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Metabolic Profiling of Hearts Exposed to Sevoflurane and Propofol Reveals Distinct Regulation of Fatty Acid and Glucose Oxidation

CD36 and Pyruvate Dehydrogenase as Key Regulators in Anesthetic-induced Fuel Shift

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ABSTRACT

Background: Myocardial energy metabolism is a strong predictor of postoperative cardiac function. This study profiled the metabolites and metabolic changes in the myocardium exposed to sevoflurane, propofol, and Intralipid and investigated the underlying molecular mechanisms.

Methods: Sevoflurane (2 vol%) and propofol (10 and 100 μM) in the formulation of 1% Diprivan® (AstraZeneca Inc., Mississauga, ON, Canada) were compared for their effects on oxidative energy metabolism and contractility in the isolated working rat heart model. Intralipid served as a control. Substrate flux through the major pathways for adenosine triphosphate generation in the heart, that is, fatty acid and glucose oxidation, was measured using [^{3}H]palmitate and [^{14}C]glucose. Biochemical analyses of nucleotides, acyl-CoAs, ceramides, and 32 acylcarnitine species were used to profile individual metabolites. Lipid rafts were isolated and used for Western blotting of the plasma membrane transporters CD36 and glucose transporter 4.

Results: Metabolic profiling of the hearts exposed to sevoflurane and propofol revealed distinct regulation of fatty acid and glucose oxidation. Sevoflurane selectively decreased fatty acid oxidation, which was closely related to a marked reduction in left ventricular work. In contrast, propofol at 100 μM but not 10 μM increased glucose oxidation without affecting cardiac work. Sevoflurane decreased fatty acid transporter CD36 in lipid rafts/caveolae, whereas high propofol increased pyruvate dehydrogenase activity without affecting glucose transporter 4, providing mechanisms for the fuel shifts in energy metabolism. Propofol increased ceramide formation, and Intralipid increased hydroxy acylcarnitine species.

Conclusions: Anesthetics and their solvents elicit distinct metabolic profiles in the myocardium, which may have clinical implications for the already jeopardized diseased heart.

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What We Already Know about This Topic

- Shifts is cardiac substrate metabolism are known to occur in diseased myocardium and after myocardial ischemia and reperfusion

What This Article Tells Us That Is New

- Sevoflurane and propofol differentially regulate myocardial fatty acid and glucose oxidation through specific intracellular mechanisms. These actions may account for the protective effects of sevoflurane on myocardial performance after ischemia and reperfusion injury

THE heart consumes vast amounts of adenosine triphosphate (ATP) to sustain contractile function, generated mainly through mitochondrial oxidation of fatty acids (70–90%) and carbohydrates (10–30%).[1] There are virtually no ATP stores in the myocardium, but a tight coupling among carbon substrate oxidation, oxidative phosphorylation, and cardiac performance allows ATP levels to remain constant over a broad range of workload.[2] The substrate preference and pathway flux for cardiac ATP production are regulated tightly through substrate–product relationships and activity modulation of metabolic enzymes, transporters, and associated regulatory enzymes.[2] Favoring the energetically economic glucose (3.17 ATP/oxygen molecule) over fatty acid oxidation (2.83 ATP/oxygen molecule) may be particularly beneficial in the mechanically stressed heart with only limited oxygen supply.[3] Pronounced metabolic shifts between fatty acid and glucose oxidation occur in a number of clinically important disease states, including obesity and diabetes,[4] heart remodeling and failure,[2] and ischemia–reperfusion injury.[5] To date, a large body of literature supports the concept that cardiac substrate metabolism critically affects the function in acute and chronic disease states[2] and that therapeutic strategies that switch the fuel preference away from fatty acids are able to enhance contractility and improve long-term outcome.[3] Hence, the modulation of cardiac energy metabolism by pharmacologic means seems a promising approach to combat perioperative contractile dysfunction and reduce cardiac complications.

We previously showed the differences in myocardial substrate metabolism between sevoflurane and propofol at the transcriptional level using high-density oligonucleotide microarrays.[6] Sevoflurane-mediated attenuation of transcripts involved in fatty acid oxidation was associated closely with improved postoperative cardiac function, as determined by transesophageal echocardiography and pulmonary artery catheter measurements, in patients undergoing off-pump coronary artery bypass graft surgery. In addition, isoflurane-induced down-regulation of transcripts involved in fatty acid oxidation was observed in Langendorff-perfused rat hearts, and the resulting metabolic remodeling triggered by brief ischemia or volatile anesthetics resembled "metabolic hibernation,"[7] which is indeed a hallmark of the preconditioned protected state of the myocardium.[8] So far, only a few studies have indirectly explored the effects of anesthetics on fuel preference and substrate shift in the heart.[6,9] However, none of these studies directly measured and compared the effects of anesthetics on metabolic flux rates of fatty acids and glucose. Therefore, we hypothesized that sevoflurane and propofol, two commonly used anesthetics, would differentially regulate the "metabolome" in the heart. To examine this, we profiled their effects on oxidative energy metabolism, intermediary metabolites, and contractility using the working rat heart model.

Materials and Methods

All experimental procedures conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (publication 85-23, revised 1996; National Academy Press, Washington DC). An expanded Materials and Methods section is available in Supplemental Digital Content 1, http://links.lww.com/ALN/A602.

Working Heart Perfusions, Left Ventricular Function, and Experimental Protocols

Male Sprague-Dawley rats (weight, 350–400 g), housed and treated according to the guidelines of the University of Alberta Animal Policy and Welfare Committee (Edmonton, Alberta, Canada) and the Canadian Council on Animal Care (Ottawa, Ontario, Canada), were deeply anesthetized with pentobarbital (150 mg/kg, intraperitoneally). Each heart was rapidly removed and perfused, as described previously.[10] Briefly, after cannulation of the aorta, a nonworking Langendorff perfusion with Krebs-Henseleit solution was initiated within 20 s. Ten minutes later, the working mode perfusion was established with a recirculating perfusate of 100 ml (37°C, pH 7.4) gassed with 95% O_2–5% CO_2 mixture consisting of a modified Krebs-Henseleit solution containing 4.7 mM KCl, 118 mM NaCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 11 mM glucose, 1.2 mM palmitate (prebound to 3% fatty acid–free bovine serum albumin), and insulin 100 mU/l. All constituents of the Krebs-Henseleit solution were purchased from Fisher Scientific (Ottawa, ON, Canada). Perfusions were performed at a constant workload (preload: 11.5 mmHg, after load: 80 mmHg). Heart rate and systolic and diastolic aortic pressures were determined, as described previously.[10] Cardiac output (ml/min) and aortic flow (ml/min) were measured using ultrasonic flow probes (Transonic T206; Transonic Systems Inc., Ithaca, NY) placed in the left atrial inflow and the aortic outflow lines. Left ventricular work (LVW) ($ml \cdot min^{-1} \cdot mmHg^{-1}$) was calculated as LVW = cardiac output × (aortic systolic pressure − preload) and served as a continuous index of left ventricular mechanical function. Coronary flow (ml/min) was calculated as the difference between cardiac output and aortic flow, and coronary vascular conductance (CVC; $ml \cdot min^{-1} \cdot mmHg^{-1}$) was computed as CVC = coronary flow/(mean aortic − pressure preload). Myocardial efficiency was defined as a ratio between LVW per tricarboxylic acid cycle produced acetyl-coenzyme A per gram dry weight. Isolated hearts were initially subjected to 45 min of perfusion for the measurement of baseline mechanical and metabolic functions. The hearts were then assigned to

