In vivo assessment of cardiac metabolism and function in the abdominal aortic banding model of compensated cardiac hypertrophy

Anne-Marie L. Seymour1†, Lucia Giles2†, Vicky Ball2, Jack J. Miller2, Kieran Clarke2, Carolyn A. Carr2, and Damian J. Tyler2*

1School of Biological, Biomedical and Environmental Sciences, University of Hull, Hull HU6 7RX, UK; and 2Department of Physiology, Anatomy and Genetics, University of Oxford, Sherrington Building, Parks Road, Oxford OX1 3PT, UK

Received 8 September 2014; revised 27 February 2015; accepted 2 March 2015; online publish-ahead-of-print 6 March 2015

Time for primary review: 38 days

Aims
Left ventricular hypertrophy is an adaptive response of the heart to chronic mechanical overload and can lead to functional deterioration and heart failure. Changes in cardiac energy metabolism are considered as key to the hypertrophic remodelling process. The concurrence of obesity and hypertrophy has been associated with contractile dysfunction, and this work therefore aimed to investigate the in vivo structural, functional, and metabolic remodelling that occurs in the hypertrophied heart in the setting of a high-fat, high-sucrose, Western diet (WD).

Methods and results
Following induction of cardiac hypertrophy through abdominal aortic banding, male Sprague Dawley rats were exposed to either a standard diet or a WD (containing 45% fat and 16% sucrose) for up to 14 weeks. Cardiac structural and functional characteristics were determined by CINE MRI, and in vivo metabolism was investigated using hyperpolarized 13C-labelled pyruvate. Cardiac hypertrophy was observed at all time points, irrespective of dietary manipulation, with no evidence of cardiac dysfunction. Pyruvate dehydrogenase flux was unchanged in the hypertrophied animals at any time point, but increased incorporation of the 13C label into lactate was observed by 9 weeks and maintained at 14 weeks, indicative of enhanced glycolysis.

Conclusion
Hypertrophied hearts revealed little evidence of a switch towards increased glucose oxidation but rather an uncoupling of glycolytic metabolism from glucose oxidation. This was maintained under conditions of dietary stress provided by a WD but, at this compensated phase of hypertrophy, did not result in any contractile dysfunction.

Keywords
Dynamic nuclear polarization • Cardiac hypertrophy • Metabolic remodelling • Pyruvate dehydrogenase • 13C magnetic resonance spectroscopy

1. Introduction
Heart failure continues to be a major cause of death in the Western World. Despite current therapies, the prognosis is poor and patient care is often costly. Patients with heart failure often have pre-existing hypertension and hypertrophy, recognized as independent risk factors in the development of heart failure.1 Left ventricular hypertrophy (LVH), the adaptive response of the heart to chronic overload, is characterized by cellular and structural remodelling,2 which may underpin the transition from compensated hypertrophy to decompensated heart failure. Studies using 31P phosphorus magnetic resonance spectroscopy (MRS) have demonstrated a marked reduction in the energy reserves in the hypertrophied heart, leading to the suggestion that the failing heart is energy depleted.3 In particular, the hypertrophied heart undergoes significant metabolic remodelling, switching from dependence upon fatty acids for energy provision to reliance on carbohydrates.4,5 This switch is underpinned by altered expression of key transcription factors including PPARα and PGC1α, which in turn cause decreased expression of the enzymes involved in fatty acid oxidation.6

† The Author 2015. Published by Oxford University Press on behalf of the European Society of Cardiology. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
Obesity is a growing threat to health owing to its association with a number of cardiovascular risk factors including hypertension, insulin resistance, and dyslipidaemia (metabolic syndrome). Exposure of the hypertrophied heart to surplus lipids and nutrients, as occurs in obesity, may result in further dysregulation of metabolism and energy provision. Ultimately, the combination of hypertrophy and obesity may accelerate the onset of contractile dysfunction, cell death, and heart failure. Studies to date have generated conflicting data in that high-lipid diets have been both beneficial and deleterious to cardiac function. An extended study using a variety of dietary manipulations including a Western diet (WD), consisting of high saturated lipid and sucrose content, demonstrated the greatest reduction in cardiac power in the WD group, as well as obesity, whereas in the high fat, low carbohydrate group, obesity was comparable, but no reduction in cardiac power occurred. However, the cellular mechanisms that underpin this deterioration in function in the heart confronted by lipid and carbohydrate overload in the setting of hypertrophy and failure are complex and incompletely understood.

Many of the experimental studies on metabolic alterations have used in vitro experimentation on isolated hearts, assessing metabolic fluxes with the use of $^{13}$C or $^{13}$C-labelled substrates. The advent of hyperpolarized magnetic resonance (MR) spectroscopy provides a powerful means to enhance the sensitivity of $^{13}$C MR investigations. In combination with the rapid dissolution of small $^{13}$C-labelled hyperpolarized substrates, this gives a novel method with which to study metabolic flux in vivo.

