率を期待できる冠状動脈であるが，右冠状動脈本幹へのバイパスはもっとも遠隔開存率が低い[87]．GEAやRAを狭窄の軽い右冠状動脈に吻合した場合の血流競合は頻度が高く，動脈グラフトよりも上行大動脈からのSVGを右冠状動脈分枝にバイパスするほうが高い早期開存率を期待できるとの考えもある．

Ⅲ．CABGの技術革新

1．ロボット手術

手術支援ロボットシステムとしては，コンソールが手術台から離れて操作できるtelesurgery（遠隔手術）が可能なことがあげられる．将来は，僻地や離島での心臓手術が可能になる可能性がある．

現在，唯一欧米にて販売されている手術支援ロボットシステムであるda Vinci（Intuitive Surgical社，サニーベール）は，生理的振動の補正機能や術者の動きを最高1/5まで縮小し鉗子に伝達できる特徴を有する．さらに，EndoWristと呼ばれる人の手首をモデルとした7軸の可動性を有する左右アームと高画質の三次元立体画像により，緻密な手術手技を内視鏡下に行いうるとされ，低侵襲心臓手術のさらなる発展性が期待されている．

MIDCABの早期成績は不良で，全国的にMIDCABが減少している．LITAの採取は胸腔鏡でも行われているが，既存の胸腔鏡用の手術器具の作業軸は，挿入部のポートの部分で規定される1軸となるため，心臓外科医が習熟するには時間がかかる．

当センターにおいても，da Vinciで内胸動脈を採取した後に第4もしくは5肋間開胸でLITA-LAD吻合を行うMIDCABを2004年9月より導入した．2005年11月現在13例に施行しているが，LITAに吻合した枝からの出血のため正中切開に変更が必要であった80歳の1例を除いて，LITA剥離は65～90分で十分な長さのグラフトを採取することができ，バイパスは全例開存していた．

LITAをLADに吻合する完全内視鏡下のCABG（TECAB：totally endocsopic coronary artery bypass）は1998年にはじめて行われ[91]，多枝バイパスを行うTECABも報告されている[92]．

2．自動吻合器

中枢側の自動吻合器は上行大動脈を部分遮断せず，小切開手術でも容易に吻合できるように開発された．はじめて臨床使用されたSymmetry bypass system（St. Jude Medical社，セントポール）は，早期吻合部閉塞を含め[93]，さまざまな合併症が増加し販売中止となっている．現在本邦で使用可能な自動吻合器は，吻合部内膜に金属を露出しないように改良されたPas-Port system（Cardica社，レッドウッドシティー）と，手縫いと同様な吻合機序をとり入れたU-clipによる結節縫合を自動的に行うSpyder（Medtronic社，ミネアポリス）の2種類である．その他，自動吻合器ではないが，大動脈部分遮断を行わずに中枢吻合を行うためのHeartstring（Bostonscientific社，サンタクララ）やEnclose（Novare社，クパチーノ）が臨床使用されている．末梢側の自動吻合器としては，MVP system（Medtronic社），C-Port（Cardica社），ATG coronary connector system（St. Jude Medical社）などが研究されており，手術視野が限られるCABGやTECABにおいて期待されている．

3．Awake CABG

気管内挿管せずに硬膜外麻酔などで行うawake OPCABは，呼吸機能低下例や坦癌例などの人工呼吸（全身麻酔）が困難な症例に行われている[94, 95]．また，両側ITAを使用したawake OPCABの症例報告もされている[96]．Awake OPCABにおいて患者選択基準は確立されていないが，$FEV_{1.0}$%が50%未満の閉塞性呼吸機能障害例はawake OPCABの適応とはならないとしている報告もある[94]．Awake OPCABの有用性に関しては，硬膜外麻酔の動脈拡張作用，不整脈抑制作用の利点もあり，今後の検討が期待される．

4．Hybrid手術

人工心肺が非常に危険な症例では吻合可能な冠状動脈のみをOPCABで行い，術後にPCIを併用するhybrid revascularizationが一部で行われている[13]．手術室に心カテーテル検査を行える装置がある部屋で，MIDCABに引き続いてLITAの確認造影とPCIを行う方法が効率的である．右冠状動脈狭窄が軽いtype Aの病変を有する患者がよい適応である．da Vinci外科手術支援ロボットシステムを使用し，側方開胸でITAとRAのcompositeグラフトでOPCABをLADと回旋枝に行い，右冠状動脈にPCIを行うことでより低侵襲の手術が行える．

おわりに

当センターにおいて，2000年から積極的に動脈グラフトを多用したOPCABを行ってきたが，

EBMとしてその有用性が明らかとなってきている．DESを使用したPCI全盛時代においてCABGが生き残るためには，低侵襲で遠隔成績のよい手術を行う必要があると考えられる．

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SUMMARY

Current Status of Coronary Artery Bypass Grafting

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The number of coronary artery bypass grafting (CABG) has reached more than 21,000 cases per year in Japan, and the operative mortality has decreased less than 1% including emergent operation. There are 2 trends in CABG. One is the revival and wide spread of off-pump CABG (OPCAB). The other is multiple arterial coronary revascularization. In 2004 and 2005, 60% of all CABG procedures in Japan were performed without cardiopulmonary bypass. For competition with percutaneous coronary intervention with drug eluting stents and better long-term outcomes, CABG with only arterial grafts was carried out in 52% of total cases and 66% of OPCAB cases. OPCAB with multiple arterial grafts has been becoming the standard CABG in Japan. We reviewed OPCAB and arterial CABG including new technology.

KEY WORDS
CABG/cardiopulmonary bypass/arterial grafts

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Undernutrition *in Utero* Augments Systolic Blood Pressure and Cardiac Remodeling in Adult Mouse Offspring: Possible Involvement of Local Cardiac Angiotensin System in Developmental Origins of Cardiovascular Disease

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Evidence has emerged that undernutrition *in utero* is a risk factor for cardiovascular disorders in adulthood, along with genetic and environmental factors. Recently, the local expression of angiotensinogen and related bioactive substances has been demonstrated to play a pivotal role in cardiac remodeling, *i.e.* fibrosis and hypertrophy. The aim of the present study was to clarify the possible involvement of the local cardiac angiotensin system in fetal undernutrition-induced cardiovascular disorders. We developed a mouse model of undernutrition *in utero* by maternal food restriction, in which offspring (UN offspring) showed an increase in systolic blood pressure (8 wk of age, $P < 0.05$; and 16 wk, $P < 0.01$), perivascular fibrosis of the coronary artery (16 wk, $P < 0.05$) and cardiac cardiomegaly (16 wk, $P < 0.01$), and cardiomyocyte enlargement, concomitant with a significant augmentation of angiotensinogen ($P < 0.05$) and endothelin-1 ($P < 0.01$) mRNA expression and a tendency to increase in immunostaining for both angiotensin II and endothelin-1 in the left ventricles (16 wk). These findings suggest that fetal undernutrition activated the local cardiac angiotensin system-associated bioactive substances, which contributed, at least partly, to the development of cardiac remodeling in later life, in concert with the effects of increase in blood pressure. (*Endocrinology* 148: 1218–1225, 2007)

IN THE EARLY 1990s, a novel hypothesis was advanced by Barker *et al.* (1) to link nutritional insults during embryonic and fetal periods not only to impaired maturation of physiological functions, but also to cardiovascular diseases in adulthood. Alterations in nutrition and endocrine status during the embryonic, fetal, and neonatal periods can trigger developmental predictive adaptive responses (2), causing permanent structural, physiological, and metabolic changes, thereby predisposing an individual to cardiovascular, metabolic, and endocrine diseases in adult life.

