

A novel role of endothelial autophagy as a regulator of myocardial fatty acid oxidation



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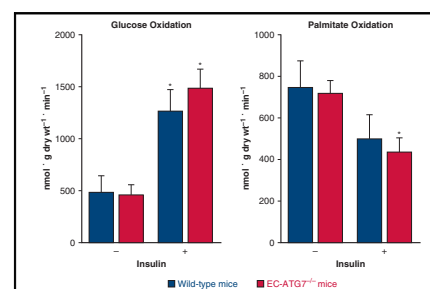
ABSTRACT

Background: We sought to determine if endothelial autophagy affects myocardial energy metabolism.

Methods: We used isolated working mouse hearts to compare cardiac function, energy metabolism, and ischemic response of hearts from endothelial cell-specific ATG7 knockout (EC-ATG7^{-/-}) mice to hearts from their wild-type littermates. We also conducted gene analyses on human umbilical vein endothelial cells incubated with scrambled small interfering RNA or small interfering ATG7.

Results: In the presence of insulin, working hearts from EC-ATG7^{-/-} mice, relative to those from wild-type littermates, exhibited greater reductions in insulin-associated palmitate oxidation indicating a diminished reliance on fatty acids as a fuel source. Likewise, palmitate oxidation was markedly lower in the hearts of EC-ATG7^{-/-} mice versus wild-type mice during reperfusion of ischemic hearts. Although hearts from EC-ATG7^{-/-} mice revealed significantly lower triacylglycerol content compared with those from wild-type mice, ATG7-silenced human umbilical vein endothelial cells demonstrated appreciably lower fatty acid binding protein 4 and 5 expression relative to those treated with scrambled small interfering RNA.

Conclusions: Disruption of endothelial autophagy reduces cardiac fatty acid storage and dampens reliance on fatty acid oxidation as a cardiac fuel source. The autophagy network represents a novel target for designing new strategies aimed at resetting perturbed myocardial bioenergetics. (J Thorac Cardiovasc Surg 2019;157:185-93)



Endothelial ATG7 loss reduces palmitate oxidation as a fuel source in response to insulin.

Central Message

The endothelial autophagy network in conjunction with FABP 4 and 5 may offer novel therapeutic targets for normalizing deranged myocardial mitochondrial bioenergetics.

Perspective

Myocardial contractile function imposes a significant metabolic demand on the body. Our results underscore the importance of endothelial cell autophagy in the regulation of fatty acid metabolism to feed the innate energy requirements of the heart. They also offer the possibility of the endothelial autophagy network as a novel target for cardiovascular therapeutics.

See Editorial Commentary page 194.

Myocardial contractile function imposes a significant metabolic demand on the heart. Under physiologic conditions, approximately 70% of the energy requirements of hearts are fueled by fatty acid oxidation with much of the remaining 30% derived from glucose oxidation.¹ Although

in the normal heart, fatty acid and glucose metabolism are intricately entwined and tightly regulated, this relationship can become progressively perturbed in heart disease, especially when there is an imbalance in the oxygen supply and demand axis.²

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Abbreviations and Acronyms

ATG7	= autophagy-related protein 7
CoA	= coenzyme A
EC	= endothelial cell
FABP	= fatty acid-binding protein
HUVEC	= human umbilical vein endothelial cell
I/R	= ischemia/reperfusion
siRNA	= small interfering RNA
TAG	= triacylglycerol
TCA	= tricarboxylic acid
WT	= wild-type

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Most of the attention has focused on understanding the myocardial metabolome within cardiomyocytes.³ In contrast, despite our appreciation that endothelial cells (ECs) and cardiomyocytes have an intimate relationship⁴ and that ECs play a fundamental role in the pathophysiology of ischemia/reperfusion (I/R),⁵ there has been relatively little reported on the EC-cardiomyocyte bioenergetic relationship and how derangements of this partnership could affect the energy demands of the myocardium and in turn myocardial contractility.

Autophagy, an essential and evolutionary well-conserved catabolic pathway that recycles cellular components to maintain cellular homeostasis and promote cellular survival, has been implicated in the pathophysiology of various cardiovascular diseases.⁶⁻⁸ We previously reported that autophagy-related protein 7 (ATG7), and accordingly autophagy, is key to the paracrine regulation of EC-released vasoactive substances,⁹ whereas disruption of ATG7 expression and therefore the autophagy machinery contributes to the pathophysiology of thrombosis¹⁰ and I/R injury.¹¹ Our group and others have further demonstrated a bidirectional regulatory relationship between autophagy and cholesterol homeostasis, thus implicating a role for autophagy in potentially regulating the metabolic network.^{12,13}

We sought to determine if endothelial autophagy may be a yet unrecognized regulator of myocardial energy metabolism. To this aim, functional and biochemical assessments were conducted on hearts isolated from EC-specific ATG7 knockout (EC-ATG7^{-/-}) mice. To provide a molecular context, concomitant gene analyses were performed with ATG7-silenced human umbilical vein endothelial cells (HUVECs).

