Myocardial FFA Metabolism During Rest and Atrial Pacing in Humans

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Running Title: Myocardial Substrate Utilization

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Abstract

There is limited in vivo data in humans evaluating myocardial fat utilization during increased heart work. This study was done to determine myocardial free fatty acid (FFA) metabolism during rest and atrial pacing, which increases cardiac work without changing arterial substrate concentration. We studied 7 healthy men and women (age=49.7±3.9 yr; BMI=23.4±1.1 kg/m², VO₂max=35.5±3.0 ml/kg/min, ejection fraction=68±3%). After 3 days of dietary control, coronary sinus, femoral arterial and venous, and peripheral venous catheters were placed. Subjects received ¹³C-bicarbonate, followed by a continuous infusion of [1-¹³C] palmitate through the end of the study. Arterial and coronary sinus blood sampling and measurements of resting coronary sinus blood flow were made during rest and atrial pacing to 120 bpm. Myocardial MVO₂ increased (p<0.05) from rest to atrial pacing. Coronary sinus FFA concentration was significantly lower than arterial through rest and atrial pacing (p=0.007). Isotopically measured myocardial palmitate uptake increased significantly from rest to atrial pacing (p=0.03). Approximately one third of palmitate delivery was extracted by the myocardium during rest and atrial pacing. Myocardial V¹³CO₂ production and palmitate oxidation increased significantly from rest (p<0.01) to atrial pacing. Net glycerol balance was significantly greater than zero during rest (p=0.04), but not different than zero during atrial pacing (p=0.13). These data suggest myocardial lipid uptake and oxidation increase with greater heart work during atrial pacing, with a similar relative proportion of fat oxidation to total myocardial energy expenditure.

Keywords: atrial pacing, stable isotopes, myocardial substrate utilization
Introduction

Myocardial substrate utilization can influence myocardial energy efficiency (8), tissue survival during ischemia (19), and cardiac efficiency (30). Additionally, alteration of myocardial substrate utilization has been reported and may be implicated in decreased ventricular performance in heart failure (2, 36), obesity (30), type 1 (4) and type 2 diabetes (35). Therefore, understanding myocardial substrate utilization is important to elucidate potential derangements in myocardial metabolic control that may contribute to disease states.

Much of the knowledge on myocardial FFA utilization comes from studies on isolated heart preparations. It is well established that the majority of myocardial energy supply in the resting state comes from the oxidation of free fatty acids (6, 38) with arterial substrate concentration influencing myocardial uptake (32). Some data indicate the isolated working heart increases reliance on CHO derived fuels more than beta-oxidation (15), while others suggest increased reliance on circulating FFA with a decrease in glucose utilization (26). Others suggest increase reliance on both glucose and FFA oxidation during exercise compare to rest in catheterized dogs (25).

Data from in vivo human studies largely corroborate findings from work in the isolated heart. The human myocardium in the basal state relies mostly on FFA for fuel (18, 22). Similar to the isolated working heart, arterial substrate concentration plays a large role in dictating myocardial substrate utilization (18, 22). During increased heart work induced by whole body exercise there is a shift in myocardial substrate utilization. Most studies investigating myocardial FFA utilization increased heart work using whole body exercise, which results in changes in arterial substrate concentration (18, 22, 44). Substrate concentration, especially FFA, influences myocardial substrate utilization (18, 22, 44). Therefore, these studies do not allow investigators to determine myocardial substrate preferences independent of changing arterial substrate supply during increased heart work. Atrial pacing increases heart work independent of changes in arterial substrate concentration, and may be used to study metabolic demands of the working heart in vivo. Several investigators reported data using atrial pacing to increase heart work in normal humans (3, 9, 17, 34). However, only 1 of these publications used
isotopic tracers to accurately determine simultaneous myocardial substrate uptake, release, and oxidation (17). As a result, there are limited data available on myocardial FFA utilization during increased heart work without changes in arterial substrate supply induced by peripheral muscular work.

This study was done to determine myocardial palmitate uptake and oxidation during rest and increased heart work performed without a change in arterial substrate concentration. An increase in cardiac work was achieved with atrial pacing instead of whole body exercise or pharmacologic intervention. This allowed us to measure myocardial substrate utilization during increased cardiac work without the influence of alterations in arterial substrate supply. We hypothesized that palmitate uptake and oxidation would increase during atrial pacing on an absolute basis, with no significant changes in uptake relative to total myocardial work.
Methods

Subjects

Seven healthy men and women were recruited for this study. Subjects gave informed consent, and were excluded if they: smoked, had diabetes, hyperlipidemia, liver or kidney disease, or were taking medications which affect glucose or lipid metabolism, and/or regularly engage in vigorous exercise (> 2 hours/week). Subjects were excluded if they had a body mass index < 20 kg/m² or > 30 kg/m². Women were taking oral contraceptives and were studied during the mid-follicular phase of their menstrual cycle to minimize effects of menstrual cycle phase on substrate metabolism. This study was approved by the Colorado Multiple Institution Review Board at the University of Colorado Denver.

