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Assessing mitochondrial respiration in isolated hearts using ^{17}O MRS

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Abstract

The application of ^{17}O MRI and MRS for the evaluation of cardiac mitochondrial function has been limited because of the challenge of detecting metabolic H_2^{17} in the vast background of naturally abundant H_2^{17}. In this study, we have developed a direct ^{17}O MRS approach to examine the feasibility and sensitivity of detecting metabolically produced H_2^{17} in isolated rat hearts perfused with $^{17}O_2$-enriched Krebs-Henseleit buffer containing normal (1.5 mM) and high (2.5 mM) calcium (Ca^{2+}) concentrations to induce high workload. Consistent with increased workload at high Ca^{2+} concentration, the measured myocardial oxygen consumption rate (MVO_2) increased by 82%. Dynamic ^{17}O MRS showed an accelerated increase in the $H_2^{17}O$ signal at high Ca^{2+} concentration, suggesting increased mitochondrial production of $H_2^{17}O$ in concordance with the increased workload. A compartment model was developed to describe the kinetics of $H_2^{17}O$ production as a function of MVO_2. The myocardial $^{17}O_2$ consumption rate ($MV^{17}O_2$) was determined by least-squares fitting of the model to the NMR-measured $H_2^{17}O$ concentration. Consistent with the measured MVO_2, the model-determined $MV^{17}O_2$ showed a 92% increase at high Ca^{2+} concentration. The increase in metabolic activity at high workload allowed the balance between ATP production and utilization to be maintained, leading to a similar phosphocreatine to ATP ratio. These results demonstrate that dynamic ^{17}O MRS can provide a valuable tool for the detection of an altered metabolic rate associated with a change in cardiac workload.

Keywords

^{17}O spectroscopy; mitochondrial function; myocardial metabolism; oxygen consumption; energy–function relationship

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INTRODUCTION

The heart is the most energy-demanding organ because of its nonstop contraction. There is increasing evidence that abnormal mitochondrial function and energy metabolism are characteristic of several prevalent cardiac diseases, such as heart failure (1–3), and ischemic and diabetic cardiomyopathy (4–6). However, our fundamental understanding of cardiac energetics in diseased hearts remains incomplete because of the limited means to investigate myocardial metabolism in intact hearts. To date, most of the studies on mitochondrial function have been performed in isolated mitochondria or with *in vitro* tissue samples. As a result of the lack of nondestructive methods to quantify mitochondrial respiration *in vivo*, an integrative understanding of the mechanisms underlying metabolic dysfunction in diseased hearts is still incomplete.

In the last 20 years, MRS has become a valuable tool for the evaluation of cardiac metabolism under various pathophysiological conditions, largely because of its noninvasive nature and the exquisitely detailed information it can offer (7,8). While ^{13}C and ^{31}P MRS allow the quantification of substrate metabolism and ATP synthesis, ^{17}O MRS provides the unique opportunity to evaluate mitochondrial respiration in intact, viable tissues through the observation of metabolic $H_2{}^{17}O$ production via the reduction of $^{17}O_2$. The use of ^{17}O MRS for the assessment of mitochondrial $H_2{}^{17}O$ production was first demonstrated, independently, in 1991 by Mateescu *et al.* (9) in mouse brain and Pekar *et al.* (10) in cat brain. Recently, ^{17}O MRS methods for the quantification of cerebral oxygen consumption have been developed in animals through the monitoring of dynamic changes of metabolically generated $H_2{}^{17}O$ from inhaled $^{17}O_2$ (11–16). However, cardiac applications of ^{17}O MRS have been limited. Only a few studies have explored the potential of detecting mitochondrial function in the heart with ^{17}O MR techniques (17–20).

Current *in vivo* ^{17}O MRS methods are complicated by several factors that can potentially affect the accuracy of the measurement of mitochondrial respiration. These include the efflux of metabolic $H_2{}^{17}O$ via normal tissue water exchange and the input of $H_2{}^{17}O$ from other metabolizing tissues. These issues can be addressed using an isolated perfused heart model. The advantages of using an isolated heart model are as follows: (i) it allows precise control of the substrates and other experimental conditions; (ii) it enables easy manipulation of mitochondrial respiration via changes in cardiac workload; (iii) it allows the simultaneous recording of cardiac function to investigate the energy–function relationship; and (iv) it eliminates systemic $H_2^{17}O$ input from other metabolizing organs. Therefore, ^{17}O MRS on isolated perfused hearts provides the opportunity to validate quantitative ^{17}O MRS methods for the accurate assessment of mitochondrial respiration in living tissues. These *ex vivo* MRS data may serve as the benchmarks for *in vivo* studies.