The renin-angiotensin system (RAS) plays an important role in primary as well as secondary forms of hypertension in both animals and humans (3). More recently, components of the RAS, such as angiotensin-converting enzyme (ACE) and angiotensin II, were revealed to be produced locally in the cardiac tissues, and termed the local cardiac RAS (4), being primary candidates for the factors promoting cardiac remodeling, mainly cardiac myocyte hypertrophy and increased extracellular matrix fibrosis, thereby deteriorating cardiac function (5). Various experimental animal models have been developed to investigate the associations between fetal undernutrition and cardiovascular disease later in life (6, 7), and a possible commitment of a systemic RAS in the developmental origins of hypertension was reported (8). Therefore, the aim of the present study was to investigate whether the local cardiac RAS is associated with the developmental origins of cardiac remodeling in offspring exposed to undernutrition *in utero*.

Recently, we developed a mouse model of undernutrition *in utero* using maternal food restriction, in which the offspring (UN offspring) developed pronounced obesity when fed a high-fat diet, accompanied by impaired hypothalamic leptin sensitivity, as compared with normally nourished offspring (NN offspring) (9). Using this model, we investigated whether fetal undernutrition affects systolic blood pressure (SBP), cardiac remodeling, and expression of local cardiac RAS-associated bioactive substances. We found that undernutrition *in utero* caused a significant increase in SBP as well

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Abbreviations: ACE, Angiotensin-converting enzyme; Ang, angiotensinogen; ANP, atrial natriuretic peptide; ARC, arcuate nucleus of the hypothalamus; AT1R, angiotensin II type 1 receptor; AT2R, angiotensin II type 2 receptor; BNP, brain natriuretic peptide; dpc, d postcoitum; ET-1, endothelin-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NN offspring, normally nourished offspring; NOx, nitrite/nitrate; PAS, periodic-acid Schiff; RAS, renin-angiotensin system; SBP, systolic blood pressure; UN offspring, offspring of undernutrition *in utero*.

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as cardiac remodeling, concomitant with a significant elevation in mRNA expression in angiotensinogen (Ang) and endothelin-1 (ET-1) in the left ventricle.

Materials and Methods

Development of a mouse model of undernutrition in utero

Undernutrition *in utero* by maternal food restriction was carried out as described previously (9). In brief, pregnant C57Bl/6 mice were purchased at 8.5 d postcoitum (dpc) from Japan Central Laboratories for Experimental Animals (Tokyo, Japan) and were divided into two groups at 10.5 dpc. Dams were housed individually with free access to water during 14-h light, 10-h dark cycles. The daily food supply of one group was restricted to 70% of the food consumed by the other group, fed *ad libitum*, based on the data of the previous day, from 10.5 dpc to the day of delivery of the pups. Dams of the food restriction group were supplied 2.5 g of extra food in the evening of 18.5 dpc, just before the night of parturition, to prevent mothers from eating their own pups. Pups were nursed by mothers fed *ad libitum* (eight pups per mother) and were weaned on to regular chow diet (RCD; Oriental Yeast Co., Tokyo, Japan) at 21.5 d of age. RCD includes 20.8% protein and 4.8% fat, with contents of sodium (0.19 g/100 g) and potassium (0.75 g/100 g). Only male pups were used for the following experiments, except for the study of fetal heart tissues. Each group in all experiments consists of offspring from at least four litters. All experimental procedures were approved by the Animal Research Committee, Kyoto University Graduate School of Medicine (Med Kyo 64116).

Measurement of SBP

At 4, 8, and 16 wk of age, SBP was measured at least five times in conscious mice (n = 8–10 for each group) using an indirect tail-cuff method (MK-2000; Muromachi Kikai Co. Ltd., Tokyo, Japan).

Neonatal leptin or monosodium glutamate treatment

Leptin (2.5 μg/g body weight·d) (PeproTech Inc., Rocky Hill, NJ) or vehicle saline was sc administered to NN offspring daily from 5.5 to 10.5 d of age, as a model of premature leptin surge (9), then SBP was measured at 8 wk. Monosodium glutamate (2 mg/g body weight·d) was sc administered to NN and UN offspring from 1.5 to 5.5 d of age, as previously described (9), for the purpose of permanent chemical injury of the arcuate nucleus of the hypothalamus (ARC) (10), then SBP was measured at 16 wk.

Morphological analysis of the kidney

For morphological analysis, whole kidneys were sampled at 8 and 16 wk, weighed and fixed in 10% formalin, and embedded in paraffin. The kidneys were cut into sections 2-μm thick and stained with hematoxylin and eosin, periodic-acid Schiff (PAS), or Masson trichrome. The stained sections were analyzed light microscopically.

Serum nitrite/nitrate (NOx) and plasma angiotensin II concentration

NOx concentration was determined by the Griess reaction using a commercial colorimetric assay kit (Cayman Chemical, Ann Arbor, MI).

The angiotensin II concentration was determined with an ELISA kit (Peninsula Laboratories, Belmont, CA), after extraction through C_{18} Sep-Pak columns (Waters Co., Milford, MA).

Urine microalbumin concentration

Urine was collected for 24 h using metabolic cages, and microalbuminuria was determined by the competitive ELISA method (Albuwell M assay kit; Exocell, Philadelphia, PA) at 16 wk of age. Urine creatinine values were assessed simultaneously by enzyme assay (MIZUHO MEDY Co., Ltd., Saga, Japan) and were used to calculate the albumin to creatinine ratio.

Morphometric analysis of the heart

The whole hearts were sampled, fixed in 10% formalin, and embedded in paraffin at 8 and 16 wk. The heart was cut into two subserial cross-sections 6-μm thick at intervals of 1 mm and stained with Sirius Red to evaluate the perivascular fibrosis of coronary arteries 100–200 μm in diameter. The perivascular fibrosis was assessed by analyses of digital images, calculating the ratio of the area of Sirius Red-stained fibrosis to the total vessel area using a KS400 image system (Zeiss, Oberkochen, Germany). To evaluate perivascular fibrosis in renal small arteries 100–200 μm in diameter, the kidneys were also sampled in the offspring at 16 wk and evaluated in the same manner as the coronary arteries.

To determine the interstitial fibrosis of the heart at 16 wk of age, we randomly selected 20 fields in two different sections and calculated the ratio of the areas of Sirius Red-stained interstitial fibrosis to the total cross-sectional areas.

Cardiomegaly was assessed by whole-heart weight to body weight ratio at 8 and 16 wk. Cardiomyocyte enlargement was estimated by measuring shortest transverse diameter in nucleated transverse sections of the myocytes. In each sample at 16 wk, 8 fields were randomly selected, and 80 cells were measured.

Quantitative RT-PCR analysis

Total RNA was extracted from whole hearts of fetal mice at 18.5 dpc and from left ventricles of the mice at 3, 8, and 16 wk, as well as from kidneys at 16 wk. The mRNA expression was measured by real-time quantitative RT-PCR using Taqman technology (Model 7000 sequence detector; Applied Biosystems, Foster City, CA). The forward and reverse primers and Fam/Tamra or Fam/MGB probes used for the targeted amplification of part of the cDNAs of murine Ang, angiotensin II type 1 receptor (AT1R), angiotensin II type 2 receptor (AT2R), ACE, renin, ET-1, atrial natriuretic peptide (ANP), and brain natriuretic peptide (BNP) are summarized in Table 1. The forward and reverse primers and Joe/Tamra probes for the murine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and ribosomal RNA coding region were purchased from Applied Biosystems. Serial dilutions of total RNA sample, isolated from mouse left ventricles or kidneys, were used to construct the standard curve for each substance. The standard curves were calculated by linear regression analysis, and threshold cycle values were used to read off relative RNA amounts. An mRNA expression value was then obtained by dividing the value for the gene of interest by the value for the ribosomal RNA or GAPDH.

At first, we assessed expression of ribosomal RNA and GAPDH mRNA based on total RNA concentration assessed by optic densitometry. The fetal undernutrition significantly decreased ribosomal RNA expression, but not GAPDH mRNA expression, in the fetal heart (data not shown). By contrast, fetal undernutrition significantly decreased GAPDH mRNA expression, but not ribosomal RNA expression, in the left ventricle after birth (data not shown). Therefore, we used GAPDH and ribosomal RNA data for analyses in the fetal heart (18.5 dpc) and in the left ventricle after birth (3, 8, and 16 wk), respectively, to compensate the variation. Because fetal undernutrition did not change GAPDH mRNA expression in the adult kidney (data not shown), GAPDH data were used for analyses in the adult kidney.