MATERIALS AND METHODS

Endothelial Cell-Specific ATG7 Knockout Mice

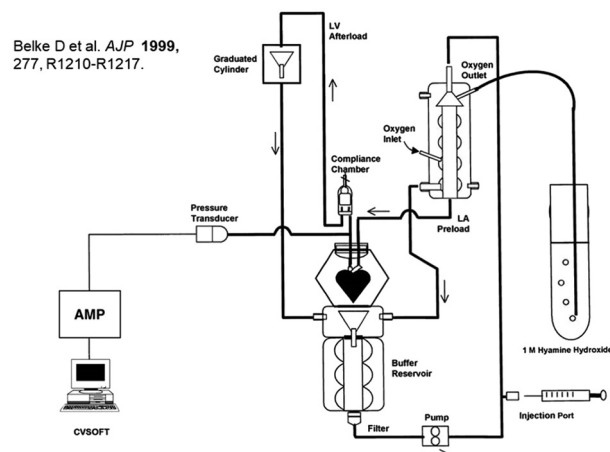
The EC-ATG7^{-/-} mice used in the studies described herein have been previously described and characterized.^{10,12,14} In short, EC-ATG7^{-/-} mice were the progeny of ATG7^{fllox/flox} and VE-Cadherin-Cre transgenic mice (The Jackson Laboratory, Bar Harbor, Me; Stock # 006137). Ex vivo experiments were conducted on hearts isolated from anesthetized (12 mg sodium pentobarbital, intraperitoneal) age-matched (22- to 36-week old) male EC-ATG7^{-/-} and wild-type (WT) littermate controls. A total of 6 WT and 8 EC-ATG7^{-/-} mice were used in the aerobic perfusion set, and 15 WT and 23 EC-ATG7^{-/-} mice were initially used in the I/R protocol before excluding 1 WT and 5 EC-ATG7^{-/-} mice from metabolic assessment because of development of arrhythmias. All animal-related protocols were approved by the University of Alberta Health Sciences Animal Welfare Committee and the St Michaels Hospital Animal Care Committee and conformed to the guidelines of the Canadian Council of Animal Care.

Ex Vivo Aerobic Heart Perfusion Protocol

Cannulated working hearts were perfused as described previously¹⁵ (Video 1) with an aerated (95% O₂/5% CO₂) modified Krebs–Henseleit bicarbonate solution maintained at 37°C and containing (in mmol/L) 118.5 NaCl, 25 NaHCO₃, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 2.5 CaCl₂, and 5 glucose with 0.8 palmitate bound to 3% bovine serum albumin as well as trace amounts of [U-¹⁴C]glucose and [9,10-³H]palmitate. At the end of 30 minutes, insulin (100 μU/mL) was added to the perfusate after which the hearts were continually perfused for a further 30 minutes. Steady-state glucose and palmitate oxidative rates were concurrently measured via quantitative collection of ¹⁴CO₂ and ³H₂O, both products of glucose and palmitate metabolism.¹⁵

Ex Vivo Ischemic-Reperfusion Heart Perfusion Protocol

Cannulated working hearts were perfused under aerobic conditions for 30 minutes with palmitate-supplemented (1.2 mmol/L) Krebs–Henseleit bicarbonate solution kept at 37°C. This was followed by 20 minutes of global no-flow ischemia then 40 minutes of aerobic reperfusion. The palmitate levels added served to simulate the enhanced fatty acid levels typically observed under pathologic I/R conditions.^{1,2} Cardiac functional parameters of heart rate, peak systolic pressure, and cardiac output were measured over time



VIDEO 1. A short video showing a brief explanation of the isolated working mouse heart perfusion. Video available at: [https://www.jtcvs.org/article/S0022-5223\(18\)32038-5/fulltext](https://www.jtcvs.org/article/S0022-5223(18)32038-5/fulltext).

with an MP100 system coupled to the AcqKnowledge software from BIOPAC Systems, Inc (Goleta, Calif). Cardiac work was calculated as the product of the corresponding peak systolic pressure and cardiac output values, whereas cardiac efficiency was assessed by normalizing cardiac work to the rate of acetyl coenzyme A (CoA) production, derived from glucose and palmitate oxidation (2 and 8 acetyl CoA molecules produced per 1 molecule of glucose and palmitate, respectively). At the end of the perfusion protocols, the hearts were freeze-clamped in liquid nitrogen and stored at -80°C until they were processed for biochemical analyses.

Triacylglycerol Assay

Lipids were extracted from frozen ventricle samples (10 mg) of murine hearts using a modified version of the Bligh and Dyer method.¹⁶ Samples were homogenized in a 2:1 chloroform-methanol mixture then mixed with a 20% volume of methanol. The suspension was centrifuged at 3500g for 10 minutes at 4°C , and the supernatant mixed with a 20% volume of 0.04% CaCl_2 . After a second spin of 2400g for 20 minutes at 4°C , the upper phase was discarded and the interface washed 3 times with a (by volume ratio) chloroform (3):methanol (48):water (47) mixture. Each sample was mixed with 50 μL methanol then dried under nitrogen at 60°C . The resultant pellet was dissolved in a mixture of (by volume ratio) tert-Butyl alcohol (3): Triton X-100 (1): methanol (1) and levels of myocardial triacylglycerol (TAG) measured with a colorimetric assay kit (Wako Chemicals, Richmond, Va).

Real-Time Polymerase Chain Reaction

HUVECs (Lonza, Walkersville, Md), cultured as previously described, were treated for 48 hours with small interfering RNA (siRNA) targeted at *ATG7* (siATG7) or scrambled siRNAs (both from Ambion, Foster City, Calif).¹⁴ Complementary DNAs, synthesized from total RNA using the Quantitect kit (Qiagen, Germantown, Md), were processed for quantitative analyses of *ATG7*, *CD36*, fatty acid-binding proteins (FABPs), and glyceraldehyde 3-phosphate dehydrogenase by real-time polymerase chain reaction using the StepOnePlus™ Real-Time polymerase chain reaction System (Applied Biosystems, Foster City, Calif).

