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ORIGINAL ARTICLE

Quantitative FDG-uptake by positron emission tomography in progressive hypertrophy of rat hearts in vivo

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Abstract
Background Quantitative myocardial fluorodeoxyglucose positron emission tomography (FDG-PET) for assessing glucose uptake in vivo is reliable in normal rat heart.
Objective To assess the applicability of myocardial FDG-PET on multiple occasions in the longitudinal disease process of progressive hypertrophy of rat heart.
Methods Six salt-sensitive Dahl rats (Dahl-S) developing progressive hypertrophy with subsequent dilated cardiomyopathy were compared with salt-resistant Dahl rats (controls). FDG-PET was applied twice at early stage (ES: 14–18 weeks) and at late stage (LS: 22–26 weeks) of hypertrophy. Standardized uptake value (SUV) was calculated for comparing between different animal weights and different injection dosages of FDG. For validating the quantitative study, radioactivity of a total of 36 tissue samples was compared with the corresponding PET values.
Results The left ventricular mass in Dahl-S increased by 17% at ES and by 25% at LS. The SUV in Dahl-S was 95% of controls at ES and reduced to 62% at LS ($P = 0.023$). The heart function started to deteriorate after LS. Linear regression analysis showed a good correlation between the radioactivity of tissue samples and PET values ($Y = 1.20X$, $P < 0.0001$, $R^2 = 0.979$).
Conclusions Small animal PET studies on longitudinal multiple occasions in vivo were feasible and useful for the repeating assessment of glucose uptake. The reduction of glucose uptake in progressive hypertrophy of heart over time may precede its progression to heart failure.

Keywords Small animal PET · Glucose metabolism · Fluorodeoxyglucose (FDG) · Quantitative analysis · Hypertrophied heart

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Introduction

In molecular imaging of myocardial metabolism, positron emission tomography (PET) for small animals has several advantages, which include noninvasive studies, quantitative assessment in vivo, repeated longitudinal studies over the disease process, and its modification with treatment [1–3]. The rat is an excellent experimental animal, particularly for in vivo chronic experiments because a number of syngeneic strains that develop heart

disease have been established [4–6]. A quantitative myocardial PET for the rat was earlier explored using two-deoxy-2-[^{18}F]fluorodeoxyglucose (FDG), which is a glucose analogue for analyzing glucose uptake in vivo [7]. Although the heart is more difficult to image than the other organs because of the heartbeat, we demonstrated the reliability of quantitative FDG-PET for a normal rat heart to assess glucose uptake in a prior study. In the present study, this approach is extended to rat heart with heart disease.

The energy production for the heart by glucose metabolism through glycolysis is well appreciated to be important in ischemic and hypertrophied heart [8–10]. In coronary artery disease, FDG-PET has been successfully used in the differentiation of ischemic but viable myocardium from irreversible myocardial necrosis [11, 12]. It is widely appreciated that hypertrophied heart is more dependent on energy production via glycolysis [8–10]. It is well recognized that glucose utilization is influenced by many factors such as insulin, glycogen storage, ischemia and reperfusion, and cardiac pressure or volume overload [13]. Therefore, glucose uptake in the progression of hypertrophy is still poorly understood and has been described as increased [14, 15], decreased [16–18], or unchanged [19, 20]. The objectives of the present study were twofold, to assess the validity and applicability of quantitative FDG-PET study on longitudinal multiple occasions for rat heart with heart disease, and to assess the change in glucose uptake in the process of developing progressive hypertrophy.

Materials and methods

Animals

Salt-sensitive Dahl (Dahl-S) rats and salt-resistant Dahl (Dahl-R) rats 6 weeks after birth were purchased and used in the present study (Japan SLC, Shizuoka, Japan). The animals were maintained at the animal care facilities at Kyoto University, Graduate School of Medicine under standard temperature and humidity. Water and food were provided ad libitum. All rats were fed an 8% salt diet from the age of 9 weeks after birth. Dahl-S rats develop hypertrophied heart caused by chronic hypertension over 14–18 weeks and subsequently dilated cardiomyopathy [5, 21]. Dahl-R rats served as controls because they maintain a normal blood pressure resistant to a high salt diet. The investigation was approved by the Kyoto University Animal Care Committee and 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).