In this study, we aimed to develop a ^{17}O MRS method on isolated perfused hearts to examine the feasibility and sensitivity of ^{17}O MRS for the detection of metabolically produced $H_2{}^{17}O$ in hearts at different workloads. A closed-loop perfusion system specifically designed for economic and efficient ^{17}O MRS studies was developed. Metabolically produced $H_2{}^{17}O$ was detected by rapid, dynamic ^{17}O MRS on a 9.4-T vertical-bore spectrometer. A compartment model that describes the kinetics of $H_2{}^{17}O$ production was also developed for the estimation of the myocardial oxygen consumption rate (MVO_2). Our results demonstrate that a high-temporal-resolution ^{17}O MRS method provides an opportunity to detect an altered mitochondrial respiration rate associated with a change in cardiac workload.

MATERIALS AND METHODS

Perfusion system

A major challenge in performing ^{17}O MRS experiments on perfused hearts is the large system volume that may reduce the detectability of metabolic $H_2{}^{17}O$ because of the dilution of metabolic $H_2{}^{17}O$ by natural abundance $H_2^{17}O$. To improve the detection sensitivity, we developed a closed-loop perfusion system with a total volume of only 26 mL (Fig. 1A). The perfusion system has two gravitational reservoirs for $^{17}O_2$- and $^{16}O_2$-oxygenated perfusate, respectively. The perfusion pressure was maintained at a constant level by adjusting the level of the overflow port in the reservoir. Re-oxygenation of the recycling perfusate was achieved via $^{17}O_2$-filled silicone tubing inserted into the perfusion line.

Buffer preparation

The formidable cost of $^{17}O_2$ requires a low-loss system for efficient oxygenation of the perfusate with $^{17}O_2$. Two approaches were attempted in the current study—a pressurized chamber (Fig. 1B) and a gas-exchange circuit (Fig. 1 C)—with the goal of maximizing $^{17}O_2$ usage for each experiment. In the gas-exchange system, oxygenation with $^{17}O_2$ was achieved by circulating the perfusate through a cartridge of $^{17}O_2$-filled silicone tubing. The high gas permeability of the silicone tubing allowed the efficient transfer of $^{17}O_2$ to the perfusate. In the second approach, we used a pressurized chamber for buffer oxygenation. On introduction of $^{17}O_2$ (70% enrichment; Rockland Technimed Ltd., Airmont, NY, USA), the pressure of the chamber was increased to about 8 psi and a magnetic stirrer operating at 700 rpm allowed the gas to quickly dissolve into the perfusate. To maximize the $^{17}O_2$ content in the perfusate, the perfusate was degassed at atmospheric pressure by bubbling helium gas to reduce the content of $^{16}O_2$ prior to oxygenation with $^{17}O_2$.

Perfusion protocol

Isolated hearts from 2-month-old male Sprague-Dawley rats were perfused with oxygenated Krebs-Henseleit buffer containing (in mM): NaCl, 118; $NaHCO_3$, 25; KCl, 5.3; $MgSO_4$, 1.2; $CaCl_2$, 1.5 or 2.5; glucose, 10. Two different calcium (Ca^{2+}) concentrations, normal at 1.5 mM (NCA, $n = 7$) and high at 2.5 mM (HCA, $n = 7$), were used to induce normal and high workloads, respectively. The left ventricular developed pressure (LVDP) and heart rate (HR) were measured via a water-filled balloon connected to a pressure transducer (ADInstruments, Colorado Springs, CO, USA). The rate–pressure product (RPP) was used as an index of ventricular workload.

At the start of the perfusion protocol, $^{16}O_2$-oxygenated perfusate was supplied for 15 min to establish metabolic equilibrium. A ^{31}P spectrum was collected to verify the metabolic status of the heart. A natural abundance ^{17}O spectrum was then collected for background subtraction. The perfusate was then switched to $^{17}O_2$-oxygenated buffer. Dynamic acquisition of ^{17}O spectra began immediately for 5 min. To reduce the negative inotropic effect caused by adenosine accumulation, theophylline (Sigma-Aldrich, St. Louis, MO, USA), an adenosine antagonist, was infused at 0.5 mL/min once the perfusate was switched to $^{17}O_2$-oxygenated buffer. At the end of the perfusion protocol, hearts were taken off the perfusion column, blotted dry and weighed.