Statistical Analysis

Data are presented as mean \pm standard error of the mean. Statistical significance was determined with an unpaired *t* test when comparing WT with EC-ATG7^{-/-} mice or paired *t* test when comparing before and after consecutive treatments within the same genotype (ie, \pm insulin or pre- and postischemia). Two-way analysis of variance with repeated measures was used for cardiac functional data assessed over the duration of perfusions.

RESULTS

Endothelial-Specific *ATG7* Deletion Is Associated With Reduced Myocardial Fatty Acid Oxidation in Response to Insulin

Under basal aerobic conditions, the cardiac work of hearts isolated from EC-ATG7^{-/-} mice appeared indistinguishable from those of their WT littermate controls and remained stable during the 60 minutes observation window (Figure 1, A). Insulin perfusion led to marked and significant increases in glucose oxidation rates: approximately 2.6-fold for hearts from WT mice and approximately 3-fold for hearts from EC-ATG7^{-/-} mice (Figure 1, B; $P = .0022$ and $.0005$, respectively). In contrast, palmitate oxidation rates were decreased by insulin (Figure 1, C) although only the change observed with EC-ATG7^{-/-} mouse hearts reached statistical significance ($P = .012$).

Tricarboxylic acid (TCA) cycle acetyl CoA production rates, in the absence and presence of insulin, did not differ between the hearts from EC-ATG7^{-/-} mice and those from their WT littermates (Figure 1, D). In the absence of insulin, the acetyl CoA for the TCA cycle in the 2 groups of hearts was fed to similar extents by the intrinsic glucose and fatty oxidation pathways (Figure 1, E). In the presence of insulin, however, hearts from the 2 groups of mice displayed enhanced contribution of glucose oxidation to energy production compared with palmitate oxidation (Figure 1, E; $P = .0013$, WT w/insulin vs basal) and ($P = .0010$, EC-ATG7^{-/-} w/insulin vs basal). Cardiac efficiency for both groups of hearts was similar (Figure 1, F).

Endothelial-Specific *ATG7* Deletion Is Associated With Augmented Cardiac Glucose Oxidation, Diminished Cardiac Fatty Acid Oxidation and Reduced Cardiac Efficiency After Ischemia/Reperfusion Insult

Cardiac work measured in the hearts from EC-ATG7^{-/-} and WT mice, before, during, and after I/R was similar with approximately 50% recovery detected in both cases (Figure 2, A). Notably, there were no functional differences between the 2 groups of hearts, although their metabolic profiles displayed distinct differences as explained next. This finding is surprising to us because we expected an improved postischemic recovery in EC-ATG7^{-/-} hearts given the fact that these hearts exhibited lower reliance on palmitate (the less efficient substrate) during reperfusion ($P = .0436$ vs WT, Figure 2, C) as opposed to greater reliance on glucose during both the preischemic period (EC-ATG7^{-/-} 2984 ± 598 vs WT 1242 ± 184 nmol/g dry weight/min; $P = .0139$) and reperfusion (recovery) (EC-ATG7^{-/-} 2804 ± 561 vs WT 900.7 ± 91 nmol/g dry weight/min; $P = .0054$) (Figure 2, B). Intact autophagy has been correlated with better recovery in cardiac I/R models.¹⁷ Although the impairment of autophagy was confined to ECs, it may have hindered postischemic recovery even in these metabolically efficient hearts. The salutary effect of decreased palmitate oxidation and increased glucose oxidation in EC-ATG7^{-/-} compared with their WT controls was most likely masked by the fact that in control hearts, palmitate oxidation was unchanged or even tending to decrease during reperfusion instead of increasing as we normally see. The reason behind this variation is unclear but may stem from some collateral effects accompanying the genetic manipulation in these mice. Although palmitate oxidation rates for the 2 groups of mice were comparable before ischemia (Figure 2, C), palmitate oxidation rates decreased only in EC-ATG7^{-/-} mice during reperfusion compared with preischemia ($P = .0312$) (Figure 2, C). Of note, the enhanced difference in glucose oxidation rates between EC-ATG7^{-/-} and WT hearts compared with the

