J Mol Med (2012) 90:31–43
DOI 10.1007/s00109-011-0804-9

ORIGINAL ARTICLE

Therapeutic inhibition of fatty acid oxidation in right ventricular hypertrophy: *exploiting Randle's cycle*

Yong-Hu Fang · Lin Piao · Zhigang Hong · Peter T. Toth · Glenn Marsboom · Peter Bache-Wiig · Jalees Rehman · Stephen L. Archer

Received: 21 April 2011 / Revised: 2 August 2011 / Accepted: 10 August 2011 / Published online: 28 August 2011

Abstract Right ventricular hypertrophy (RVH) and RV failure are major determinants of prognosis in pulmonary hypertension and congenital heart disease. In RVH, there is a metabolic shift from glucose oxidation (GO) to glycolysis. Directly increasing GO improves RV function, demonstrating the susceptibility of RVH to metabolic intervention. However, the effects of RVH on fatty acid oxidation (FAO), the main energy source in adult myocardium, are unknown. We hypothesized that partial inhibitors of FAO (pFOXi) would indirectly increase GO and improve RV function by exploiting the reciprocal relationship between FAO and GO (Randle's cycle). RVH was induced in adult Sprague-Dawley rats by pulmonary artery banding (PAB). pFOXi were administered orally to prevent (trimetazidine, 0.7 g/L for 8 weeks) or regress (ranolazine 20 mg/day or trimetazidine for 1 week, beginning 3 weeks post-PAB) RVH. Metabolic, hemodynamic, molecular, electrophysiologic, and functional comparisons with sham rats were performed 4 or 8 weeks post-PAB. Metabolism was quantified in RV working hearts, using a dual-isotope technique, and in isolated RV myocytes, using a Seahorse Analyzer. PAB-induced RVH did not cause death but reduced cardiac output and treadmill walking distance and elevated plasma epinephrine levels. Increased RV FAO in PAB was accompanied by increased carnitine palmitoyltransferase expression; conversely, GO and pyruvate dehydrogenase (PDH) activity were decreased. pFOXi decreased FAO and restored PDH activity and GO in PAB, thereby increasing ATP levels. pFOXi reduced the elevated RV glycogen levels in RVH. Trimetazidine and ranolazine increased cardiac output and exercise capacity and attenuated exertional lactic acidemia in PAB. RV monophasic action potential duration and QTc interval prolongation in RVH normalized with trimetazidine. pFOXi also decreased the mild RV fibrosis seen in PAB. Maladaptive increases in FAO reduce RV function in PAB-induced RVH. pFOXi inhibit FAO, which increases GO and enhances RV function. Trimetazidine and ranolazine have therapeutic potential in RVH.

Keywords Pulmonic stenosis · Monophasic action potential duration · QTc interval · Myocardial fibrosis · Right heart failure

Electronic supplementary material The online version of this article (doi:10.1007/s00109-011-0804-9) contains supplementary material, which is available to authorized users.

Y.-H. Fang · L. Piao · Z. Hong · P. T. Toth · G. Marsboom · S. L. Archer (✉)
Medicine/Cardiology, University of Chicago,
5841 South Maryland Avenue (MC6080),
Chicago, IL 60637, USA
e-mail: sarcher@medicine.bsd.uchicago.edu

P. Bache-Wiig
Department of Biology, University of Wisconsin,
Madison, WI, USA

J. Rehman
Department of Medicine (Cardiology), University of Illinois,
Chicago, IL, USA

Introduction

Right ventricular hypertrophy (RVH) and RV failure are major determinants of prognosis and functional state in patients with congenital heart disease, such as pulmonic stenosis, or pulmonary artery hypertension (PAH) [1]. The mechanism of RV failure is not fully understood, although recent data in rats and humans suggest a role for RV ischemia and maladaptive metabolic changes [2, 3], specifically a switch from glucose oxidation (GO) to

anaerobic glycolysis [3–5]. In experimental RVH, whether induced by pulmonary artery banding (PAB) or pulmonary arterial disease, there is increased RV glycolysis, evidenced by increased uptake of fluorodeoxyglucose on positron emission tomography (PET) and by direct measurement of metabolism in isolated RV working hearts [3, 4]. One cause of impaired GO in RVH is increased expression of pyruvate dehydrogenase kinase isoforms (PDKs), which inhibit pyruvate dehydrogenase (PDH). This metabolic switch is associated with impaired cardiac function (impaired RV contractility and decreased cardiac output) and cardiac electrical remodeling (action potential and QT interval prolongation) [3]. Both the contractile and electrical consequences of the glycolytic shift in RVH can be improved by inhibition of PDK with dichloroacetate [3].

However, little is known about the role of fatty acid oxidation (FAO) in RVH. This is potentially important because FAO is the major source of ATP production (60–90%) in normal adult hearts, whereas glucose metabolism (glycolysis and GO) is considered a secondary source, accounting for 10–40% of energy production [6]. Moreover, there is a reciprocal relationship between FAO and GO, called Randle's cycle, such that inhibiting one increases the other [7]. The rationale for increasing GO and inhibiting FAO is that FAO uses 12% more oxygen than GO to generate a given amount of ATP [8]. Consequently, FAO inhibition could enhance metabolic efficiency. The importance of Randle's cycle has not been assessed in RVH, but if operant, it would offer the possibility of utilizing clinically available partial inhibitors of FAO (pFOXi), ranolazine and trimetazidine, to indirectly increase GO and improve RV function.

Here, we measure FAO in RVH and test the ability of two chemically unrelated pFOXi to regress or prevent RVH. Trimetazidine, an inhibitor of the mitochondrial enzyme long-chain 3-ketoacyl coenzyme A (CoA) thiolase (3-KAT), is used in Europe to treat refractory ischemia in patients with coronary artery disease [9, 10]. In myocardial ischemia, trimetazidine reduces FAO and secondarily promotes GO thereby reducing intracellular acidosis and preserving ATP levels [11]. Trimetazidine has beneficial effects on mitochondrial function and Ca^{2+} handling in rat myocardium [12, 13]. Ranolazine is approved for the treatment of refractory ischemia in the USA [14–16]. There is controversy whether ranolazine's benefits reflect inhibition of FAO and activation of PDH vs inhibition of sodium currents and reduction of sodium-dependent calcium overload [17–20]. However, in left ventricular ischemia–reperfusion models, ranolazine stimulates GO by activating PDH [18, 19]. In canine left heart failure, ranolazine also improves abnormal depolarization and contractility [21]. Dose-dependent inhibition of repolarizing potassium channels and sodium channels by ranolazine can result in prolongation of the QTc interval [22]. The therapeutic role of pFOXi in RVH has not yet been evaluated. We demonstrate that increased FAO occurs in RVH and can be corrected by either trimetazidine or ranolazine. Both therapies markedly improve RV function without prolonging QTc.

Methods

Pulmonary artery banding model The University of Chicago Institutional Animal Care and Use Committee approved all protocols. Adult male Sprague-Dawley rats weighing 200–230 g were anesthetized with 3% isoflurane and intubated. The PAB model has been described previously [3]. Briefly, the main pulmonary artery (PA) was carefully dissected from the ascending aorta via a limited median sternotomy. A 1.3-mm diameter needle was placed parallel to the main PA and ligated with a 4-0 silk suture. The needle was then withdrawn to create a fixed PA stenosis. Age- and sex-matched control rats underwent a sham surgery.

Two protocols were used to study pFOXi in RVH:

1. Prevention protocol: Trimetazidine (0.7 g/L drinking water) was started on the same day as PAB surgery and endpoints were studied after 8 weeks [23].
2. Regression protocol: Ranolazine (20 mg/day) or trimetazidine (0.7 g/L drinking water) was administrated for 1 week, beginning 3 weeks after PAB.

All the chemicals were obtained from Sigma-Aldrich (St Louis, MO, USA) unless otherwise noted.

RVH, histology, electrocardiograms and thermodilution cardiac output, qRT-PCR, and RV O_2 consumption (on biopsy) These techniques are performed as in reference [3] and are briefly described in the Online supplement.

Measurement of plasma epinephrine, glucose, insulin, and fatty acid concentration These assays are described in the Online supplement.

RV Langendorff perfusion and measurement of monophasic action potential duration The RV Langendorff model and technique for measuring RV monophasic action potential duration (MAPD) have been described and are summarized in the Online supplement [3].

Metabolism in the isolated RV working heart model The rat was intubated and ventilated with 3% isoflurane. The heart was rapidly excised and the aorta was cannulated and perfused retrograde with oxygenated Krebs–Henseleit buffer for 10 min in the Langendorff mode. The main PA and superior vena cava were isolated and cannulated. The inferior

vena cava was ligated. Then the Langendorff perfusion system was switched to a sealed, RV working heart system. The left heart worked against an 80-mmHg aortic retrograde pressure without preload. To initiate flow to the RV, perfusate was delivered to the superior vena cava cannula at a constant preload (11 mmHg). The right ventricle worked against a 23-mmHg PA afterload. Glycolysis and GO were measured using a Krebs–Henseleit perfusate containing 11 mM [5-^{3}H/U-^{14}C] glucose, 1.2 mM palmitate, 3% albumin, and 100 U/ml insulin. Glycolysis and GO were evaluated by quantitative collection of $^{3}H_2O$ (glycolysis) and $^{14}CO_2$ (GO). To measure FAO, separate experiments were performed using a perfusate containing ^{14}C palmitate. The release of $^{14}CO_2$ was analyzed to quantify rates of FAO [24].