To validate the ^{17}O MRS results, the oxygen content (pO_2)of the perfusion medium in the supply line and in coronary effluent was measured with a blood-gas analyzer (Radiometer ApS, Copenhagen, Denmark). MVO_2 was calculated experimentally from the measured coronary flow rate and the difference in pO_2 between the perfusate and the coronary effluent (21). These measurements were performed during the acquisition of the ^{31}P spectrum. All

procedures were approved by the Institutional Animal Care and Use Committee at Case Western Reserve University.

^{31}P and dynamic ^{17}O MRS

^{31}P and ^{17}O MR spectra were acquired on a Bruker AVANCE-400 9.4-T vertical-bore spectrometer (Bruker Biospin Co., Billerica, MA, USA) with a 20-mm broad-band probe. Field homogeneity was adjusted by shimming on the proton signal to a linewidth of 30–50 Hz. ^{31}P spectra were acquired at 162 MHz with 90° radiofrequency pulses, TR = 1.3 s, 12 K dataset and 64 averages. Spectrum analysis was performed using NMR-dedicated software MestRec (Mestrelab Research SL, Santiago de Compostela, Spain). Raw free induction decay signals were converted into frequency domain spectra by Fourier transformation following the application of an exponential filter (15-Hz line broadening) to enhance the signal-to-noise ratio. Relative ATP and phosphocreatine (PCr) levels were quantified by fitting the β-ATP and PCr peaks with Lorentzian curves, respectively, followed by the integration of these two peak areas. The PCr to ATP ratio was calculated as an index of the high energetic status of the heart.

Sequential ^{17}O spectra were acquired at 54 MHz with 90° radiofrequency pulses, TR = 300 ms, 1 K dataset and 64 averages. The temporal resolution for ^{17}O acquisition was 20 s. For spectrum quantification, the natural abundance ^{17}O signal was digitally subtracted. The raw MRS signal was processed by exponential filtering with a line broadening of 30 Hz, followed by Fourier transformation to obtain frequency domain spectra. $H_2{}^{17}O$ was quantified by fitting the resonance peaks with Lorentzian curves, followed by the integration of the peak area. Assuming an average water concentration of 55 M in the whole NMR tube, the $H_2{}^{17}O$ concentration was calculated using the natural abundance ^{17}O spectrum as the reference.

Kinetic analysis

To quantify the mitochondrial respiration rate from ^{17}O MRS data, we developed a compartment model to describe the transport and production of metabolic $H_2{}^{17}O$ during the perfusion protocol. The model included three compartments: the heart; the fluid in the NMR tube that surrounded the heart; and the recirculating perfusate (Fig. 2).

According to the mass balance principle, the $H_2{}^{17}O$ content in the heart (C_h; μmol/g wet weight) is governed by:

$$\mathrm{HW}\cdot\frac{\mathrm{dC}_h}{\mathrm{dt}}=2\mathrm{MV}^{17}\mathrm{O}_2\cdot\mathrm{HW}+\mathrm{FL}\cdot C_r-\mathrm{FL}\cdot\frac{C_h}{\mathrm{p}} \quad [1]$$

where HW is the wet heart weight (g), $MV^{17}O_2$ is the myocardial $^{17}O_2$ consumption rate (μmol/min/g wet weight), FL is the coronary flow rate (mL/min) measured during the experiment, C_r is the $H_2{}^{17}O$ concentration in the recirculating perfusate (μmol/mL) and p is the myocardium–blood partition coefficient of water. A myocardium–blood partition coefficient of 0.91 mL/g wet weight was used in the current study (22).

The mass balance of the $H_2{}^{17}O$ content in the NMR tube (C_t; μmol/mL) can be described as:

$$V_t\frac{\mathrm{dC}_t}{\mathrm{dt}}=\mathrm{FL}\left(\frac{C_h}{\mathrm{p}}-C_t\right) \quad [2]$$

where V_t is the fluid volume in the NMR tube. V_t was maintained at 6 mL in the current study.