Echocardiography

Echocardiography for rat hearts was repeated every 4 weeks from 12 weeks after birth until sacrifice or animal death to obtain information regarding heart size and myocardial wall thickness. Ether was used for anesthetizing rats during the echocardiographic assessment. Both M-mode and two-dimensional (2D) echocardiograms were obtained using a 5–12 MHz ultraband sector transducer. (SONOS 5500, Agilent Technologies, Andover, MA, USA). Images were obtained from the left parasternal windows in a right lateral decubitus position. The following parameters were measured and calculated from M-mode tracing: left ventricular end-diastolic dimension (LVDd: mm), left ventricular end-systolic dimension (LVDs: mm), fractional shortening (FS: %), and ejection fraction (EF: %) [22]. Wall thickness of four segments (anterior, septal, lateral, and posterior walls) was measured on short axis 2D images to correct for the partial volume effect. The left ventricular mass volume (LV mass) was calculated from Penn's method with parameters of echocardiography just prior to the first and second PET studies [23].

Phantom study and correction of partial volume effect

The methods for acquiring dynamic and serial multiplane images and for correcting for the partial volume effect were described elsewhere [7]. In brief, image acquisitions were performed using a PET scanner for animal use (SHR-7700L, Hamamatsu Photonics, Hamamatsu, Japan) [24]. The system achieved the spatial resolution of 2.7 mm in plane and 3.3 mm in the axial direction as the full width at half maximum [25]. The details of the constitution of the PET scanner were described elsewhere [7]. Radioactive recovery was assessed using different sizes of hollow phantom filled with a homogeneous solution of FDG, which showed that more than 30 mm of hollow phantom has nearly 100% radioactive recovery. Recovery coefficients (RC) curve reconstructed from the phantom study done in the previous study was used to correct for the partial volume effect [7].

PET study and image reconstruction

The method for PET study and image reconstruction was described earlier [7]. In brief, rats were allowed to feed until 1 h prior to the PET study. The rats were anesthetized with a pentobarbital injection intraperitoneally (1 mg/kg). After an intravenous injection line was cannulated through the tail vein, the rats were immobilized and fixed on the tray about 30° oblique to the axial line of the scanner to obtain the short axial section of

the heart image. Following a transmission scan for attenuation correction, serial dynamic scans (2 min × 30 frames) were performed for 60 min following the injection of FDG with the mean dosage of $5.1 \times 10^5 \pm 1.1 \times 10^5$ kBq/kg (3.7×10^5–7.4×10^5 kBq/kg).

Static FDG images were reconstructed from the last five dynamic images. Serial two-dimensional images that were reconstructed included four to five short axial heart images. Of four to five images, one middle image was selected for multiple ROI sampling consisting of 10 to 12 regions, and PET values per pixel of each ROI consistent with four segmental walls (anterior, septal, lateral, and posterior walls) were measured. Subsequently, the partial volume effect of PET values calculated in the four segmental walls was corrected, according to wall thickness by echocardiogram and the RC curve by phantom study performed earlier [7]. The mean value of FDG uptake was re-calculated from the PET values of the segmental wall and cross calibration factor. Thereafter to correct for the differences in the injection dosage of FDG and weight between animals and between the two studies conducted on different occasions, FDG uptake was expressed as a standardized uptake value (SUV) as follows and was compared between the studies [26]:

$$\mathrm{SUV} = \frac{\text{radioactivity in the heart by PET (Bq/ml)} \times \text{body weight (g)}}{\text{injection dosage of FDG (Bq)}}.$$

Experimental protocol

Experimental protocols were designed as follows. A total of 12 rats (six Dahl-S and six Dahl-R rats) had FDG-PET studies on two different occasions at the early stage (ES) of hypertrophy between 14 and 18 weeks and at the late stage (LS) between 22 and 26 weeks according to the schedule of multiple animal PET study protocols ongoing together.

Validation of quantitative assessment by third FDG-PET study

Following the second PET study, two Dahl-S rats and one Dahl-R rat died. Accordingly, the remaining nine rats had a third PET study additionally to validate the quantitative analysis. Immediately following the third PET study, all the animals were killed. Five cc of blood was sampled from the inferior vena cava to measure blood glucose, non-esterified free fatty acid (NEFA), and serum insulin levels. Their hearts were processed as described elsewhere [7]. The hearts were divided into three short axial sections. The middle segment of the transverse section in heart tissue at the time of being killed was divided into four segmental tissue samples from the anterior, septal, inferior, and lateral walls according to the anatomical location. After the weights of the tissue samples were measured, the concentrations of FDG uptake were measured with an auto-gamma counter (Perkin Elmer Life Science, Boston, MA, USA). Radioactivity per gram in the four segmental walls was calculated from these numbers. Radioactivity of a total of 36 tissue samples (4 × 9 rats) was compared with the corresponding PET value following the correction made for the partial volume effect.

Statistics

All data are expressed as the mean and standard deviation. Linear regression analysis and correlation coefficients were calculated as required. For a multiple group comparison, analysis of variance was used with a Sheffe F test for post hoc testing. A probability (P value) of less than 0.05 was considered to be significant.