Metabolism in RV myocytes The heart was isolated and cannulated for Langendorff perfusion, and myocytes were enzymatically dispersed (see Online supplement). Four groups were studied: sham, PAB, and PAB+1 week of either trimetazidine or ranolazine in vivo treatment.

Myocytes were plated into 24-well plates (~2×10^4/plate; Seahorse Bioscience, Billerica, MA, USA) and incubated in XF assay media containing 1.2 mM palmitate or 5 mM glucose. The plate was incubated at 37°C for 2 h with or without trimetazidine (200 μM). The oxygen consumption rate (OCR) was measured using a Seahorse XF24 Extracellular Flux Analyzer. The OCR protocol consisted of multiple, 6-min cycles, (2 min each of mixture, delay, and measurement). After measuring basal OCR, oligomycin (0.5 μM) and carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP, 5 μM) were injected sequentially. Metabolic data were normalized to total protein concentration.

Exercise capacity The exercise capacity was calculated by measuring the total running distance on a treadmill (Simplex II Instrument; Columbus Instruments, Columbus, OH, USA). The treadmill speed started at 10 m/min and increased by 5 m/min every 5 min. The maximum speed was set as 30 m/min.

Post-exertional serum lactate level Serum lactate levels were measured on tail vein blood using a Lactate Colorimetric Assay Kit (Abcam, Cambridge, MA, USA).

ATP production RV ATP production was measured on 10 mg RV tissue using an ATP Colorimetric/Fluorometric Assay Kit (Abcam, Cambridge, MA, USA).

PDH activity PDH activity was quantified by immunocapturing PDH with anti-PDH antibody immobilized on a dipstick following the manufacturer's instructions (MitoSciences Eugene, OR, USA). RV (50 mg) was homogenized and resuspended in the lysis buffer. Detergent (1/10 volume) was added to the buffer containing 25 μg protein. The tip of the dipstick was placed into the buffer for 5 min. PDH activity was visualized as a band at the designated area of the dipstick by coupling PDH-dependent NADH production to NBT reduction in the presence of diaphorase. The colored precipitate was quantified using Image J (NIH, Bethesda, MD, USA).

Analysis of glycogen from RV myocardium Myocardial glycogen content was determined by measurement of glucose after digestion of 100 mg of powdered ventricular tissue with 30% KOH and conversion to glucose (by 2 N H_2SO_4). Glucose was measured using a glucose assay kit (Sigma-Aldrich, St. Louis, MO, USA).

RV collagen Sirius red staining was used to measure RV collagen in frozen sections. Briefly, cryosections were fixed for 20 min in acetone at −20°C. After rehydration in distilled water, slides were stained for 90 min with 2 mg/ml of Direct Red 80 dye dissolved in saturated picric acid (Sigma Aldrich, St. Louis, MO, USA). Slides were then washed with 0.01 N HCl, dehydrated, and mounted with DPX mounting solution (Sigma Aldrich, St. Louis, MO, USA). Extravascular collagen was quantified using the Zeiss Axiovision 4.6 software. After setting the detection threshold, all slides were analyzed with the same settings. Perivascular areas were excluded from the analysis.

Statistics Values were expressed as mean±SEM. Sample size is noted on each figure. Prism5 (GraphPad Software, Inc., La Jolla, CA, USA) was used for data analysis. Comparisons between groups were performed with an ANOVA or paired *t* test, as appropriate. Post hoc testing was performed with a Bonferroni's correction for multiple comparisons. A value of $P<0.05$ was considered statistically significant.

All authors had access to the data and read and approved the manuscript in its current form.

Results

Trimetazidine and ranolazine reduce RVH and improve RV function without causing QTc prolongation

RVH There was similar RVH 4 and 8 weeks after PAB, evident both as cellular hypertrophy of RV myocytes and as an increase in RV mass, measured by the RV/LV+ septum ratio (Fig. 1). Trimetazidine, begun at the time of PAB, reduced RVH (Fig. 1a–c). Likewise, ranolazine, begun 3 weeks after PAB, regressed RVH ($P<0.001$; Fig. 1d–f).

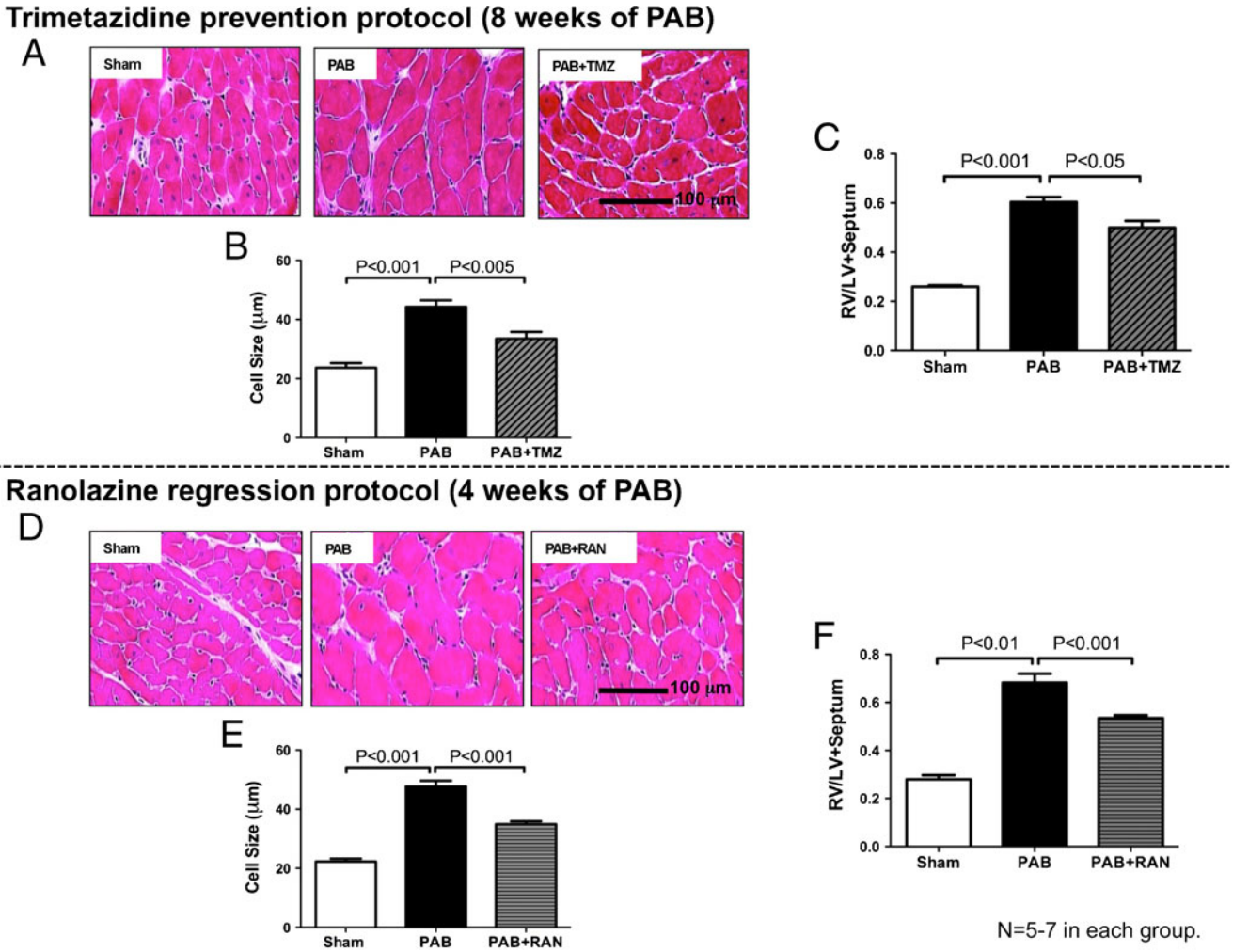

Fig. 1 pFOXi prevent and regress PAB-induced RVH. **a**, **b**, **d**, **e** Representative hematoxylin and eosin photomicrographs and mean data showing cardiomyocyte hypertrophy in RVH. Both trimetazidine (given in a prevention protocol) and ranolazine (given in a regression protocol) reduce RV cardiomyocyte size in PAB. **c**, **f** The RV/LV+septum ratio is similarly increased at 4 and 8 weeks post-PAB and is reduced by both pFOXi

Cardiac electrophysiology Neither PAB nor pFOXi therapy significantly altered the heart rate (Supplemental Fig. 1). The QTc interval on surface EKG, which was prolonged in RVH, was shortened by trimetazidine, while ranolazine had no effect (Fig. 2c, f). Consistent with the QTc prolongation, MAPD, recorded from the RV epicardium, was prolonged in RVH ($P<0.05$; Supplemental Fig. 2). We investigated the molecular basis for impaired cardiac repolarization and demonstrated reduced expression of the repolarizing, voltage-dependent potassium channel Kv1.5 in PAB vs sham RV ($P<0.001$; Supplemental Fig. 2). Long-term therapy with trimetazidine shortened both QTc (Fig. 2c) and MAPD while increasing Kv1.5 expression (Supplemental Fig. 2).