Similarly, the change in $H_2{}^{17}O$ content in the recirculating perfusate (C_r; μmol/mL) can be described as:

$$V_r \frac{dC_r}{dt} = FL\,(C_t - C_r) \quad [3]$$

where V_r is the volume of the circulating perfusate. The initial perfusate volume, including both the reservoir and the tubing, was 20 mL. With the infusion of theophylline at 0.5 mL/min, the total recirculating perfusate (V_r) can be described by the following equation:

$$V_r = 20 + 0.5t$$

The model output was the NMR signal arising from both the heart and the fluid in the NMR tube, i.e.:

$$S_{NMR} = HW \cdot C_h + V_t C_t \quad [4]$$

The only unknown parameter in Equations [1–3] is the myocardial $^{17}O_2$ consumption rate ($MV^{17}O_2$), which was determined from the least-squares fitting of the model output (Equation [4]) to the NMR-measured metabolic $H_2{}^{17}O$ accumulation.

Statistical analysis

All data are presented as means±standard deviation. Comparisons of datasets were performed using unpaired Student's *t*-test. Differences in mean values were considered to be significant at a probability level of <5%.

RESULTS

Perfusate oxygenation with $^{17}O_2$ gas

Both the closed-loop system and the pressurized chamber allowed efficient oxygenation of the buffer with $^{17}O_2$ gas. With the closed-loop system, the pO_2 of 40 mL of perfusate was increased to >400 mmHg within 2 min of circulation and at the cost of only 30 mL of $^{17}O_2$ gas. The efficiency of the pressurized chamber was slightly higher, with the pO_2 of 50 mL of perfusate increased to >500 mmHg within 2 min and at the cost of 30 mL of $^{17}O_2$ gas.

Contractile function

The body weight and heart weight were similar between the two experimental groups. The average heart weights were 1.71±0.27 g and 1.91±0.14 g for the NCA and HCA groups, respectively (p = not significant). The average HR was also similar between the two groups: 339±43 beats/min for the NCA group and 321±24 beats/min for the HCA group (p = not significant). The HR remained constant during data acquisition (Fig. 3A).

LVDP decreased slightly from 59±24 mmHg at the beginning of ^{17}O perfusion to 48±23 mmHg at the end of 5 min of ^{17}O MRS data acquisition in the NCA group. This reduction in LVDP was even more pronounced in the HCA group: from 92±25 mmHg to 59±26 mmHg (Fig. 3B). As a result, RPP changed from 16 800±5900 mmHg/min to 14 300±5400 mmHg/min for the NCA group, and from 28 200±9400 mmHg/min to 19 400±8700 mmHg/min for the HCA group (Fig. 3 C). LVDP and RPP were statistically higher in the HCA group for the first 2 min of ^{17}O MRS data acquisition.

^{31}P MRS and measured oxygen consumption rate

A representative ^{31}P spectrum is shown in Fig. 4A. Both groups showed normal and similar PCr to ATP ratios: 1.49±0.16 for the NCA group and 1.45±0.20 for the HCA group (Fig. 4B, p = not significant). The measured MVO_2 in the HCA group (6.23±0.19 μmol/min/g wet weight) increased by 82% relative to that of the NCA group (3.42±0.50 μmol/min/g wet weight; $p < 0.05$; Fig. 4 C).

Dynamic ^{17}O MRS and model-fitted $^{17}O_2$ consumption rate

Representative dynamic ^{17}O spectra are shown in Fig. 5A. Both groups showed a progressive increase in the $H_2{}^{17}O$ resonance peak (Fig. 5B), suggesting steady production of mitochondrial $H_2{}^{17}O$. As shown in Fig. 5B, hearts perfused with higher Ca^{2+} concentration demonstrated an accelerated increase in the $H_2{}^{17}O$ signal.

Model-fitted $H_2^{17}O$ curves are also shown in Fig. 5B. The myocardial $^{17}O_2$ consumption rate ($MV^{17}O_2$), determined from the least-squares fitting of the model to group-averaged MRS data, increased from 1.15 μmol/min/g wet weight in the NCA group to 2.21 μmol/min/g wet weight in the HCA group, a 92% increase, which is comparable with the overall increase in MVO_2 (82%).