Results

Echocardiographic examination

Echocardiograms were repeated every 4 weeks from 12 weeks following birth until animal death or the third PET. As two Dahl-S and one Dahl-R rats died after the second PET study (at LS) prior to 28 weeks, and one Dahl-S rat and two Dahl-R rats had third PET between 28 weeks and 32 weeks for tissue sampling for the validation study, echocardiographic data were available only for nine rats at 28 weeks and six rats at 32 weeks. The results of the serial echocardiographic examinations were summarized in Figs. 1a–d. The left ventricular end-diastolic dimensions (LVEDD) were comparable between Dahl-S and Dahl-R (controls) throughout the observational period. The left ventricular end-systolic dimensions (LVESD) were comparable as well between the groups until 24 weeks, but started to increase at 28 weeks in Dahl-S rats. As a result, ejection fraction (EF) and fractional shortening (FS) were maintained in the normal range in both groups until 24 weeks but they started to decrease in Dahl-S group at 28 weeks. At 32 weeks, LVESD, EF, and FS were significantly reduced in Dahl-S rats when compared with Dahl-R rats (LVESD: 3.7 mm ± 0.5 mm vs. 5.3 mm ± 0.1 mm: $P = 0.0026$, EF: 72% ± 3% vs. 88% ± 3%: $P = 0.0027$: FS: 34 ± 2 vs. 53% ± 5%: $P = 0.0019$). The mean wall thickness of the four segmental walls at end-diastole and end-systole increased in

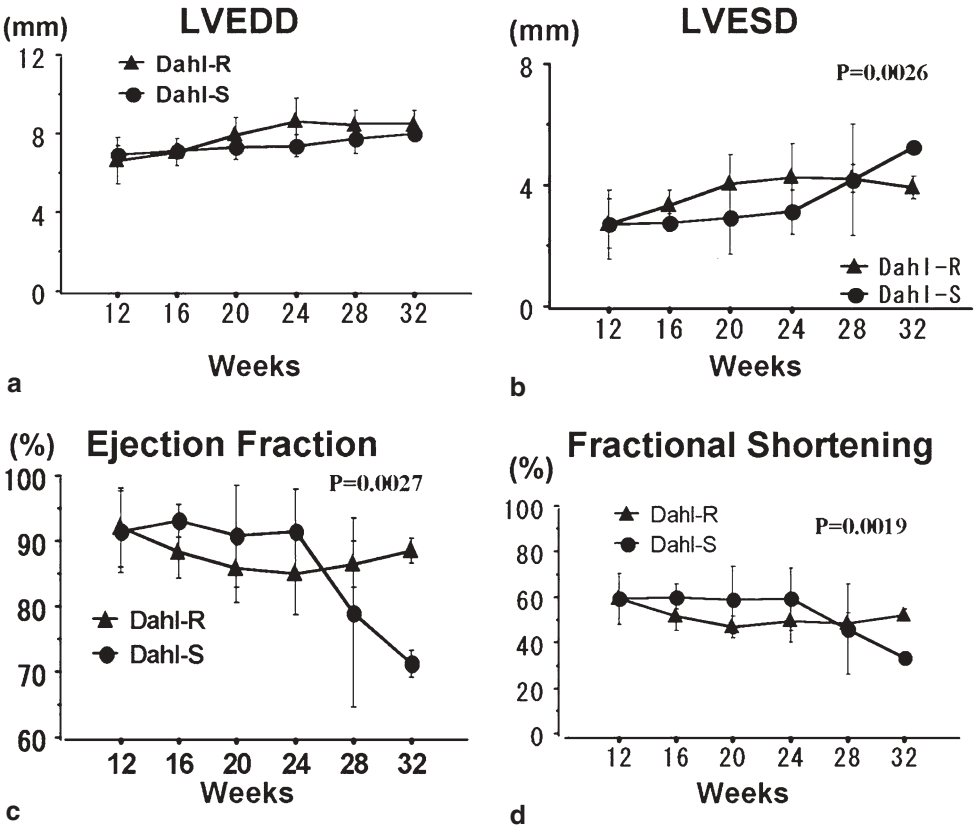

Fig. 1 Serial echocardiograms every 4 weeks from 12 weeks after birth until sacrifice or animal death. LVEDD were similar throughout the study period (**a**). On the other hand LVESD in Dahl-S rats started to increase around 28 weeks (**b**). As a result, ejection fraction and fractional shortening, indicators of systolic function, started to deteriorate around 28 weeks (**c**, **d**). *Dahl-S* Dahl sensitive rat, *Dahl-R* Dahl resistant rat, *ES* early stage of hypertrophy, *LS* late stage of hypertrophy, *LVEDD* left ventricular end-diastolic dimensions, *LVESD* left ventricular end-systolic dimensions