The aim of this study was therefore to investigate the in vivo structural, functional, and metabolic remodelling that occurs during the time course of cardiac hypertrophic development in the setting of excess nutrient supply. In particular, the impact of a WD on the structure, function, and metabolism of the in vivo heart exposed to abdominal aortic banding (AAB) was determined through the use of CINE MRI and hyperpolarized $^{13}$C MRS to assess pyruvate dehydrogenase (PDH) flux and incorporation of pyruvate into the tricarboxylic acid (TCA) cycle. This study utilized the relatively mild model of physiological hypertrophy generated by AAB as it allowed us to explore the metabolic alterations that occurred over a period of time as hypertrophy developed. The AAB model was chosen over genetic models like the Spontaneously Hypertensive Rat (SHR), because the SHR has a loss of function mutation in the gene encoding for fatty acid translocase (FAT/CD36), a sarcolemmal fatty acid transporter that is crucial for the transport of long chain fatty acids (LCFA) across the plasma membrane. Such a mutation severely impacts the metabolic phenotype induced by cardiac hypertrophy. The AAB model was also chosen over more severe hypertrophy models, such as the transverse aortic constriction (TAC) model, as they can result in a very rapid onset of overt heart failure, which would have limited our ability to examine the metabolic changes that occur during the highly clinically relevant period of compensated hypertrophy.

## 2. Methods

$[1-^{13}]$C and $[2-^{13}]$Cpyruvic acid were obtained from Sigma-Aldrich Company Ltd (Dorset, UK). Male Sprague Dawley rats (Body weight 200–250 g; $n = 48$) were obtained from Harlan UK. All animals were housed on a 12:12-h light–dark cycle and studies were performed between 7 a.m. and 1 p.m., during the early absorptive (fed) state. All investigations conformed to Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act (HMSO) of 1986, to institutional guidelines and to the

### Table 1 Dietary composition of standard and WDs

| Calorie composition (%) | Standard chow | WD* |
|-------------------------|--------------|-----|
| Carbohydrates           | 69           | 35  |
| Total protein           | 19           | 20  |
| Total fat               | 11           | 45  |
| Saturated fat           | 6            | 16  |
| Monounsaturated fat     | 4            | 20  |
| Total polyunsaturated fat | 1          | 8   |

*Contains carbohydrate from sucrose (16%), rice, and starch.
Metabolism and function in the hypertrophied rat heart

2.3 In vivo assessment of pyruvate dehydrogenase flux and oxidative metabolism

PDH flux was determined using hyperpolarized \([1-^{13}C]\)pyruvate and incorporation of pyruvate into the TCA cycle evaluated using hyperpolarized \([2-^{13}C]\)pyruvate. Hyperpolarized pyruvate was generated using \(\sim 40\) mg of either \([1-^{13}C]\)pyruvic acid or \([2-^{13}C]\)pyruvic acid doped with \(15\) mM trityl radical (OXO63, Oxford Instruments, Abingdon, UK) and \(3\) \(\mu\)L Dotarem (1:50 dilution, Guerbet, Birmingham, UK) in a prototype polarizer system, with \(45\) min of microwave irradiation as previously described. The sample was subsequently dissolved in a pressurized and heated alkaline solution, containing \(2.4\ \text{g/L sodium hydroxide and 100 mg/L EDTA dipotassium salt (Sigma-Aldrich)}\), to yield a solution of \(80\) mM hyperpolarized sodium \([1-^{13}C]\)pyruvate or \([2-^{13}C]\)pyruvate with a polarization of \(\sim 20\), respectively, and at physiological temperature and pH.

Immediately following dissolution, \(1\) mL of the \([^{13}C]\) hyperpolarized substrate was injected intravenously into the anaesthetized animal over \(10\) s. Sixty individual ECG-gated \([^{13}C]\) MR pulse-acquire cardiac spectra were acquired over \(1\) min after injection (repetition time, \(1\) s; excitation flip angle, \(17^\circ\); pyruvate/61 bicarbonate), demonstrates that the pyruvate signal (B) originates primarily from the blood in the chambers of the heart, while the downstream metabolic signals, in this example bicarbonate (C), are originating from the front wall of the left ventricle.

Figure 1 Cardiac hyperpolarized \([^{12}C]\) signal localization is achieved via the high local sensitivity of the RF surface coil used and the high metabolic rate of the heart relative to other organs/tissues within the sensitive region of the coil (A, LV, left ventricular lumen; RV, right ventricular lumen; myo, myocardial tissue). This figure, acquired with a 3D spectral-spatial, echo planar spectroscopic imaging sequence (matrix: \(32 \times 32 \times 12\); field of view: \(64 \times 64 \times 45.5\) mm\(^2\); acquired resolution: \(2 \times 2 \times 4\) mm\(^2\); echo time (TE): \(16.3\) ms, repetition time (TR): \(1\) RR interval (\(\sim 150\) ms); flip angle: \(17^\circ\); pyruvate/61 bicarbonate), demonstrates that the pyruvate signal (B) originates primarily from the blood in the chambers of the heart, while the downstream metabolic signals, in this example bicarbonate (C), are originating from the front wall of the left ventricle.