DISCUSSION

Oxygen utilization in the mitochondrial respiratory chain is an important index of cardiac metabolic status. Although ^{15}O positron emission tomography (PET) has been the standard imaging modality for the assessment of oxygen consumption for more than 20 years, its application has been limited (23). In ^{15}O PET, all ^{15}O-labeled oxygen contributes to the measured signal, regardless of its chemical form. Hence, data interpretation is complicated, and requires complex compartmental modeling to determine the oxygen consumption rate. Frequently, a multitracer study is necessary to account for blood volume and blood flow in modeling (22). By contrast, ^{17}O MRS provides specificity by measuring only nascent mitochondrial $H_2{}^{17}O$ generated from the reduction of $^{17}O_2$. Therefore, it allows more direct and straightforward quantification of the mitochondrial respiration rate.

Previously, *in vivo* cardiac ^{17}O MRS/I studies have been reported which used either ^{17}O spectroscopic imaging for the direct detection of $H_2{}^{17}O$ (19), or indirect $H_2{}^{17}O$ detection via T_2 or $T_{1\rho}$ imaging (17,20). These studies demonstrated the feasibility of ^{17}O MRS/I for the quantification of the oxygen consumption rate in the heart. Here, we report the first ^{17}O MRS study on isolated perfused hearts. The use of an isolated perfused heart model eliminates the complications arising from the systemic circulation. It also allows better control of the physiological conditions and cardiac function. Such simplification can provide valuable benchmarks for future *in vivo* development.

Oxygen demand in the heart is primarily governed by its energy expenditure, i.e. workload. In the current study, the cardiac workload was altered by changing the Ca^{2+} concentration in the perfusate. A higher Ca^{2+} level caused a higher Ca^{2+} influx during contraction, leading to increased contractile force, but similar HR (Fig. 3). As a result, RPP was increased significantly in the HCA group. This increase in cardiac workload must be fueled by a corresponding increase in ATP generation. The ^{17}O MRS-observed increase in the kinetics of $H_2{}^{17}O$ generation reflected an increase in the mitochondrial respiration rate (Fig. 5), which is coupled to ATP synthesis under normal physiological conditions. In addition, the increase in the measured oxygen consumption rate provides further evidence of increased mitochondrial respiration (Fig. 4). This increase in metabolic activity allowed the balance

between ATP production and utilization to be maintained, as demonstrated by the ^{31}P MRS data (Fig.4).

Several studies have reported a linear relationship between oxygen consumption and workload (RPP) both *in vivo* and *ex vivo* (24,25). In the current study, the ratios of oxygen consumption between normal- and high-workload hearts were 1.82 and 1.92, measured by the analytical method and by ^{17}O MRS, respectively. The ratio of RPP between the two groups was 1.68 at the beginning of ^{17}O MRS acquisition and decreased to 1.35 towards the end. These results were similar to those reported by Yamamoto *et al.*(24) in an *in vivo* PET ^{15}O study on dogs. The authors reported ratios of 2.10 and 2.27 between isoproterenol-stimulated and baseline workload for RPP and MVO_2, respectively. The decrease in RPP ratio at the end of ^{17}O MRS acquisition may be caused by reduced metabolic efficiency because of insufficient re-oxygenation.

A major challenge in performing ^{17}O MRS studies in isolated hearts is the dilution of mitochondrial $H_2{}^{17}O$, in combination with the low sensitivity of ^{17}O MRS. This problem is less pronounced for *in vivo* studies as the total blood volume of a rat is less than 30 mL (26). In addition, the recirculation of metabolic $H_2{}^{17}O$ generated in other organs also contributes to the increase in ^{17}O signal acquired from the heart. By contrast, a perfusion system designed for MRS studies typically has a perfusion volume of more than 1 L. The large system volume can quickly dilute the nascent $H_2{}^{17}O$ generated in the mitochondria, rendering the signal increase undetectable. To improve the detection sensitivity, we switched to a smaller system volume of only 26 mL on introduction of ^{17}O-oxygenated perfusate. As a result, there were elevated levels of metabolic byproducts which may have an impact on the myocardial contractility. Among these metabolic byproducts is adenosine, which is a potent agent that produces a negative inotropic effect in both atrial and ventricular myocardium. To counteract the adenosine effect, theophylline, an adenosine antagonist, was employed to maintain normal ventricular function (27). Although there was still a trend of functional decrease, especially at high Ca^{2+} concentration, it was much less pronounced relative to perfusion without theophylline (data not shown).