Table 1 Echocardiographic assessment

	Stage of hypertrophy			
	Dahl-S rats ($n = 6$)		Dahl-R rats ($n = 6$)	
	ES	LS	ES	LS
LVED dimensions	7.25 ± 0.86	7.99 ± 0.78	7.31 ± 0.75	8.25 ± 0.82
Wall thickness, LVED (mm)				
Anterior wall	2.19 ± 0.34	3.18 ± 0.27**	1.91 ± 0.16	1.96 ± 0.20
Inferior wall	2.12 ± 0.48	3.31 ± 0.45**	2.01 ± 0.26	2.10 ± 0.16
Lateral wall	2.49 ± 0.33	2.82 ± 0.38	2.27 ± 0.38	2.41 ± 0.31
Septal wall	2.43 ± 0.53	2.84 ± 0.32	2.06 ± 0.20	2.23 ± 0.36
Mean of four walls	2.31 ± 0.43	3.02 ± 0.39*	2.06 ± 0.28	2.17 ± 0.30
Wall thickness, LVES (mm)				
Anterior wall	3.18 ± 0.27	3.51 ± 0.58	2.56 ± 0.25	2.54 ± 0.34
Inferior wall	3.31 ± 0.45	3.67 ± 0.68	2.68 ± 0.31	2.54 ± 0.36
Lateral wall	3.44 ± 0.57	4.00 ± 0.65	3.04 ± 0.42	2.89 ± 0.41
Septal wall	3.57 ± 0.71	3.65 ± 0.46	2.67 ± 0.16	2.65 ± 0.31
Mean of four walls	3.38 ± 0.51	3.71 ± 0.59	2.73 ± 0.34	2.65 ± 0.37
LV mass (cm^3)	1.35 ± 0.31	1.73 ± 0.44	1.16 ± 0.22	1.38 ± 0.38

Results are shown as mean ± standard deviation
Dahl-S Dahl sensitive rat, *Dahl-R* Dahl resistant rat, *ES* early stage of hypertrophy, *LS* late stage of hypertrophy, *LVED* Left ventricular end-diastole, *LVES* Left ventricular end-systole, *LV mass* left ventricular mass volume
* $P < 0.05$, ** $P < 0.01$, at LS compared with ES

Dahl-S from 2.31 mm at ES to 3.02 mm at LS ($P < 0.05$), and from 3.20 at ES to 3.71 at LS (P = NS), whereas these were not changed in Dahl-R from 2.06 mm at ES to 2.17 mm at LS, and from 2.77 at ES to 2.65 at LS (Table 1). The left ventricular dimension of end-diastole (LVEDD) increased in Dahl-R (controls) from 7.31 mm ± 0.75 mm at ES to 8.25 mm ± 0.82 mm at LS. The LVEDD in Dahl-S increased as well from 7.25 mm ± 0.86 mm at ES to 7.99 mm ± 0.78 mm at LS. The left ventricular mass volume (LV mass) was calculated from the wall thickness and LVEDD. The LV mass was increased by 17% at ES in Dahl-S (1.35 cm^3 ± 0.31 cm^3)

when compared with that in Dahl-R ($1.16\,cm^3 \pm 0.22\,cm^3$) and was increased by 25% at LS (Dahl-S vs. Dahl-R = $1.73\,cm^3 \pm 0.44\,cm^3$ vs. $1.38\,cm^3 \pm 0.38\,cm^3$) although these increases did not reach statistically significant levels.

Quantitative analyses of FDG uptake

The SUV was calculated from the animal weight and the injection dosage of FDG to compare the PET studies between animals and the different study occasions. In the early stage of hypertrophy (at ES), SUV in Dahl-S group was 35.4 ± 9.0, which is approximately 95% of that (37.1 ± 5.2) in control group. The SUV at LS in Dahl-R was significantly increased to 61.7 ± 12.5 during the transition from ES to LS. On the other hand the SUV in the Dahl-S group remained at 38.1 ± 12.5 at LS. As a result, it was significantly reduced to 62% of that in control group ($P = 0.023$; Fig. 2, Table 2).