2.4 CINE MRI

Cardiac structure and function were determined in vivo by CINE MRI at \(4\), \(9\), and \(14\) weeks post-surgery. A \(72\) mm quadrature birdcage transmit/recieve radio frequency coil was used to obtain MR images (Rapid Biomedical, Rimpar, Germany). Long- and short-axis scout images were acquired so that true short-axis images could be planned using a segmented, ECG-triggered FLASH sequence. CINE-MR images, consisting of \(28–35\) frames per heart cycle, were acquired in \(7–10\) contiguous slices in the short-axis orientation covering the entire heart. The imaging parameters were as follows: field of view = \(51.2 \times 51.2\) mm, matrix size = \(256 \times 256\), slice thickness = \(1.6\) mm, TE/TR = \(1.4/3.6\) ms, 0.5 ms/17.5\(^\circ\) Gaussian RF excitation pulse, and 6 averages. The total experimental time was \(\sim 50\) min per animal. Heart rate remained stable throughout the procedure. End-diastolic (ED) and end-systolic (ES) frames were selected as those with the largest and smallest cavity volumes, respectively. Epicardial and endocardial borders were outlined using the freehand drawing function of Image (National Institutes of Health, USA). Measurements from all slices were summed to calculate ED volume (VED), ES volume (VES), stroke volume (SV = VED − VES), ejection fraction (EF = SV/VED), cardiac output (CO = SV × heart rate), and cardiac index (CO/body weight). LV mass was calculated as myocardial area × slice thickness × myocardial specific gravity (1.05).

2.5 Serum analyses

Blood samples were collected from the saphenous vein at all time points immediately prior to \([1-^{13}C]\)pyruvate hyperpolarization studies. Whole blood was centrifuged to obtain plasma (15 \(600\) g for \(10\) min at \(4^\circ\)C) and analysed for plasma metabolites [glucose, lactate, triglycerides (TAGs), low-density lipoproteins (LDLs), high-density lipoproteins (HDLs), cholesterol, and \(\beta\)-hydroxybutyrate] using an ABX Pentra 400 (Horiba ABX Diagnostics). At the 14-week time point, plasma insulin levels were assessed on post mortem plasma using an insulin ELISA kit (Mercodia, Sweden).

2.6 Tissue metabolite analyses

After the MR investigations at 14 weeks post-surgery, animals were euthanized with an overdose of isoflurane (5% isoflurane with 2 L/min oxygen), and heart tissue, abdominal fat tissue, and kidneys were harvested, weighed, and, where appropriate, freeze clamped with Wollenberger tongs cooled in liquid nitrogen. Tissues were stored at \(-80^\circ\)C until further analysis. Tissues were then ground into a fine powder using a liquid nitrogen cooled pestle and mortar, extracted as appropriate for the assay and analysed as described below.

2.7 Cardiac TAG assay

TAG was extracted from \(\sim 25–50\) mg of powdered cardiac tissue following mixing and overnight suspension in 8 mL of Folch Solution (CHCl3:MeOH; 2:1) and 2 mL of distilled water. The aqueous phase was subsequently removed leaving the organic layer, which was evaporated to dryness and re-suspended in 1 mL of cold ethanol to form the sample solution. Approximately 100 \(\mu\)L of the sample solution was evaporated to dryness overnight and the sample re-suspended in 20 \(\mu\)L of cold ethanol from which the TAG concentration was measured spectrophotometrically using a Randox triglyceride assay kit (Randox Laboratories Ltd).

2.8 Western blotting

Frozen tissue was crushed and lysis buffer added before tissue was homogenized, a protein assay established the protein concentration of each lysate. The same concentration of protein from each sample was loaded on to 12.5% SDS–PAGE gels and separated by electrophoresis. A primary antibody for PDH kinase 4 (PDK4) was kindly donated by Prof. Mary Sugden.
independent of any dietary modification. Quantitatively, left ventricular hearts of animals subjected to AAB over time.

3. Results

All data are presented as mean ± SD. Statistical significance of the effects of AAB and diet on the acquired data were assessed using a two-way ANOVA at each individual time point. In cases where the interaction term was significant, the differences were evaluated in more detail by separately analysing the effects of AAB and diet using a Bonferroni corrected, two-sample unpaired t-test assuming equal variances. Statistical significance was considered at the $P \leq 0.05$ level.

3.1 Structural remodelling

In vivo CINE MRI was used to study the extent of structural remodelling in hearts of animals subjected to AAB over time. Figure 2 shows representative images of hypertrophied and control hearts in systole and diastole after 14 weeks of exposure to standard chow (Figure 2A and B) or WD (Figure 2C and D) with evident enlargement of the ventricle in AAB hearts independent of any dietary modification. Quantitatively, left ventricular mass (LVM) was markedly augmented in the AAB group compared with

![Figure 2](https://example.com/fi2e.png)

**Figure 2** Representative in vivo CINE MR images of sham and AAB hearts at 14 weeks post-surgical induction of cardiac hypertrophy during systole and diastole, (A and B) on standard chow diet, (C and D) on WD.

In terms of body weight, there was no significant difference between AAB and control animals at 4 or 9 weeks irrespective of diet (Table 2). However by 14 weeks, both WD fed groups showed a significantly greater body weight than their standard chow counterparts (WD 480 ± 40 g vs. chow 440 ± 30 g), with signs of increased adiposity evidenced by larger abdominal fat deposits. This weight gain was independent of the hypertrophy present in the AAB group.

Initially systolic and diastolic volumes were enlarged in AAB groups at 4 weeks post induction of hypertrophy (Figure 3), which resulted in a reduction in ejection fraction (Figure 4). However by 14 weeks, these alterations had resolved, indicating a transition from an initial acute phase of hypertrophic induction to a more compensated stage. End-systolic volumes were reduced in the WD-fed animals at the 4-week time point, resulting in an elevated ejection fraction; however, this also resolved with time and no structural differences were seen between chow and WD-fed animals at 14 weeks (Figures 3 and 4).