To date, the MR detection of $H_2{}^{17}O$ has used two approaches, i.e. direct detection via ^{17}O MRS/I and indirect detection via ^{1}H MRI. The indirect approach relies on ^{17}O-induced changes in $T_{1\rho}$ or T_2. It can be readily implemented on a clinical scanner at low field. However, the absolute quantification of $H_2{}^{17}O$ concentration can be complicated because of the sensitivity of the proton relaxation time to changes in the biochemical and physiological milieu in tissues (28). In addition, the relatively high radiofrequency power required by ^{1}H spin-locking or ^{17}O decoupling can also limit its application to patients. In comparison, direct ^{17}O MRS/I has the advantage of being highly specificfor $H_2{}^{17}O$, especially at high magnetic field with significantly improved signal-to-noise ratio. Potentially, the natural abundance $H_2{}^{17}O$ signal can be used as an internal reference. However, because of the high water content in blood, changes in blood volume can lead to changes in the natural abundance $H_2{}^{17}O$ signal. We observed a >15% decrease in the natural abundance $H_2{}^{17}O$ signal when the perfusion pressure was reduced from 100 cm water column to 40 cm (data not shown). Such changes are probably caused by changes in vascular volume in response to perfusion pressure. Therefore, care must be taken when using the natural abundance $H_2{}^{17}O$ signal as a reference.

The current study has several limitations. First, a simplified compartment model was used to estimate the oxygen consumption rate. Because of our inability to measure the ^{17}O enrichment of the oxygen gas in the perfusate, current parameter estimation can only determine the metabolic rate of ^{17}O-labeled oxygen rather than the total oxygen consumption. Although model-determined $^{17}O_2$ consumption rates showed agreement with

experimentally measured MVO_2, the robustness of parameter estimation needs to be evaluated further. As the acquired ^{17}O signal comprised signals from both the heart and the perfusate in the MR tube, further validation of the model can be accomplished by measuring $H_2{}^{17}O$ in the heart and perfusate separately, either by MRI or with the assistance of other methods, such as mass spectrometry. In addition, given the short T_1 relaxation time (~5 ms) of ^{17}O, a shorter TR (~20 ms) can be used to improve the signal-to-noise ratio and temporal resolution of data acquisition, which will lead to improved parameter estimation.

Second, the current system was not optimized for longer time durations. The need for cost-savings and for the maximization of detection sensitivity has rendered it a challenge to maintain normal cardiac function during the time course of data acquisition. The decrease in ventricular function at high Ca^{2+} concentration may have been caused by hypoxia because of increased oxygen extraction at high workload and the inability of the system to maintain adequate re-oxygenation of the perfusate. As a result, the model-fitted $MV^{17}O_2$ reflects an average index of oxygen consumption during the time course of ^{17}O MRS acquisition. Nevertheless, these preliminary results demonstrate that ^{17}O MRS on an isolated perfused heart can provide a valuable approach for the validation of *in vivo* ^{17}O studies, as well as for the investigation of metabolic regulation in cardiac mitochondria under various pathophysiological conditions.

In conclusion, this study has demonstrated the feasibility and sensitivity of dynamic ^{17}O MRS for the detection of the altered metabolic rate associated with changes in cardiac workload. In combination with kinetic modeling, this high-temporal-resolution MRS method allows the quantification of the mitochondrial respiration rate in beating hearts. When combined with ^{31}P magnetization transfer for the quantification of ATP synthesis, ^{17}O MRS provides a unique opportunity to evaluate the coupling between mitochondrial oxidation and phosphorylation in a nondestructive manner.

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Abbreviations used

HCA	high calcium concentration group
HR	heart rate
LVDP	left ventricular developed pressure
MVO_2	myocardial oxygen consumption rate
$MV^{17}O_2$	myocardial $^{17}O_2$ consumption rate
NCA	normal calcium concentration group
PCr	phosphocreatine
PET	positron emission tomography
RPP	rate–pressure product