General Experimental Design

After preliminary testing, subjects participated in one metabolic trial. Diet was controlled for 3 days prior to the study via the metabolic kitchen on the General Clinical Research Center (GCRC). During the metabolic trial, a right heart catheterization was combined with the measurement of palmitate uptake and oxidation at rest and under conditions of increased cardiac work with atrial pacing.

Preliminary Testing

Subjects reported to the GCRC for the screening procedures following a 12 hour overnight fast. They were given a health and physical exam, following by a fasting blood draw. Body composition was determined using Dual Energy X-ray Absorptiometry (DEXA) analysis (Lunar DPX-IQ, Lunar Corporation, Madison, WI). Resting metabolic rate (RMR) was measured using indirect calorimetry via a metabolic cart system (Sensormedics 2900, Sensormedics, Yorba Linda, CA). Subjects rested supine for 30 minutes, then a ventilated canopy was placed over their head and measurements continue for 15-20 min. Metabolic rate was calculated from the flow rate of expired air, in conjunction with measuring differences in the oxygen (O₂) and carbon dioxide (CO₂) concentrations using standard equations (43). Subjects were included in the study if they had normal fasting glucose, defined by the American Diabetes Association
as fasting serum glucose concentration <100 mg/dl. On a separate screening day, subjects arrived on the GCRC and completed a VO$_2$max test using the Balke treadmill test. A resting echocardiogram was also performed to exclude subjects with myocardial or valvular disease and left ventricular hypertrophy (Sonos 5500, Royal Philips Electronics, Netherlands) and to verify a normal left ventricular ejection fraction (LVEF).

**Diet Control**

Three day diet records were analyzed for each subject to determine macronutrient and energy content of their diet. Dieticians used the RMR with an activity factor of 1.4, combined with each subject's typical dietary intake, to make a 3 day diet which was provided to each subject prior to the metabolic study. The diet followed the American Heart Association recommendations for macronutrient composition (30% fat, 15% protein, and 55% carbohydrate). Subjects were asked to maintain normal daily physical activity during the period of dietary control, and refrain from planned exercise for 3 days prior to the metabolic study. Pre-study nutritional control ensured subjects were close to energy balance, and therefore, differences in energy status and glycogen stores prior to testing were controlled for between subjects. Subjects spent the evening before the metabolic study on the General Clinical Research Center (GCRC) at University of Colorado Hospital to ensure compliance with the overnight fast.

**Metabolic Study**

Subjects were taken to the cardiac catheterization laboratory on the morning of the metabolic study after an overnight fast where catheters were placed into the right internal jugular vein for coronary sinus access (6 Fr, Cordis Multipurpose A-2, Johnson and Johnson Corporation, Piscataway, NJ) and Doppler flow (0.014 Doppler Flow Wire, size 300, Cardiometrics Inc, Mountain View, CA), femoral artery for arterial blood (Cordis 4 Fr arterial sheath, Johnson and Johnson Corp, Piscataway, NJ), right femoral vein for pulmonary artery access (8Fr venous sheath, Boston Scientific, Natick, MA and 7.5 Fr VIP
thermodilution pulmonary artery catheter, Baxter-Edwards, Dearfield, IL), right femoral vein for atrial pacer wire (6 Fr venous sheath, Boston Scientific and Cordis 6Fr atrial pacing wire, Johnson and Johnson), and a peripheral venous catheter for isotope infusion using standard catheterization techniques. Heparin was administered at 15 units/hr to maintain patency of the arterial line during the 3-hour study. After insertion of the coronary sinus catheter, an angiogram was performed using 30 cc of an nonionic contrast agent to measure the diameter of the coronary sinus and to determine the position of the multipurpose catheter for both blood sampling and positioning of the Doppler flow wire. This angiogram was performed prior to the initiation of the isotope infusion and there was an interval of at least 60 minutes between the angiogram and the measurement of coronary sinus flow velocity with the Doppler wire. Initial cardiac hemodynamic measurements were made with a pulmonary artery catheter with the determination of cardiac output by thermodilution and Fick methods and the recording of right atrial, pulmonary artery and pulmonary capillary wedge pressures. Systemic arterial pressure was determined from the femoral arterial sheath. Determination of rate-pressure product, mean arterial pressure, and systemic and pulmonary vascular resistances were calculated by standard methods.