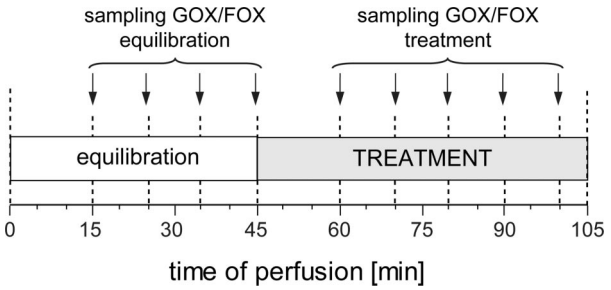

Fig. 1. Study protocol. All hearts were equilibrated for 45 min and aerobically perfused for an additional 60 min, during which sampling of the perfusate was performed to measure glucose oxidation (GOX) and fatty acid oxidation (FOX) rates. Hearts were assigned to one of the six treatment groups, as outlined in the Materials and Methods section.

one of the six groups and aerobically perfused for an additional 60 min, as outlined in figure 1: (1) control: time-matched perfusion without treatment, (2) SEVO: sevoflurane administered at 2 vol%, (3) PROP10: propofol 10 μM in the formulation of Diprivan® 1% (AstraZeneca, Mississauga, ON, Canada), (4) INTRA10: corresponding Intralipid (Sigma-Aldrich, Oakville, ON, Canada) control, (5) PROP100: propofol 100 μM, or (6) INTRA100: corresponding Intralipid control. Additional hearts (n = 8) were harvested at the end of equilibration (baseline). At the end of the perfusions, all hearts were immediately frozen in liquid nitrogen with Wollenberger clamps for later determinations of dry weights and molecular analyses.

Measurement of Metabolic Flux Rates: Glucose and Fatty Acid Oxidation

Glucose and fatty acid oxidation were measured simultaneously by perfusing hearts with [U-^{14}C]glucose and [9,10-^{3}H]palmitate, respectively, as described previously.[11] The total myocardial $^{14}CO_2$ production and $^{3}H_2O$ production were determined at 10-min intervals. The rates (expressed as micromolar per gram dry weight per minute) were calculated for each time interval and were averaged for the period of baseline and the period of treatment perfusions.

Measurements of Intracellular Calcium

The measurements of intracellular [Ca^{2+}] were performed in separate experiments (n = 5), as described previously.[10] The hearts were loaded with 5 μM fluorescent [Ca^{2+}] indicator indo-1AM (TEFLabs, Austin, TX) dissolved in dimethyl sulfoxide (final concentration <0.25%) for 25 min during the working mode perfusion (Supplemental Digital Content 2, fig. 1, http://links.lww.com/ALN/A603). Any remaining extracellular indo-1AM was flushed from the hearts for 5 min using a short period of Langendorff perfusion before the working mode perfusion was reestablished with fresh modified Krebs-Henseleit solution and a second perfusion circuit. Indo-1AM fluorescence was measured from a small area (~0.3 cm^2) of the epicardial surface of the left ventricle-free wall using a spectrofluorometer (Photon Technology International, London, ON, Canada). Signals were acquired at 500 Hz, and the ratio of indo-1AM fluorescence emitted at 405 and 485 nm (F405:F485 ratio) was calculated to provide an index of intracellular [Ca^{2+}].

Determinations of Nucleotides, Short-chain CoAs, Long-chain CoAs, and Ceramides

Nucleotides and cofactors (including adenosine, adenosine monophosphate, adenosine diphosphate, ATP, guanosine 5′-triphosphate, and nicotinamide adenine dinucleotide), short-chain CoAs (including free CoA, acetyl-CoA, malonyl-CoA, succinyl-CoA, and propionyl-CoA), long-chain acyl-CoA (16:0; 18:0; 18:1), and ceramide levels were measured after tissue extraction and high performance liquid chromatography.

Acylcarnitine Profiling Using Mass Spectrometry

Tissue levels of 32 acylcarnitine species were measured using electrospray ionization tandem mass spectrometry. Briefly, acylcarnitines were extracted from the heart tissue with methanol and quantified using eight isotopically labeled internal standards (Cambridge Isotopes Laboratories, Andover, MA). Precursor ions of m/z 85 in the mass range of m/z 150–450 were acquired on a PE SICEX API 365 LC-ESI-MS/MS instrument (Applied Biosystems, Foster City, CA).

Determination of Tissue Triglyceride Content and Incorporation of [9,10-^{3}H]Palmitate into Triglycerides

After chloroform–methanol extraction of lipids from the cardiac tissue, triglyceride content was quantified colorimetrically with the enzymatic assay kit L-Type Triglyceride M (Wako Pure Chemical Industries, Richmond, VA). Incorporation of [9,10-^{3}H]palmitate into triglycerides was counted.

Western Blotting of Key Metabolic Enzymes

The ratios of phosphorylated-to-total of acetyl-CoA carboxylase and of adenosine monophosphate-activated protein kinase, as well as malonyl-CoA decarboxylase and pyruvate dehydrogenase (PDH) kinase isozyme 4 expression levels in the cardiac tissue were determined by Western blotting, chemiluminescence, and densitometric image analysis.