Tissue concentrations of FDG and their relation with PET values by linear regression analysis

The radioactivity of 36 tissue samples ranged from 3353 kBq/g to 13712 kBq/g (8721 kBq/g ± 2215 kBq/g). The PET values in 36 segments after the partial volume effect was corrected ranged from 3621 kBq/ml to

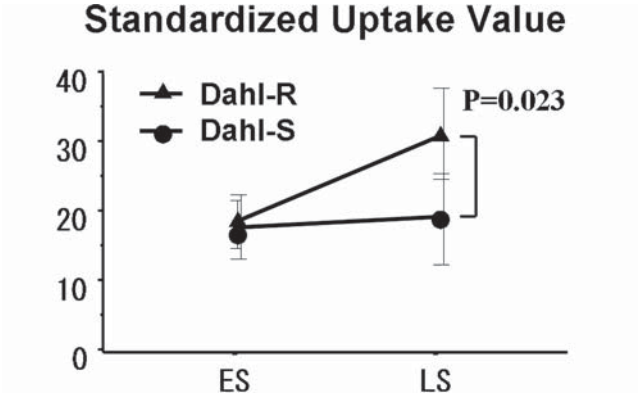

Fig. 2 SUV was calculated from the animal weight and the injection dosage. In the early stage of hypertrophy (at ES), SUV in Dahl-S group was approximately 95% of that in control group. SUV in Dahl-R was significantly increased from at ES to at LS, whereas SUV in Dahl-S group was not increased. As a result, it was significantly reduced to 62% of that in control group. *SUV* systemic uptake value, *Dahl-S* Dahl sensitive rat, *Dahl-R* Dahl resistant rat, *ES* early stage of hypertrophy, *LS* late stage of hypertrophy

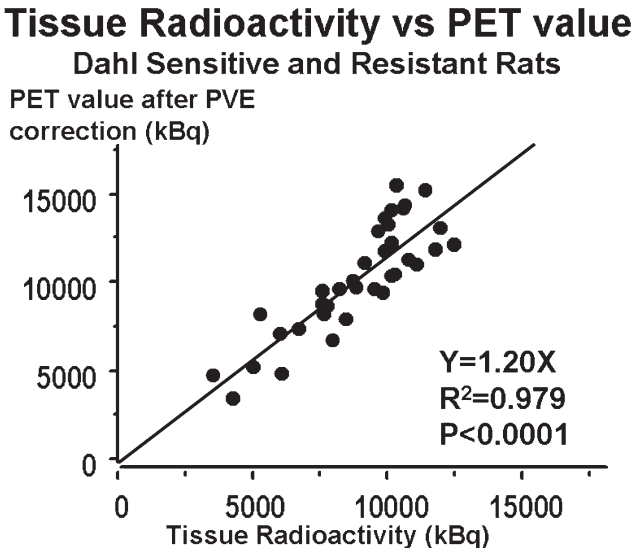

Fig. 3 Scattergram and linear regression analysis between tissue fluorodeoxyglucose (FDG) uptake and injected dosage of FDG per rat weight

Table 2 Standardized uptake value of four segmental walls

	Stage of hypertrophy			
	Dahl-S rats ($n = 6$)		Dahl-R rats ($n = 6$)	
	SUV at ES	SUV at LS	SUV at ES	SUV at LS
Name of segmental wall				
Anterior wall	38.9 ± 10.6	41.8 ± 16.5	41.2 ± 7.5	66.1 ± 15.6*,**
Inferior wall	34.5 ± 8.4	37.2 ± 12.1	33.6 ± 6.0	61.0 ± 13.5*,**
Lateral wall	34.4 ± 7.4	37.1 ± 12.8	36.2 ± 3.3	63.9 ± 13.1*,**
Septal wall	34.0 ± 11.1	36.2 ± 9.1	37.2 ± 6.3	55.8 ± 9.8*,**
Mean of four walls	35.4 ± 9.0	38.1 ± 12.5	37.1 ± 5.2	61.7 ± 12.5*,**
LV mass (cm^3)	1.35 ± 0.31	1.73 ± 0.44	1.16 ± 0.22	1.38 ± 0.38
Total uptake (cm^3)	48.9 ± 18.4	62.2 ± 13.2	43.3 ± 11.2	85.7 ± 28.7*,**

Results are shown as mean ± standard deviation

Dahl-S Dahl sensitive rat, *Dahl-R* Dahl resistant rat, *SUV* systemic uptake value, *ES* early stage of hypertrophy, *LS* late stage of hypertrophy, *LV mass* left ventricular mass volume, *total uptake* mean SUV × LV mass

* $P < 0.01$ compared with SUV at ES. ** $P < 0.01$ compared with SUV value of Dahl-S rats at LS

15996 kBq/ml (10439 kBq/ml ± 3092 kBq/ml). There was no significant difference between the four segmental walls in Dahl-S rats, Dahl-R rats or all animals. Linear regression analysis demonstrated a significant correlation between PET values and radioactivity of tissues in a total of 36 segments (Fig. 3; $Y = 1.20X$, $r = 0.979$; $P < 0.0001$).