Following these measurements, blood was withdrawn from the arterial and coronary sinus catheters for background palmitate and blood $^{13}$CO$_2$ enrichment. Expired air was collected for background breath$^{13}$CO$_2$ enrichment. Then through the peripheral venous catheter a priming bolus of [1-14C]acetate, NaH$^{13}$CO$_3$, and NaH$^{14}$CO$_3$ was initiated containing 1.5 µmol/kg of NaH$^{13}$CO$_3$ and 10nCi/kg of NaH$^{14}$CO$_3$. A continuous infusion was started of [1-$^{13}$C] palmitate at 0.012 mg/kg/min and [1-$^{14}$C]acetate at 0.2 nCi/kg/min and continued until the end of the study. We followed methods for the acetate correction factor which have been previously published (33). Additionally, we infused [3,3,3]$^2$H-lactate and [6,6]$^2$H-glucose to measure myocardial glucose and lactate exchange which are reported separately. Measurements of resting coronary sinus blood flow were determined from Doppler flow velocities in the coronary sinus and hemodynamic measurements were made with the pulmonary artery catheter at minutes 30 and 45 of the infusion. Indirect calorimetry was performed for 15 minutes starting at 50 minutes of the infusion. Simultaneous arterial and coronary sinus blood sampling was performed at 60,
70, 80, and 90 minutes of rest. Atrial pacing was then started at a heart rate of 120 bpm in an attempt to standardize myocardial oxygen consumption (MVO$_2$). Measurements of paced coronary sinus blood flow, cardiac output by the thermodilution and Fick methods and intracardiac filling pressures were made at minutes 15 and 55 of pacing. Simultaneous arterial and coronary sinus blood samples were obtained following 20, 30, 40 and 50 minutes of atrial pacing. Blood was obtained for glucose, lactate, FFA, glycerol, plasma catecholamines, insulin, glucagon, isotopic enrichment of palmitate, arterial blood gasses, hemoglobin and hematocrit, and O$_2$ content. Another angiogram of the coronary sinus was performed with intravenous contrast during atrial pacing after completion of all coronary sinus Doppler flow velocity measurements and blood sampling. At the completion of the study, all catheters were removed and the subjects returned to the GCRC for 7 hours of observation before discharge. Three of the subjects were tested when the [1-$^{14}$C] acetate was not available for infusion, therefore the NaH$^{14}$CO$_3$ prime was also not given. Mean values for acetate correction were used to estimate the acetate correction factor in these 3 subjects.

**Metabolite and hormone analyses**

Insulin (42) (Clinical Assays Gamma Coat RIA) and glucagon (1) were determined by radioimmunoassay. Catecholamines were determined by high performance liquid chromatography with electrochemical detection (10). Standard enzymatic assays were used to measure lactate (Sigma Kit #826), glycerol (Boehringer Mannheim Diagnostics), and FFA (NEFA Kit, Wako).

**Gas chromatography/mass spectroscopy methods**

Metabolite isotopic enrichment were measured using gas chromatography-mass spectrometry (GCMS; GC model 5890 series II and MS model 5989A, Hewlett-Packard). The [1-$^{13}$C]palmitate isotopic enrichments were measured by derivatization to the fatty acid methyl ester to allow easy volatilization by gas chromatography. The instrumentation measured total FFA concentration by monitoring oleate, palmitate, stearate, linoleate, and palmitoleate (98% of FFA content) and isotopic enrichment of palmitate
by gas chromatography-mass spectrometry. Measurement of $^{13}\text{CO}_2$ enrichments was determined by isotope ratio mass spectroscopy (IRMS). Measurement of breath $^{14}\text{CO}_2$ specific activity was performed via liquid scintillation counting (Beckman LS 6000TA).

Calculations

The rates of appearance (Ra) and disappearance (Rd) of palmitate were calculated using equations defined by Steele, modified for stable isotopes (45).

$$Ra (\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \left( F - V \left[ \frac{(C_1+C_2)}{2} \left( \frac{IE_2-IE_1}{(t_2-t_1)} \right) \right] \right) / \left( \frac{(IE_2+IE_1)}{2} \right)$$

$$Rd (\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = Ra - V \left[ \frac{(C_2-C_1)}{(t_2-t_1)} \right]$$

Myocardial VO$_2$ (ml/min) = arterial – coronary sinus O$_2$ content x coronary sinus blood flow

Coronary Sinus O$_2$ content (ml/L) = 1.34 x [coronary sinus Hb] x O$_2$ saturation (%)

Coronary Sinus blood flow (ml/min) = cross sectional area (CSA) x mean flow velocity = coronary sinus diameter$^2$ x 0.785 x (mean flow velocity) x heart rate. The mean flow velocity was determined by the area under the curve of the CS Doppler flow velocity.