Detergent-free Fractionation of Lipid Rafts/Caveolae from Heart Tissue

As described previously,[12] 0.9 ml of 500 mM Na_2CO_3 was added to 100 mg frozen powdered heart tissue and homogenized on ice using a chilled Polytron (3 × 10 s). The samples were sonicated on ice (3 × 20 s) and centrifuged at 1,000*g* for 10 min at 4°C. The supernatants were collected, and protein concentrations were measured by the Bradford assay (Bio-Rad, ON, Canada). One hundred microliters of the samples were diluted with equal volumes of cold 90% sucrose in 2-(*N*-morpholino)ethanesulfonic acid–buffered saline (25 mM 2-(*N*-morpholino)ethanesulfonic acid, 150 mM $NaCl_2$, pH 6.5) in the bottom of a Beckman MLS-50 rotor centrifuge tube (Beckman Coulter, Mississauga, ON, Canada). The total volume was brought to 1.2 ml by adding

cold 45% sucrose in 2-(*N*-morpholino)ethanesulfonic acid–buffered saline/Na_2CO_3 (1:1; v:v) and mixing gently. The samples were gently overlaid with 2.4 ml of cold 35% sucrose in 2-(*N*-morpholino)ethanesulfonic acid-buffered saline/Na_2CO_3, followed by 1 ml of cold 5% sucrose in 2-(*N*-morpholino)ethanesulfonic acid-buffered saline/Na_2CO_3. The samples were centrifuged in a Optima MAX-XP ultracentrifuge using the MLS-50 rotor (Beckman Coulter) at 175,000*g* for 18 h at 4°C. Three hundred microliters of fractions were removed from the top of the tube and stored at −80°C before Western blotting (48-μl samples, 10% acrylamide separating gel, 4% stacking gel) with the following primary antibodies: rabbit anti-CD36 (ab36977; Abcam, Cambridge, MA), rabbit anti-glucose transporter 4 (GLUT4) (Cell Signaling Technology, Danvers, MA), rabbit anti-caveolin-1 (610059; BD Biosciences, Mississauga, ON, Canada), mouse anti-caveolin-3 (610420; BD Biosciences), mouse anti-flotillin (610820; BD Biosciences), and rabbit anti–early endosome antigen 1 (ab2900; Abcam). All solutions contained protease (P8340; Sigma) and protein phosphatase (P5726 from Sigma plus 524628 from Calbiochem [Cabiochem/EMD Chemicals Inc., Gibbstown, NJ], distributed in Canada by VWR International, Edmonton, AB, Canada) inhibitor cocktails. Gel densitometry data (acquired using the public domain image processing program ImageJ##) of Western blots were normalized to total fraction amounts and given as percent intensity. To ensure similar loading, equal amounts of protein (Bradford assay) were used for lipid raft isolations. Also, CD36 and GLUT4 expressions were determined in total fractions.

Pyruvate Dehydrogenase Complex Activity Assay

PDH activity (pyruvate + CoA − SH + NAD^+ → acetyl − CoA + NADH + H^+ + CO_2) was measured by the radioisotopic coupled enzyme assay based on a previously described method,[13] which determines the PDH_{active}:PDH_{total} ratio. This ratio gives an indication of the phosphorylation state of PDH E1α subunit, which is subjected to regulation by PDH kinase isozyme 4 and PDH phosphatase. The multistep assay is described in detail in Supplemental Digital Content 1, http://links.lww.com/ALN/A602. Briefly, in a first step, duplicate tissue samples are homogenized in "active" (promoting preservation of native endogenous phosphorylation of sample PDH) and "total" buffer (promoting maximal sample PDH activity). In a second step, [^{14}C]oxaloacetate is generated from [^{14}C]aspartate using glutamate oxaloacetate transaminase. In a third step, chemical reactions to obtain active activity, that is, PDH activity under native PDH phosphorylation, and total activity, that is, maximal PDH activity under complete dephosphorylation, are performed. In a fourth step, [^{14}C]oxaloacetate is used to convert PDH reaction product acetyl-CoA into labeled [^{14}C]citrate and excess [^{14}C]oxaloacetate back into [^{14}C]aspartate. Finally, [^{14}C]aspartate is absorbed from the reaction mixtures, and scintillation counting of supernatant [^{14}C]citrate is performed and used to extrapolate the formation of acetyl-CoA from standard curves.

http://rsbweb.nih.gov/ij/. Accessed December 1, 2008.

Statistical Analysis

All values are expressed as mean (SD) for the indicated number of independent observations (n). The significance of the difference among groups was determined by ANOVA followed by the Student-Newman-Keuls method for *post hoc* analysis or by Kruskal-Wallis ANOVA on ranks followed by the Dunn method for *post hoc* multiple comparisons, depending on the underlying data distribution (nonparametric methods were necessary for some of the data presented in the Supplemental Digital Content 2, http://links.lww.com/ALN/A603). Repeated-measures, two-way ANOVA followed by a Holm-Sidak procedure for multiple comparisons was used to analyze time- and treatment-dependent changes in hemodynamic and metabolic variables. To test the association between changes in LVW and changes in fatty acid oxidation and glucose oxidation, linear regression analysis was performed. The correlation coefficient r and P value were reported. Differences were considered significant if $P < 0.05$. SigmaStat (version 3.5; Systat Software, Inc., Chicago, IL) was used for the analyses.

Results

Distinct Regulation of Fatty Acid and Glucose Oxidation in Sevoflurane- and Propofol-perfused Working Hearts

Sevoflurane at 1 minimum alveolar anesthetic concentration decreased palmitate oxidation after 1 h of perfusion by 23% compared with time-matched control hearts ($P = 0.011$), whereas no change in glucose oxidation was observed (fig. 2A). In contrast, propofol (100 μM) significantly increased glucose oxidation by 21% when compared with time-matched control ($P < 0.001$) and the Intralipid control hearts ($P = 0.013$), but it did not affect palmitate oxidation (figs. 2B and C). Acetyl-CoA turnover was markedly reduced in sevoflurane-perfused hearts ($P = 0.024$), but it was unchanged in propofol-perfused hearts (fig. 2). During the 1-h period of the metabolic measurements in the isolated working rat heart model of the current study, fatty acid oxidation decreased slightly, and glucose oxidation increased in untreated control hearts (fig. 2). However, these changes in energy metabolism have been reported previously for this model and reflect the metabolic adaptations of the isolated working heart to the *ex vivo* conditions.[14] Taken together, isolated working rat hearts exposed to sevoflurane and propofol show distinct regulation of fatty acid and glucose oxidation.

Left Ventricular Work and Energy Metabolism in Sevoflurane- and Propofol-perfused Hearts

Sevoflurane only marginally affected heart rate and peak systolic pressure, but it markedly reduced cardiac output, coronary flow, and LVW because of a decrease in stroke volume (Supplemental Digital Content 3, table 1, http://links.lww.com/ALN/A604). Conversely, propofol at even high concentrations did not affect the measured parameters. Despite the marked reduction in LVW in sevoflu-