Immunohistochemistry of angiotensin II, ET-1, and renin

Six-micrometer-thick sections of the paraffin-embedded whole heart were incubated for overnight at 4 C with rabbit antiserum against angiotensin-II (1:500) (T-4007; Peninsula Laboratories), ET-1 (1:500) (T-4050; Peninsula Laboratories), or goat antiserum against renin (1:1600) (kindly donated by Professor Tadashi Inagami, Vanderbilt University School of Medicine, Nashville, TN) (11). Normal goat or rabbit serum (Dako Co., Carpinteria, CA) was used as negative controls. Staining was detected using an avidin-biotin-peroxidase method kit (ELITE ABC; Vector Laboratories, Burlingame, CA) with 3,3′-diaminobenzidine as previously described (12).

Statistical analysis

Values were expressed as means ± SEM. The significance of differences was assessed with Student's *t* test. *P* values < 0.05 were regarded as significant.

TABLE 1. Forward/reverse primers and FAM/Tamra or FAM/MGB probes used in the quantitive PCR analysis

	Primers (5′–3′)
Ang	
Forward	CAGCACCCTACTTTTCAACACCTA
Reverse	TGTTGTCCACCCAGAATTCATG
FAM/MGB probe	TCCAAGGAACGATGAGAG
ACE	
Forward	AATCGGCCTACTGGACCATGT
Reverse	GGCCATCTTTAGCAGGTAATTGAT
FAM/MGB probe	ACCAATGACATAGAGAGTG
AT1R	
Forward	GATCGCTACCTGGCCATTGT
Reverse	GTGACTTTGGCCACCAGCAT
FAM/MGB probe	CCGATGAAGTCTCGC
AT2R	
Forward	TGCTGGGATTGCCTTAATGAA
Reverse	TCAGGACTTGGTCACGGGTAAT
FAM/MGB probe	AGCAACGTGTTACTTTG
Renin	
Forward	CACTACGGATCAGGGAGAGTCAA
Reverse	CAGCTCGGTGACCTCTCCAA
FAM/MGB probe	CAGGACTCGGTGACTGT
ET-1	
Forward	CTTCTGCCACCTGGACATCAT
Reverse	TGGTGAGCGCACTGACATCTA
FAM/MGB probe	AGCGCGTCGTACCGTA
ANP	
Forward	GCCATATTGGAGCAAATCCT
Reverse	GCAGGTTCTTGAAATCCATCA
FAM/Tamra probe	TGTACAGTGCGGTGTCCAACACAGAT
BNP	
Forward	CCAGTCTCCAGAGCAATTCAA
Reverse	GCCATTTCCTCCGACTTTT
FAM/Tamra probe	TGCAGAAGCTGCTGGAGCTGATAAGA

Ang, GenBank accession no. BC019496; Strausberg *et al.*, 2002. ACE, BC083109; Strausberg *et al.*, 2002. AT1R, BC036175; Strausberg *et al.*, 2002. AT2R, AK086334; Carninci *et al.*, 1999. Renin, NM_031192; Wilson *et al.*, 1977. ET-1, BC029547; Strausberg *et al.*, 2002. ANP, D70837; Tamura *et al.*, 1996. BNP, D82049; Ogawa *et al.*, 1994.

Results

SBP at 4, 8, and 16 wk

There was no significant difference in SBP between UN and NN offspring at 4 wk. However, the SBP of UN offspring was significantly higher than that of NN offspring at 8 wk ($P < 0.05$), and the elevation of SBP in UN offspring continued at least until 16 wk ($P < 0.01$) (Fig. 1A).

SBP after neonatal leptin or monosodium glutamate treatment

There was no significant difference in SBP between NN offspring with neonatal leptin treatment (90.5 ± 1.4 mm Hg, n = 10) and those with neonatal vehicle treatment (86.3 ± 1.6 mm Hg, n = 10) at 8 wk. The significant elevation of SBP in UN offspring, as compared with NN offspring, at 16 wk was not blocked by chemical injury of the ARC by neonatal monosodium glutamate treatment (108.1 ± 5.2 mm Hg, n = 9 *vs.* 88.8 ± 5.0 mm Hg, n = 8; $P < 0.05$).

Serum NOx concentration and plasma angiotensin II concentration

The serum NOx concentration of UN offspring was significantly lower than that of NN offspring at 8 wk ($P < 0.05$) (Table 2). Such a tendency was also observed at 16 wk, but the difference was not statistically significant (Table 2).

The plasma angiotensin II concentration of UN offspring was similar to that of NN offspring at 8 wk (Table 2). At 16 wk, the plasma angiotensin II concentration of UN offspring was higher than that of NN offspring, but the difference was not significant (Table 2).

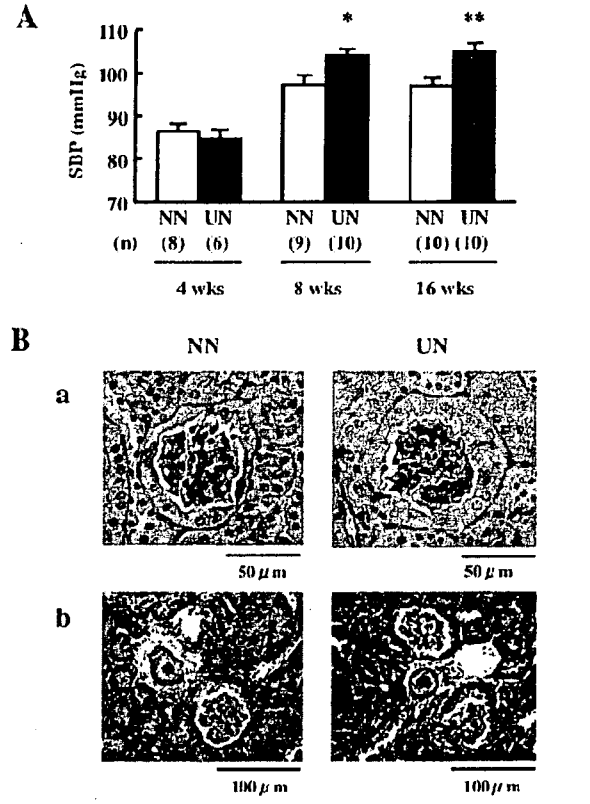

FIG. 1. SBP (A), PAS (Ba), and Masson trichrome (Bb) staining of kidney in NN offspring and UN offspring. *Columns* and *error bars* represent the mean and SEM of SBP. *, $P < 0.05$; **, $P < 0.01$ *vs.* NN offspring. Original magnification was ×400 (Ba) or ×200 (Bb). wks, Weeks of age.

Urine microalbuminuria

There was no significant difference in urine microalbumin concentration between UN and NN offspring at 16 wk (25.03 ± 2.06 μg/mg creatinine, n = 7 *vs.* 22.52 ± 1.65 μg/mg creatinine, n = 8).

Morphological analysis of the kidney

At 16 wk of age, the ratio of renal weight to body weight (mg/g) in UN offspring (5.58 ± 0.32, n = 20) was similar to that of in NN offspring (5.69 ± 0.27, n = 20).

Microscopic observation of hematoxylin and eosin (data not shown), PAS (Fig. 1Ba), and Masson trichrome (Fig. 1Bb) staining of kidneys from UN offspring at 8 and 16 wk showed no histological abnormalities as compared with NN offspring including nephron numbers.

TABLE 2. Serum NOx concentrations and plasma angiotensin II concentrations

	8 wk		16 wk	
	NN	UN	NN	UN
NOx (μM)	29.1 ± 3.1 (n = 9)	19.0 ± 2.5[a] (n = 10)	19.0 ± 2.8 (n = 10)	14.2 ± 2.2 (n = 10)
Angiotensin II (pg/ml)	43.3 ± 3.5 (n = 14)	43.4 ± 4.9 (n = 11)	118.9 ± 14.0 (n = 8)	179.1 ± 38.7 (n = 8)

Values are the mean ± SEM.
[a] $P < 0.05$ *vs.* NN.