Myocardial Fractional Extraction (%) =

$$\left( \frac{Art.Ep \times [Art]}{[CS] \times (Art \text{ Hct}/CS \text{ Hct})} \right) \times 100$$

Myocardial substrate uptake =

myocardial fractional extraction x arterial concentration x CS blood flow

Whole body $V^{13}\text{CO}_2$ production (mmol/kg/min) = $[\text{ECO}_2 \times \text{VCO}_2]/\text{FAR}/\text{body weight}$

Myocardial $V^{13}\text{CO}_2$ production (mmol/kg/min) =

$$[(C_{csCO}_2 \times ^{13}\text{CO}_2) - (C_{aCO}_2 \times ^{13}\text{CO}_2a)] \times \text{CS blood flow/body weight}$$

Whole body palmitate oxidation (mg · kg$^{-1}$ · min$^{-1}$) = $[\text{ECO}_2 \times \text{VCO}_2]/ E p \times \text{FAR}/\text{body weight}$

Myocardial palmitate oxidation (mg · kg$^{-1}$ · min$^{-1}$) = $[\text{EBCO}_2 \times \text{MVCO}_2]/ E p/\text{body weight}$

Myocardial palmitate release ($\mu$mol/min) =

Tracer measured palmitate uptake – arterio-coronary sinus palmitate difference

Fractional acetate recovery (FAR): $V^{14}\text{CO}_2$ production (dpm/min)/ acetate infusion rate (dpm/min)
where $F$ represents isotope infusion rate, $IE_1$ and $IE_2$ are isotopic enrichments at sampling time points 1 ($t_1$) and 2 ($t_2$), respectively. $C_1$ and $C_2$ are metabolite concentrations at $t_1$ and $t_2$; $V$ is the estimated volume distribution of palmitate (40 ml/kg) which has been previously published (14). SACO$_2$ is the specific activity of CO$_2$, ECO$_2$ is the isotopic enrichment of breath CO$_2$, EBCO$_2$ is the isotopic enrichment of blood CO$_2$, VCO$_2$ is whole body breath CO$_2$ production, MVCO$_2$ is myocardial CO$_2$ production, $E_p$ is the plasma palmitate enrichment, $C_{csCO_2}$ is the coronary sinus CO$_2$ content, $^{13}$CO$_{2a}$ is the enrichment of coronary sinus CO$_2$, $C_{aCO_2}$ is the arterial CO$_2$ content, $^{13}$CO$_{2a}$ is the enrichment of arterial CO$_2$, FAR is the fractional acetate recovery. All isotopic enrichments were corrected for background enrichments from blood samples taken before isotope infusion.

Myocardial exchange of glycerol and triglyceride were determined by the arterio-coronary sinus difference corrected for differences in hematocrit x coronary blood flow. Blood PCO$_2$, PO$_2$, pH, and hemoglobin were measured on both arterial and venous samples and used in the calculations by Douglas et al. for determination of blood CO$_2$ content (11). CO$_2$ solubility and apparent dissociation constant were estimated from the equations of Kelman (20). Hemoglobin concentration, saturation and content of O$_2$ were directly measured using the OSM-3 Hemocytometer (Radiometer, Copenhagen). Blood O$_2$ content was calculated with hemoglobin concentration, and saturation was determined from an equation from Nunn (29), as described previously (5).

Myocardial respiratory quotient was calculated from the ratio of coronary sinus – arterial CO$_2$ difference and arterial - coronary sinus O$_2$ difference. When RQ was below 0.70 or above 1.0, a theoretical limit was assumed. Myocardial oxygen extraction ratio (OER, %) was used to calculate the relative proportion of tracer measured FFA uptake to myocardial oxygen consumption, as described by Lassers et al. (21).

Statistics

Data are presented as mean ± SEM. Differences between rest and atrial pacing measures were analyzed using a repeated measures ANOVA. Differences in arterial and coronary sinus metabolite concentrations were analyzed using paired Student’s t-test (SPSS, Chicago, IL). Linear regression
analyses were performed to correlate arterial substrate concentration and myocardial uptake or release. An alpha level of 0.05 was used throughout.
Results

Subject Characteristics

Anthropometric data for subjects are reported in Table 1. Subjects were weight stable in the 6 months prior to participation in this research study. The men and women in this study were in the healthy BMI range with a mean age of 49.7 ± 3.9 yrs. VO_{2}\text{max} was average for this age range, and left ventricular size and ejection fraction were also normal as determined by echocardiography.

Hemodynamics

Cardiac output was 5.3±0.2 L/min at rest and didn’t change significantly with atrial pacing (Table 2). Heart rate increased significantly from 70±4 to 108±5 bpm from rest to atrial pacing. Coronary sinus oxygen saturation (31±1 %) and arterial-coronary sinus oxygen content different (4.1±0.4 vol%) also did not change significantly from rest to atrial pacing. There were no significant changes in cardiac filling pressures, systemic BP, and vascular resistance during atrial pacing compared to rest.

Indirect Calorimetry

Whole body oxygen consumption, CO_{2} production, and respiratory exchange ratio (RER) were not significantly different from rest to atrial pacing. Myocardial oxygen consumption increased significantly from rest to atrial pacing (p<0.05). Myocardial RQ was also not significantly different during rest (0.83 ± 0.03) compared to atrial pacing (0.78 ± 0.04).

