation [34,35]. We therefore speculate that the impairment of flow-mediated coronary vasodilation due to endothelial dysfunction could be an additional mechanism of reduced CFR in the prediabetic stage of OLETF rats.

4.4. Limitations of this study

Intermediate but not close correlation between morphological changes in coronary arterioles and elevated CVR during hyperemia may suggest the existence of multiple factors affecting coronary function that were not assessed in the present study. Although macroangiopathy is another important cause of the impairment of coronary arterial function in DM, we did not perform functional or histopathological evaluation of the epicardial large coronary arteries. However, it was reported that a minor degree of atherosclerosis (coronary stenosis less than 40%) produced no or only minor impairment in CFR and CVR [37]. Also, in a previous study of CFR in diabetic patients, impairment in CFR was observed in the absence of differences in severity of coronary artery diseases compared with nondiabetic patients [4]. Thus, it is more likely that coronary microcirculation abnormality might be a major factor in the reduced CFR and increased CVR seen in this study. In addition, it is unlikely that epicardial coronary stenosis developed significantly by 30 weeks in our model, since there was no further decrease in CFR from 15 to 30 weeks.

Because it is technically difficult to directly evaluate coronary vasodilator reserve in response to endothelium-dependent agents such as acetylcholine or bradykinin in vivo in the rat model, in our study coronary arterial function was defined by dipyridamole-induced hyperemia, which is primarily an endothelium-independent response. However, it has been reported that some vasodilators exert indirect endothelium-dependent vasodilation due to a flow-mediated mechanism that is secondary to direct smooth muscle relaxation [34,35]. Therefore, coronary endothelial dysfunction in OLETF rats might have been demonstrated indirectly in the present study by the reduced CFR as defined by dipyridamole. Nevertheless, the lack of a direct
assessment of coronary endothelial function is a major limitation of our study.

DM commonly affects the autonomic nervous system, which can be an additional explanation for impaired arterial vasodilator reserve. Cardiovascular reflex abnormalities include a higher resting HR or inadequate alteration in HR and blood pressure in response to various stimuli [36]. However, there were no significant differences in HR or MAP at baseline between the two groups at any weeks of age. None of the changes in HR or MAP in response to dipyridamole infusion was significantly different between the two groups. Whereas TPRI was reduced by dipyridamole in both groups at all ages, CI tended to be increased (5 and 10 weeks) or was significantly increased during dipyridamole infusion. Therefore, it is possible that the increased fluid volume per se from the dipyridamole solution (infused at 0.8 ml/kg/min for 10 min) might have masked the subtle differences in the cardiovascular autonomic response, including the HR responses, between the two groups.

Left ventricular function, especially diastolic function, is another determinant of coronary arterial function. Although CI was found significantly reduced in OLETF rats at 30 weeks of age, we did not evaluate diastolic function in the present study. We have shown that myocardial collagen accumulation, probably due to metabolic abnormalities in early stage of DM, could impair left ventricular diastolic filling [9]. Thus, incorporation of left ventricular diastolic function might have provided a better explanation for impaired CFR in DM.

It was demonstrated that true CVR should be defined as $(P_a - P_e)/MBF$, where $P_a$ is arterial pressure, and $P_e$ is the effective back pressure such as left-ventricular end diastolic pressure (LVEDP) or left atrial pressure (LAP) [38]. In this study, however, we calculated CVR as the ratio of MAP to MBF, thus assuming that the effective back pressure is negligible. We can not exclude the possibility that differences in LVEDP or LAP and their responses to dipyridamole existed between the two groups with potentially different left ventricular systolic and diastolic function, which might influence CVR evaluation.

4.5. Conclusions

Functional impairment and histopathological remodeling of coronary arterioles were demonstrated at the prediabetic stage of type II DM model. This study directly correlated morphological alterations of coronary arterioles with functional deterioration during development of microangiopathy in DM.

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References