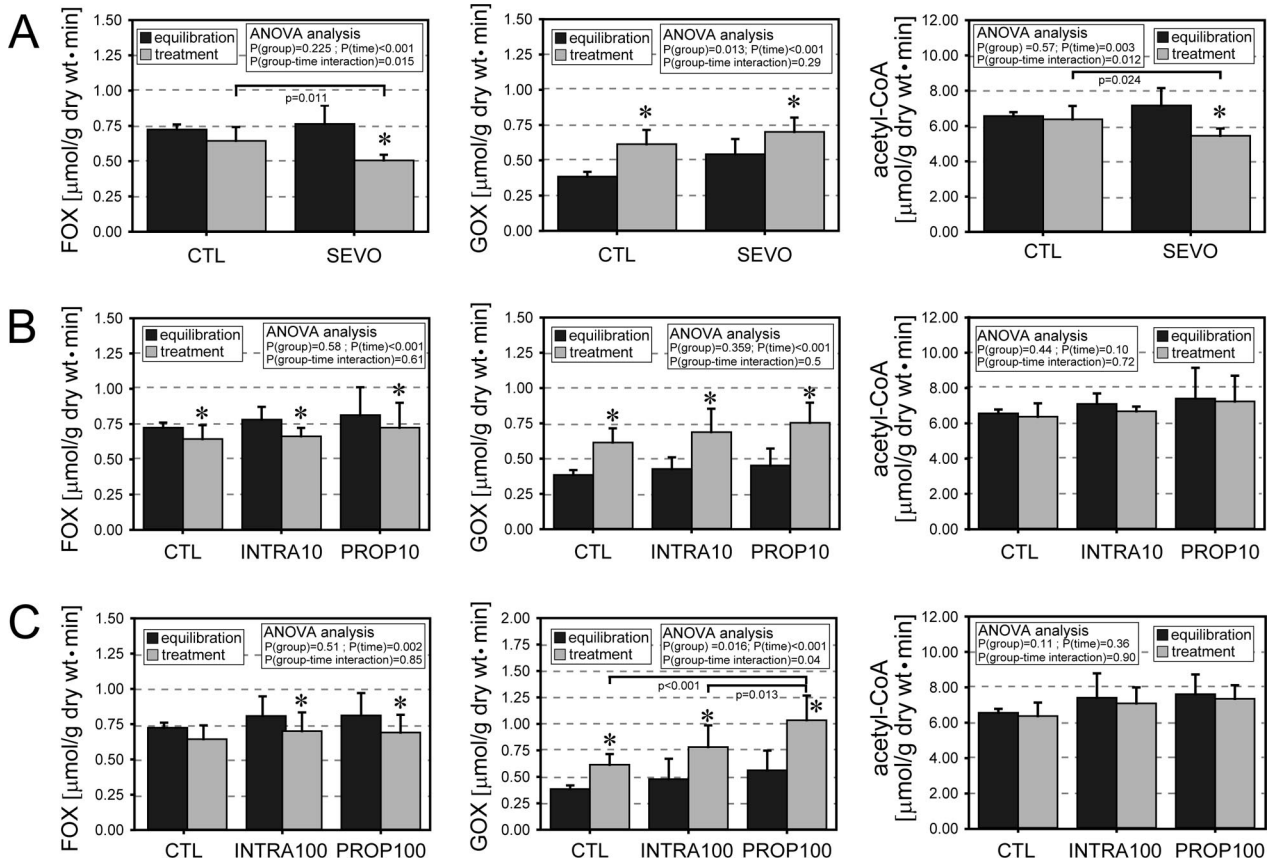

Fig. 2. Fatty acid oxidation (FOX), glucose oxidation (GOX), and total acetyl-CoA production in sevoflurane- and propofol-treated working rat hearts. (*A*) Sevoflurane (2 vol%) significantly reduced FOX and acetyl-CoA turnover but did not change GOX. (*B*) Propofol 10 μM did not lead to significant metabolic changes. (*C*) Propofol 100 μM increased GOX. Two-way repeated-measures ANOVA *P* values (group, time, and group-time interaction) are indicated in the *box*; *horizontal bars* and *P* values indicate significantly different comparisons for factor "treatment"; * Significantly different from corresponding equilibration value ($P < 0.05$; *post hoc* analysis, comparisons for factor "time"). CTL = time-matched control group (n = 5); INTRA10/INTRA100 = corresponding Intralipid control groups (n = 4 and n = 7, respectively); PROP10/PROP100 = propofol 10/100 μM (n = 4 and 8, respectively); SEVO = sevoflurane (2 vol%; n = 9); wt = weight.

rane hearts, myocardial efficiency was similarly maintained in sevoflurane and propofol hearts (Supplemental Digital Content 3, table 1, http://links.lww.com/ALN/A604). Because the decreased stroke volume was not accompanied by changes in systolic and diastolic Ca^{2+} levels (Supplemental Digital Content 2, fig. 2, http://links.lww.com/ALN/A603), our results suggest that sevoflurane desensitized contractile filaments to Ca^{2+} in the heart. When CVC was normalized to LVW, sevoflurane was found to significantly increase the conductance, whereas increasing concentrations of propofol had the opposite effect (fig. 3A; Supplemental Digital Content 3, table 1, http://links.lww.com/ALN/A604). LVW directly and closely correlated with changes in fatty acid and glucose oxidation ($r = 0.474$ and $r = 0.475$, respectively, $P = 0.003$; figs. 3B and C). ATP levels remained unchanged with both anesthetics (Supplemental Digital Content 2, fig. 3, http://links.lww.com/ALN/A603). Collectively, these findings provide evidence that sevoflurane, as opposed to propofol administration, leads to reduced mechanical work of the left ventricle, which is accompanied by a decrease in oxidative energy metabolism.

Sevoflurane Redistributes CD36—the Principal Fatty Acid Transporter in the Heart—from the Functional Lipid Raft/Caveolae Fraction to the Nonfunctional Nonraft Membrane and Endosomal Fractions

A series of additional experiments served to elucidate the molecular mechanisms underlying changes in fatty acid oxidation and glucose oxidation. First, sevoflurane decreased the myocardial triglyceride levels ($P = 0.009$) and reduced the incorporation of radioactive palmitate into triglycerides (fig. 4A and Supplemental Digital Content 2, fig. 4, http://links.lww.com/ALN/A603) ($P = 0.004$). Second, there were no changes in the expression and phosphorylation levels of key metabolic enzymes involved in the regulation of fatty acid and glucose metabolism after the administration of sevoflurane or propofol (Supplemental Digital Content 3, table 2, http://links.lww.com/ALN/A604). Third, the expression of CD36, the principal transporter of fatty acids in cardiomyocytes, was unchanged between groups and during the 1-h experimental period of metabolic measurements (Supplemental Digital Content 2, fig. 5A, http://links.lww.com/ALN/A603). However, lipid rafts/caveolae isolation by sucrose density gradi-