Substrate Concentration

Atrial pacing did not significantly change glycerol, epinephrine, norepinephrine, and insulin concentrations compared to rest (Table 2). However, triglyceride and glucagon concentrations increased during atrial pacing compared to rest (p<0.05).
Cardiac Hemodynamic Measurements:
Atrial pacing increased coronary sinus blood flow from rest to atrial pacing (p<0.05). There was an expected significant increase in heart rate with atrial pacing. Although a target heart rate of 120 bpm was sought, three subjects developed intermittent AV nodal Wenkebach resulting in a slight decrease in mean pacing heart rate for the group. There were no significant changes with atrial pacing in systemic arterial pressure, right atrial and pulmonary capillary wedge pressure, and cardiac output. Since heart rate increased without a change in cardiac output, mean stroke volume decreased from 77±7 ml at rest to 53±7 ml with atrial pacing (p <0.05). Systemic vascular resistance was unchanged but there was a 61% increase in the rate-pressure product (RPP) with atrial pacing. The RPP, an indicator of myocardial O₂ consumption, was 9587±640 mmHg-bpm at rest and 15,419±1131 mmHg-bpm with pacing (p < 0.005). There was a slight decrease in systolic pulmonary artery pressure with pacing. There were no significant changes in mixed venous O₂ saturation, arteriovenous O₂ content difference, coronary sinus O₂ saturation, and arterial-coronary sinus O₂ content difference with pacing.

Tracer Kinetics
Arterial palmitate enrichment was stable over time during rest and atrial pacing (Figure 1). Coronary sinus palmitate enrichment was significantly decreased compared to arterial palmitate enrichment at rest (p=0.01) but was not significantly different during atrial pacing. Whole body palmitate appearance did not change significantly during the study with Ra at 2.9±0.6 µmol/kg/min at rest and 2.8±0.3 during atrial pacing.

Palmitate Metabolism
Arterial and coronary sinus free fatty acid (FFA) and palmitate concentrations did not change significantly during rest and atrial pacing (Figure 2A). Throughout rest and atrial pacing, coronary sinus FFA and palmitate concentrations were significantly lower than arterial concentration (p<0.001). Arterial-coronary sinus FFA concentration difference did not change significantly during atrial pacing compared to
rest (Figure 2B). Figure 2C shows isotopically measured myocardial palmitate uptake which increased significantly from rest to atrial pacing (p=0.04). The myocardium released palmitate at rest, which did not change significantly during atrial pacing (Figure 2D). Approximately one third of palmitate delivery was extracted by the myocardium at rest, which did not change significantly during atrial pacing (Figure 2E). The heart accounted for a significantly greater proportion of whole body palmitate disappearance during atrial pacing compared to rest (Figure 2F, p=0.04). The relative proportion of tracer measured FFA uptake to myocardial oxygen consumption (OER) was 48±11 % at rest and 43±11 % during atrial pacing.

**Palmitate Oxidation**

Whole body and myocardial acetate recovery factor was determined in 4 of the 7 subjects in this study. Average acetate recovery factor data were used for the subjects in which an individual recovery factor was not determined. The acetate correction factor used was 72.2% at rest, and 87.4% during atrial pacing. Whole body V^{13}CO_2 production was stable at rest and did not change significantly during atrial pacing (Figure 3). Approximately 100% of the 1-^{14}C acetate label was recovered across the heart during rest and atrial pacing, so tissue label recovery was not applied. Myocardial V^{13}CO_2 production was significantly lower than whole body values during rest (Figure 3, p=0.0001) and atrial pacing (p=0.0002). Myocardial V^{13}CO_2 production was stable at rest, and increased significantly (p=0.04) during atrial pacing. Similarly, whole body palmitate oxidation was stable at rest and did not change significantly during atrial pacing (Figure 4). Myocardial palmitate oxidation was significantly lower than whole body values throughout rest (p=0.004) and atrial pacing (p=0.002). Myocardial palmitate oxidation was stable at rest and increased significantly during atrial pacing (p=0.05).

**Glycerol and Triglyceride Net Balance**

Net glycerol release was significantly greater than zero during rest (p=0.04), and was not significantly different from zero during atrial pacing (p=0.13). Net glycerol balance was non-significantly decreased during atrial pacing (Figure 5, p=0.08). These data indicate the myocardium was taking up glycerol on a
net basis during rest, but not during atrial pacing. There was no significant net triglyceride exchange during rest (p=0.43), and no significant change in net triglyceride balance across the heart during atrial pacing compared to rest (p=0.36).
Discussion

We performed this study to test the hypothesis that myocardial palmitate uptake and oxidation would increase from rest to atrial pacing on an absolute basis, with similar relative proportion to total substrate uptake by the heart. These data support our hypothesis and suggest that myocardial lipid uptake and oxidation increase with an increase in heart work during atrial pacing, with a similar relative proportion of fat oxidation to total myocardial energy expenditure. The similar proportion of energy expenditure from lipid likely reflects unchanged concentration of substrates delivered to the myocardium.