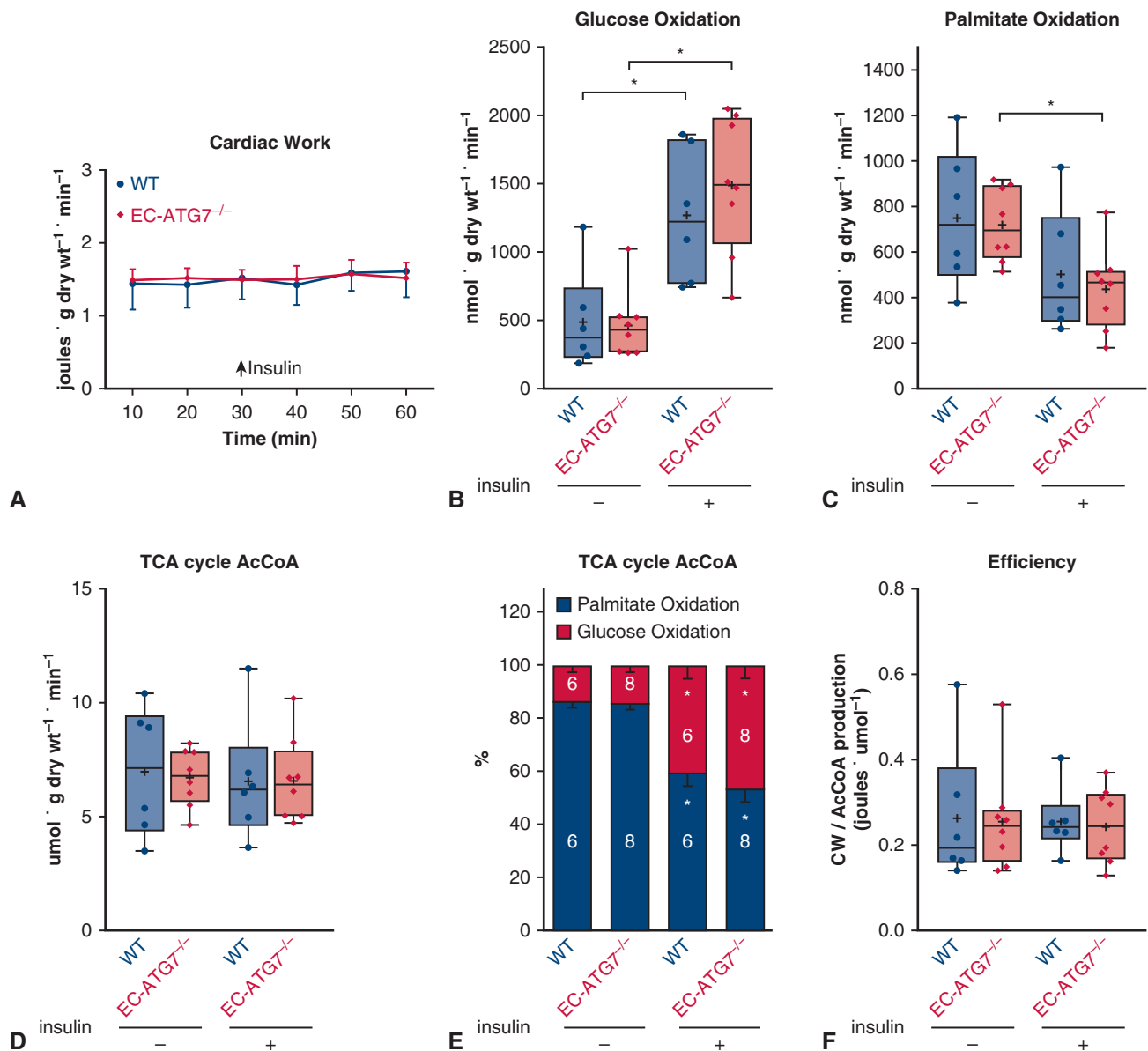


FIGURE 1. Endothelial-specific ATG7 deletion is associated with reduced myocardial fatty acid oxidation in response to insulin. The functional and metabolic capacities of hearts from EC-ATG7^{-/-} mice and their WT littermates were measured or calculated under aerobic conditions using the isolated working heart system in the absence and presence of insulin. A, Cardiac work during 60 minutes of ex vivo working heart perfusion, not significantly different using 2-way analysis of variance with repeated measures. B, glucose oxidation ($P = .0022$, WT w/insulin vs basal), ($P = .0005$, EC-ATG7^{-/-} w/insulin vs basal). C, Palmitate oxidation rates ($P = .012$, EC-ATG7^{-/-} w/insulin vs basal). D, TCA cycle acetyl CoA production from glucose and palmitate oxidation combined calculated as explained in “Materials and Methods.” E, Percent contribution of glucose and palmitate oxidation to total TCA cycle acetyl CoA production presented as stacked bars; n numbers are shown in the figure. The error bars represent standard error of the mean of % acetyl CoA individually produced by glucose ($P = .0013$, WT w/insulin vs basal) and ($P = .0010$, EC-ATG7^{-/-} w/insulin vs basal) and palmitate oxidation ($P = .0013$ WT w/insulin vs basal) and ($P = .0010$, EC-ATG7^{-/-} w/insulin vs basal). F, Cardiac efficiency calculated as explained in “Materials and Methods.” Data in B, C, D, and F are presented as box-and-whiskers plots where the upper and lower borders of the box represent the upper and lower quartiles, the horizontal line inside the box represents the median, the upper and lower whiskers represent the maximum and minimum values of nonoutliers, and the + sign represents the mean. WT n = 6, EC-ATG7^{-/-} n = 8 for all parameters displayed in A to F; * $P < .05$ versus corresponding data collected in the absence of insulin, paired t test. WT, Wild-type; TCA, tricarboxylic acid.

aerobic perfusions above (with insulin) may have been accentuated by the higher palmitate concentration used (and thus metabolism) in the I/R set, which in turn would

suppress glucose oxidation in normal hearts (ie, WT) unlike hearts with presumably impaired fatty acid oxidation (ie, EC-ATG7^{-/-}).