Blood glucose, non-esterified free fatty acid, and insulin level

The levels of blood glucose, NEFA, and insulin were measured from blood samples obtained at the time of being killed. The mean level of blood glucose was 89 mg/dl ± 35 mg/dl (Dahl-S vs. Dahl-R = 99 mg/dl ± 35 mg/dl vs. 81 mg/dl ± 37 mg/dl). The mean levels of NEFA and blood insulin were 3508 mEq/l ± 1065 mEq/l (4054 mEq/l ± 1195 mEq/l vs. 3071 mEq/l ± 785 mEq/l) and 17.0 mU/ml ± 9.0 mU/ml (20.2 mU/ml ± 10.1 mU/ml vs. 14.4 mU/ml ± 8.2 mU/ml). These levels were not significantly different between the two groups.

Discussion

The PET study for small animals provides a unique opportunity to assess molecular imaging of myocardial metabolism in the longitudinal disease process in vivo with quantitative analyses. Dahl-S rats are a classical animal model for developing hypertensive hypertrophy of heart culminating in dilated cardiomyopathy at the end of life [5, 21]. In the present study, PET studies were applied noninvasively for rats on multiple occasions in vivo. The results showed that SUV of the control heart of Dahl-R rats increased with growth from 14–18 weeks to 22–26 weeks following birth. On the other hand, the SUV of Dahl-S rat did not change with progression of the hypertrophy. As a result, the glucose uptake of the heart in Dahl-S rats was relatively reduced from the early to late stages of progressive hypertrophy in comparison with the control group of Dahl-R rats. The second PET study (LS) between 22 weeks and 26 weeks appeared to have been conducted just prior to the deterioration of heart function in Dahl-S group by serial echocardiography. It is speculated that glucose uptake was reduced prior to the failure of heart function because of long-standing hypertension. In the literature of animal studies, it is suggested that substrate use of hypertrophied heart because of long-standing hypertension is characterized by a shift from fatty acid to glucose so that it is more dependent on glucose utilization [10, 14, 27]. However, it has been argued that the decline of energy production by fatty acid oxidation was not fully compensated for by glucose utilization [13]. In addition the glucose uptake in the hypertrophied heart may be impaired by relative insensitivity to insulin [28]. These observations were drawn mostly from animal studies by in vitro or ex-vivo studies (isolated Langendorf preparations). Many issues of glucose metabolism in the heart have been poorly understood because of the numerous factors affecting it, particularly in the in vivo experiments [28]. The present study was conducted as an in vivo experiment over a long period with the same animals. It is supposed that reduction of glucose uptake (not necessarily glucose utilization) by the hypertrophied heart is more relevant to the time point when the heart is about to deteriorate in this particular experimental model.

The PET study has been applied to patients with cardiac hypertrophy. Although myocardial oxygen consumption was significantly increased in these patients, PET study in patients with hypertrophied heart because of chronic hypertension failed to demonstrate any change of glucose uptake overall [19]. Another PET study in patients with hypertrophic cardiomyopathy showed that impairment of glucose metabolism preceded the reduction of myocardial blood flow [17]. In the clinical setting, patients with different stages and various etiologies of hypertrophy are mixed up, and so the condition of glucose metabolism might be widely different in each patient.

It is obvious that an ideal modality for in vivo evaluation would be noninvasive, quantitative, reproducible, and applicable to both humans and experimental animals [1, 2]. Magnetic resonance imaging (MRI) is another potentially attractive option for visualizing heart metabolism in the in vivo animal experiments, although the spatial resolution of clinical MRI may be insufficient for small animals. In fact, glucose uptake in the hypertrophied heart has been assessed by ^{31}P-nuclear magnetic resonance spectroscopy using isolated perfused rabbit hearts [16]. The study showed that glucose uptake was maintained in the early stage of progressive hypertrophy (so-called compensated hypertrophy). In the late stage of hypertrophy (so-called uncompensated hypertrophy), glucose uptake was significantly reduced when compared with that in the control animals [16]. It must be taken into account that the reduction of glucose uptake in the study may be affected by the nonphysiological conditions of an ex-vivo experimental model.