There were no changes in whole body oxygen consumption or RER during the period of atrial pacing compared to rest. Therefore, atrial pacing was successful in increasing heart work only, without a change in whole body energy expenditure, substrate utilization, or arterial concentration of glucose, lactate, and FFA. Furthermore, systemic sympathetic nervous system activity, as determined by plasma catecholamine concentrations, was not significantly different during atrial pacing.

Myocardial RQ and oxygen extraction ratio data suggest the relative proportion of lipid to total energy expenditure was not significantly different during atrial pacing compared to rest. These data are similar to data published by Camici et al. who reported unchanged myocardial RQ during atrial pacing at a similar heart rate (9). In their study, myocardial RQ increased only during maximal atrial pacing at a heart rate of 159 bpm despite similar arterial substrate concentration. Therefore, changes in myocardial substrate use during atrial pacing may be intensity dependent. However, others reported myocardial RQ was not significantly different at the end of 2 hours of whole body exercise compared to rest with alterations in substrate concentration (18, 22). Therefore, during moderate intensity exercise or atrial pacing, RQ data from this study and others suggest that the relative proportions of carbohydrate and fat to total myocardial energy expenditure are similar compared to rest. However, the sources of carbohydrate and lipids may be different with increased contributions from lactate, and potentially from intramyocellular triglyceride, which will be discussed below.

Myocardial palmitate extraction in the current study was similar to others reporting 40-74% isotopically measured fractional extraction in resting human myocardium (18, 40, 44). Fractional
extraction of FFA has been reported to be unchanged during atrial pacing (34), and to either not change (22), or decrease during whole body exercise (18, 22). Therefore, our data showing similar myocardial extraction during pacing compared to rest are consistent with the literature. Unlike others (22), we did not find a significant relationship between arterial FFA concentration and FFA uptake or A-CS FFA difference during either rest or atrial pacing. This is likely due to the small variation in fasting FFA concentration in the current study.

As during rest (7, 44), myocardial substrate utilization during whole body exercise in humans is heavily influenced by arterial substrate concentration (22, 24). Lassers et al. found that changes in myocardial FFA utilization during 2 hours of submaximal exercise were directly proportional to alterations in FFA concentration (22). They infused nicotinic acid to decrease FFA concentration and found a proportional decrease in FFA uptake along with increased myocardial carbohydrate oxidation. Similarly, Kaijser et al. reported that the relative proportions of myocardial substrate utilization changed during 2 hours of cycle ergometry at 50% of VO2max relative to the changes in substrate concentration (18). At the beginning of exercise lactate concentration increased, and FFA concentration decreased which were reflected in their myocardial uptake. By the end of the 2 hours of exercise, glucose concentration had decreased significantly, along with myocardial glucose uptake. Arterial FFA and lactate concentrations were increased at the end of exercise relative to rest, while the uptake of lactate was doubled and that of FFA determined isotopically decreased. Myocardial RQ was not significantly different during exercise compare to rest, suggesting that increased lactate uptake replaced the contribution of glucose, and decreased FFA uptake during exercise was compensated for by increased reliance on intramyocellular lipids (18). These data indicate that arterial substrate concentration plays an important role in myocardial substrate utilization during rest and increased heart work. Additionally, duration of increased heart work may influence substrate selection. Unlike our data, others (9, 27) reported unchanged plasma FFA, but increased glucose uptake during short duration atrial pacing (6-16 min) with similar arterial substrate concentrations. This comparison suggests that an increase in heart work may only stimulate FFA uptake after a sustained period of time, with short changes in heart work fueled by alterations in carbohydrate
utilization. Therefore, our data are consistent with the literature and reveal that when heart work is increased in isolation by atrial pacing, the absolute uptake of FFA increases proportionally to myocardial energy requirements.

Some (18, 22), but not all previous studies (9) have reported net glycerol release across the myocardium. Kaijser et al. found that net glycerol release was not different from zero at rest, but switched to significant net glycerol release during cycle ergometry at 50% of maximum workload (18). These authors suggested intramyocellular triglyceride was the source of glycerol release during exercise. Net glycerol release during atrial pacing was not reported by Camici et al., 1989 (9). This may be due to the different pacing strategy used by Camici which was only performed for 4 minutes per step, and for 16 total minutes. By comparison, we performed atrial pacing for 50 minutes at one pacing stress and were able to achieve steady state. Our data showed net myocardial glycerol uptake during rest, which was not significantly different than zero during atrial pacing. Therefore, our data are unclear as to the role of intramyocardial triglyceride utilization during increased heart work. If the myocardium increases reliance on intramyocellular triglyceride (IMTG) during prolonged increases in heart work, this would be opposite that reported for skeletal muscle IMTG, where decreased IMTG utilization has been reported in humans during prolonged exercise (41).