Perivascular fibrosis of the coronary artery and renal small artery

At 8 wk of age, the ratio of coronary perivascular fibrosis to total vessel area in UN offspring had tended to increase as compared with that in NN offspring; however, the difference was not significant (Fig. 2B). At 16 wk of age, the ratio of coronary perivascular fibrosis to total vessel area was significantly higher in the UN offspring than NN offspring ($P < 0.05$) (Fig. 2, A and B). By contrast, the ratio of perivascular fibrosis to total vessel area in renal small arteries of UN offspring was similar to that in NN offspring at 16 wk of age (Fig. 2C).

Interstitial fibrosis of the heart

Interstitial fibrosis of the heart in UN offspring at 16 wk was similar to that in NN offspring (Table 3A).

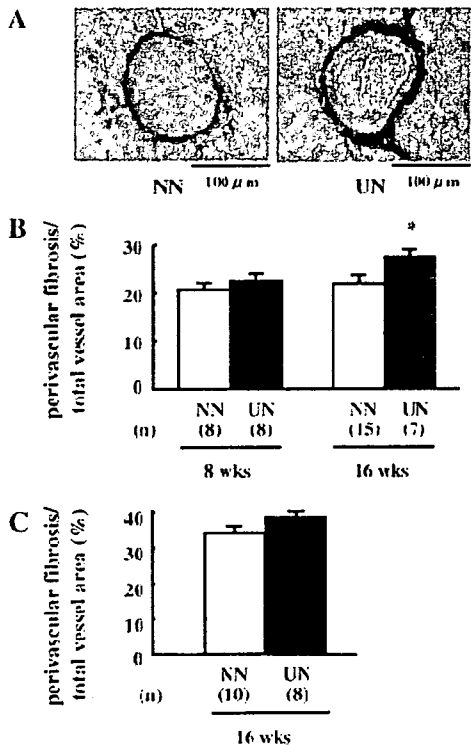

FIG. 2. Perivascular fibrosis in coronary and renal small arteries of NN and UN offspring. Representative cross-sections of coronary perivascular fibrosis at 16 wk of age (A). Collagen fibril was stained red with Sirius Red stain. Original magnification was ×400. Digital image analysis of perivascular fibrosis of coronary (B) and renal small arteries (C) as described in *Materials and Methods*. *Columns* and *error bars* represent the mean and SEM of the ratio of the area of Sirius Red-stained fibrosis to total vessel area (%). *, $P < 0.05$ *vs.* NN offspring. wks, Weeks of age.

Cardiomegaly and cardiomyocyte enlargement

The ratio of heart weight to body weight and transverse diameter of the cardiomyocytes were significantly higher in the UN offspring than NN offspring at 16 wk ($P < 0.01$) (Table 3B), in parallel with the increased perivascular fibrosis of coronary artery (Fig. 2B). However, cardiomegaly was not detected in UN offspring at 8 wk (Table 3B).

The mRNA expression of local cardiac RAS-associated bioactive substances in the left ventricles at 3, 8, and 16 wk

There were no significant changes in Ang, ACE, AT1R, AT2R, ET-1, ANP, or BNP mRNA expression between NN and UN offspring at 3 wk (Figs. 3 and 4).

At 8 wk, a significant decrease was observed in Ang mRNA expression in UN offspring ($P < 0.01$) (Fig. 3). By contrast, a significant increase was detected in AT2R ($P < 0.01$), ET-1 ($P < 0.01$), and BNP ($P < 0.01$) (Figs. 3 and 4) at 8 wk; whereas ANP mRNA expression had a tendency to increase, but not significantly (Fig. 4).

At 16 wk, a significant increase was observed in the mRNA expression of Ang ($P < 0.05$), AT2R ($P < 0.05$), and ET-1 ($P < 0.01$), but not in that of other substances (Figs. 3 and 4).

The renin mRNA expression in the left ventricles at 3, 8, and 16 wk was less than detection sensitivity of quantitative RT-PCR analysis (<0.00024-fold, compared with the whole kidney as a positive control).

Immunohistochemistry of angiotensin II, ET-1, and renin in the left ventricle

Immunostaining of both angiotensin II and ET-1 were mainly observed in cardiomyocytes of the left ventricle at 16 wk (Fig. 5, A and B). There occurred a tendency to increase in immunostaining for angiotensin II as well as ET-1 in UN offspring, as compared with NN offspring (Fig. 5, A and B).

Immunohistochemistry detected a few renin positive cells (one to two cells per slide) in the perivascular interstitial area (Fig. 5C). There was no apparent difference in the renin staining between NN and UN offspring at 16 wk (Fig. 5C).

The mRNA expression of local cardiac RAS-associated bioactive substances in the whole fetal heart at 18.5 dpc

A significant increase was observed in the mRNA expression of Ang ($P < 0.05$), ACE ($P < 0.01$), and ET-1 ($P < 0.05$) in the whole fetal heart at 18.5 dpc, but not in that of other substances (Table 4).

Discussion

In the present study, maternal food restriction caused a significant increase in SBP. However, neither the plasma

TABLE 3. Interstitial fibrosis of the heart (A) and cardiomegaly and cardiomyocyte enlargement (B)

	8 wk		16 wk	
	NN	UN	NN	UN
A. Interstitial fibrosis of the heart (%)[a]			0.782 ± 0.041 (n = 15)	0.751 ± 0.065 (n = 7)
B. Cardiomegaly and cardiomyocyte enlargement				
HW (mg)	127.1 ± 3.8	120.0 ± 3.0	158.9 ± 5.6	177.3 ± 6.2[b]
BW (g)	23.9 ± 0.2	23.2 ± 0.3	31.4 ± 0.5	30.6 ± 0.5
HW/BW (mg/g)	5.31 ± 0.13 (n = 24)	5.20 ± 0.14 (n = 19)	5.05 ± 0.12 (n = 14)	5.79 ± 0.18[c] (n = 16)
Cardiomyocyte diameter (μm)			14.3 ± 0.3 (n = 10)	16.6 ± 0.3[c] (n = 10)

Values are the mean ± SEM. HW, Heart weight; BW, body weight.
[a] No significant difference.
[b] $P < 0.05$ *vs.* NN.
[c] $P < 0.01$ *vs.* NN.

angiotensin II concentration (Table 2) nor the microalbumin concentration in UN offspring produced significant changes, although basal plasma angiotensin II concentration at 16 wk was higher than other reports (13). A significant decrease in the plasma NOx concentration of UN offspring was observed at 8 wk, as compared with that of NN offspring (Table 2). The decrease in the plasma NOx concentration of UN offspring was also observed at 16 wk, although it was not significant (Table 2). These observations suggested a possible involvement of endothelial dysfunction in the elevation of blood pressure in UN offspring, which is relevant to previous reports (14, 15). Histological examinations detected no abnormal findings in the renal tissues of UN offspring at 8 and 16 wk, although some investigators have demonstrated a possible involvement of small nephron numbers and/or a small number and size of glomeruli in increases in blood pressure during adulthood (16, 17).