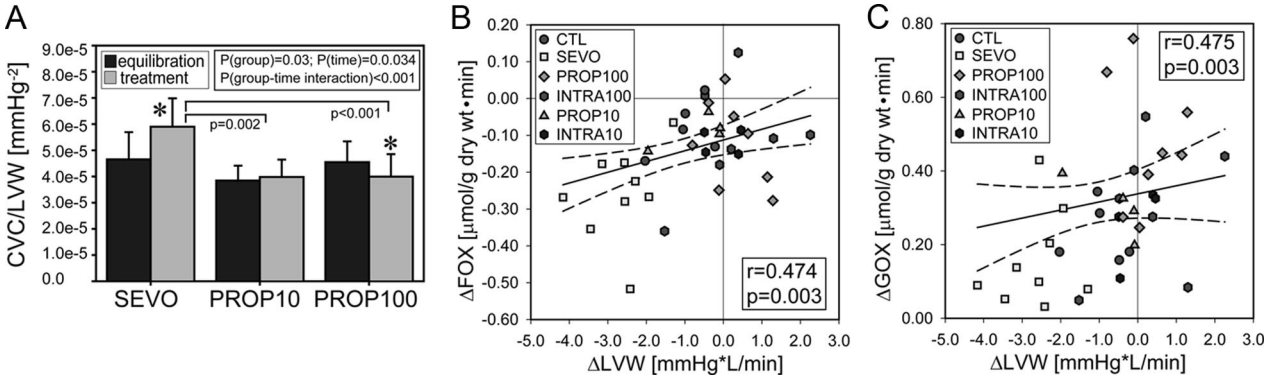

Fig. 3. Coronary vascular conductance, cardiac work, and metabolic shifts. (*A*) Coronary vascular conductance corrected by left ventricular work significantly increased after sevoflurane treatment, whereas the opposite occurred during propofol administration. Two-way repeated-measures analysis of variance *P* values (group, time, and group–time interaction) are indicated in the *box*; *horizontal bars* and *P* values indicate significantly different comparisons for factor "treatment"; * Significantly different from corresponding equilibration value ($P < 0.05$; *post hoc* analysis, comparisons for factor "time"). (*B*, *C*) Linear regression plots between changes in LVW and changes in fatty acid oxidation (*B*) and glucose oxidation (*C*) in the working hearts after 1 h of treatment. *Dashed lines* indicate 95% confidence intervals. CTL = time-matched control group (n = 5); CVC = coronary vascular conductance; FOX = fatty acid oxidation; GOX = glucose oxidation; INTRA10/INTRA100 = corresponding Intralipid control groups (n = 4 and 7, respectively); LVW = left ventricular work; PROP10/PROP100 = propofol 10/100 μM (n = 4 and n = 8, respectively); SEVO = sevoflurane (2 vol%; n = 9); wt = weight.

ent revealed significant redistribution of functional CD36 from the plasma membrane lipid rafts to the nonfunctional nonraft fractions, suggesting reduced CD36-mediated uptake of fatty acids into the heart as a potential mechanism of the observed reduced fatty acid oxidation in sevoflurane-treated hearts (figs. 4B and C). To determine whether fatty acid uptake into mitochondria by the carnitine palmitoyltransferase 1^2 and CD36 pathways[15] or dysfunctional β-oxidation was involved in the reduced fatty acid oxidation[16] in sevoflurane hearts, short- and long-chain CoA esters and 32 acylcarnitine species were profiled. None of these metabolites showed significant differences in sevoflurane-treated hearts when compared with time-matched perfused control hearts (Supplemental Digital Content 2, fig. 6, http://links.lww.com/ALN/A603, and Supplemental Digital Content 4, table, http://links.lww.com/ALN/A605). In accordance with these data, mitochondrial levels of CD36 were unaffected (Supplemental Digital Content 2, fig. 5B, http://links.lww.com/ALN/A603). Sevoflurane did not affect the distribution of GLUT4 in lipid rafts or PDH activity when compared with control hearts (Supplemental Digital Content 2, figs. 5C and D, http://links.lww.com/ALN/A603). These data provide evidence that reduced fatty acid oxidation in sevoflurane hearts is indeed the result of a decreased fatty acid uptake at the plasma membrane because of a shift of functional CD36 from the lipid rafts/caveolae to nonfunctional CD36 in plasma membrane or endosomes.

Propofol at High Concentrations Dephosphorylates Pyruvate Dehydrogenase and Increases Ceramide Formation, whereas Intralipid Increases Hydroxy-acylcarnitine Species in the Heart

To investigate the mechanisms of increased glucose oxidation in high propofol concentrations, GLUT4 expression and its content in lipid raft/caveolae fractions (active form of the transporter) were determined together with PDH complex phosphorylation levels and glycogen tissue content. PDH active-to-total ratio was markedly increased by propofol 100 μM (fig. 5A), indicative of decreased phosphorylation of the PDH complex, whereas glycogen tissue levels were unchanged (Supplemental Digital Content 2, fig. 4C, http://links.lww.com/ALN/A603). Propofol did not alter GLUT4 expression (Supplemental Digital Content 2, fig. 7A, http://links.lww.com/ALN/A603) or its predominant lipid raft localization (figs. 5B and C), but it enhanced ceramide formation at 100 μM (fig. 6A). Consistent with metabolic flux measurements, propofol or its solvent Intralipid did not affect the CD36 distribution in lipid rafts (Supplemental Digital Content 2, fig. 7B, http://links.lww.com/ALN/A603). Intralipid decreased acetylcarnitine levels (fig. 6B), but it increased hydroxy-acylcarnitine species, which was partly offset by concomitant propofol administration (fig. 6C). Based on the observed depletion of short-chain acylcarnitines (C2–C14) and accumulation of hydroxy-acylcarnitines, it can be concluded that Intralipid inhibits 3-hydroxy acyl-CoA-dehydrogenase (Supplemental Digital Content 2, fig. 8, http://links.lww.com/ALN/A603).[16] Compared with Intralipid, propofol also reduced tissue levels of succinyl-CoA, a potent inhibitor of carnitine palmitoyltransferase 1 (Supplemental Digital Content 2, fig. 6D, http://links.lww.com/ALN/A603). These data provide evidence that propofol and its vehicle Intralipid exert distinct metabolic effects in the heart.

Discussion

In this study, two clinically important anesthetics, sevoflurane and propofol, were analyzed for their effects on the cardiac metabolome. For this purpose, the isolated working