3.2 Functional remodelling

Functional assessment in vivo using MRI permitted the study of individual animals serially over the 14-week time course, which showed no indication of cardiac dysfunction in the AAB groups at any stage as reflected by the cardiac index (Figure 4). In addition, no effect of AAB was observed on the stroke volume or cardiac output at any time point (Figure 4), suggesting an enlarged yet still compliant heart in the AAB groups. Heart rate remained stable at all stages of hypertrophic development (Table 2) and did not differ from the sham groups, and at no stage over the time course of the experiment did the AAB animals show any signs of heart failure (breathlessness or fatigue).

In contrast, a small elevation in cardiac output was observed in both WD fed groups at 4 and 14 weeks compared with standard chow counterparts, with a trend towards increased cardiac output also visible at 9 weeks ($P = 0.07$, Figure 4). When normalized to body weight to generate the cardiac index (Figure 4), this significant elevation only remained at 4 weeks, suggesting that this was in some way related to the weight gain seen in these animals. In addition, dietary modification had no impact on heart rate (Table 2).

3.3 Metabolic remodelling

3.3.1 In vivo assessment of PDH flux

PDH flux was determined in vivo through incorporation of the hyperpolarized $^{13}$C label from [1-$^{13}$C]pyruvate into bicarbonate as previously demonstrated. Rates of $^{13}$C label incorporation into bicarbonate in AAB groups at the different time points were not significantly different from their respective controls (Figure 6A), implying little effect of AAB on PDH flux in vivo and thus glucose oxidation over the time course of hypertrophic development. At all time points however, dietary manipulation resulted in a marked reduction in $^{13}$C label incorporation into bicarbonate, indicating that PDH flux was reduced in the WD groups compared with their respective standard chow groups as would be predicted from the Randle cycle.

Label incorporation into alanine was unaffected by either AAB or dietary manipulation (data not shown). However, the rate of $^{13}$C label incorporation into lactate was markedly enhanced in AAB
groups by 9 weeks and sustained by 14 weeks (Figure 6B), independent of diet. These findings highlight that metabolic remodelling during the progression of cardiac hypertrophy is a continuous process with an increase in glycolytic metabolism as compensated hypertrophy develops.

3.3.2 In vivo assessment of TCA cycle metabolites

Hyperpolarized [2-13C]pyruvate was used to determine incorporation of pyruvate into TCA cycle intermediates. Within the AAB groups, there was no change in the [13C]metabolite/[2-13C]pyruvate ratio for either citrate or glutamate, independent of diet (Figure 7A and B). Given that PDH flux was unaltered in the hypertrophied hearts, these observations would indicate that there was no increase in the flux of glucose into the TCA cycle during this phase of hypertrophic development. These findings are thus suggestive of a compensatory phase of cardiac hypertrophy with no adverse impact from the high-fat, high-sucrose content of the WD.

However, there was a substantial reduction in the ratio of [1,13C]acetyl carnitine/[2-13C]pyruvate within the WD-fed groups (Figure 7C). Previous findings have proposed a role for glycolytically derived acetyl-carnitine to sustain and buffer the mitochondrial pool of acetyl-CoA, and therefore, a reduction in 13C label incorporation may indicate a reduced buffering capacity driven by enhanced utilization of the available carnitine for mitochondrial fatty acid transport.27

Figure 3 Cardiac structural parameters assessed in control and AAB animals at 4, 9, and 14 weeks post-surgical induction of cardiac hypertrophy. (A) LVM, (B) heart weight to body weight ratio, (C) end-diastolic volume, and (D) end-systolic volume. *P < 0.05 in WD groups compared with standard chow groups and §P < 0.05 in AAB groups compared with sham control groups. Group sizes as indicated on individual bars.
3.4 Serum metabolite levels

Serum metabolite concentrations are summarized in Table 3. Few differences were observed between AAB and their respective control groups at 4, 9, or 14 weeks, identifying the fact that AAB and its associated hypertension per se does not influence serum nutrient levels. However, as might be anticipated, dietary manipulation did have a significant impact, with animals subjected to WD feeding repeatedly exhibiting elevated plasma TAG and LDL levels and reduced plasma lactate levels. Plasma insulin levels were also assessed at the 14-week time point (Table 3) and showed a significant elevation in the WD-fed groups irrespective of AAB, indicating a level of insulin resistance induced by the WD.