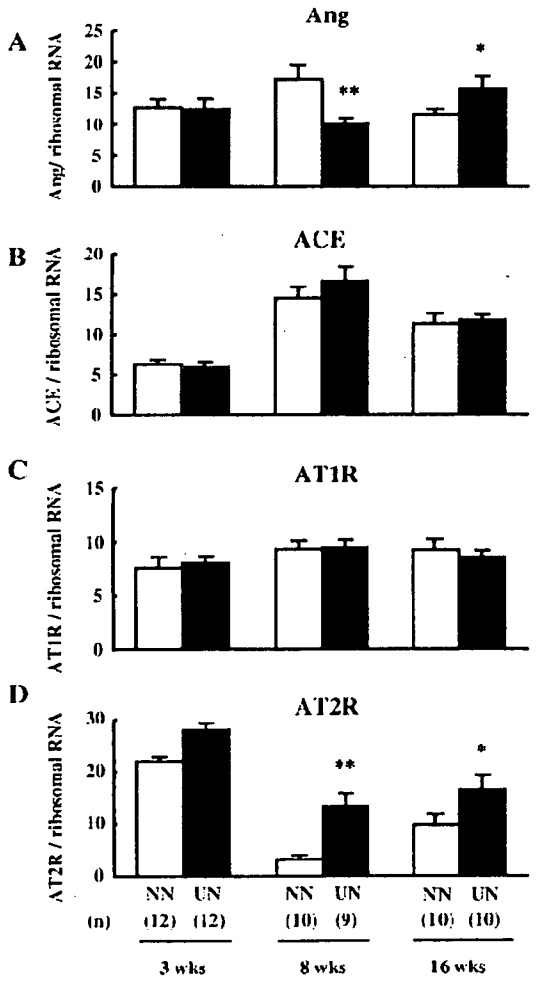

FIG. 3. The mRNA expression of Ang (A), ACE (B), AT1R (C), and AT2R (D) in the murine left ventricle at 3, 8, and 16 wk. *Columns* and *error bars* represent the mean and SEM of the mRNA expression in NN and UN offspring, measured by quantitative RT-PCR with real time TaqMan technology as described in *Materials and Methods*. *, $P < 0.01$; **, $P < 0.05$ *vs.* NN offspring. wks, Weeks of age.

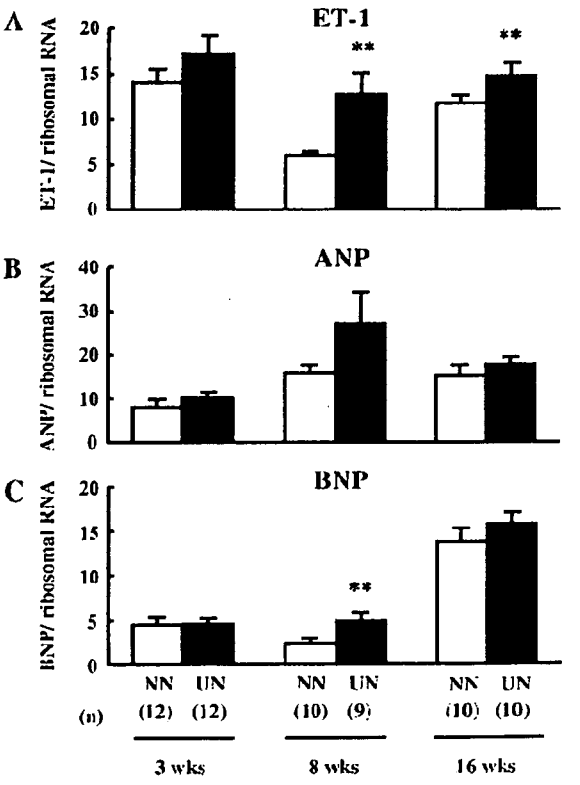

FIG. 4. The mRNA expression of ET-1 (A), ANP (B), and BNP (C) in the murine left ventricle at 3, 8, and 16 wk. *Columns* and *error bars* represent the mean and SEM of the mRNA expression in NN and UN offspring measured by quantitative RT-PCR with real time TaqMan technology as described in *Materials and Methods*. **, $P < 0.01$ *vs.* NN offspring. wks, Weeks of age.

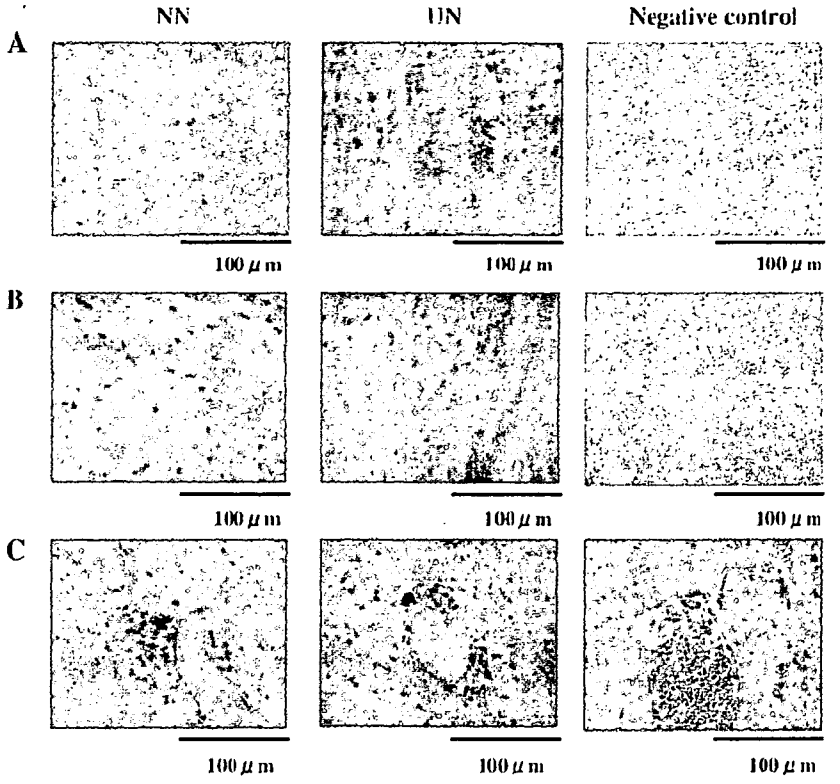

FIG. 5. Immunohistochemistry for angiotensin II (A), ET-1 (B), and renin (C) in the left ventricles of NN offspring (*left panels*) and UN offspring (*middle panels*) at 16 wk. Negative controls of NN offspring using normal rabbit serum (for angiotensin II and ET-1) or goat serum for renin are shown in *right panels*. Original magnification was ×400.

Using the same animal model, we recently reported pronounced obesity in UN offspring on a high-fat diet compared with NN offspring (9). We found premature onset of the neonatal leptin surge, *i.e.* a transient increase in serum leptin levels during the neonatal period, in UN offspring. We also demonstrated that the premature leptin surge programs hypothalamic low sensitivity to circulating leptin, a potent antiobesity hormone, causatively contributing to pronounced obesity on a high-fat diet in adulthood, by showing that an artificial premature leptin surge model produced hypothalamic low sensitivity to circulating leptin and pronounced obesity on a high-fat diet (9). However, in the present study, an artificial premature leptin surge did not increase SBP in NN offspring. Moreover, artificial premature leptin surge did not augment cardiac remodeling (Kawamura, M., and H. Itoh, unpublished observations). We also revealed that chemical injury of the ARC by neonatal monosodium glutamate treatment during the neonatal period cancelled the acceleration of obesity on the high-fat diet in UN offspring (9).

TABLE 4. The mRNA expression of Ang, AT1R, AT2R, ACE, ET-1, ANP, and BNP in the murine fetal whole heart at 18.5 dpc

	NN (n = 10)	UN (n = 10)
Ang/GAPDH	0.38 ± 0.05	0.74 ± 0.17[a]
AT1R/GAPDH	0.25 ± 0.02	0.31 ± 0.03
AT2R/GAPDH	4.88 ± 0.55	6.53 ± 1.11
ACE/GAPDH	0.10 ± 0.01	0.18 ± 0.03[b]
ET-1/GAPDH	2.13 ± 0.22	3.22 ± 0.44[a]
ANP/GAPDH	17.32 ± 1.93	22.58 ± 2.63
BNP/GAPDH	0.811 ± 0.07	0.70 ± 0.04

Values are the mean ± SEM (arbitrary units).
[a] $P < 0.05$; [b] $P < 0.01$ *vs.* NN offspring.

However, a significant increase in SBP was not blocked by monosodium glutamate treatment in the present study. The mechanisms leading to increased blood pressure in adult UN offspring with undernutrition *in utero* are currently not entirely clear.