The quality of in vivo experiments depends largely on the quality of imaging and the reliability of the quantitative analysis. The reliability of quantitative myocardial FDG-PET study was clearly demonstrated in the present study as it was demonstrated as well in the earlier study using a normal rat heart [7]. The equations

between radioactivity of tissue samples and their corresponding PET values were quite similar between the earlier study using normal rats "$Y = 1.17X$" and the present study using the diseased model "$Y = 1.20X$". Although myocardial PET has an additional partial volume effect associated with heartbeat, the good quality of the quantitative analysis was again validated [7]. These results allow for application of this method to other disease models of the heart itself and their modification with treatment. As PET study provides three-dimensional tomographic images of radiotracer distribution within a living human or animal, quantitative mass analyses for radiotracer distribution have been achieved with appropriate probes and probing techniques [1, 2]. An advantage of quantitative small animal PET imaging was reported recently for assessing the volume of graft survival following islet cell transplantation in mouse in vivo by labeling it with radioactive tracers [29]. Graft survival after cell transplantation is another potential application of PET study in small animal experiments in combination with the appropriate probing technique. Measurement of the pharmacokinetics of new drugs and their treatment effect on various disease processes is another area of application for small animal PET studies in vivo [1, 2]. In this way, small animal PET studies, including the type of the present study, have moved animal research in a number of areas including metabolism from in vitro study into in vivo integrative biological study [30].

A limitation of the present study is that the observation focused solely on the particular disease model of Dahl-S rats that develop progressive hypertrophy. Another limitation is that myocardial FDG-PET in the study reflects only glucose uptake. To evaluate the myocardial metabolic state including glucose utilization rate, other associated parameters such as blood sugar level, NEFA, and insulin should also be considered. Although they were measured at the time of being killed, they were not controlled well on the occasion of the two PET studies at ES and LS. In order to calculate the glucose utilization rate in myocardium, hyperinsulinemic euglycemic clamp technique is ideal for controlling the blood glucose level [31, 32], Instead of it, it would also be better to correct SUV by blood glucose level at the time of each experiment if available [32]. Unfortunately, these were not measured in the present study because drawing a few milliliters of blood is frequently fatal for diseased rats under anesthesia. Although these levels, at the time of being killed, following the third PET for quantitative study showed no differences between groups, glucose levels between animals showed relatively wide variations. Further studies are obviously required to establish on how to manage blood glucose, NEFA and insulin levels on different occasions and in different animals in this type of in vivo repeated PET studies.

In summary, the reliability of quantitative myocardial FDG-PET was reconfirmed in hypertrophied rat heart. Small animal PET is an excellent diagnostic and experimental tool in vivo for analyzing myocardial metabolism in longitudinal multiple studies of the disease process in the same animals.

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References

1. Tai YC, Laforest R. Instrumentation aspects of animal PET. Annu Rev Biomed Eng 2005;7:255–85.
2. Dobrucki LW, Sinusas AJ. Molecular cardiovascular imaging. Curr Cardiol Rep 2005;7:130–5.
3. Di Carli MF, Prcevski P, Singh TP, Janisse J, Ager J, Muzik O, et al. Myocardial blood flow, function, and metabolism in repetitive stunning. J Nucl Med 2000;41:1227–34.
4. Dahl LK, Knudsen KD, Heine MA, Leitl GJ. Effects of chronic excess salt ingestion: modification of experimental hypertension in the rat by variations in the diet. Circ Res 1968;22:11–8.
5. Kihara Y, Sasayama S. Transitions from compensatory hypertrophy to dilated failing left ventricle in Dahl–Iwai salt-sensitive rats. Am J Hypertens 1997;10:78s–82s.
6. Mulvany MJ, Halpern W. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ Res 1977;41:19–26.
7. Handa N, Magata Y, Tadamura E, Mukai T, Nishina T, Miwa S, et al. Quantitative fluorine 18 deoxyglucose uptake by myocardial positron emission tomography in rats. J Nucl Cardiol 2002;9:616–21.
8. Nascimben L, Ingwall JS, Lorell BH, Pinz I, Schultz V, Tornheim K, et al. Mechanisms for increased glycolysis in the hypertrophied rat heart. Hypertension 2004;44:662–7.
9. Schönekess BO, Allard MF, Henning SL, Wambolt RB, Lopaschuk GD. Contribution of glycogen and exogenous glucose to glucose metabolism during ischemia in the hypertrophied rat heart. Circ Res 1997;81:540–9.
10. Christe ME, Rodgers RL. Altered glucose and fatty acid oxidation in hearts of the spontaneously hypertensive rat. J Mol Cell Cardiol 1994;26:1371–5.
11. Tillisch J, Brunken R, Marshall R, Schwaiger M, Mandelkern M, Phelps M, et al. Reversibility of cardiac wall-motion abnormalities predicted by positron tomography. N Engl J Med 1986;314:884–8.
12. Tamaki N, Yonekura Y, Yamashita K, Saji H, Magata Y, Senda M, et al. Positron emission tomography using fluorine-18 deoxyglucose in evaluation of coronary artery bypass grafting. Am J Cardiol 1989;64:860–5.
13. Depre C, Vanoverschelde JL, Taegtmeyer H. Glucose for the heart. Circulation 1999;99:578–88.
14. Kagaya Y, Kanno Y, Takeyama D, Ishide N, Maruyama Y, Takahashi T, et al. Effects of long-term pressure overload on regional myocardial glucose and free fatty acid uptake in rats:

a quantitative autoradiographic study. Circulation 1990;8: 1353–61.
15. Hajri T, Ibrahimi A, Coburn CT, Knapp FF Jr, Kurtz T, Pravenec M, et al. Defective fatty acid uptake in the spontaneously hypertensive rat is a primary determinant of altered glucose metabolism, hyperinsulinemia, and myocardial hypertrophy. J Biol Chem 2001;276:23661–6.
16. Friehs I, Moran AM, Stamm C, Colan SD, Takeuchi K, Cao-Danh H, et al. Impaired glucose transporter activity in pressure-overload hypertrophy is an early indicator of progression to failure. Circulation 1999;100:II187–93.
17. Tadamura E, Tamaki N, Matsumori A, Magata Y, Yonekura Y, Nohara R, et al. Myocardial metabolic changes in hypertrophic cardiomyopathy. J Nucl Med 1996;37:572–7.
18. Perrone-Filardi P, Bacharach SL, Dilsizian V, Panza JA, Maurea S, Bonow RO. Regional systolic function, myocardial blood flow and glucose uptake at rest in hypertrophic cardiomyopathy. Am J Cardiol 1993;72:199–204.
19. Laine H, Katoh C, Luotolahti M, Yki-Jarvinen H, Kantola I, Jula A, et al. Myocardial oxygen consumption is unchanged but efficiency is reduced in patients with essential hypertension and left ventricular hypertrophy. Circulation 1999;100: 2425–30.
20. Nienaber CA, Gambhir SS, Mody FV, Ratib O, Huang SC, Phelps ME, et al. Regional myocardial blood flow and glucose utilization in symptomatic patients with hypertrophic cardiomyopathy. Circulation 1993;87:1580–90.
21. Yuasa S, Nishina T, Nishimura K, Miwa S, Ikeda T, Hanyu M, et al. A rat model of dilated cardiomyopathy to investigate partial left ventriculectomy. J Cardiol Surg 2001;16: 40–7.
22. Douglas PS, Reichek N. Estimation of wall stress and left ventricular mass by noninvasive techniques and clinical implications. Cardiovasc Clin 1986;17:103–28.
23. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man: anatomic validation of the method. Circulation 1977;55:613–8.
24. Watanabe M, Uchida H, Okada H, Shimizu K, Satoh N, Yoshikawa E, et al. A high resolution PET for animal studies. IEEE Trans Med Imaging 1992;11:577–80.
25. Watanabe M, Okada H, Shimizu K, Omura T, Yoshikawa E, Kosugi S, et al. A high resolution animal PET scanner using compact PS-PMT detectors. IEEE Trans Nucl Sci 1997; 44:1277–82.
26. Graham MM, Peterson LM, Hayward RM. Comparison of simplified quantitative analyses of FDG uptake. Nucl Med Biol 2000;27:647–55.
27. Yonekura Y, Brill AB, Som P, Yamamoto K, Srivastava SC, Iwai J, et al. Regional myocardial substrate uptake in hypertensive rats: a quantitative autoradiographic measurement. Science 1985;227:1494–6.
28. Friehs I, Cao-Danh H, Nathan M, McGowan FX, del Nido PJ. Impaired insulin-signaling in hypertrophied hearts contributes to ischemic injury. Biochem Biophys Res Commun 2005;331:15–22.
29. Kim SJ, Doudet DJ, Studenov AR, Nian C, Ruth TJ, Gambhir SS, et al. Quantitative micro positron emission tomography (PET) imaging for the in vivo determination of pancreatic islet graft survival. Nat Med 2006;12:1423–8.
30. Phelps ME. Positron emission tomography provides molecular imaging of biological processes. Proc Natl Acad Sci USA 2000;97:9226–33.
31. Santuré M, Pitre M, Nadeau A, Bachelard H. Effect of troglitazone on vascular and glucose metabolic actions of insulin in high-sucrose-fed rats. Metabolism 2003;52:978–86.
32. Yokoyama I, Inoue Y, Moritan T, Ohtomo K, Nagai R. Simple quantification of skeletal muscle glucose utilization by static 18F-FDG PET. J Nucl Med 2003;44:1592–8.