| Table 2 In vivo structural and functional characteristics of hearts from control and abdominal aortic banded animals at 4, 9, and 14 weeks post induction of cardiac hypertrophy |
|-----------------------------------------------|
| **Sham—Chow** | **Sham—WD** | **AAB—Chow** | **AAB—WD** |
| 4 Weeks | 4 Weeks | 4 Weeks | 4 Weeks |
| n | 11 | 8 | 10 | 8 |
| Body weight/g | 340 ± 20 | 350 ± 20 | 340 ± 20 | 340 ± 20 |
| End-diastolic volume/μL | 550 ± 50 | 570 ± 80 | 620 ± 70§ | 610 ± 50§ |
| End-systolic volume/μL | 120 ± 20 | 100 ± 30* | 160 ± 40*** | 140 ± 20*** |
| Ejection fraction/% | 78 ± 4 | 83 ± 5** | 74 ± 5† | 77 ± 3*** |
| Stroke volume/μL | 430 ± 50 | 470 ± 50 | 450 ± 60 | 470 ± 50 |
| Heart rate/bpm | 350 ± 20 | 360 ± 40 | 360 ± 30 | 360 ± 30 |
| Cardiac output/mL min⁻¹ | 150 ± 20 | 170 ± 20* | 160 ± 20 | 170 ± 20* |
| 9 Weeks | 9 Weeks | 9 Weeks | 9 Weeks |
| n | 11 | 9 | 10 | 8 |
| Body weight/g | 400 ± 30 | 420 ± 30 | 420 ± 20 | 400 ± 40 |
| End-diastolic volume/μL | 640 ± 50 | 660 ± 60 | 660 ± 80 | 710 ± 60 |
| End-systolic volume/μL | 150 ± 20 | 130 ± 30 | 160 ± 30‡ | 170 ± 30‡ |
| Ejection fraction/% | 76 ± 4 | 81 ± 3* | 75 ± 5† | 77 ± 4*‡ |
| Stroke volume/μL | 490 ± 60 | 530 ± 40* | 500 ± 80 | 550 ± 60* |
| Heart rate/bpm | 380 ± 40 | 360 ± 30 | 360 ± 30 | 350 ± 40 |
| Cardiac output/mL min⁻¹ | 170 ± 30 | 190 ± 30 | 180 ± 30 | 190 ± 30 |
| 14 Weeks | 14 Weeks | 14 Weeks | 14 Weeks |
| n | 9 | 6 | 8 | 6 |
| Body weight/g | 440 ± 30 | 490 ± 40** | 450 ± 30 | 480 ± 40*** |
| End-diastolic volume/μL | 640 ± 100 | 760 ± 90 | 740 ± 120 | 730 ± 70 |
| End-systolic volume/μL | 140 ± 30 | 160 ± 40 | 180 ± 50 | 170 ± 20 |
| Ejection fraction/% | 78 ± 2 | 79 ± 4 | 75 ± 5 | 77 ± 4 |
| Stroke volume/μL | 500 ± 70 | 600 ± 70 | 560 ± 110 | 560 ± 70 |
| Heart rate/bpm | 350 ± 40 | 350 ± 30 | 360 ± 40 | 380 ± 30 |
| Cardiac output/mL min⁻¹ | 180 ± 20 | 210 ± 20* | 200 ± 30 | 210 ± 30* |

Data expressed as mean ± SD.

*P < 0.05 for effect of diet.

**P < 0.01 for effect of diet.

***P < 0.001 for effect of diet.

‡P < 0.05 for effect of AAB.

†P < 0.01 for effect of AAB.

§P < 0.001 for effect of AAB.

3.5 PDK4 expression and cardiac TAG concentrations

In vitro analysis of the key inhibitory enzyme PDK4 at the 14-week time point reflected the in vivo hyperpolarized 13C MR findings with no alteration in expression in AAB or control groups within the same dietary group but a marked elevation of PDK4 expression in WD groups (Figure 8A). In vitro analysis of cardiac TAG levels demonstrated that they were unaffected by either AAB or the WD (Figure 8B), indicating no elevated deposition of lipids in the cardiac tissue.

4. Discussion

Using the novel, non-invasive technique of hyperpolarized MRS, this in vivo study has investigated the temporal metabolic, structural, and functional changes that are associated with the development of cardiac hypertrophy in the setting of excess nutrient supply. AAB caused a mild hypertrophic response in the heart without any functional detriment, a response that was unaffected by dietary manipulation. Although there was clear evidence of structural remodelling as early as 4 weeks post surgical induction of cardiac hypertrophy, the metabolic alterations that developed with the progression of hypertrophy determined by hyperpolarized 13C MR were not observed until 9 weeks and highlighted a switch towards increased glycolytic metabolism rather than towards enhanced glucose oxidation.
4.1 Structural and functional impact of AAB

CINE MRI is a dynamic technique that allows measurement of both structural remodelling and cardiac function in vivo. The findings have clearly shown significant structural alterations in the left ventricle of AAB hearts. In line with the aim of this work to explore the structural, functional, and metabolic remodelling that occurs during the time course of cardiac hypertrophic development, the degree of hypertrophy induced in this model (14–17%) was smaller than that produced by the more severe ascending aortic constriction (30–45%), TAC (33%), and SHR (56%) models of cardiac hypertrophy, which more rapidly progress to heart failure but the degree of hypertrophy was clearly evident both in the increased cardiac mass as well as the initially enlarged ventricular volumes and was consistent with previous studies employing this model. The development of hypertrophy did not appear to be affected by the alteration in diet, although the animals maintained on the WD did show a greater weight gain by 14 weeks. This was primarily a result of increased fat mass, but the weight gain was small and could not be considered as representative of an obese model.

Although there was an increase in both end-systolic and end-diastolic dimensions in the AAB group at 4 weeks post surgery, resulting in a reduced ejection fraction, this appeared to be a transient event. These alterations may reflect the acute response of the heart to the

Figure 4 Functional characteristics of control and AAB hearts in vivo. (A) Ejection Fraction, (B) stroke volume, (C) cardiac output, and (D) cardiac index in sham and AAB animals at 4, 9, or 14 weeks post-surgical induction of cardiac hypertrophy, exposed to standard chow or WD. *P < 0.05 in WD groups compared with standard chow groups and §P < 0.05 in AAB groups compared with sham control groups. Group sizes as indicated on individual bars.
hypertrophic pressure overload stimulus, which resolved into a compensated phase over the subsequent 10 weeks. *In vitro* studies of this model have shown early expression of ANF, a marker of hypertrophy, within a week of AAB but no evidence of heart failure at this phase.31 Over time, there is little evidence of functional impairment in the AAB groups suggesting development of a compensated model of LVH by 14 weeks. This does not preclude the possibility of diastolic dysfunction, which we were unable to assess in this study. It is possible that the study of alterations in diastolic function may offer a more sensitive way to explore the effects of AAB within the setting of excess nutrient supply.