There were no significant changes in the mRNA expression of cardiac RAS-associated bioactive substances at 3 wk (Figs. 3 and 4). On the other hand, at 8 wk, the mRNA expression of ET-1, a factor promoting cardiac remodeling (18, 19), was significantly elevated in the left ventricles of UN offspring (Fig. 4A). However, several anticardiac remodeling phenomena were observed at the same time in the left ventricles as follows. The Ang mRNA expression was significantly decreased (Fig. 3A), concomitantly with the significant increase of AT2R (Fig. 3D), which suppresses cardiac remodeling (20). ANP and BNP are secreted from the heart and antagonize RAS through a decrease in blood pressure, diuresis, anticardiac hypertrophy, and anticardiac fibrosis, *etc.* (21, 22). The significant elevation of BNP mRNA expression in the left ventricles of UN offspring at 8 wk, in parallel with a tendency for an increase in ANP mRNA expression, suggested protective effects on cardiac tissues against the acceleration of cardiac remodeling. Therefore, changes that both promote and suppress cardiac remodeling are simultaneously observed in the left ventricles of UN offspring at 8 wk. These findings lead us to speculate that a kind of compensatory mechanism might be operating, thereby protecting the heart from ominous cardiac transformation at 8 wk, which was relevant to the finding that neither cardiac hypertrophy (Table 3B) nor augmentation of perivascular

fibrosis (Fig. 2) was observed with a significant increase in SBP (Fig. 1).

At 16 wk, a significant augmentation of cardiac remodeling, *i.e.* cardiac hypertrophy (Table 3B) and perivascular fibrosis (Fig. 2), was observed in UN offspring. It is a further aim of the study to assess the movement and/or thickness of the ventricular wall by ultrasound examination.

In the present study, we first demonstrated that undernutrition *in utero* significantly increased the mRNA expression of both Ang (Fig. 3A) and ET-1 (Fig. 4A) in the left ventricles of UN offspring at 16 wk, concomitantly with the augmentation of cardiac hypertrophy and perivascular fibrosis. Angiotensin II is derived from Ang and plays a central role in the local cardiac RAS in the augmentation of cardiac remodeling (4, 5). ET-1 has been found to induce hypertrophy of cardiomyocytes (18), as well as cardiac fibrosis (19). ET-1 has a close association with the local cardiac RAS in the process of cardiac remodeling (23, 24). In the present study, the significant elevation of both Ang and ET-1 mRNA levels in the left ventricle of UN offspring was observed at 16 wk. The immunostaining of both angiotensin II and ET-1 showed a tendency to increase in UN offspring compared with NN offspring at 16 wk. These findings suggested a possible decompensation of cardiac homeostasis in response to various portentous factors, as a result of fetal undernutrition, including an increase in blood pressure. A significant elevation in the AT2R mRNA expression, which suppresses cardiac remodeling by antagonizing the effects of signaling through the AT1R (20), was observed in UN offspring at 8 and 16 wk, but the increase relative to NN offspring was much lower at 16 wk than at 8 wk (Fig. 3D). Long-term observations are necessary to prove that 16 wk is the beginning of decompensation of cardiac homeostasis in this animal model. Nevertheless, these findings suggested a possible involvement of local cardiac RAS activation in the developmental origins of cardiac remodeling.

Rather stable expression was observed in ACE and AT1R after birth in UN offspring. Ang mRNA expression decreased at 8 wk and increased at 16 wk. More detailed molecular investigation is necessary to clarify the regulatory mechanism of each substance.

A few renin positive cells were detected in the left ventricle at 16 wk (Fig. 5C), although mRNA expression was below detection sensitivity of quantitative RT-PCR. This discrepancy was relevant to the recent observation that cardiac renin was predominantly derived from circulation (25). There was no apparent difference in cardiac renin immunostaining between NN and UN offspring at 16 wk. It is an interesting study to investigate whether cardiac renin uptake is involved in developmental origins of cardiac remodeling.

A significant augmentation of mRNA expression of Ang, ACE, and ET-1 was observed in the whole fetal heart at 18.5 dpc (Table 4). A possible association of these changes with local cardiac RAS activation in adulthood is a future aim of the study.

In summary, using a mouse model of fetal undernutrition, we here demonstrated the possible involvement of the local cardiac RAS in the developmental origins of cardiac disorders, represented by cardiac remodeling, by a longitudinal assessment of the expression of local cardiac RAS-associated bioactive substances from the fetal to adult periods. This study also highlighted the local cardiac RAS as a promising target for prophylactic intervention in the developmental origins of cardiovascular disease.

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Impaired flow-mediated vasodilatation and insulin resistance in type 2 diabetic patients with albuminuria

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ABSTRACT

An elevated urinary albumin excretion is associated with an increased risk of cardiovascular disease due to atherosclerosis, but the pathophysiological mechanism underlying this association is poorly understood. We studied 217 diabetic patients, that is, 121 normoalbuminuric patients, 71 microalbuminuric patients, and 25 macroalbuminuric patients. We evaluated flow-mediated dilatation of brachial artery (%FMD, one endothelial function marker associated with endogenous NO production), von Willebrand factor (vWF, endothelial activation marker), high-sensitive CRP (hsCRP, a low-grade inflammation marker), asymmetric dimethyl arginine (ADMA, an endogenous inhibitor of NO synthesis), and insulin sensitivity by steady-state plasma glucose method. %FMD was apparently decreased in microalbuminuric and macroalbuminuric patients compared with normoalbuminuric patients ($p < 0.001$). Moreover, %FMD was significantly correlated with the degree of albuminuria ($r = -0.38$, $p < 0.05$). On the other hand, vWF and hsCRP did not show significant difference between normoalbuminuric patients and microalbuminuric patients. In diabetic patients with macroalbuminuria, ADMA was significantly elevated compared to those with normoalbuminuria. Insulin sensitivity was significantly associated with urinary albumin excretion rate. These results suggested that endothelial dysfunction which may be due to impaired NO production and insulin resistance underlie the association between diabetic nephropathy and atherosclerosis in diabetic patients.

1. Introduction

Elevated urinary albumin excretion rate (UAER) is strongly associated with an increased risk of cardiovascular diseases, which is independent of conventional risk factors including hypertension, hyperlipidemia, and smoking, among individuals with and without type 2 diabetes [1,2]. This suggests that elevated UAER may be associated with atherosclerosis by the unidentified mechanism.

The endothelium plays a crucial role in the maintenance of vascular tone and structure, and endothelial dysfunction is a key feature of atherosclerosis. Nitric oxide (NO) is one of the important endothelium-derived vasoactive mediators. NO is involved in a wide variety of regulatory mechanisms of cardiovascular system, including vascular tone and vascular structure [3].

Flow-mediated endothelium-dependent vasodilatation (FMD) method is based on the endothelial stimulus of increased shear stress (the tangential force on the vessel wall exerted by flowing blood). Increased shear stress is caused by post-ischemic hyperemia and elicits a slow Ca^+-independent two to threefold increase in NO production [4,5]. Indeed,

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Celemajer et al. reported that flow mediate vasodilatation was mainly blocked by N-monomethyl-L-arginine (an inhibitor of endothelial NO synthetase) [6].

To clarify the contribution of impaired NO production in vascular endothelium to the association between atherosclerotic disease and diabetic nephropathy, we examined FMD by ultrasonography. In addition, we measured asymmetric dimethyl arginine (ADMA), an endogenous NO synthesis inhibitor [3]. Since low-grade inflammation is another key feature of the pathophysiology of atherosclerosis [7], we further examined high-sensitive CRP, which is an inflammation marker, to investigate whether this feature is involved in the association between atherosclerotic disease and diabetic nephropathy.

It has recently been indicated that microalbuminuria and atherosclerosis are closely associated with insulin resistance [8–10], implying that insulin resistance may underlie these pathophysiological conditions although the causative relationship remains unknown. In the present study, we further examined insulin sensitivity in the type 2 diabetic patients with different stage of albuminuria and analyzed the correlation between insulin sensitivity and FMD, to investigate whether elevated UAER and endothelial dysfunction may be associated with insulin resistance.