Another explanation for net glycerol uptake during rest, but not during atrial pacing, may be due to glycerol release from lipoprotein derived triglyceride hydrolysis during pacing. Triglyceride extraction by the heart has been reported during rest and atrial pacing (9). More recently, Nelson et al., 2007 reported tracer measured myocardial triglyceride utilization in 6 individuals, most of whom were diagnosed with coronary artery disease, that accounted for 17% of myocardial fatty acid uptake (28). So it is possible that changes in lipoprotein-mediated triglyceride uptake may explain variability in our net glycerol release data. Others reported increased net glycerol release across the myocardium during exercise at 50% VO\textsubscript{2}max compared to rest without a change in net triglyceride balance (18, 22). Our triglyceride balance data shown in Figure 5B argue against plasma triglyceride degradation contributing to myocardial net glycerol balance. Intramyocellular triglyceride degradation may therefore explain the
switch from net glycerol uptake to no net glycerol exchange during atrial pacing. Myocardial glycerol release has been reported in an isolated perfused rat heart model which did not contain triglycerides in the perfusion medium (12). This glycerol release likely originated from degradation of intramyocellular triglyceride. The authors also reported isotopically measured palmitate uptake was greater than net chemical uptake, providing evidence that myocardial FFA release may originate from intramyocellular triglyceride. Thus, the literature is unclear regarding degradation of intramyocardial triglyceride stores. Future studies are needed measuring myocardial isotopic glycerol and triglyceride exchange during rest and atrial pacing and/or exercise to determine if the relative importance of circulating triglyceride vs. intramyocardial triglyceride stores to energy expenditure.

There are several limitations to our study. During atrial pacing we observed a 118% increase in coronary sinus blood flow but no increase in cardiac output compared to the resting state. Despite the increase in heart rate, stroke volume decreased due to Starling forces, a concept that was demonstrated in early studies with atrial pacing in humans (31). Therefore, while atrial pacing increases myocardial oxygen consumption and does not influence arterial concentration, it is not a completely physiological model since increased heart work is performed without an increase in cardiac output. However, the lack of increased systemic blood flow and the lack of skeletal muscle and adipose tissue activity are responsible for the unchanged arterial FFA concentrations observed in this study. In a similar metabolically controlled model with increased cardiac work from either exercise or pharmacologic stimulation, the findings may be different. Further, we increased heart rate to approximately double myocardial oxygen consumption. It is unknown if our data would be similar with more dramatic increases in heart rate and thus, myocardial work. However, our data suggest that if arterial substrate concentrations are unchanged, the further increase in myocardial work would also be achieved without a significant change in the relative proportion of substrates to total myocardial energy demand. We infused a small amount of heparin to maintain patency of the arterial line in each study. The amount of heparin used to mobilize lipoprotein lipase, which would increase FFA concentration, is approximately 60 units/kg, or 4,400 units for the average subject in this study (16). We only infused 15 units per hour, and therefore
we did not observe changes in FFA concentration outside the normal increase with duration of fasting. Only half of our subjects received the [1-\textsuperscript{14}C]acetate infusion to quantify the whole body and myocardial acetate correction factor in this study. We applied mean values to the 3 subjects who did not receive this infusion. However, since we recovered all of the acetate across the heart, as has been reported before for working muscle (39), the lack of agreement between mean and individual acetate correction factors would only affect the rates of whole body fat oxidation in this study.

The determination of FFA substrate utilization by the human myocardium under a variety of physiologic conditions may have important clinical relevance to disease states such as heart failure, diabetes, and coronary artery disease. Plasma FFAs are elevated in patients with heart failure and there is enhanced total body FFA turnover and oxidation (23). There also appears to be enhanced myocardial beta-oxidation of FFA in patients with cardiomyopathy (13). However, there is a greater oxygen cost in the production of ATP when FFAs are used preferentially to glucose and lactate for energy metabolism. Despite this apparent increased myocardial oxygen cost with FFA utilization, pharmacologic approaches that decrease serum FFA levels have been associated with impaired cardiac performance in patients with heart failure (37). Thus the role of FFA utilization in patients with cardiac dysfunction has yet to be elucidated; however, it is clear that the pathophysiology of myocardial dysfunction is associated with abnormalities in myocardial lipid metabolism. Determination of the fate of FFAs in the normal human myocardium under a variety of physiologic conditions is an important step in pursuing the metabolic derangements and potential therapeutic approaches in cardiac muscle disease.