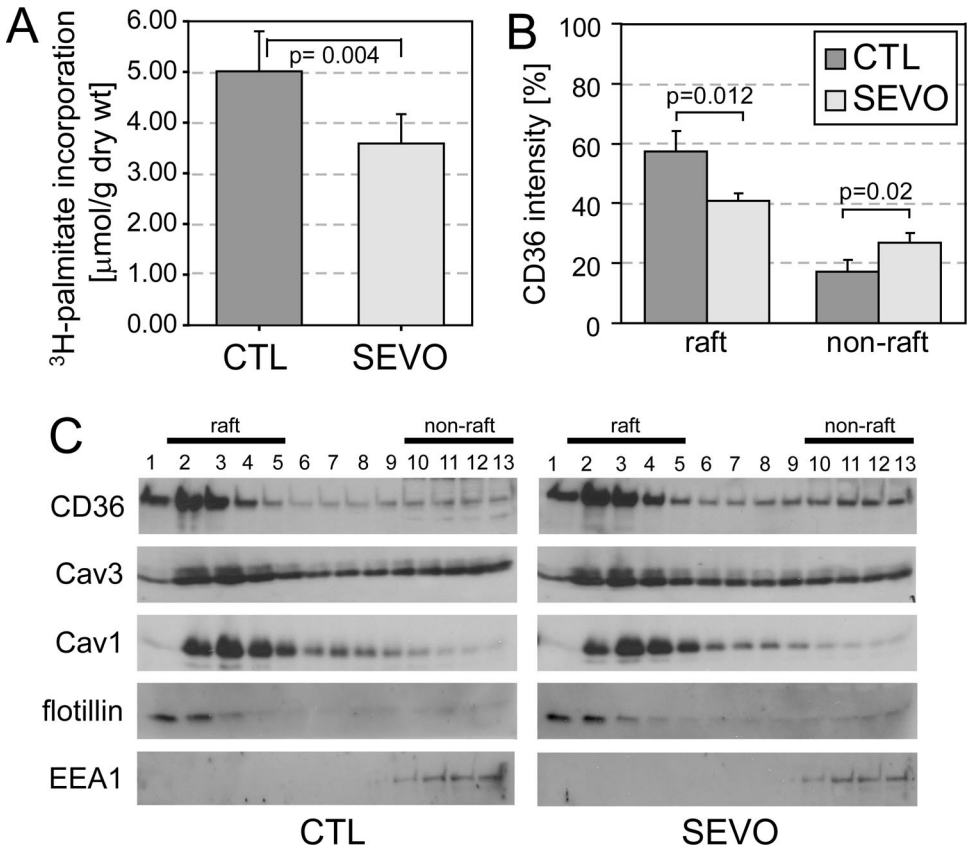

Fig. 4. Effects of sevoflurane (SEVO; 2 vol%; n = 5) on [^{3}H]palmitate incorporation into triglycerides and CD36 distribution between lipid rafts (functional CD36) and nonraft fractions (nonfunctional CD36). (*A*) Sevoflurane treatment markedly reduced [^{3}H]palmitate incorporation. (*B*) Sevoflurane redistributed CD36 from lipid rafts (fractions 2–5) to nonraft fractions (fractions 10–13), as measured by densitometry (n = 5 for each group). (*C*) Representative Western blots depicting the shift of CD36 elicited by sevoflurane from raft to nonraft fractions in the absence of changes of lipid raft markers caveolin-3 (Cav3), caveolin-1 (Cav1), flotillin, and nonraft marker early endosomal antigen 1 (EEA1). CTL = time-matched control group (n = 5).

rat heart model, which closely mimics the *in vivo* physiology of the heart and allows accurate measurements of metabolic flux rates, was used. Herein, we show the following salient findings. First, sevoflurane and propofol distinctly regulate fatty acid and glucose oxidation in the heart. While sevoflurane only marginally affects heart rate and systolic pressure, it markedly reduces LVW by decreasing stroke volume, most probably due to a desensitization of the contractile apparatus to Ca^{2+}. Nonetheless, myocardial efficiency is well preserved because reduced LVW is accompanied closely by lower myocardial energy consumption because of decreased fatty acid oxidation. In contrast, high (100 μM) but not lower concentrations of propofol increase glucose oxidation with only marginal effects on LVW or systolic or diastolic Ca^{2+} concentrations, providing evidence that sevoflurane, as opposed to propofol, reduces mechanical work and oxidative energy metabolism. Interestingly, while sevoflurane increases CVC per delivered work, propofol decreases it, an effect that may be due to the strong reactive oxygen species scavenging activity of propofol.[17] Second, our comprehensive analyses of metabolites reveal that sevoflurane does not affect mitochondrial fatty acid transport or β-oxidation but reduces the amount of functional CD36, the principal transporter of fatty acids through the sarcolemma, located in lipid rafts.[2,18] In contrast, propofol dephosphorylates and activates pyruvate dehydrogenase, the master regulatory enzyme of the glucose oxidation pathway,[2] and in addition increases formation of lipotoxic ceramides.[19–21] Finally, the propofol solvent Intralipid itself increases hydroxy-acylcarnitine species, which are known to accumulate in diabetic[22] and ischemic[23] hearts. Together, anesthetics and their solvents elicit distinct metabolic profiles in the myocardium, which may have potential clinical implications for the diseased heart.

CD36 and Fatty Acid Oxidation

The flux through the fatty acid metabolism pathway is regulated at two major points, that is, entry of fatty acids into the cell and into the mitochondria for β-oxidation. Fatty acids traverse the sarcolemma by either passive diffusion or protein-mediated transport, and CD36 seems to be the major fatty acid transporter protein in the heart.[2] The physiologic role of CD36 in regulating fatty acid metabolism was demonstrated in transgenic and knockout mice, where CD36 overexpression in the skeletal muscle enhanced fatty acid oxidation and decreased plasma lipids,[24] whereas CD36 deletion in the heart impaired fatty acid uptake, which was