Cardiac index and exercise capacity Cardiac index was reduced both 4 and 8 weeks post-PAB ($P<0.001$; Fig. 2a, d). Consistent with this, maximal treadmill distance was decreased in PAB vs sham rats at both time points ($P<0.001$; Fig. 2b, e). Both trimetazidine and ranolazine treatment (given in prevention and regression protocols, respectively) improved cardiac index and treadmill walking distance (Fig. 2). The rats in the trimetazidine regression protocol were allowed to age an additional month, compared to rats in the ranolazine regression protocol, accounting for their shorter walking time at baseline. However, this interprotocol difference had no impact on the analysis of the effects of the FAOi within its own protocol, where the comparator was an age-matched, untreated, PAB rat.

In sham rats, neither trimetazidine nor ranolazine altered RV myocyte size, RV/LV+septum ratio, cardiac index, or treadmill distance (data not shown).

Metabolic effects of trimetazidine and ranolazine in RVH RV O_2 consumption per gram progressively decreased

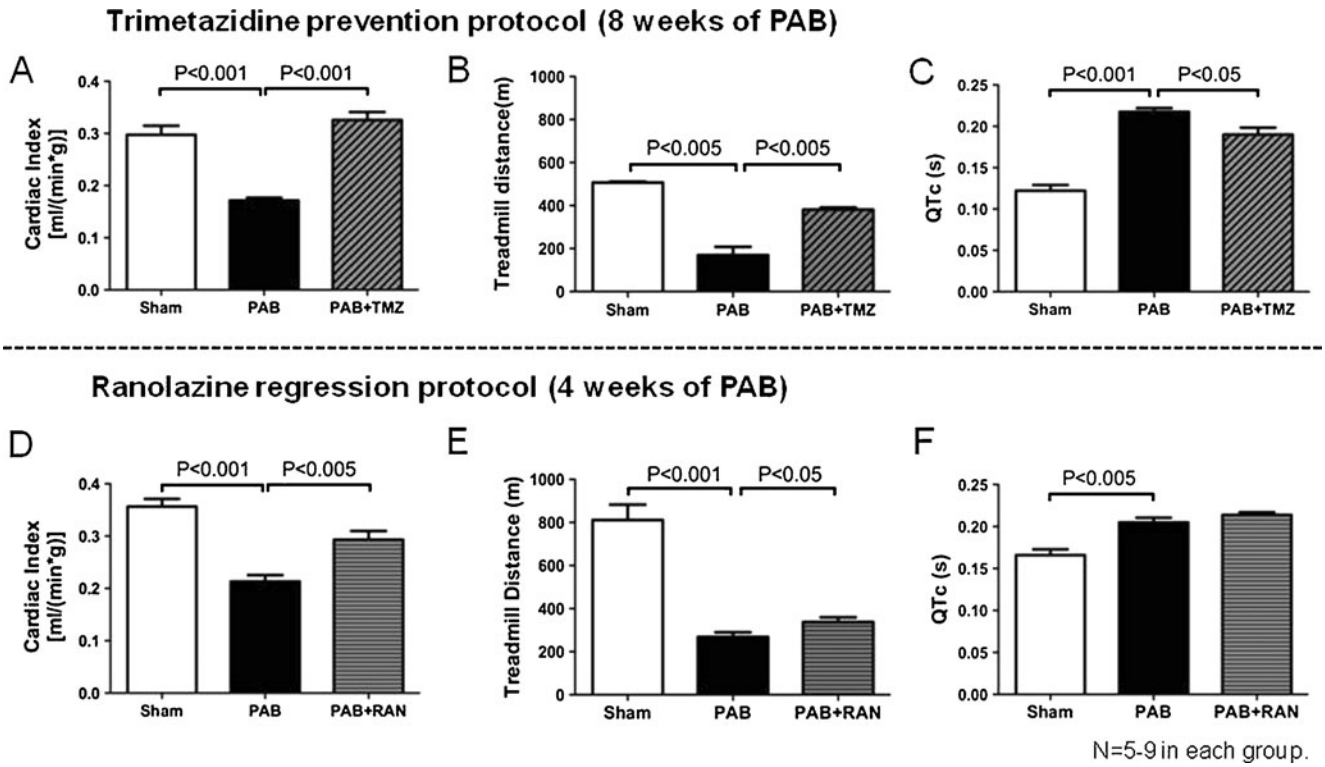

Fig. 2 Trimetazidine and ranolazine improve cardiac index and exercise performance in RVH without prolonging the QTc interval. Trimetazidine and ranolazine improve cardiac index (**a**, **d**) and increase treadmill distance walked (**b**, **e**) in PAB-induced RVH. RVH increases the QTc interval (**c**, **f**). Trimetazidine shortens, whereas ranolazine does not alter, the QTc interval in RVH (**c**, **f**)

from 4 to 8 weeks post-PAB ($P<0.005$ vs sham; Fig. 3a, c). Both trimetazidine and ranolazine treatments increased RV O_2 consumption compared to age-matched, untreated PAB rats ($P<0.005$ and $P<0.001$, respectively; Fig. 3a, c). Consistent with improved O_2 consumption, trimetazidine and ranolazine increased RV ATP levels in PAB ($P<0.05$; Fig. 3b, d).

Serum lactate levels did not differ among the groups at rest (data not shown), but increased with exercise in the PAB group ($P<0.05$; Fig. 3e), suggesting that RVH resulted in RV ischemia under stress conditions but not at baseline. Both trimetazidine and ranolazine reduced exercise-induced serum lactate levels ($P<0.05$; Fig. 3e).

Metabolism in the RV working heart This assay allowed quantitative measurement of both glucose and FA metabolism in a working RV exposed to defined substrate, both excluding confounding effects of the LV and allowing assessment of substrate preference. RV FAO increased in PAB vs sham hearts ($P<0.01$; Fig. 4c). Hearts from rats treated with trimetazidine for 8 weeks or ranolazine for 1 week had significantly lower FAO rates vs hearts from untreated PAB rats ($P<0.05$; Fig. 4c). RV glycolysis also increased after PAB vs sham ($P<0.05$; Fig. 4d) and this was decreased by long-term treatment with trimetazidine ($P<0.05$; Fig. 4d). Ranolazine tended to decrease glycolysis in the PAB group, but this did not achieve statistical significance (Fig. 4d). However, both trimetazidine and ranolazine significantly increased the coupling of GO to glycolysis vs the untreated PAB group ($P<0.005$ and $P<0.001$, respectively; Fig. 4e).

Metabolism in RV myocytes This assay used pure preparations of freshly dispersed RV myocytes, thus ensuring that metabolic changes in PAB were not confounded by changes in metabolism of vascular cells or fibroblasts. We assessed the effects of pFOXi on cardiomyocyte OCR (Fig. 5a, b) both by administration of trimetazidine or ranolazine during the assay and by studying myocytes dispersed from RVs of rats that had received long-term oral pFOXi treatment. FAO was measured by using palmitate as the only substrate. Basal FAO was increased in RV myocytes from PAB vs sham ($P<0.001$; Fig. 5e), consistent with findings in the RV working heart model. When palmitate was used as the substrate, PAB RV myocytes also had a higher O_2 consumption in response to the ionophore and uncoupling agent, FCCP (Fig. 3c). Both acute addition of the pFOXi to the medium and chronic oral pFOXi therapy reduced FAO in RVH myocytes, compared to treatment-naive control cells ($P<0.001$; Fig. 5e, f).