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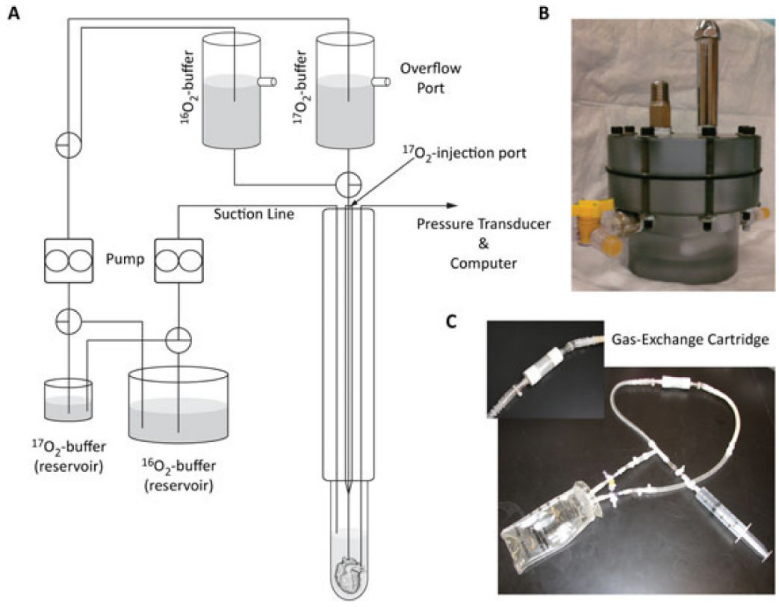

Figure 1.

(A) Schematics of the perfusion system. Two gravitational reservoirs with overflow ports supply the heart with $^{16}O_2$- and $^{17}O_2$-oxygenated buffer. Re-oxygenation of the buffer during ^{17}O MRS acquisition is accomplished via $^{17}O_2$-filled silicone tubing embedded in the water-jacketed perfusion column. Pressurized chamber (B) and closed-loop gas-exchange system (C) for efficient oxygenation of the perfusate with $^{17}O_2$.

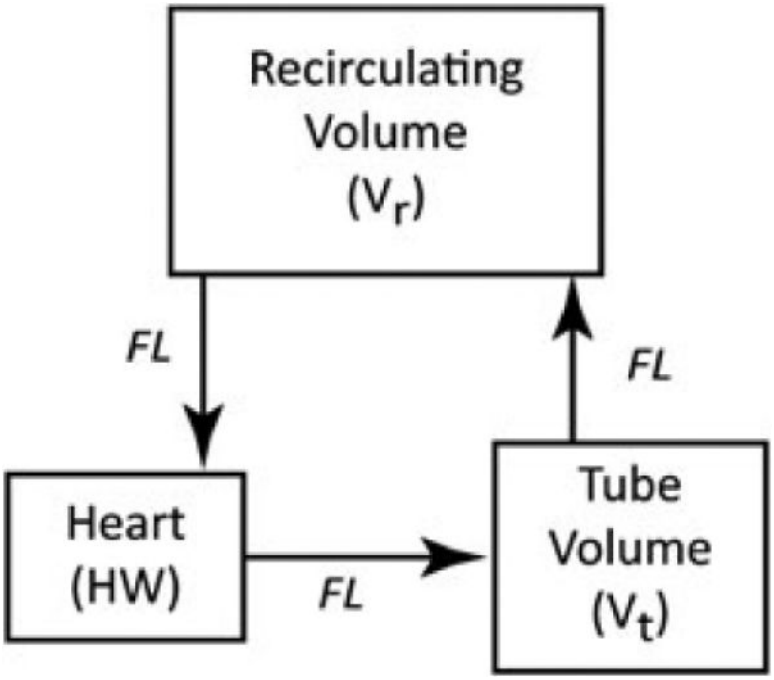

Figure 2.

Three-compartment model of $H_2^{17}O$ generation and recirculation for the estimation of mitochondrial respiration from ^{17}O MRS data. FL, coronary flow rate; HW, wet heart weight.

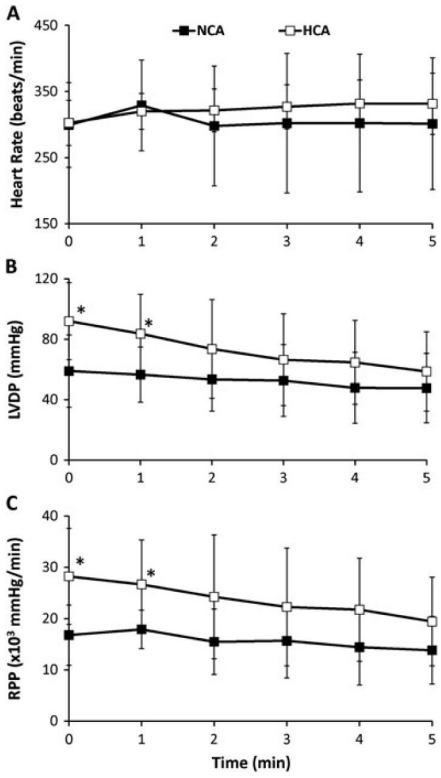

Figure 3.