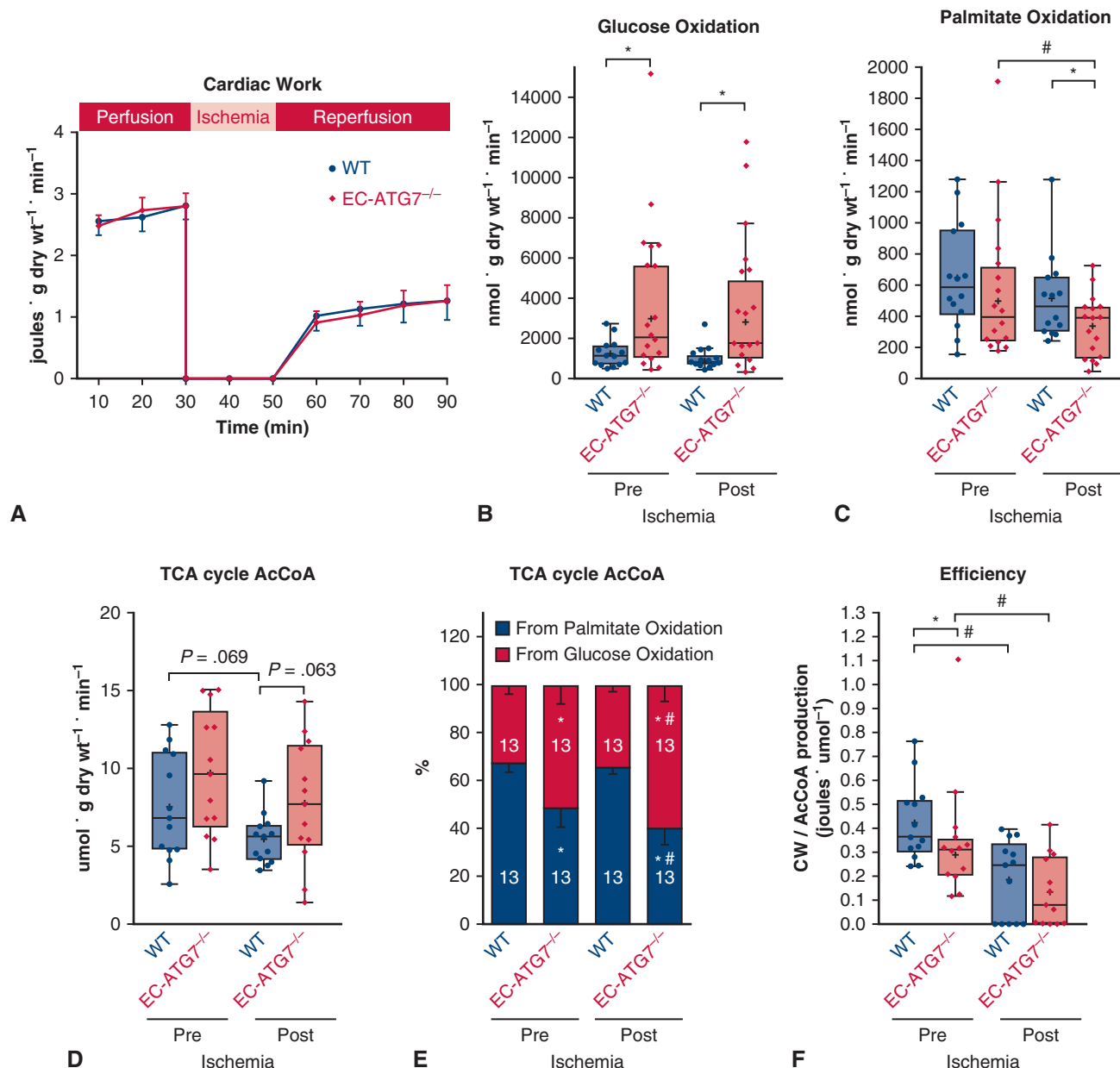


FIGURE 2. Endothelial-specific ATG7 deletion is associated with augmented cardiac glucose oxidation, diminished cardiac fatty acid oxidation, and reduced cardiac efficiency after I/R insult. The functional and metabolic capacities of hearts from EC-ATG7^{-/-} mice and their WT littermates were measured or calculated under I/R conditions in isolated working hearts both pre- and postischemia. A, Cardiac work during 90 minutes of ex vivo working heart perfusion, not significantly different using 2-way analysis of variance with repeated measures, WT n = 14, EC-ATG7^{-/-} n = 23. B, Glucose oxidation, WT n = 14, EC-ATG7^{-/-} n = 23. EC-ATG7^{-/-} versus WT, $P = .0139$ and $P = .0054$ in the preischemia and postischemia periods, respectively. C, Palmitate oxidation rates WT n = 14, EC-ATG7^{-/-} n = 17. $P = .0436$ for EC-ATG7^{-/-} versus WT during reperfusion. $P = .0312$ for postischemia versus preischemia EC-ATG7^{-/-}. D, TCA cycle acetyl CoA production from glucose and palmitate oxidation combined calculated as explained in methods, WT n = 13, EC-ATG7^{-/-} n = 13. E, Percent contribution of glucose and palmitate oxidation to total TCA cycle acetyl CoA production presented as stacked bars, n numbers are shown in the figure. The error bars represent standard error of the mean of % acetyl CoA individually produced by glucose and palmitate oxidation (EC-ATG7^{-/-} vs WT, $P = .0493$ and $P = .0030$ in the preischemia and postischemia periods, respectively. $P = .0276$ for postischemia vs preischemia EC-ATG7^{-/-}). F, Cardiac efficiency calculated as explained in “Materials and Methods.” WT n = 13, EC-ATG7^{-/-} n = 13. $P = .0305$ for EC-ATG7^{-/-} versus WT in the preischemia period, $P < .0001$ between post- and preischemic rates for both of the 2 genotypes. Data in B, C, D, and F are presented as box-and-whiskers plots where the upper and lower borders of the box represent the upper and lower quartiles, the horizontal line inside the box represents the median, the upper and lower whiskers represent the maximum and minimum values of nonoutliers, and the + sign represents the mean. Outliers were excluded from statistical tests. * $P < .05$ versus corresponding data from the hearts of the WT group within the same period, unpaired t test; # $P < .05$ versus corresponding preischemia data, paired t test. WT, Wild-type; TCA, tricarboxylic acid.