GO was also studied using the Seahorse Extracellular Flux Analyzer by using glucose (5 mM) as the sole substrate in the

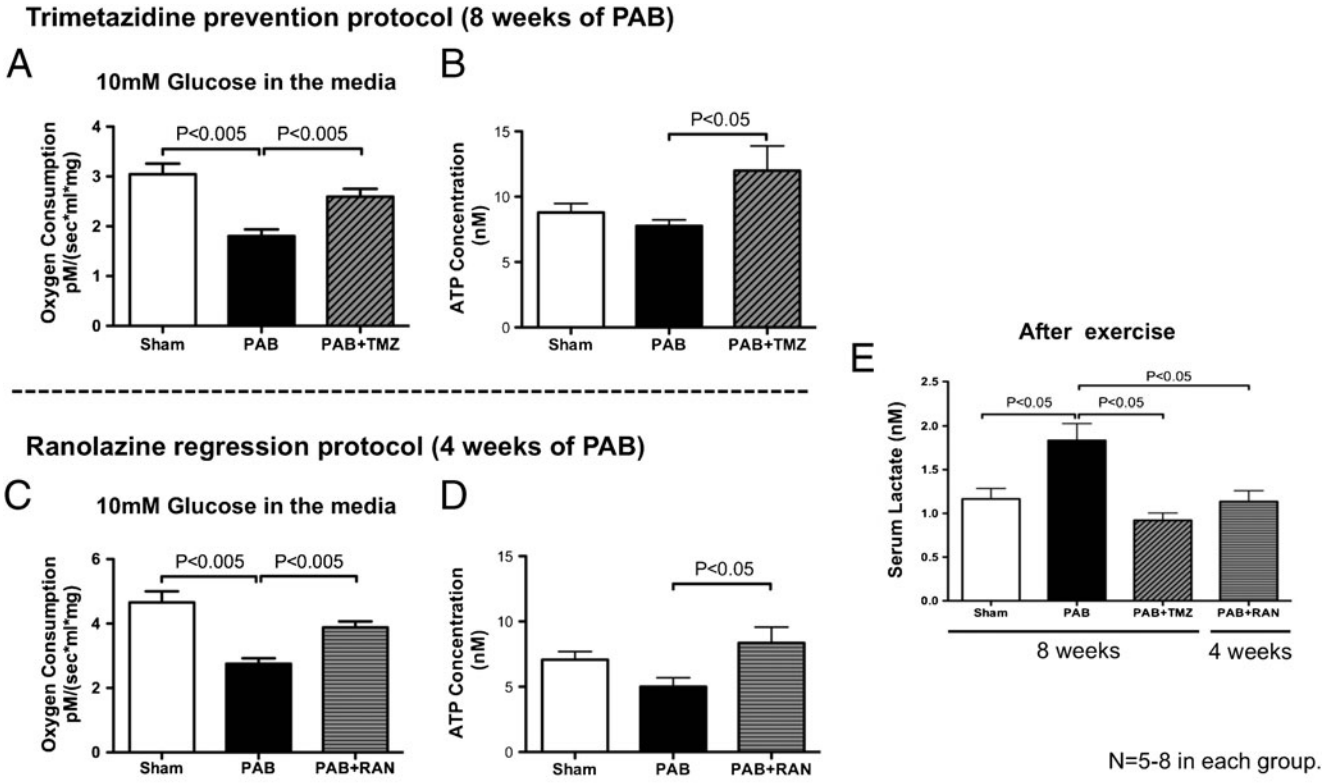

Fig. 3 Trimetazidine and ranolazine improve RV GO and ATP production and reduce plasma lactate levels in PAB. **a**, **c** In RV whole tissue samples, O_2 consumption is depressed in RVH, and this is restored by long-term treatment with either pFOXi. These measurements were made with glucose as the only substrate and thus reflect GO. **b**, **d** Although RV ATP levels are not significantly reduced in RVH vs sham, both pFOXi increase RV ATP levels. **e** After 30 min of exercise, serum lactate levels increase in PAB rats. Long-term treatment with either pFOXi reduces exertional lactic acidemia

medium. Basal GO was decreased in PAB group vs sham ($P<0.05$; Fig. 5f). The maximal O_2 consumption in the presence of the uncoupling agent, FCCP, was also reduced (Fig. 5d). Chronic pFOXi treatments increased GO in PAB RV myocytes ($P<0.01$ and $P<0.05$, respectively; Fig. 5f).

Metabolic expression profile in the RV To further characterize the metabolic changes in RVH, we assessed the mRNA expression of the glucose transporter, Glut1, the glucose-phosphorylating isoenzymes, hexokinase 1 and 2 (HKI and HKII) and lactate dehydrogenase A (LDHA), which converts pyruvate to lactate. Both Glut1 and HKI mRNA were significantly increased in PAB ($P<0.001$; Fig. 6a, b, h, i), consistent with the elevated rates of glycolysis measured in RVH. Changes in the expression of HKII and LDHA did not reach statistical significance in PAB (Supplemental Fig. 3a, c and Fig. 6c, j). In vivo therapy with trimetazidine or ranolazine reduced the gene expression of Glut1, HKI, and LDHA in PAB ($P<0.05$; Fig. 6). The protein expression of Glut1 and HKI in the RV was also increased by PAB (Fig. 6d–f, k–m). There was a trend for trimetazidine and ranolazine to decrease Glut1 expression, but this was not statistically significant (Fig. 6d, e, k, l).

The expression of key FAO enzymes, carnitine palmitoyltransferase 1 and 2 (CPT1 and CPT2), was also assessed. CPT1 protein expression in the RV was increased after PAB, whereas CPT2 remained unchanged (Fig. 7a–f). Trimetazidine decreased CPT1 and CPT2 expression (Fig. 7a–c).

RV PDH activity PDH activity in the RV was reduced in RVH. Both trimetazidine and ranolazine restored RV PDH activity ($P<0.01$; Fig. 7g, h), consistent with the observed increases in RV GO caused by pFOXi (Figs. 3, 4, and 5).

RV glycogen content Because inhibition of GO in the setting of enhanced glucose uptake would be expected to increase glycogen stores, we performed a glycogen assay. Glycogen concentration of the RV myocardium was slightly increased in RVH. Both trimetazidine and ranolazine lowered glycogen concentration, but only trimetazidine treatment yielded a statistically significant reduction ($P<0.05$; Supplemental Fig. 4a).

RV collagen content The collagen content was significantly increased in RVH, thus indicating increased fibrosis, and both

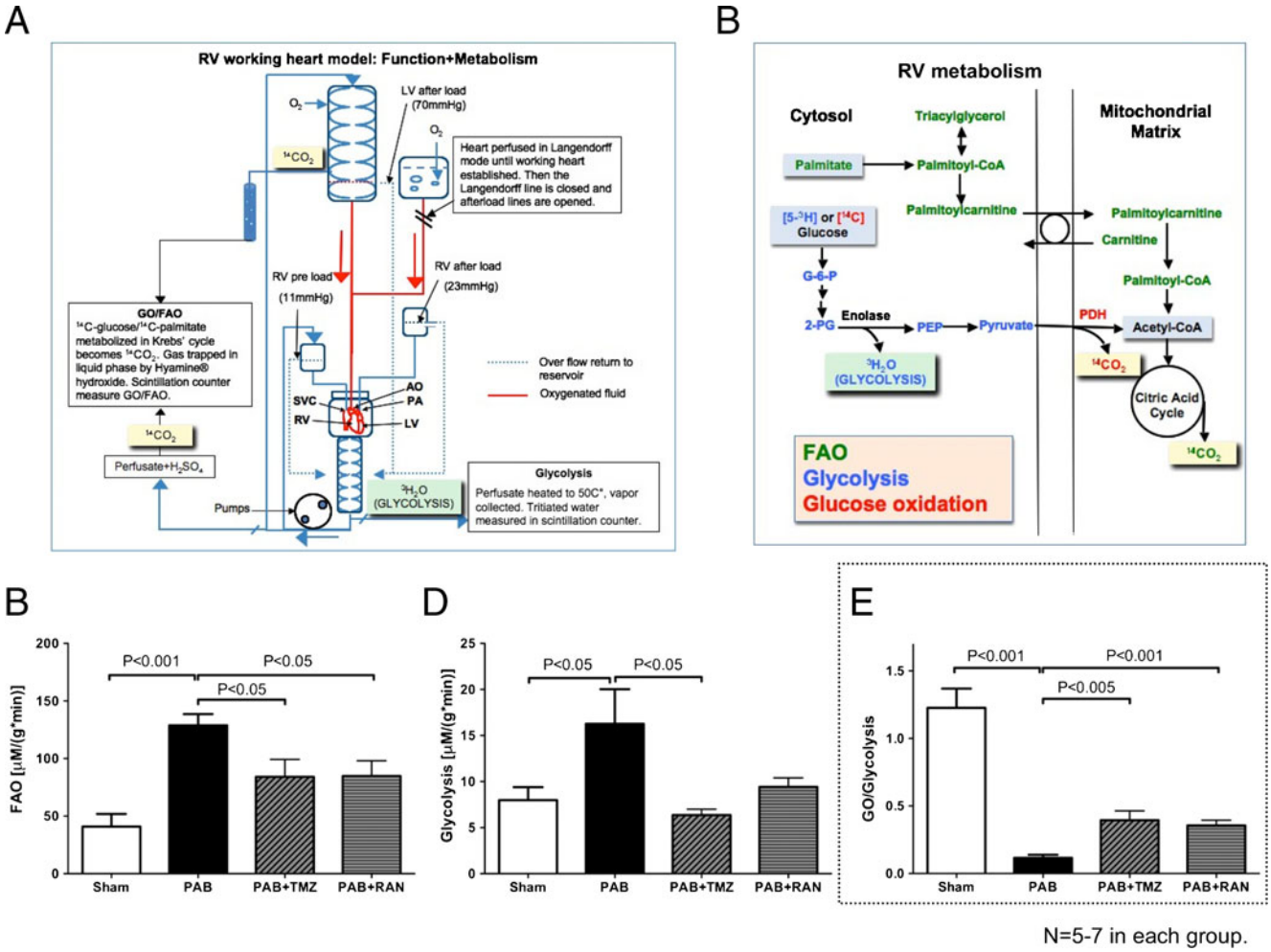

Fig. 4 Increased RV FAO and reduced GO/glycolysis coupling in RVH is corrected by pFOXi. **a** The schematic illustrates the RV working heart model and shows the use of the dual-isotope technique to quantify metabolism. Oxidative metabolism of ^{14}C-labeled glucose or ^{14}C-labeled palmitate generates labeled $^{14}CO_2$ that is quantified to measure GO and FAO, respectively (in separate experiments). ^{3}H-labeled glucose allows collection of ^{3}H-labeled water as a measure of glycolysis. **b** The diagram shows the key metabolic steps in the measurement of RV FAO, glycolysis, and GO. **c** In the RV working heart model, FAO is increased in PAB group and decreases with pFOXi treatment. **d** Glycolysis is increased in the PAB group and decreases with pFOXi treatment. **e** GO/glycolysis coupling is decreased in PAB and improves in pFOXi-treated groups

pFOXi decreased the collagen content ($P<0.001$; Supplemental Fig. 4b, c).