### 4.2 Metabolic remodelling with the development of hypertrophy

Metabolic remodelling is a frequently observed feature of cardiac hypertrophy and heart failure. Many studies have identified a switch from fatty
acid oxidation towards a reliance on glucose metabolism for energy generation in the hypertrophied and failing heart. However, the majority of these studies have used in vitro systems in the isolated perfused heart with defined substrate concentrations in crystalloid buffers. In this in vivo study, the heart has been exposed to the natural composition of metabolites within the blood—giving a more biologically relevant picture of metabolic remodelling and the functional consequences. Here we have used the novel in vivo method of hyperpolarized MRS to probe the remodelling of pyruvate metabolism in the hypertrophied heart exposed to a nutrient-rich environment over time. Our findings have identified an apparent uncoupling of glucose metabolism as an early event in hypertrophic development. Throughout the time course of the experiment, no change in flux through PDH was observed, which is in contrast to single time point studies on the SHR model where an 85% increase in PDH flux was observed. The studies on the SHR model have shown a marked increase in PDH flux, supported by in vitro assay measurements of PDH activity. In part, the differences observed between these models could be due to a lesser degree of hypertrophic remodelling in the AAB model compared with the SHR model or may imply that the increased reliance on glucose oxidation is a later event in the progression of hypertrophy. In addition, the SHR model is known to have multiple genetic mutations that contribute to the hypertensive phenotype. A major mutation is a loss of the sarcolemmal fatty acid transporter, FAT/CD36, which is likely to lead to a reduction in fatty acid oxidation and a consequential increase in glucose oxidation and PDH flux. It is therefore highly likely that the metabolic alterations seen in the previously study were impacted by this lack of FAT/CD36 and the ability to draw conclusions on the balance between the cause and effect of the hypertension in the light of these genetic changes is extremely challenging.

Previous studies measuring PDH activity in tissue extracts from the AAB model have identified a 69% reduction in the percentage of PDH in the active fraction. However, the hyperpolarized MRS method is unique in measuring in vivo flux through PDH in contrast to in vitro assay systems, which only assess the proportion of enzyme in the active form. Although there was no alteration in PDH flux in vivo, we have observed an elevation in glycolytic flux by 9 weeks as detected by greater incorporation of the hyperpolarized $^{13}$C label into lactate. The level of enhanced label incorporation into lactate (26% at 9 weeks and 30% at 14 weeks) was smaller than that seen in previous hyperpolarized MRS studies investigating cancer treatment (lactate signal levels 50% higher

![Figure 7](https://academic.oup.com/cardiovascres/article-abstract/106/2/249/318926)
in untreated vs. treated tumours)\textsuperscript{35} or acute ischaemia (lactate signal levels 138% higher in ischaemic hearts vs. control hearts)\textsuperscript{36} but when combined with the lack of alteration in PDH flux, it would suggest that, beyond the initial acute response to the hypertrophic stimulus, there was a degree of uncoupling of glucose oxidation from glycolysis. Lopaschuk and colleagues\textsuperscript{37} have suggested that this may occur as a compensatory response under conditions such as ischaemia or more chronic situations such as hypertrophy. Enhanced [1-13C]lactate generation was still observed at the 14-week time point suggesting, in the absence of any functional deterioration, that the observed level of glycolytic uncoupling was not a significantly deleterious adaptation. However, this does not preclude this uncoupling from becoming a detrimental factor as the LVH progresses into heart failure.

### 4.3 The effects of dietary modification

The application of a WD appears to have had little impact on cardiac structure, suggesting that, unlike other studies,\textsuperscript{30,38,39} exposure to a WD for this time period does not induce hypertrophy. Functionally, the WD led to a small but significant increase in stroke volume and cardiac output. Therefore, at this stage, the WD does not cause any cardiac dysfunction but rather a small degree of hyperfunction.

Further, the combination of cardiac hypertrophy with dietary manipulation using a WD does not appear to have caused any functional deterioration, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of saturated fats in the diet without affecting the sugar composition had a major impact on PDH flux, consistent with other studies employing high-fat/high-sugar diets.\textsuperscript{40,41} However, controversy exists in this area, as augmenting the percentage of satu
Although predicted from in vitro experimentation, hyperpolarized MRS offers a unique way to visualize this effect in vivo. No concomitant change in [1-13C]lactate production was observed with the dietary-induced decrease in PDH flux. Elevated insulin levels were observed in the WD-fed groups at the 14-week time point, which when coupled with the unaltered glucose levels, indicates a degree of insulin resistance induced by the high-fat, high-sugar diet.