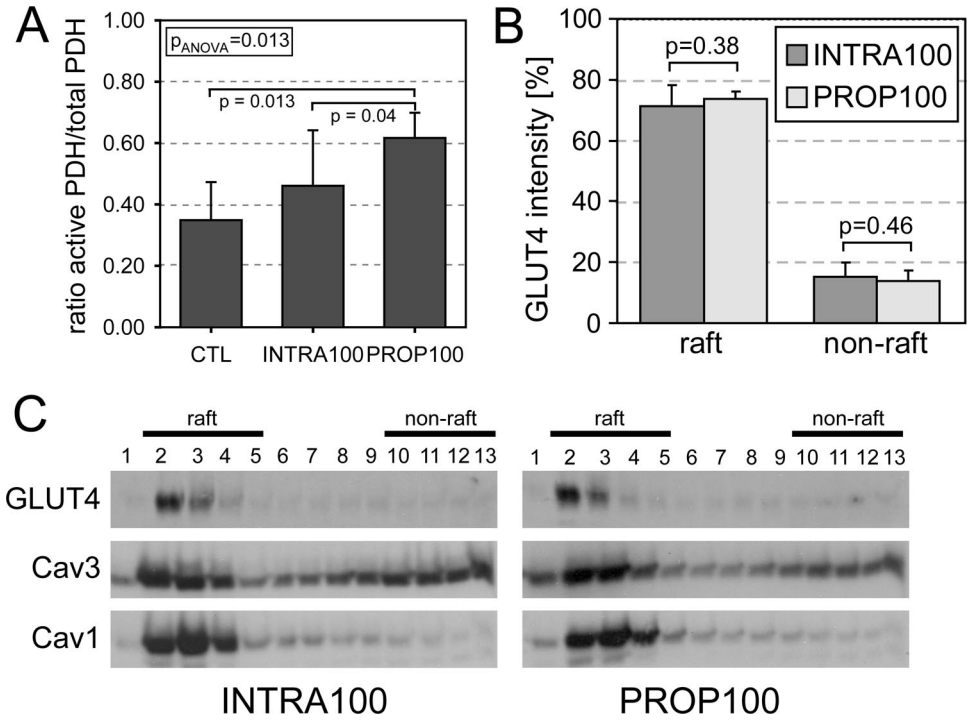

Fig. 5. Effects of propofol and Intralipid on pyruvate dehydrogenase (PDH) complex activity and the glucose transporter GLUT4 in lipid rafts. (*A*) Propofol (100 μM; PROP100) increased the ratio of active (dephosphorylated) to total PDH E1α subunit compared with time-matched perfused (CTL) and Intralipid control (INTRA100) hearts. (*B*) Propofol did not alter the distribution of GLUT4 from lipid rafts (fractions 2–5) to nonraft fractions (n = 5 for each group), as measured by densitometry. (*C*) Representative Western blots showing no changes in GLUT4 distribution. Caveolin-3 (Cav3) and caveolin-1 (Cav1) were used as lipid raft markers. PROP100 = propofol 100 μM (n = 5); INTRA100 = corresponding Intralipid control (n = 5).

compensated by increased glucose oxidation.[25] After entering the cell, the nonesterified fatty acids are bound to fatty acid-binding proteins and esterified to acyl-CoAs by acyl-CoA synthase, where they proceed toward triglyceride synthesis (10–30%) or undergo mitochondrial β-oxidation (70–90%) in the heart.[2] Transport of long-chain acyl-CoAs into the mitochondrial matrix is achieved by the carnitine-dependent transport system, in which the first step, mediated by carnitine palmitoyltransferase 1, has a major regulatory function in fatty acid oxidation.[2] Carnitine palmitoyltransferase 1 is strongly inhibited by malonyl-CoA, which serves as the key regulatory metabolite in the heart and is formed from extramitochondrial acetyl-CoA, through the actions of acetyl-CoA carboxylase and degraded by malonyl-CoA decarboxylase, ensuring a rapid turnover.[2] Another route for entry of fatty acids into mitochondria, albeit still controversial, involves mitochondrial CD36.[15] Once fatty acyl-CoAs have gained entry into mitochondria, they undergo the reactions of β-oxidation. Our results provide evidence for the first time that sevoflurane regulates cardiac fatty acid oxidation at the sarcolemma by specifically decreasing fatty acid transport through altered targeting of CD36 to lipid rafts/caveolae. In our experiments, CD36 total expression levels were unchanged after 1 h of sevoflurane treatment. Hence, CD36 turnover is not a factor in the observed decrease of fatty acid transport. Also, mitochondrial levels of CD36 were unaffected. The decreased [^{3}H]palmitate incorporation into cardiac triglycerides further supports reduced fatty acid transport as the cause of decreased fatty acid oxidation. No changes in malonyl-CoA levels or changes in expression and/or phosphorylation of acetyl-CoA carboxylase, adenosine monophosphate-activated protein kinase, or malonyl-CoA decarboxylase levels were observed. Because no changes in long-chain acyl-CoAs (which are mainly intramitochondrial) or levels of acylcarnitine species were detected, it can be concluded that sevoflurane did not affect transport of fatty acids into mitochondria or myocardial β-oxidation. Regulation of CD36 activity involves three major components: lipid raft/caveolae association,[26,27] intracellular translocation,[28] and protein turnover.[29] Caveolae are a subset of lipid rafts, which are specialized membrane microdomains compartmentalizing cellular processes. They can be isolated according to their detergent resistance as well as through detergent-free approaches.[30,31] Active plasma membrane CD36 was shown to distribute entirely within lipid raft membrane fractions, whereas intracellular CD36 was found in nonraft fractions.[18] Raft association of CD36 within the plasma membrane regulates fatty acid uptake activity.[27] Because translocation of CD36 from the intracellular storage pool to the sarcolemma is regulated by cardiac contraction,[32] it is likely that sevoflurane-induced decrease in cardiac work is causally related to the observed reduced fatty acid oxidation. Targeting of CD36 to lipid rafts might also involve interactions with caveolin-1.[33] Indeed, isoflurane was reported pre-

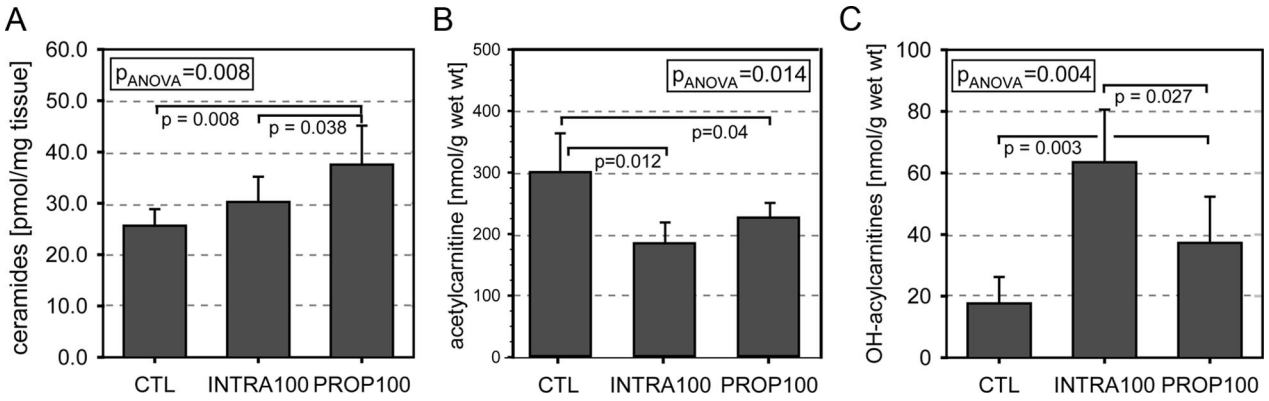

Fig. 6. Accumulation of ceramides and hydroxy-acylcarnitine species and depletion of acetylcarnitine after perfusion with propofol or Intralipid. (*A*) Propofol significantly increased ceramide levels compared with control and Intralipid hearts. (*B*) Both Intralipid and propofol significantly decreased acetylcarnitine levels compared with control. (*C*) Both Intralipid and propofol increased hydroxy-acylcarnitines (OH-acylcarnitines) levels compared with control hearts. CTL = control group with time-matched perfusion (n = 5); PROP100 = 100 μM propofol (n = 8); INTRA100 = corresponding Intralipid control (n = 7).