Plasma epinephrine, insulin, glucose, and fatty acid levels To assess circulating systemic markers of metabolism in PAB, plasma glucose and insulin were measured at the end of the protocols. Consistent with the induction of mild right heart failure in PAB, epinephrine levels were increased in RVH ($P<0.01$; Supplemental Fig. 5a). Neither trimetazidine nor ranolazine treatment lowered epinephrine levels. Plasma fatty acid levels tended (P=NS) to decrease in RVH. Both trimetazidine and ranolazine increased plasma fatty acid, but only ranolazine yielded statistically significant differences ($P<0.001$; Supplemental Fig. 5d). There are no significant differences in glucose or insulin levels among groups (Supplemental Fig. 5b, c).

Discussion

This study provides direct evidence that FAO is increased in the RV myocytes in a PAB model of RVH (Fig. 4c). Moreover, elevated FAO in RVH is demonstrated to be maladaptive, since inhibiting FAO increases RV ATP concentrations and improves RV function and exercise capacity in vivo. Mechanistically, we confirm that the Randle cycle is operating in RVH, evident from the fact that chemically distinct FAO inhibitors (trimetazidine and ranolazine) enhance GO (Fig. 4e). We used multiple assays to prove that the putative FAO inhibitors were indeed inhibiting FAO in the RV at the doses used.

A key strength of our methodology is the use of multiple, quantitative techniques to measure RV metabolism, including a micropolarimeter for biopsy specimens

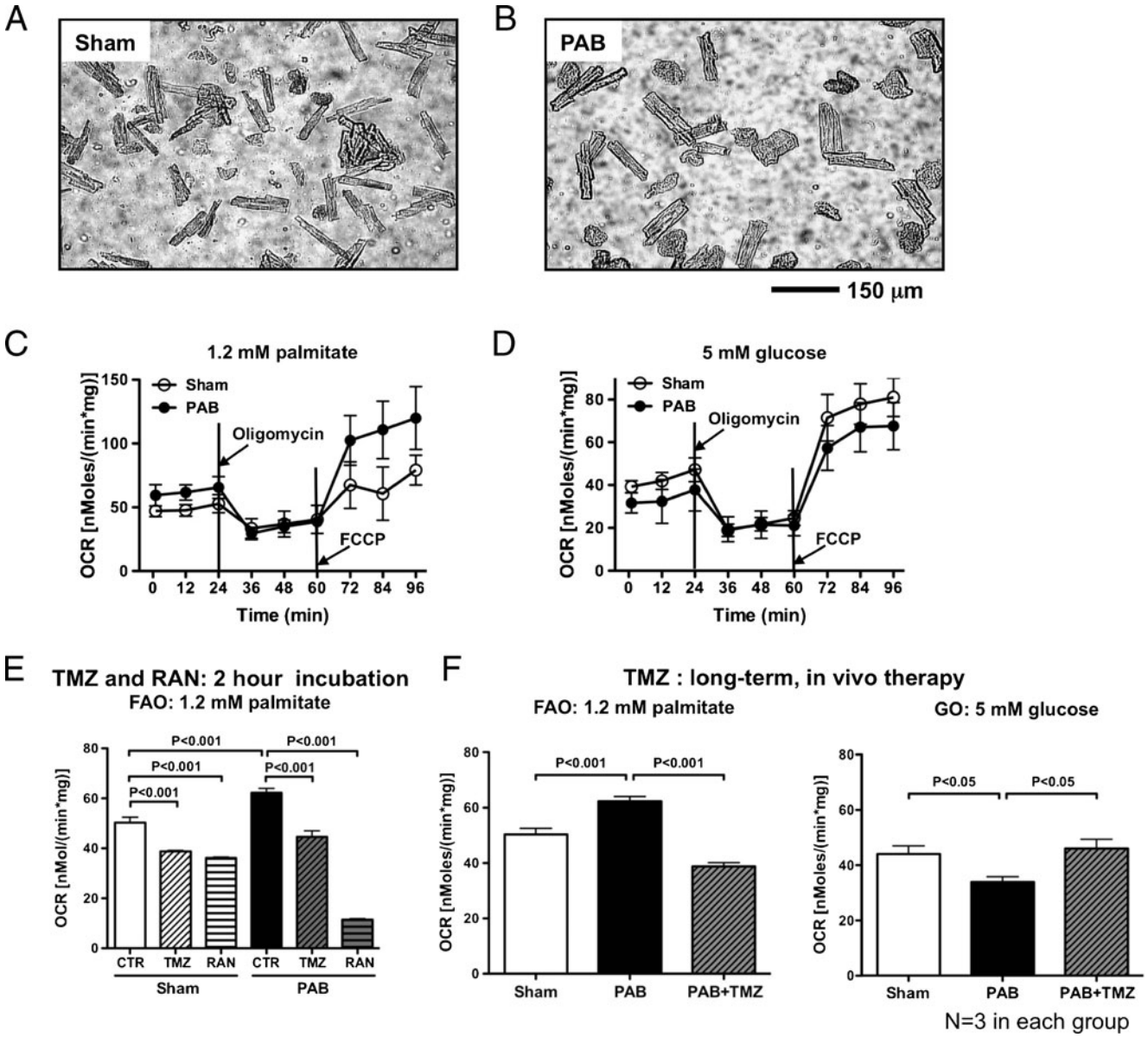

Fig. 5 pFOXi reduce FAO and improve GO in isolated RV myocytes in PAB. **a**, **b** Images of freshly isolated RV cardiomyocytes from sham (**a**) and PAB (**b**) rats. **c**, **d** The time course curves of OCR with oligomycin and FCCP treatment when palmitate alone (**c**) or glucose alone (**d**) is used as the energy substrate. **e** Acute in vitro treatment with trimetazidine or ranolazine decreases FAO in PAB cardiomyocytes. **f** In RV myocytes isolated from rats that received long-term in vivo treatment with trimetazidine, there is increased GO and decreased FAO relative to control PAB cardiomyocytes

(Fig. 3), the dual-isotope technique, in the RV working heart model (Fig. 4), and the Seahorse® Analyzer, for isolated adherent RV myocytes (Fig. 5). Each of these techniques yielded concordant results, showing that inhibiting the elevated FAO that occurs in PAB-induced RVH, by two distinct pFOXi, reciprocally increases GO (Fig. 8). The in vivo studies provide preclinical evidence for the tolerability and therapeutic efficacy of both ranolazine and trimetazidine in the RV pressure overload model. The use of the RV working heart model allowed us to exclude potential confounding metabolic effects of the LV that might occur in vivo. The use of the Seahorse® Extracellular Flux Analyzer provided the ability to localize the metabolic changes to the RV myocyte, excluding the possibility that the metabolic changes observed in the more integrative models reflected other cardiac cells, such as vascular cells or fibroblasts.

Since the observed RV O_2 consumption reflects both the RV's intrinsic metabolic enzyme profile and substrate that is provided experimentally, we measured RV metabolism of cardiomyocytes in media containing either glucose or palmitate. In media containing only glucose, net O_2 consumption is reduced in RVH, consistent with impaired GO