2. Methods

2.1. Study subjects

We studied 217 patients with type 2 diabetes who were <75 years of age. Patients with a current acute illness (including clinically significant infectious disease) were excluded from this study. Twenty-four-hour urine collections were performed for two consecutive days to determine the stage of diabetic nephropathy. Creatinine clearance (Ccr) was calculated from the 24-h urine sample and serum creatinine levels. The patients were divided into three groups according to the UAER, as follows: normoalbuminuria (UAER <30 mg/day), microalbuminuria (30 ≤ UAER < 100 mg/day) and macroalbuminuria (UAER ≥ 300 mg/day). To exclude diabetic patients with nondiabetic kidney disease, we excluded patients with hematuria or abnormal urinary sediments. This study was conducted with the approval of National Cardiovascular Center Trust Ethics Committee, and patients gave written informed consent before participation.

2.2. Brachial artery flow-mediated dilatation

Using ultrasonography, arterial endothelium and smooth muscle function were measured by examining brachial artery responses to endothelium-dependent and endothelium-independent stimuli. Ultrasoundsonographic measurements were carried out according to the method described by Celemajer et al. [6]. Brachial artery diameter was measured from B-mode ultrasound images using 10-MHz liner array transducer (ProSound SSD-5500; Aloka, Japan) while an ECG trace was simultaneously recorded. The right brachial artery was scanned in longitudinal sections 1–10 cm above elbow, after at least 15 min of rest in the supine position, the skin surface was marked and the arm was kept in the same position during the study.

Baseline measurements of the diameter were carried out. Endothelium-dependent vasodilatation (flow-mediated dilatation) was determined by scans during reactive hyperemia. A pneumatic cuff placed around the forearm was inflated to 220 mmHg and was deflated after 4.5 min. The diameter of the brachial artery was scanned and recorded after dilation. After 10 min rest, the second control scan of the diameter was recorded. Then, sublingual glyceryl trinitrate spray (300 μg) was administered and 3.5 min later a final scan of the diameter was recorded.

Measurements of the vessel diameter were taken from the anterior to posterior "m" line (interface between the media and adventitia) at end-diastole, coincident with the R wave on a continuously recorded ECG. The diameters at four cardiac cycles were measured for each scan, and these results were averaged. Determinations of the FMD were carried out 45–60 s after the cuff release to measure a maximal diameter. Vasodilatation by reactive hyperemia or glyceryl trinitrate (NTG) was expressed as the percent change in diameter compared with the baseline values.

2.3. Insulin sensitivity test

Glucose utilization in response to insulin was evaluated with a newly modified steady-state plasma glucose (SSPG) method with octreotide acetate (Sandostatin; Novartis) after an overnight fasting period of 12 h [11]. Sandostatin (9.8-pmol bolus followed by a constant infusion of 73.5 pmol/h) and Humulin R insulin (45 pmol/kg bolus followed by a constant infusion at a rate of 4.62 pmol/(kg min); Eli Lilly) were infused intravenously for 120 min. Glucose in a final 12% solution containing KCl (0.5 μmol/(kg min)) was infused at a rate of 0.033 mmol/(kg min) (6 mg/(kg min)) through an antecubital vein via a constant infusion pump. Blood samples were drawn routinely at 0 and 120 min (9:00 and 11:00 a.m.) for the determination of glucose, insulin, and lipids. The value of glucose at 120 min (SSPG) was used as a marker of insulin sensitivity to glucose utilization. High SSPG levels showed peripheral insulin resistance.

Another marker of insulin resistance (IR) was estimated by calculating homeostasis model assessment (HOMA-IR) index ((fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l))/22.5) [12].

2.4. Measurement of vWF, hsCRP, and ADMA

vWF was determined in citrated plasma using a homemade enzyme-linked immunosorbent assay. Data are given as the percentage of pooled human plasma (set at 100%). Serum hsCRP concentration was determined by latex nephelometry method (SRL, Tokyo, Japan). Serum ADMA concentration was determined by high-performance liquid chromatography method (SRL, Tokyo, Japan).

2.5. Statistical analysis

Values are expressed as means ± S.D. Statistical analysis was performed by use of ANOVA followed by Scheffes' test. The

Table 1 – Characteristics of diabetic patients with normoalbuminuria, microalbuminuria, and overt nephropathy

Parameter	Stage of nephropathy		
	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
n	121	71	25
Age (years)	62 ± 9	65 ± 8	66 ± 7
Men/women	76/45	34/37	12/13
Duration of diabetes (years)	12 ± 8	14 ± 8	18 ± 8*
BMI (kg/m^2)	25.0 ± 3.7	25.1 ± 3.7	25.1 ± 3.9
SBP (mmHg)	128 ± 13	133 ± 15	141 ± 19*
DBP (mmHg)	74 ± 10	73 ± 9	76 ± 10
FBS (mmol/l)	7.4 ± 1.4	7.5 ± 1.5	7.5 ± 1.9
HbA1c (%)	8.3 ± 1.5	8.9 ± 1.7*	8.8 ± 1.4
HOMA-IR	1.62 ± 0.98	1.71 ± 2.06	2.29 ± 1.47
Total cholesteroyl (mmol/l)	4.86 ± 0.90	4.86 ± 0.90	4.73 ± 0.75
Serum creatinine (μmol/l)	70 ± 20	60 ± 20	110 ± 40
Urinary albumin (mg/day)	10 ± 7	85 ± 79**	583 ± 576**
Creatinine clearance (ml/s)	1.43 ± 0.52	1.50 ± 0.63	0.73 ± 0.43**
ACEI or ARB (yes/no)	36/85	24/47	11/14*
Statin (yes/no)	45/76	25/46	10/15
Current smoker (yes/no)	11/110	7/64	6/19

*$p < 0.05$; **$p < 0.01$ vs. normoalbuminuria, mean ± S.D.

strength of correlation between variables was tested by linear correlation and multiple regression analysis. $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. *Patients characteristics*

Table 1 shows the clinical characteristics of three groups. There was no significant difference in age, gender, BMI, FBS and total cholesterol among the three groups. HbA1c of diabetic patients with microalbuminuric patients was significantly higher than normoalbuminuric patients. Systolic blood pressure of macroalbuminuric patients was significantly higher than normo- and micro-albuminuric patients. Creatinine clearance was significantly decreased in macroalbuminuric patients compared with normo- and micro-albuminuric patients. There is no significant difference in rate of patients taking ACE/ARB between normo- and micro-albuminuric patients whereas the rate of patients taking ACE/ARB of macroalbuminuric patients were significantly large compared with other two groups. On the other hand, there is no significant difference in rate of patients taking statin among three groups.

3.2. *%FMD of diabetic patients*

We studied the endothelial function by FMD using brachial artery echography. %FMD (Δhyperemia) of diabetic patients with microalbuminuria (4.5 ± 3.7%) and macroalbuminuria (4.2 ± 2.4%) was apparently decreased compared with those of diabetic patients with normoalbuminuria (6.6 ± 3.7%) (Fig. 1A). Moreover, %FMD was significantly correlated with UAER in normo- and micro-albuminuric patients independent of age, HbA1c, and systolic blood pressure by multiple regression analysis ($r = -0.38$, $p < 0.05$) (Fig. 2). Dilatation of brachial artery by NTG (ΔNTG) showed no difference among three groups (Fig. 1B).