viously to increase caveolae formation and caveolin-1 content,[34] and isoflurane cardioprotection required caveolin-3 and caveolae.[35] However, under our experimental conditions, no changes in caveolin-1 or -3 targeting to lipid rafts/caveolae were measured as a result of sevoflurane treatment. Also, caveolin-1 knockout mice had increased cardiac fatty acid uptake,[36] and mice with deletion of caveolin-3, the major heart isoform, had normal uptake.[37]

Pyruvate Dehydrogenase and Glucose Oxidation

We here provide for the first time evidence that propofol increases cardiac substrate flux through the glucose oxidation pathway by decreasing PDH complex phosphorylation. During activation of glucose transport, GLUT4 is translocated toward caveolae, which is important for its stability and function,[38] but propofol did not affect caveolin or GLUT4 association with lipid raft/caveolae, whereas increased cytosolic adenosine diphosphate may have stimulated the phosphofructokinase-1 step in the glycolysis pathway.[2] Regulation of PDH activity, a key regulatory enzyme of glucose oxidation, involves reversible phosphorylation and substrate–product relationships. Mitochondrial PDH kinases (in the heart PDH kinase isozyme 4) phosphorylate serine residues of the E1α subunit and inactivate the PDH complex, whereas PDH phosphatases have the opposite effect.[39] The PDH reaction substrate pyruvate inhibits PDH kinases, whereas the reaction products acetyl-CoA and reduced nicotinamide adenine dinucleotide are stimulatory.[2] PDH phosphatases require Mg^{2+}, and the PDH phosphatase 1 isoform is stimulated by Ca^{2+}.[39] Our PDH enzymatic assay results suggest that propofol decreased PDH complex phosphorylation, and although the degree of phosphorylation of the PDH complex sets the maximum rate of pyruvate oxidation, the actual flux is determined by substrate and product concentrations within the mitochondrial matrix.[2] Propofol is known to increase mitochondrial Ca^{2+}, which could have enhanced PDH phosphatase activity.[40] Because propofol did not affect flux through the fatty acid oxidation pathway (nor CD36 targeting to lipid rafts), it is unlikely that signals from that metabolic pathway regulated glucose oxidation flux through mechanisms related to the "Randle cycle."[2]

Clinical Implications

Metabolic derangements in the heart, which occur during ischemic heart disease and diabetes, strongly impact cardiac efficiency and contractile function, likely affecting perioperative morbidity and mortality. Sevoflurane provides organ protection by pharmacologic preconditioning,[6,41,42] whereas propofol may have cardioprotective effects because of reactive oxygen species scavenging, specifically at higher concentrations.[17] Consistent with the previous observations of metabolic changes at the transcriptional level in human hearts,[6] our current study on anesthetic-induced changes at the metabolite level lends support to the preferred use of sevoflurane in a number of specific clinical scenarios. First, in the reperfused ischemic heart, there is accelerated fatty acid oxidation and excessive proton production because of uncoupling of glycolysis, leading to decreased cardiac efficiency. Our findings suggest that sevoflurane could improve these derangements and offer additional benefits by markedly reducing cardiac work and energy demand and increasing CVC. The reduction of fatty acid oxidation by sevoflurane is likely to spare oxygen and to relatively increase glucose oxidation,[2] resulting in more oxygen-efficient ATP generation and decreasing the imbalance between glycolysis and glucose oxidation to reduce the acidosis of cardiac ischemia. Second, cardiac lipotoxicity, the ectopic accumulation of lipids and lipid intermediates in the myocardium, can lead to myocyte dysfunction. Cardiac lipotoxicity plays a pivotal role in the pathogenesis of diabetic cardiomyopathy.[19,20,43] Lipid accumulation in the heart is thought to arise from an imbalance between fatty acid uptake and utilization, which is mainly β-oxidation in the case of the heart, giving rise to accumulation of triglycerides and ceramides as well as other free fatty acid metabolites. Interestingly, many animal models of cardiac lipotoxicity report increased pro-

apoptotic ceramide levels. Accordingly, the reduction of ceramide levels in the glycosylphosphatidylinositol (GPI)-anchored human lipoprotein lipase (LpL^{GPI}) mouse model was able to improve abnormal energy substrate utilization.[44] In other experimental models,[20,21] channeling of excess fatty acid toward triglyceride synthesis could prevent lipotoxicity, although excess triglyceride accumulation is also likely to harm. Rescue of cardiomyopathy in transgenic mice with cardiac-specific overexpression of peroxisome proliferator-activated receptor α by CD36 deficiency impressively exemplifies the crucial role of excess fatty acid uptake in the lipotoxic process.[45] Mismatch between fatty acid uptake and utilization is also the cause of lipotoxicity-induced cardiomyopathy in transgenic mice with cardiac-specific overexpression of long-chain acyl-CoA synthetase[46] and some mouse models of mitochondrial dysfunction.[47] Together, various perturbations in lipid homeostasis contribute to cardiac lipotoxicity, and its correction is accompanied by improved energy metabolism. Hence, down-regulation of fatty acid oxidation and CD36 function accompanied by lowering of triglyceride accumulation by sevoflurane may be particularly beneficial in the diabetic heart. Our data further support the notion that propofol and its solvent Intralipid differentially influence cardiac mitochondrial metabolism. Intralipid-induced accumulation of hydroxy-acylcarnitine species may result from an imbalance between the flavin adenine dinucleotide- and nicotinamide adenine dinucleotide-linked steps of β-oxidation.[48] Based on the previous reports from ischemic hearts,[23] hydroxy-acylcarnitine species can be regarded as markers of dysfunctional fatty acid metabolism.[22] In fact, the acylcarnitine profiles as observed in our experiments show that Intralipid or its byproducts block 3-hydroxyl-acyl-CoA dehydrogenase,[16] a pivotal step in the oxidation of fatty acids. On the other side, propofol induced ceramide accumulation, suggesting increased flux through serine palmitoyltransferase[49] or sphingomyelinase,[50] potentially mediated by increased mitochondrial Ca^{2+} levels[40] and disinhibition of carnitine palmitoyltransferase 1 by reduced succinyl-CoA levels. Although stimulation of pyruvate oxidation is regarded as beneficial within the diabetic heart, and our study shows a stimulatory effect of high propofol on glucose oxidation, ceramide accumulation by propofol is likely to be detrimental.[19,43,44] Finally, our observations raise the interesting possibility that lipotoxic ceramides, combined with Intralipid-induced inhibition of β-oxidation, might be involved causally in the pathogenesis of the so-called propofol infusion syndrome,[51] a rare cause of propofol-induced multiorgan failure closely related to mitochondrial failure.

Conclusions

In this study, we measured the changes in flux through the major pathways for energy metabolism in the working heart as a result of sevoflurane and propofol treatment. Novel mechanisms related to CD36 lipid raft targeting and activation of PDH were discovered. Our findings demonstrate that anesthetics profoundly and directly modulate the metabolome of the heart and further suggest that these changes could be of relevance, especially in patients with preexisting metabolic disorders.

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