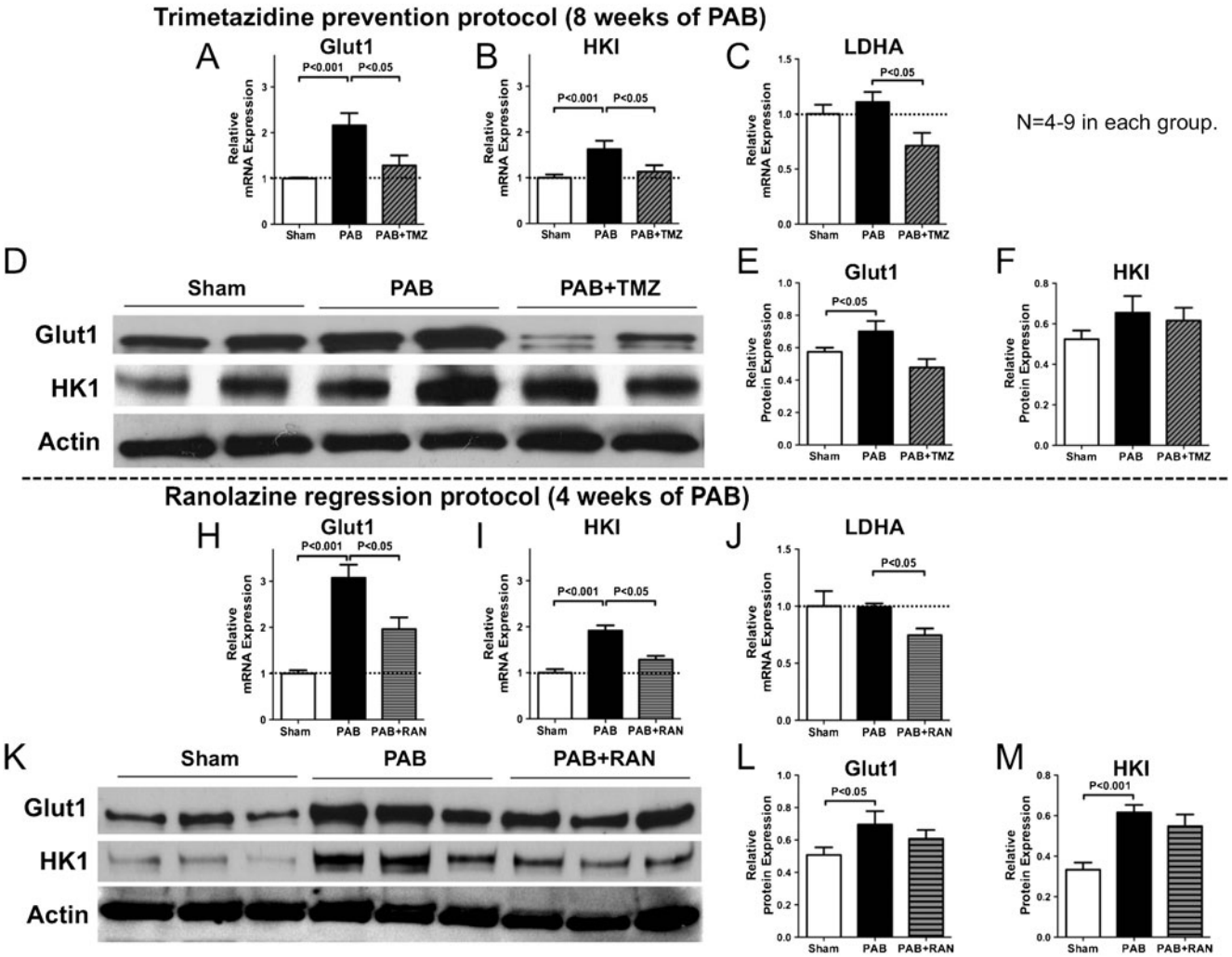

Fig. 6 Increased expression of Glut1 and HKI in RVH. **a**, **b**, **h**, **i** RV Expression of Glut1 and HKI mRNA increase in PAB and decrease after in vivo treatment with pFOXi. **c**, **j** The mRNA expression of LDHA is unchanged in PAB; however, trimetazidine and ranolazine reduce its expression. **d**, **f**, **k**, **m** Immunoblots confirm the increase in Glut1 and HKI in PAB. Both TMZ and RAN tend to decrease Glut1

(Figs. 4e and 5f). When palmitate is present, it is clear that FAO is increased in RVH (Figs. 4c and 5e). In vivo, it appears that the net effect of the increase in FAO and suppression of GO in RVH is a metabolic shift to glycolysis and a reduction in O_2 consumption per gram of RV (Fig. 3a, c). This increase in glycolysis is sufficient for survival but is inadequate to maintain resting cardiac output or offer the reserve to support exercise, without development of lactic acidemia (Fig. 3e). Thus, the severe PAB we studied recapitulates the borderline RV reserve seen clinically in patients with severe RVH due to pulmonic stenosis.

The molecular fingerprint of the RV's reliance on glycolysis is evident in RVH in the increased expression of Glut1 and HKI, both at the mRNA and protein levels. This is consistent with more glucose being transported into the cytosol to support the less energetically efficient glycolytic metabolism of RVH. In RVH, this glycolysis produces more lactate, detectable systemically during exercise (Fig. 3e), and reduces RV contractility. As expected, RV glycogen increases in the presence of suppressed GO and glycogen levels fall with pFOXi therapy (Supplemental Fig. 4a). Consistent with restoration of GO, pFOXi therapy reduces the expression of glycolytic genes (Fig. 6). As would be expected with increased FAO, RV expression of CPT1, a key regulatory enzyme, is elevated in RVH (Fig. 7b, e). The expression of CPT1 decreases with chronic pFOXi therapy, consistent with the measured reduction in FAO achieved by trimetazidine and ranolazine (Figs. 4 and 7).

One point that has great potential clinical relevance is that the depression in RV function in PAB is metabolically mediated. Despite the persistence of the mechanical obstruction to RV outflow, RV function is substantially improved by pFOXi, whether trimetazidine is given as a preventative therapy or ranolazine is initiated to regress

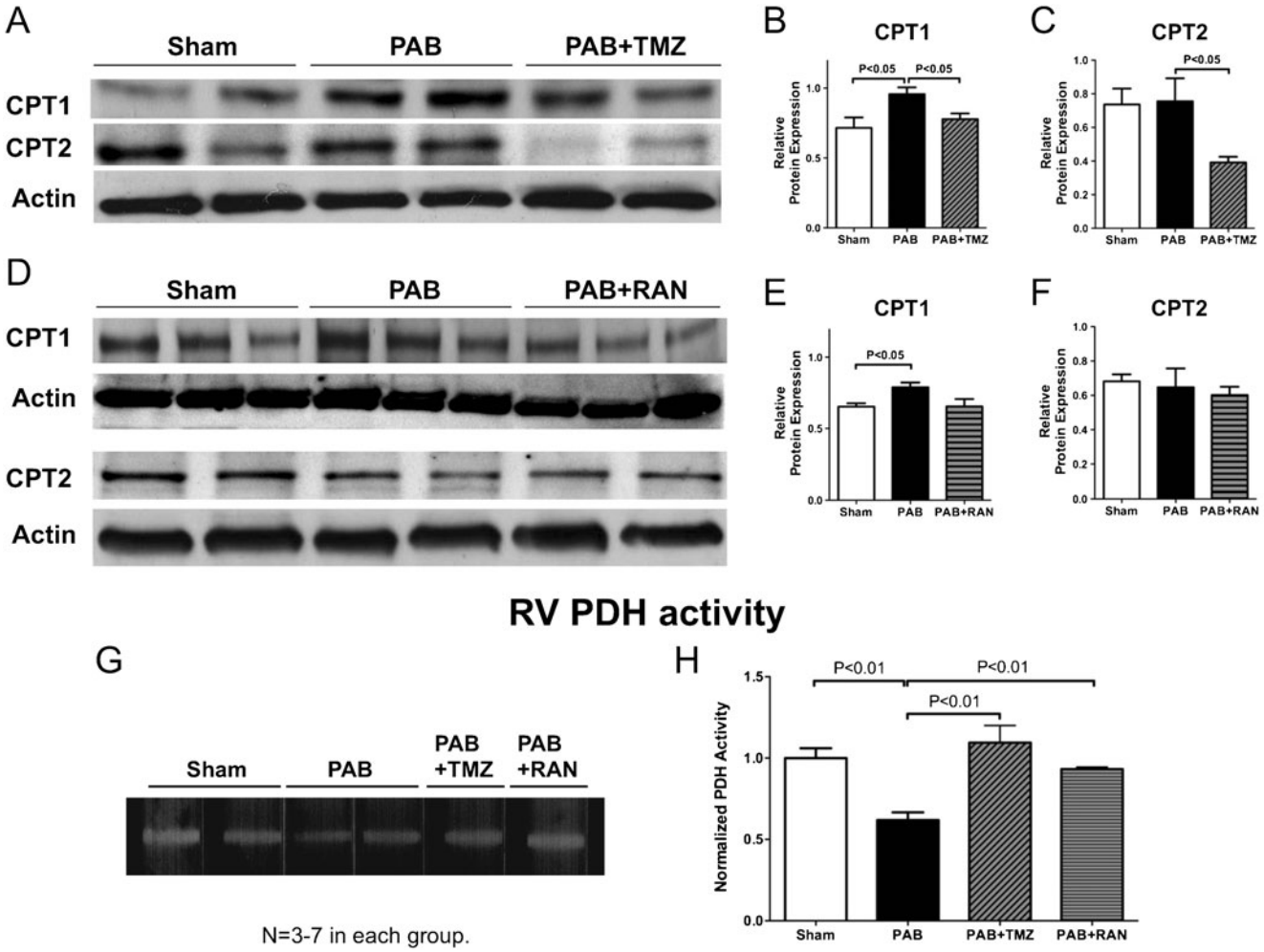

Fig. 7 pFOXi correct the increased CPT1 expression and decreased PDH activity in RVH. **a**–**f** The protein expression of CPT1 is increased in PAB RVs, whereas CPT2 remains unchanged. Trimetazidine, but not ranolazine, decreases both CPT1 and CPT2 (upper membrane in **d** is identical to the one shown in Fig. 6k, but has been reprobed with CPT1 antibody). **g**, **h** Representative and mean data showing decreased RV PDH activity in RVH and restoration of PDH activity by long-term pFOXi therapy

RVH. It is remarkable that as little as a week of ranolazine has beneficial hemodynamic and functional effects in established RVH (Figs. 1 and 2). This is encouraging from a translational aspect, since patients typically present with established RVH. In this regard, the PAB model is relevant to patients with pulmonic stenosis.