3.3. *vWF, hsCRP, and ADMA of diabetic patients*

We studied other atherosclerotic markers, that is, vWF, hsCRP, and ADMA. There was no significant difference of the levels of vWF and hsCRP between normoalbuminuric and microalbu-

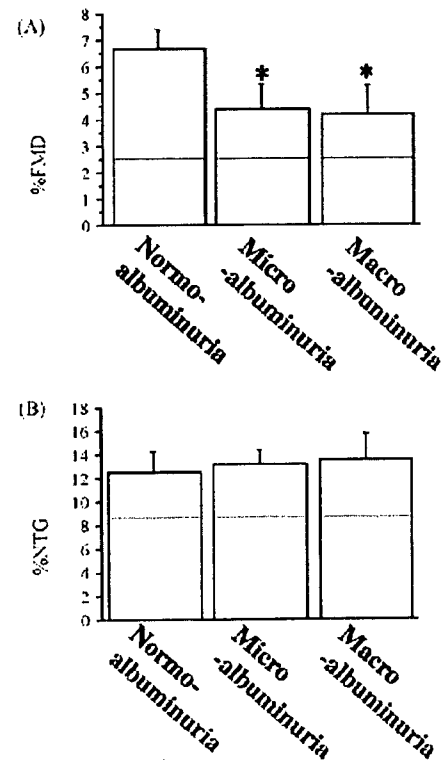

Fig. 1 – %FMD (A) and %NTG (B) in diabetic patients with normoalbuminuria, microalbuminuria and macroalbuminuria. Each value means (means ± S.D.), *$p < 0.001$.

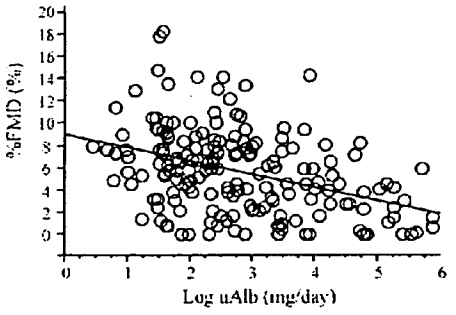

Fig. 2 – Correlation between degree of UAE and %FMD in normo- and micro-albuminuric diabetic patients. There was a significant correlation between both variables ($r = -0.38$, $p < 0.05$, $n = 192$).

minuric patients (Table 2). Although the levels of ADMA in microalbuminuric patients did not show significant difference compared with normoalbuminuric patients (Table 2), the levels of ADMA in macroalbuminuric patients were significantly elevated compared with normoalbuminuric patients (Table 2).

3.4. *Insulin sensitivity of diabetic patients*

We studied the insulin sensitivity by SSPG method. The levels of SSPG had weak but significant correlation with both %FMD ($r = -0.175$, $p < 0.05$) and UAER ($r = 0.181$, $p < 0.05$) independent of age, HbA1c, and systolic blood pressure (Fig. 3A, B).

4. Discussions

There were two main findings from this investigation in type 2 diabetic patients. First, diabetic micro- and macro-albuminuric patients showed significant reduction of %FMD compared with normoalbuminuric patients. This finding suggests that the endothelial dysfunction may account for the association between atherosclerosis and albuminuria in diabetic patients. Second, the level of SSPG was significantly associated with both UAER and %FMD. This finding suggests that insulin resistance may play a role in both atherosclerosis and nephropathy in type 2 diabetic patients.

In diabetic patients, %FMD is decreased compared with healthy control [13,14]. These reports indicated that diabetes mellitus is associated with endothelial dysfunction due to

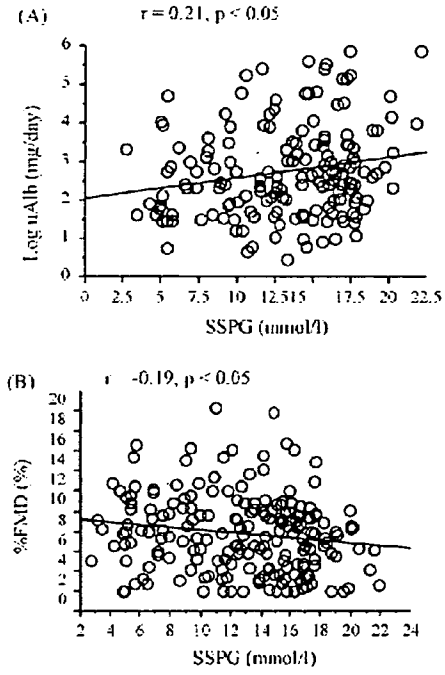

Fig. 3 – Correlation between SSPG and UAE (A), and correlation between SSPG and %FMD (B) in normo- and micro-albuminuric patients.

impaired NO production. However the involvement of endothelial dysfunction in diabetic nephropathy has been unclarified. We demonstrated that microalbuminuric and macroalbuminuric patients showed significant decreased %FMD compared with normoalbuminuric patients. In contrast, there was no significant difference of vWF between normoalbuminuric patients and microalbuminuric patients. vWF is a product of vascular endothelial cell, and induces coagulation and platelet aggregation [15]. These findings suggest that endothelial dysfunction due to impaired NO production is specifically induced in micro- and macroalbuminuric patients. One recent report showed that coronary endothelium-dependent dilatation was impaired in a rat model of spontaneous albuminuria [16] supporting this hypothesis. It has been reported that renal NO production was decreased in rodent diabetic model [17]. This report suggests that decrease of NO production may play a role in the

Table 2 – Parameters of atherosclerosis in diabetic patients with normoalbuminuria, microalbuminuria, and overt nephropathy

Parameter	Stage of nephropathy		
	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
von Willebrand factor (%)	147 ± 44	146 ± 44	143 ± 41
High-sensitive CRP (ng/ml)	976 ± 1401	951 ± 1110	1113 ± 1187
ADMA (nmol/ml)	0.45 ± 0.06	0.47 ± 0.07	0.55 ± 0.11*

*$p < 0.001$ vs. normoalbuminuria, mean ± S.D.

progression of diabetic nephropathy as well as atherosclerosis. We investigated serum ADMA levels in diabetic patients. There was no significant difference of ADMA levels between normo- and micro-albuminuric patients, suggesting that the reduction of %FMD in microalbuminuric patients might not be resulted from the elevation of ADMA. However, in macroalbuminuric patients, ADMA level was significantly higher than normoalbuminuric patients. Vallance et al. reported that the level of ADMA was elevated in patients with chronic renal failure and suggested the involvement of this in coronary artery disease [18]. They indicate that the elevation of ADMA might be associated with atherosclerosis in patients with chronic renal disease [18]. Thus, this finding suggests that the elevation of ADMA might be associated with atherosclerotic change in diabetic patients with macroalbuminuria.

An association between chronic low-grade inflammation and development of atherosclerotic disease has been observed in basic and clinical studies [7,19–21]. Furthermore, diabetic patients have higher CRP levels than normal subjects, suggesting that chronic inflammation may contribute diabetic atherosclerotic complication [22]. An association between micro- and macro-albuminuria and inflammation has also been reported [23,24]. However, several other studies showed that inflammatory molecules were not associated with micro- and macro-albuminuria [25–27]. Thus the knowledge of this association is still controversial. Also we could not demonstrate the association between CRP and development of microalbuminuria in this study. Our data suggested that chronic low-grade inflammation might not be involved in the association between atherosclerosis and microalbuminuria. However, since this study was performed by cross-sectional analysis and other inflammatory marker was not measured, further study is necessary for demonstrating this hypothesis.

Insulin resistance has been reported to play an important role in the development and progression of atherosclerotic coronary disease [8,9]. Recently the association between insulin resistance and microalbuminuria was also reported [10]. Nakamura et al. demonstrated that administration of pioglitazone to diabetic patients attenuated UAER [28]. In this study, we showed that both the UAER and %FMD were significantly correlated to the level of SSPG. These findings suggest that insulin resistance may be involved in both the elevated urinary albumin excretion and endothelial dysfunction due to impaired NO production. However, HOMA-IR, another insulin sensitivity marker which reflects insulin sensitivity in both the liver and the periphery, did not show significant difference among three groups, suggesting that particularly peripheral insulin resistance may be important for the pathogenesis of atherosclerosis and diabetic nephropathy.

In summary, we showed that %FMD of micro- and macro-albuminuric patients was decreased compared with those of normoalbuminuric patients, without showing significant difference in other various atherosclerotic markers. Furthermore, the level of SSPG was significantly correlated to UAER and %FMD. These findings suggest that endothelial dysfunction which may be due to impaired NO production underlies the mechanism of association between elevated urinary albumin excretion and atherosclerosis in diabetic patients, and that peripheral insulin resistance might be possibly involved in both diabetic nephropathy and atherosclerosis.

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