Contractile function during $^{17}O_2$-enriched buffer perfusion. (A) Heart rate. (B) Left ventricular developed pressure (LVDP). (C) Rate–pressure product (RPP). *p<0.05. HCA, high calcium concentration group; NCA, normal calcium concentration group.

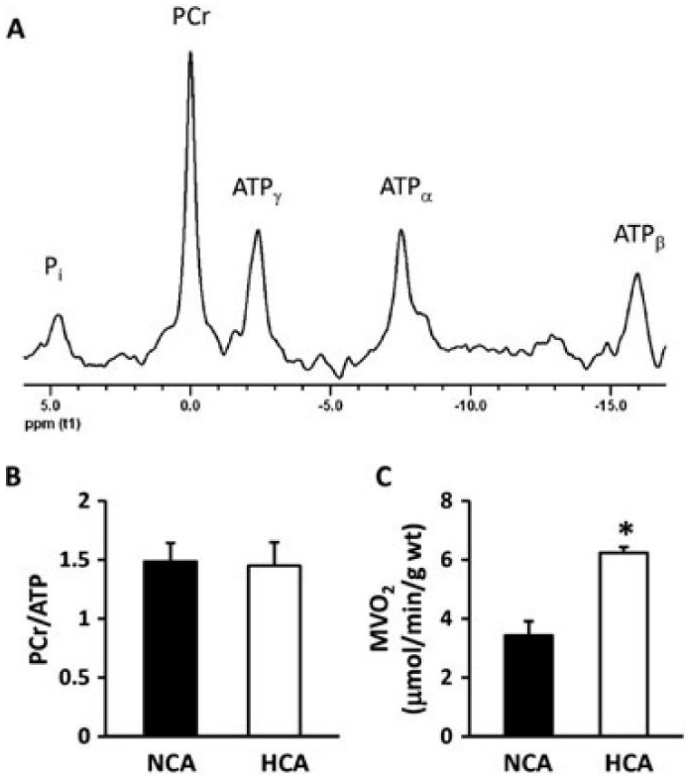

Figure 4.

(A) Representative ^{31}P NMR spectrum. (B) Phosphocreatine (PCr) to ATP ratio. (C) Measured oxygen consumption rate (MVO_2). HCA, high calcium concentration group; NCA, normal calcium concentration group.

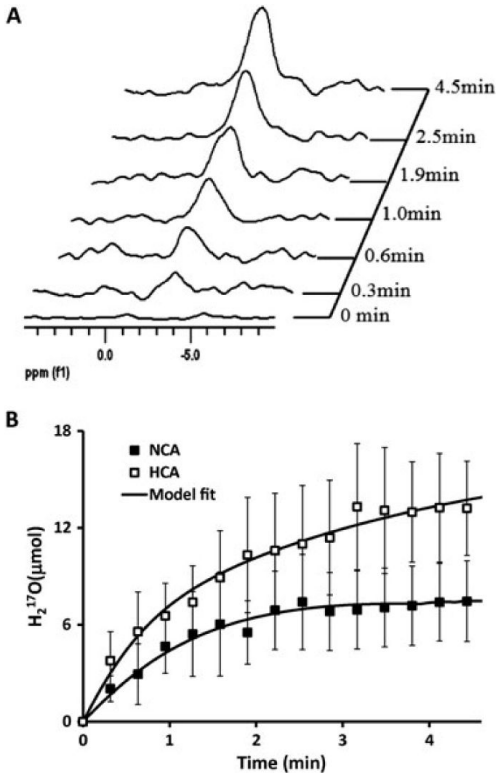

Figure 5.

Production of mitochondrial $H_2^{17}O$ measured by ^{17}O MRS. (A) Representative dynamic ^{17}O spectra. (B) Average time course of $H_2^{17}O$ accumulation and results of kinetic analysis. HCA, high calcium concentration group; NCA, normal calcium concentration group.