In a prior study, we demonstrated that a glycolytic switch in RV metabolism in various RVH models (whether associated with PAB or with pulmonary arterial hypertension) resulted, in part, from PDK-mediated inhibition of PDH [3]. The current study identifies an additional mechanism for the suppression of PDH activity and GO, namely, activation of the Randle cycle. In PAB RVH, high rates of FAO inhibit PDH (Fig. 8). Conversely, the decrease in PDH activity in RVH is corrected by oral pFOXi therapy in vivo (Fig. 7). The consequences of restoring PDH activity is that both trimetazidine and ranolazine increase GO (Figs. 4e and 5f). Likewise, the interpretation that increased FAO is maladaptive is supported by the observation that both trimetazidine and ranolazine elevate RV ATP production (Fig. 3b, d) and improve cardiac function (Fig. 2a, c). The precise metabolic measurements of RV metabolism ensure that the concordant therapeutic effects of two distinct pFOXi are almost certainly the result of FAO inhibition (Figs. 4c and 5c).

The Randle cycle in RVH

The reciprocal relationship between FAO and GO reflects, in part, the production of citrate during FAO. Citrate inhibits phosphofructokinase (PFK), causing accumulation of glucose-6-phosphate. This inhibits HK and decreases GO (Fig. 8). Another important component of Randle's cycle is the inhibition of PDH by the acetyl CoA generated from FAO (Fig. 8). Consistent with this, the decreased PDH activity in RVH improves with pFOXi therapy (Fig. 7). This

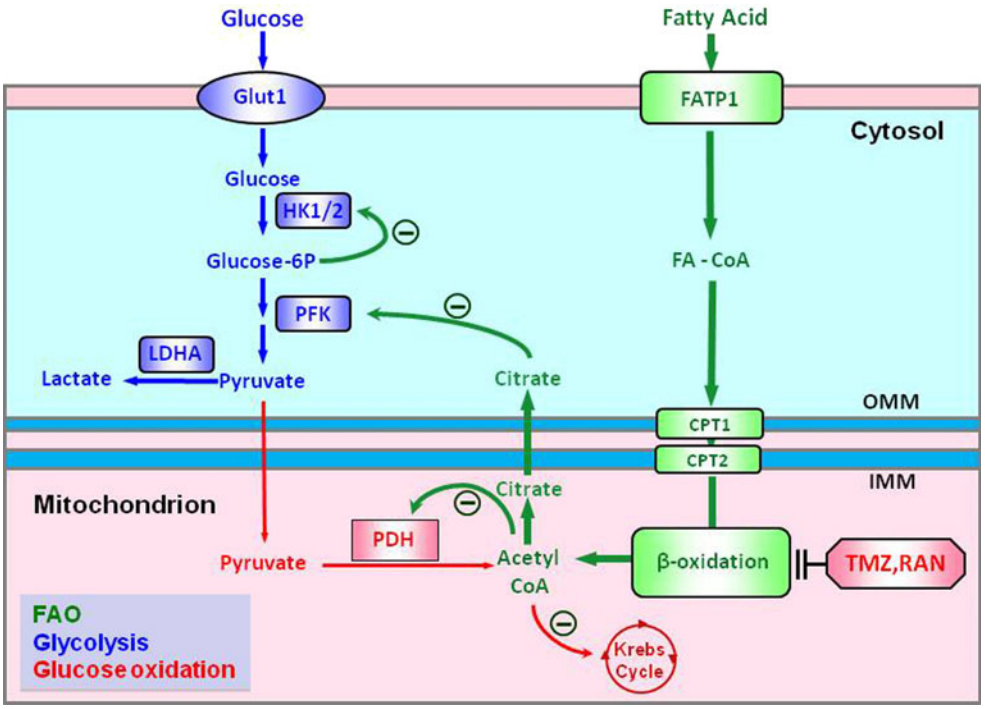

Fig. 8 The Randle cycle in RVH The inhibition of β-FAO by trimetazidine and ranolazine increases PDH activity and improves GO. This reciprocal relationship between GO and FAO is referred to as Randle's cycle

"Randle cycle"-mediated inhibition of PFK and HK should also inhibit glycolysis [7]. However, in PAB, it seems that the increased FAO is insufficient to block increased glycolysis (Fig. 4d). This may reflect a competing process that enhances glycolysis, such as PDK activation [3]. It is unknown whether combining PDK inhibitors and FAO inhibitors would have benefit in RVH, although this merits future study because of the potential for synergistic increases in PDH activity.

Trimetazidine and ranolazine reverse the metabolic changes in RVH

Trimetazidine inhibits the long-chain isoform of the final enzyme in β-FAO, 3-ketoacyl CoA thiolase (IC_{50}=75 nmol/L) [10]. This mechanism is important to trimetazidine's ability to increase GO in patients with ischemic heart disease [10]. In patients with idiopathic dilated cardiomyopathy, PET studies, using palmitate and acetate tracers, show that trimetazidine decreases FAO and causes a compensatory increase in GO [25], consistent with our findings in RVH. Trimetazidine also prevents β-adrenergic receptor desensitization, elevation of brain natriuretic factor and cardiac hypertrophy in rats with left heart failure induced by aortic stenosis [26]. Guarnieri and Muscari reported the beneficial effect of trimetazidine in monocrotaline-induced RVH [13]. They found that trimetazidine increases net O_2 consumption and enhances cardiac mitochondrial function, while reducing the formation of oxygen free radicals [27]. Our findings support and extend their work by measuring the metabolic changes that they inferred and by demonstrating the beneficial effect of trimetazidine on cardiac function.

The pFOXi not only improve RV metabolism, they also reduce RVH. It is unclear how much of their benefit relates to RVH regression vs enhancement of GO. Both effects lessen the RV ischemia, which may initiate the metabolic reprogramming of the hypertrophied RV, as recently postulated [28]. Interestingly, in acute studies where isolated RV myocytes were exposed to pFOXi for only 2 h, there was an immediate metabolic shift towards GO in RVH. This suggests that pFOXi can acutely restore GO in RVH, independent of their ability to regress RVH.

Ranolazine is approved for refractory angina in the USA [29]. Although the molecular targets of ranolazine are not clear, ranolazine has been shown to inhibit FAO and activate PDH in perfused, normoxic rat hearts [19]. In the ischemia and/or reperfusion model, ranolazine improves cardiac work by activating PDH and stimulating GO [18]. However, to our knowledge, the beneficial effect of ranolazine on the hypertrophied right ventricle has not yet been studied. In the present study, we directly confirmed that ranolazine partially inhibits FAO and improves GO in RVH (Figs. 3c and 4e). Consistent with these effects, ranolazine increases O_2 consumption and ATP production (Fig. 3c, d), reduces the expression of glycolytic mediators such as Glut1, HKI, and LDHA, and decreases lactate production (Figs. 3e and 6h–j). As a result, the cardiac function is significantly improved by the treatment of ranolazine (Fig. 2d, e).

The effects of pFOXi in RVH seem largely related to their ability to alter cardiac metabolism, rather than changes

in peripheral metabolism or attenuation of the mild autonomic nervous system activation that occurs in this adaptive RVH model. Neither trimetazidine nor ranolazine has dramatic effects on the humoral metabolic profile in PAB. Neither agent altered plasma epinephrine, insulin, nor glucose levels (Supplemental Fig. 5a–c).

Safety

Prolongation of the QT interval is a concern for any cardiovascular therapy. RVH itself leads to prolongation of the QTc due to downregulation of repolarizing Kv channels in the RV myocyte, including Kv1.2, Kv1.5, and Kv4.1 [3]. This electrical remodeling is reversible with restoration of GO [3]. Consistent with this, the prolongation of MAPD and QTc, due in part to downregulation of RV Kv1.5 channels, is improved significantly by trimetazidine (Fig. 2 and Supplemental Fig. 2). Ranolazine had no effect on QTc. These preclinical data suggest that the pFOXi should not exacerbate RVH-induced QTc prolongation, should it be encountered in human RVH. Another encouraging sign for future translational studies is that RV fibrosis (which admittedly is very mild in PAB) was reduced by pFOXi therapy (Supplemental Fig. 4b, c).

Limitations

The key strengths of the PAB model are that it mimics the RVH seen in certain congenital heart diseases, such as pulmonic stenosis, and permits the study of RVH in the absence of confounding changes in the pulmonary vasculature. The RVH in PAB is somewhat "adaptive," meaning that it is better tolerated than RVH of similar severity in models with concomitant pulmonary arterial hypertension [3]. However, PAH-induced RVH is not benign. These rodents have reduced cardiac index and exercise capacity, and when they exercise, their blood lactate levels increase (Fig. 3). Nonetheless, additional studies will be necessary to determine the effects of pFOXi in RVH resulting from pulmonary vascular disease.