During reperfusion, TCA cycle acetyl CoA production rates in EC-ATG7^{-/-} hearts tended to be greater than those in WT hearts (Figure 2, D; $P = .063$). This heightened energy drive appeared to be fueled by elevations in glucose oxidation rates that increased significantly, most likely as a result of the decrease in palmitate use. Both before ischemia and during reperfusion, EC-ATG7^{-/-} hearts exhibited lower contribution of palmitate oxidation in the feeding of TCA cycle with acetyl CoA (Figure 2, E; $P = .0493$ and $P = .0030$ vs WT preischemia and WT postischemia, respectively). The greater energy consumption in EC-ATG7^{-/-} versus WT hearts before ischemia to maintain comparable level of cardiac work translated into lower cardiac efficiency compared with WT hearts (Figure 2, F; $P = .0305$). Both animal groups displayed decreased

efficiency postischemia compared with preischemic values due to the largely declined function (Figure 2, F; $P < .0001$).

Endothelial-Specific ATG7 Deletion Lowers Cardiac TAG Stores and Expression of Endothelial Fatty Acid Binding Proteins

Because the TAG levels in the heart ventricles from EC-ATG7^{-/-} mice were approximately half of those detected in the WT littermates (Figure 3, A; $P = .0234$), we hypothesized that endothelial-specific deletion of ATG7 may be associated with dysfunctional lipid transport. Figure 3, B and C, show the confirmation of ATG7 knock-down in HUVECs treated with siRNA against ATG7 ($P = .0002$). Real-time polymerase chain reaction analyses indicated that CD36 expression in