Label incorporation into acetylcarnitine was markedly reduced in the WD-fed animals from 4 weeks onwards providing early evidence of the effect of dietary composition on substrate metabolism and storage. Given the role of carnitine as a co-factor in mitochondrial fatty acid transport, this suggests that carnitine availability has become limiting following enhanced fatty acid utilization promoted by an increase in dietary fatty acid availability. The implications of a reduction in the buffering of pyruvate-derived acetyl-CoA into acetylcarnitine remains unclear at this point but does not appear to affect the development of hypertrophy nor have any deleterious effect on normal function.

5. Conclusions

This in vivo study examining the metabolic alterations that occur during the development of compensatory hypertrophy suggests a switch towards increased glycolytic hypertrophy rather than enhanced glucose oxidation as previously described. This appears to be irrespective of dietary manipulation with a WD, which despite causing a significant reduction in flux through PDH and evidence suggesting promoted fatty acid utilization, did not correlate with any structural or functional deterioration in the heart.

Conflict of interest: none declared.
Funding
This work was supported by the British Heart Foundation in the form of an Intermediate Basic Science Research Fellowship (FS/10/002/28078), a Senior Basic Science Research Fellowship (FS/14/17/30634) and a 4-year PhD Studentship. This work was also supported by EPSRC in the form of a 4-year PhD studentship, and GE Healthcare in the form of equipment support. Funding to pay the Open Access publication charges for this article was provided by the British Heart Foundation.