This study does not directly address why upregulated FAO is detrimental to RV function in RVH. We suspect that the negative consequences of increased FAO in RVH relate to the increased O_2 requirements per mole of ATP generated from fatty acids vs glucose [8], the suppressive effect of increased FAO on GO (mediated by "Randle's cycle" [7]), or a combination of these two factors.

Although ranolazine may also act in part through inhibition of sodium channels [20, 22], we interpret the concordant effects of ranolazine and trimetazidine on FAO and RV function as favoring the interpretation that it is inhibition of FAO that primarily determines benefit. Moreover, at the doses used, we did not observe QTc prolongation, arguing against significant ranolazine-induced sodium channel inhibition as the major cause of benefit in RVH.

Conclusion

In RVH induced by PAB, there is a metabolic shift from GO to FAO and glycolysis, which contributes to reduced RV function. Trimetazidine and ranolazine inhibit FAO and thereby improve GO, RV function, and the functional capacity. Exploitation of FAO inhibition as an indirect means of enhancing RV GO is appealing, in part, because two pFOXi, trimetazidine and ranolazine, are clinically available. This should lower the barrier for clinical trials of trimetazidine or ranolazine in RVH and RV failure.

Acknowledgements Dr. Archer is supported by NIH-RO1-HL071115 and 1RC1HL099462-01, and the American Heart Association (AHA).

Disclosures None.

References

1. Rajabi M, Kassiotis C, Razeghi P, Taegtmeyer H (2007) Return to the fetal gene program protects the stressed heart: a strong hypothesis. Heart Fail Rev 12:331–343
2. Bogaard HJ, Natarajan R, Henderson SC, Long CS, Kraskauskas D, Smithson L, Ockaili R, McCord JM, Voelkel NF (2009) Chronic pulmonary artery pressure elevation is insufficient to explain right heart failure. Circulation 120:1951–1960
3. Piao L, Fang YH, Cadete VJ, Wietholt C, Urboniene D, Toth PT, Marsboom G, Zhang HJ, Haber I, Rehman J et al (2010) The inhibition of pyruvate dehydrogenase kinase improves impaired cardiac function and electrical remodeling in two models of right ventricular hypertrophy: resuscitating the hibernating right ventricle. J Mol Med 88:47–60
4. Oikawa M, Kagaya Y, Otani H, Sakuma M, Demachi J, Suzuki J, Takahashi T, Nawata J, Ido T, Watanabe J et al (2005) Increased [18F]fluorodeoxyglucose accumulation in right ventricular free wall in patients with pulmonary hypertension and the effect of epoprostenol. J Am Coll Cardiol 45:1849–1855
5. Rich S, Pogoriler J, Husain AN, Toth PT, Gomberg-Maitland M, Archer SL (2010) Long-term effects of epoprostenol on the pulmonary vasculature in idiopathic pulmonary arterial hypertension. Chest 138:1234–1239
6. Stanley WC, Lopaschuk GD, Hall JL, McCormack JG (1997) Regulation of myocardial carbohydrate metabolism under normal and ischaemic conditions. Potential for pharmacological interventions. Cardiovasc Res 33:243–257
7. Randle PJ, Priestman DA, Mistry SC, Halsall A (1994) Glucose fatty acid interactions and the regulation of glucose disposal. J Cell Biochem 55(Suppl):1–11
8. Abozguia K, Clarke K, Lee L, Frenneaux M (2006) Modification of myocardial substrate use as a therapy for heart failure. Nat Clin Pract Cardiovasc Med 3:490–498
9. Gunes Y, Guntekin U, Tuncer M, Sahin M (2009) Improved left and right ventricular functions with trimetazidine in patients with heart failure: a tissue Doppler study. Hear Vessel 24:277–282
10. Kantor PF, Lucien A, Kozak R, Lopaschuk GD (2000) The antianginal drug trimetazidine shifts cardiac energy metabolism from

fatty acid oxidation to glucose oxidation by inhibiting mitochondrial long-chain 3-ketoacyl coenzyme A thiolase. Circ Res 86:580–588
11. McClellan KJ, Plosker GL (1999) Trimetazidine. A review of its use in stable angina pectoris and other coronary conditions. Drugs 58:143–157
12. Meng D, Feng L, Chen XJ, Yang D, Zhang JN (2006) Trimetazidine improved Ca^{2+} handling in isoprenaline-mediated myocardial injury of rats. Exp Physiol 91:591–601
13. Guarnieri C, Muscari C (1990) Beneficial effects of trimetazidine on mitochondrial function and superoxide production in the cardiac muscle. Cardiovasc Drugs Ther 4(Suppl 4):814–815
14. Wang P, Fraser H, Lloyd SG, McVeigh JJ, Belardinelli L, Chatham JC (2007) A comparison between ranolazine and CVT-4325, a novel inhibitor of fatty acid oxidation, on cardiac metabolism and left ventricular function in rat isolated perfused heart during ischemia and reperfusion. J Pharmacol Exp Ther 321:213–220
15. Stanley WC (2002) Partial fatty acid oxidation inhibitors for stable angina. Expert Opin Investig Drugs 11:615–629
16. Fragasso G, Spoladore R, Cuko A, Palloshi A (2007) Modulation of fatty acids oxidation in heart failure by selective pharmacological inhibition of 3-ketoacyl coenzyme-A thiolase. Curr Clin Pharmacol 2:190–196
17. Samudio I, Harmancey R, Fiegl M, Kantarjian H, Konopleva M, Korchin B, Kaluarachchi K, Bornmann W, Duvvuri S, Taegtmeyer H et al (2010) Pharmacologic inhibition of fatty acid oxidation sensitizes human leukemia cells to apoptosis induction. J Clin Invest 120:142–156
18. McCormack JG, Barr RL, Wolff AA, Lopaschuk GD (1996) Ranolazine stimulates glucose oxidation in normoxic, ischemic, and reperfused ischemic rat hearts. Circulation 93:135–142
19. Clarke B, Wyatt KM, McCormack JG (1996) Ranolazine increases active pyruvate dehydrogenase in perfused normoxic rat hearts: evidence for an indirect mechanism. J Mol Cell Cardiol 28:341–350
20. Fraser H, Belardinelli L, Wang L, Light PE, McVeigh JJ, Clanachan AS (2006) Ranolazine decreases diastolic calcium accumulation caused by ATX-II or ischemia in rat hearts. J Mol Cell Cardiol 41:1031–1038
21. Undrovinas AI, Belardinelli L, Undrovinas NA, Sabbah HN (2006) Ranolazine improves abnormal repolarization and contraction in left ventricular myocytes of dogs with heart failure by inhibiting late sodium current. J Cardiovasc Electrophysiol 17(Suppl 1):S169–S177
22. Antzelevitch C, Belardinelli L, Zygmunt AC, Burashnikov A, Di Diego JM, Fish JM, Cordeiro JM, Thomas G (2004) Electrophysiological effects of ranolazine, a novel antianginal agent with antiarrhythmic properties. Circulation 110:904–910
23. Sentex E, Helies-Toussaint C, Rousseau D, Lucien A, Ferrary E, Grynberg A (2001) Influence of trimetazidine on the synthesis of complex lipids in the heart and other target organs. Fundam Clin Pharmacol 15:255–264
24. Lopaschuk GD, Spafford MA, Davies NJ, Wall SR (1990) Glucose and palmitate oxidation in isolated working rat hearts reperfused after a period of transient global ischemia. Circ Res 66:546–553
25. Tuunanen H, Engblom E, Naum A, Nagren K, Scheinin M, Hesse B, Juhani Airaksinen KE, Nuutila P, Iozzo P et al (2008) Trimetazidine, a metabolic modulator, has cardiac and extracardiac benefits in idiopathic dilated cardiomyopathy. Circulation 118:1250–1258
26. Tabbi-Anneni I, Helies-Toussaint C, Morin D, Bescond-Jacquet A, Lucien A, Grynberg A (2003) Prevention of heart failure in rats by trimetazidine treatment: a consequence of accelerated phospholipid turnover? J Pharmacol Exp Ther 304:1003–1009
27. Guarnieri C, Muscari C (1988) Beneficial effects of trimetazidine on mitochondrial function and superoxide production in the cardiac muscle of monocrotaline-treated rats. Biochem Pharmacol 37:4685–4688
28. Piao L, Marsboom G, Archer SL (2010) Mitochondrial metabolic adaptation in right ventricular hypertrophy and failure. J Mol Med 88:1011–1020
29. Wilson SR, Scirica BM, Braunwald E, Murphy SA, Karwatowska-Prokopczuk E, Buros JL, Chaitman BR, Morrow DA (2009) Efficacy of ranolazine in patients with chronic angina observations from the randomized, double-blind, placebo-controlled MERLIN-TIMI (Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST-Segment Elevation Acute Coronary Syndromes) 36 Trial. J Am Coll Cardiol 53:1510–1516