Conclusions

These data suggest that in normal healthy humans, increased heart work \textit{in vivo} without a change in arterial substrate concentration results in a similar proportion of FFA utilization relative to myocardial energy expenditure. The similar proportion of energy expenditure from fat sources during rest and atrial pacing likely reflects unchanged arterial concentration of substrates delivered to the myocardium.
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References


**Figure Legends**

Figure 1. Arterial and coronary sinus palmitate enrichment during rest and atrial pacing. Values are means ± SEM. ¥ = significantly different than arterial, p<0.05.

Figure 2. Arterial and coronary sinus palmitate concentration (A), arterial-coronary sinus palmitate difference (B), and myocardial net palmitate uptake (C), myocardial palmitate release (D) myocardial palmitate fractional extraction (E), and % of palmitate Rd from myocardial palmitate uptake (F) during rest and atrial pacing in men and women. Values are means ± SEM. ¥ = significantly different than arterial, p<0.05, § = significantly different than rest, p<0.05.

Figure 3. Whole body and myocardial V\(^{13}\)CO\(_2\) oxidation during rest and atrial pacing. Values are means ± SEM. § = significantly different than rest, p<0.05, # = significantly different than whole body, p<0.05.

Figure 4. Whole body and myocardial palmitate oxidation during rest and atrial pacing. Values are means ± SEM. § = significantly different than rest, p<0.05, # = significantly different than whole body, p<0.05.

Figure 5. Net myocardial glycerol (A) and triglyceride (B) balance during rest and atrial pacing. Values are means ± SEM.
Table 1. Subject demographics.

<table>
<thead>
<tr>
<th>Values</th>
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</thead>
<tbody>
<tr>
<td>N (M/W)</td>
<td>7 (5/2)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>49.7 ± 3.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.6 ± 4.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.9 ± 2.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4 ± 1.1</td>
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<tr>
<td>% Body Fat</td>
<td>28.7 ± 1.5</td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)</td>
<td>35.5 ± 3.0</td>
</tr>
<tr>
<td>LV Ejection Fraction (%)</td>
<td>68 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SEM.
Table 2. Hemodynamics and concentrations of hormones and substrates during rest and atrial pacing in men and women. Values are means ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Atrial Pacing</th>
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</thead>
<tbody>
<tr>
<td>Heart Rate (bpm)</td>
<td>70 ± 4</td>
<td>108 ± 5§</td>
</tr>
<tr>
<td>Cardiac Output (L/min)</td>
<td>5.3 ± 0.25</td>
<td>5.6 ± 0.44</td>
</tr>
<tr>
<td>(Thermodilution)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary Sinus blood flow (ml/min)</td>
<td>196±34</td>
<td>430±102§</td>
</tr>
<tr>
<td>MVO₂ (ml/min)</td>
<td>24±2</td>
<td>65±18§</td>
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<tr>
<td>Glucose (mg/dl)</td>
<td>83.5±2.4</td>
<td>83.9±2.5</td>
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<tr>
<td>Lactate (mmol/L)</td>
<td>0.67±0.03</td>
<td>0.75±0.1</td>
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<tr>
<td>Glycerol (umol/L)</td>
<td>108.7±30.3</td>
<td>114.6±28.6</td>
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<tr>
<td>Triglyceride (mg/dl)</td>
<td>95.8±12.3</td>
<td>100.8±12.3 §</td>
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<tr>
<td>Epinephrine (pg/ml)</td>
<td>61.4±12.4</td>
<td>62.6±16.1</td>
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<td>Noriepinephrine (pg/ml)</td>
<td>398.6±86.6</td>
<td>391.7±96.5</td>
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<tr>
<td>Insulin (pg/ml)</td>
<td>4.8±0.5</td>
<td>4.6±0.7</td>
</tr>
<tr>
<td>Glucagon (pg/ml)</td>
<td>68.2±4.7</td>
<td>72.2±4.7 §</td>
</tr>
</tbody>
</table>

§ = significantly different than rest at p<0.05.
Arterial and Coronary Sinus Palmitate Enrichment Over Time in Lean Men and Women

Palmitate Enrichment (MPE)

Time (min)

Rest

Atrial Pacing

70 90 p30 p50

Arterial

Coronary Sinus
Arterial-Coronary Sinus FFA Difference Over Time in Lean Men and Women
Myocardial Palmitate Uptake Over Time in Lean Men and Women

![Graph showing myocardial palmitate uptake over time with markers for rest and atrial pacing.](image)
Myocardial Palmitate Release Over Time in Lean Men and Women
Whole Body and Myocardial $^{13}$CO$_2$ Production Over Time in Lean Men and Women

- **Whole Body $^{13}$CO$_2$**
- **Myocardial $^{13}$CO$_2$**

![Graph showing the production of $^{13}$CO$_2$ over time with markers for rest and atrial pacing.](image)
Whole Body and Myocardial Palmitate Oxidation Over Time in Lean Men and Women

- Whole Body Oxidation
- Myocardial Oxidation

Time (min)

Rest
Atrial Pacing