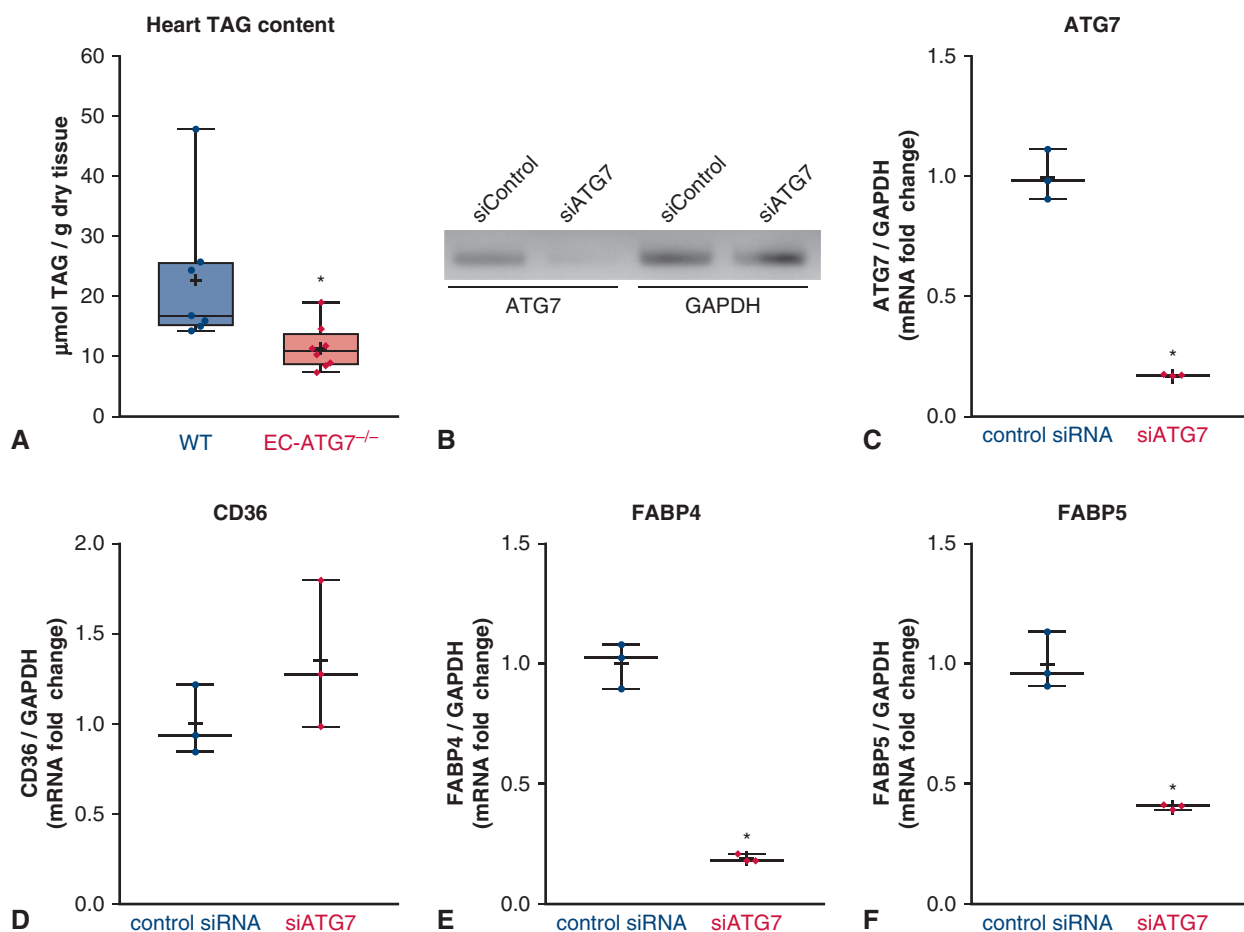


FIGURE 3. Endothelial-specific ATG7 deletion lowers cardiac TAG stores and expression of endothelial fatty acid binding proteins. A, TAG levels in freshly harvested, flash-frozen ventricles from EC-ATG7^{-/-} mice and their WT littermates, $n = 7-8$. $P = .0234$ between the 2 groups. B, Agarose gel electrophoresis of real-time polymerase chain reaction from total RNA of HUVEC cells treated with siRNA showing successful knock-down of ATG7 in cells treated with siATG7. ATG7 ($P = .0002$) (C), CD36 ($P = .2494$) (D), FABP4 ($P = .0001$) (E), and FABP5 ($P = .0010$) (F), expression in siATG7- and scrambled (control) siRNA-treated HUVECs. GAPDH served as the housekeeping gene. Real-time polymerase chain reaction results represent the mean of 3 independent experiments conducted in triplicates. Data in A and C to F are presented as box-and-whiskers plots where the upper and lower borders of the box represent the upper and lower quartiles, the horizontal line inside the box represents the median, the upper and lower whiskers represent the maximum and minimum values of nonoutliers, and the + sign represents the mean. * $P < .05$ versus corresponding WT or scrambled siRNA-treated group, unpaired t test. TAG, Triacylglycerol; ATG7, autophagy-related protein 7; siRNA, small interfering RNA.

ATG7-silenced HUVECs did not differ appreciably from that in scrambled siRNA-transfected HUVECs (Figure 3, D, $P = .2494$). The expression of FABP4 and FABP5, on the other hand, was significantly lower in siATG7-treated HUVECs relative to that in the scrambled siRNA-transfected cells (Figure 3, E and F; $P = .0001$ and $.0010$, respectively).

DISCUSSION

Normal functioning hearts have high energy demands that are supported mainly by fatty acid and glucose oxidation.² Evidence to date suggests that the pathophysiology of several chronic cardiac disorders may stem from, or are exacerbated, by anomalies in myocardial energy substrate metabolism.¹⁸ For example, in the diabetic and ischemic heart, there is an increased reliance on fatty acids oxidation compared with glucose, whereas in the failing heart and ischemic heart, mitochondrial oxidative phosphorylation is suppressed in relation to glycolysis with uncoupling of

glycolysis and glucose oxidation, all of which contribute to cardiac deficiency and contractile dysfunction.^{1,19,20} Therefore, identifying regulatory checkpoints in the metabolic network of the heart can help in the designing of new therapeutics for the modulation of cardiac energy metabolism.^{1,21}

We report in this work that disruption of normal EC autophagic flux decreases EC FABP expression and suggest that this molecular anomaly may account in part for the lower TAG levels detected in hearts from EC-ATG7^{-/-} mice relative to those from their WT littermate controls. The smaller myocardial TAG pool may partially explain why hearts isolated from EC-ATG7^{-/-} mice appeared less reliant on palmitate oxidation as a fuel source during both insulin stimulation and after ischemia induction in our I/R model.

The heart is a metabolic “omnivore” that consumes a relatively wide range of energy substrates to sustain its demanding contractile function.¹ These include, in addition to fatty acid and glucose, lactate, amino acids, and ketone