References
1. Ope LH, Commerford PJ, Gersh BJ, Pfeffer MA. Controversies in ventricular remodel-
2. Hennek J, Molkentin JD. Regulation of cardiac hypertrophy by intracellular signalling
3. Neubauer S. The failing heart—an engine out of fuel. New Engl J Med 2007;356:
4. Doenst T, Pytel G, Schrepper A, Amorim P, Farber G, Shing Y, Mohr FW, Schwarzer M. Decreased rates of substrate oxidation ex vivo predict the onset of heart failure and con-
5. Filmore N, Lopaschuk GD. Targeting mitochondrial oxidative metabolism as an ap-
6. Madrazo JA, Kelly DP. The PPAR trio: regulators of myocardial energy metabolism in
7. Lopaschuk GD, Folmes CD, Stanley WC. Cardiac energy metabolism in obesity. Circ Res 2007;101:335–347.
8. Boudina S, Sena S, O'Neill BT, Tathireddy P, Young ME, Abel ED. Reduced mitochondrial oxidative capacity and increased mitochondrial uncoupling impair myocardial ener-
9. Chiu HC, Kovacs A, Ford DA, Hsu FF, Garcia R, Herrero P, Saffitz JE, Schaffer JE. A novel mouse model of lipotoxic cardiomyopathy. J Clin Invest 2007;111:813–822.
10. Wilson CR, Tran MK, Salazar KL, Young ME, Taegtmeyer H. Western diet, but not high
11. Akki A, Seymour AM. Western diet impairs metabolic remodelling and contractile effi-
12. Allard MF, Schonekess BO, Henning SL, English DR, Lopaschuk GD. Contribution of oxida-
13. Chatham JC, Seymour AM. Cardiac carbohydrate metabolism in Zucker diabetic fatty
14. Atherton HJ, Dodd MS, Heathcote LC, Schroeder MA, Griffin JL, Radda GK, Clarke K, Tyler DJ. Role of pyruvate dehydrogenase inhibition in the development of hypertrophy in the hypertrophic rat heart: a combined magnetic resonance imaging and hyperpolar-
15. Dodd MS, Ball DR, Schroeder MA, Le Page LM, Atherton HJ, Heathcote LC, Seymour A-M, Ashrafian H, Watton H, Clarke K, Tyler DJ. In vivo alterations in cardiac metabolism and function in the spontaneously hypertensive rat heart. Cardiovas Res 2012;93:69–76.
16. Schroeder MA, Cochlin LE, Heathcote LC, Clarke K, Radda GK, Tyler DJ. In vivo assessment of pyruvate dehydrogenase flux in the heart using hyperpolarized carbon-13 magnetic resonance imaging. Proc Natl Acad Sci USA 2010;105:12051–12056.
17. Cassidy PJ, Schneider JE, Grieve SM, Lygate C, Neubauer S, Clarke K. Assessment of motion gating strategies for mouse magnetic resonance at high magnetic fields. J Magn Reson Imaging 2004;19:229–237.
18. Tyler DJ, Lygate CA, Schneider JE, Cassidy PJ, Neubauer S, Clarke K. CINE-MR imaging of the normal and infarcted rat heart using an 11.7 T vertical bore MR system. J Cardiovasc Magn Reson 2006;8:279–333.
19. Ardenkjaer-Larsen JH, Fridlund B, Gram A, Hansson G, Hansson L, Lerche MH, Servin R, Thansing M, Golman K. Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR. Proc Natl Acad Sci USA 2001;100:10158–10163.
20. Naressi A, Cautener C, Ciares J, de Beer R, Graversen-Denilby D. Java-based graphical user interface for MRLUI, a software package for quantification of in vivo-medical magnetic resonance spectroscopy signals. Comput Biol Med 2001;31:269–286.
21. Atherton HJ, Schroeder MA, Dodd MS, Heathcote LC, Carter EE, Cochlin LE, Nagel S, Sibson NR, Radda GK, Clarke K, Tyler DJ. Validation of the in vivo assessment of pyruvate dehydrogenase activity using hyperpolarized 13C MR. NMR Biomed 2011;24:201–208.
22. Zierhut ML, Yen YF, Chen AP, Bok R, Albers MJ, Zhang V, Tropp J, Park I, Vigneron DB, Kurhanewicz J, Hund RE, Nelson SJ. Kinetic modeling of hyperpolarized 13C-pyruvate metabolism in normal rats and TRAMP mice. J Magn Reson 2010;202:85–93.
23. Schneider JE, Cassidy PJ, Lygate C, Tyler DJ, Wiesmann F, Grieve SM, Hibbert K, Clarke K, Neubauer S. Fast, high-resolution in vivo cine magnetic resonance imaging and normal and failing mouse hearts on a vertical 11.7 T system. J Magn Reson Imaging 2003;18:691–701.
24. Schneider JE, Wiesmann F, Lygate CA, Neubauer S. How to perform an accurate assess-
25. Boehm EA, Jones BE, Radda GK, Veech RL, Clarke K. Increased uncoupling proteins and decreased efficiency in palmitate-perfused hyperthyroid rat heart. Am J Physiol Heart C 2001;280:H1977–H1983.
26. Randi P, Garlind PB, Hales CN, Newsholme EA. The glucose-fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1963;1:785–789.
27. Schroeder MA, Atherton HJ, Dodd MS, Lee P, Cochlin LE, Radda GK, Clarke K, Tyler DJ. The cycling of acetyl-coenzyme A through acetylaminic buffered carbohydrate substrate supply: a hyperpolarized 13C magnetic resonance study. Circ Cardiovasc Imaging 2012;5:201–209.
28. Schunkert H, Dzau VJ, Tang SS, Hirsch AT, Apstein CS, Lorell BH. Increased rat cardiac angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy. Effects on coronary resistance, contractility, and relaxation. J Clin Invest 1990;86:1913–1920.
29. Weinberg EO, Schoen FJ, George D, Kagaya Y, Douglas PS, Litwin SE, Schunkert H, Benedict CR, Lorell BH. Angiotensin-converting enzyme inhibition prolongs survival and modifies the transition to heart failure in rats with pressure overload hypertrophy due to ascending aortic stenosis. Circulation 1994;90:1410–1422.
30. Rockman HA, Ross RS, Harns AN, Knowlton KU, Steinhilber ME, Field LJ, Ross JR, Chien KR. Segregation of atrial-specific and inducible expression of an atrial natriuretic factor transgene in vivo and murine model of cardiac hypertrophy. Proc Natl Acad Sci USA 1991;88:8277–8281.
31. Akki A, Smith K, Seymour AM. Compensated cardiac hypertrophy is characterised by a decline in palmitate oxidation. Mol Cell Biochem 2008;313:215–224.
32. Stanley WC, Raczec FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. Physiol Rev 2005;85:1093–1129.
33. Turer AT, McIver CR, Newgard CB, Podorensky MV. Energetics and metabolism in the failing heart: important but poorly understood. Curr Opin Clin Nutr 2010;13:458–465.
34. Seymour AM, Chatham JC. The effects of hypertrophy and diabetes on cardiomyocyte dehydrogenase activity. J Mol Cell Cardiol 1997;29:2771–2778.
35. Day SE, Kettunen MI, Gallagher FA, Hu DE, Lerche M, Wolber J, Golman K, Ardenkjaer-Larsen JH, Bridle KM. Detecting tumor response to treatment using hyperpolarized 13C magnetic resonance imaging and spectroscopy. Nat Med 2007;13:1382–1387.
36. Schroeder MA, Atherton HJ, Ball DR, Cole MA, Heathcote LC, Griffin JL, Clarke K, Radda GK, Tyler DJ. Real-time assessment of Krebs cycle metabolism using hyperpolarized 13C magnetic resonance spectroscopy. FASEB J 2009;23:2529–2538.
37. Lopaschuk GD, Wambelt RB, Barr RL. An imbalance between glycolysis and glucose oxidation is a possible explanation for the detrimental effects of high levels of fatty acids during aerobic reperfusion of ischemic hearts. J Pharmacol Exp Ther 1999;293:135–144.
38. Qin F, Siwik DA, Luptak I, Hou X, Wang L, Higuchi A, Weisbrod RM, Ouchi N, Tu VH, Calamara TD, Miller EJ, Verbeuren TJ, Walsh K, Cohen RA, Colucci WS. The polyphe-
39. Vasanji Z, Cantor EJ, Juric D, Moyen M, Notticadan T. Alterations in cardiac contractile performance and sarcoplasmic reticulum function in sucrose-fed rats is associated with insulin resistance. Am J Physiol Cell Ph 2006;291:C727–C780.
40. Okere IC, Young ME, McElfresh TA, Chess DJ, Sharov VG, Sabbah HN, Hoot BD, Ermbrnger P, Chandler MP, Stanley WC. Low carbohydrate/high-fat diet attenuates angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy. Effects on coronary resistance, contractility, and relaxation. J Clin Invest 1990;86:1913–1920.
41. Rennison JH, McElfresh TA, Okere IC, Vazquez EJ, Patel HV, Foster AB, Patel KK, Chen Q, Benedict CR, Lorell BH. Angiotensin-converting enzyme inhibition prolongs survival and modifies the transition to heart failure in rats with pressure overload hypertrophy due to ascending aortic stenosis. Circulation 1994;90:1410–1422.