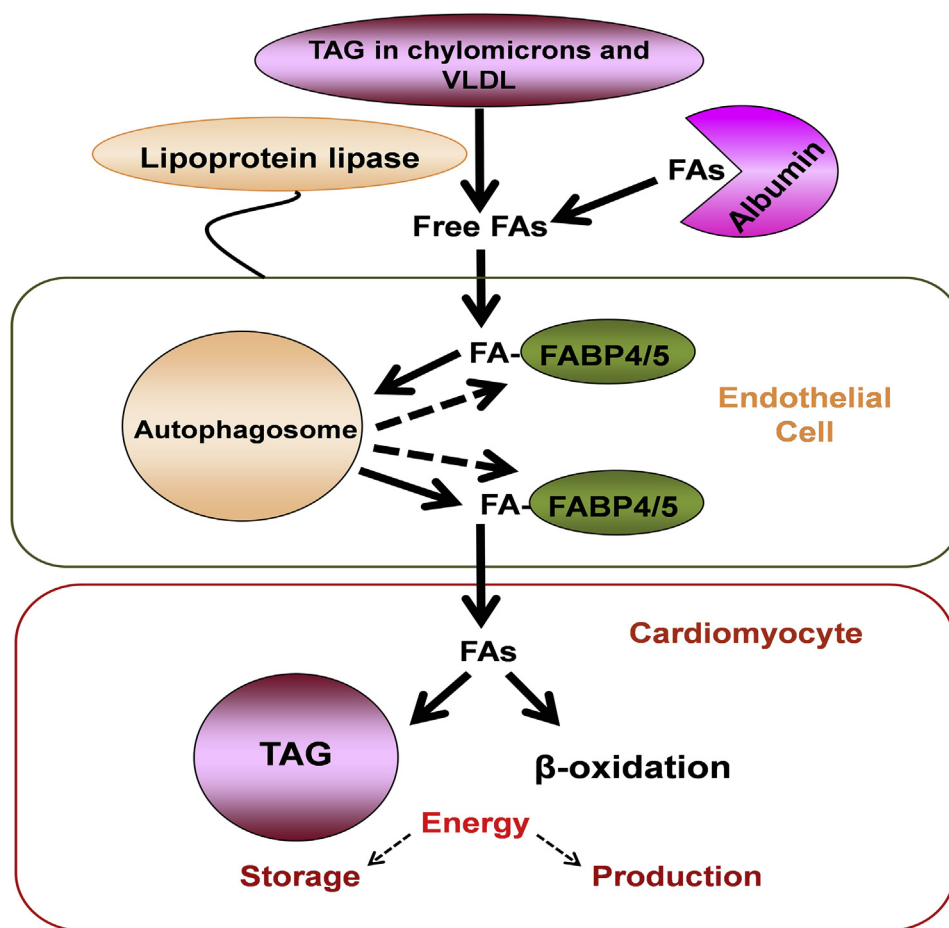


FIGURE 4. A proposed scheme of endothelial-mediated fatty acid delivery to cardiomyocytes. Free fatty acids (FAs) liberated from albumin or hydrolysis of TAG from very-low density lipoprotein or chylomicrons require association with fatty acid binding proteins FABP 4/5 inside the ECs that may have a relationship with normal autophagic flux involving trafficking of these fatty acids inside autophagosomes or through effects of autophagy on FABP 4/5 expression that eventually influence endothelial-mediated delivery of FAs to underlying cardiomyocytes for either energy production by fatty acid oxidation or energy storage as TAG. TAG, Triacylglycerol.

bodies, contributing only by minor proportions compared with fatty acids and glucose,^{1,22} particularly under the normal or ischemic conditions investigated in our study. In fact, exogenous fatty acids are the major source of cardiac energy through the processes of fatty acid β -oxidation and synthesis of endogenous TAG stores.^{1,23} Fatty acids are delivered to the myocardium as circulating free fatty acids bound to albumin or packed into the TAG constituent of chylomicrons or very-low-density lipoproteins (Figure 4).^{23,24} We have used in this study physiologically relevant concentrations of glucose and fatty acids that take into consideration and compensate for the absence of chylomicrons and very low-density lipoprotein in our ex vivo system.^{1,23,25,26}

The crosstalk between ECs and cardiomyocytes forms a critical regulatory pathway for normal cardiac development and growth.⁴ Accordingly, deviations from the norm, no matter how small, could have significant implications on cardiomyocyte phenotype, metabolism, growth, contractility, rhythmicity, and survival.⁴ Indeed, dysfunctional autocrine and paracrine signals at the endothelial level are known to play a role in triggering and promoting I/R-associated irregularities.⁵ Therefore, it is somewhat surprising that given the basic high energy needs of the heart and our evolving understanding of the pathological foundation of multiple coronary and vascular diseases, the delivery of energy substrates from ECs to cardiomyocytes remains a poorly explored and understood biological entity.

Milieu changes in free fatty acid and TAG levels trigger lipid droplet formation and degradation that ensure EC metabolic homeostasis while also serving as fatty acid reservoirs for the cells nearby.²⁷ FABP4/5 double-knockout mice, lacking the main FABPs in ECs, show attenuated cardiac fatty acid uptake accompanied by enhanced glucose uptake.²⁸ These observations suggest that ECs can regulate their intracellular lipid levels, buffer the circulatory fatty acid content, and assist in the translocation of fatty acids from ECs to the neighboring cardiomyocytes. However, unlike cardiomyocytes that impose the greatest energy demands among the cells of the heart, mitochondrial content of ECs is relatively low and these cells rely primarily on glycolysis as their fuel source.²⁹ Therefore, it is highly likely that the impact ECs have on cardiac energy metabolism, particularly fatty acid and glucose oxidation, is limited to their role in substrate delivery. Accordingly, the metabolic rates that we report most likely represent cardiomyocyte utilization of coronary EC-delivered substrates.

As mentioned earlier, our group has reported extensively on how abnormalities in the autophagic network are associated with functional, biochemical, and molecular changes that have been implicated in a wide range of cardiovascular anomalies.⁶⁻¹¹ This includes showing that EC autophagy plays a critical role in restricting lipid

accumulation within the vasculature and consequently can alter the downstream atherosclerotic burden.¹² These results linking autophagy to lipid homeostasis and atherogenesis not only align well with those previously reported³⁰⁻³³ but also extend the findings of Rambold and colleagues,³⁴ who described the significance of autophagy in fatty acid trafficking and toxicity. The observations made in the current body of experiments add to the existing literature by demonstrating that an intact endothelial autophagy network contributes to matching energy substrate availability with energy demands of the heart.

FABPs are lipid chaperones that are ubiquitously expressed in cells and tissues acutely involved in fatty acid trafficking, signaling, and metabolism.³⁵ Compared with the hearts and skeletal muscles of WT mice, the corresponding samples from FABP4/5 double-knockout mice exhibited significantly lower uptake of a fatty acid analogue and markedly greater uptake of a glucose analogue.²⁸ Because *ATG7* silencing appreciably dampened cardiac TAG content and lowered EC FABP4 and FABP5 expression, it would not be unreasonable to posit that the absence of autophagy leads to a decline in fatty acid translocation and in turn a diminished TAG reservoir resulting in less fatty acid oxidation-associated energy production. However, we cannot exclude the possibility that alternative roles of the *ATG7* protein in ECs that extend beyond its known role in autophagy may have led to the altered cardiac energy substrate preference. Although our I/R model did not show beneficial effects on recovery in EC-*ATG7*^{-/-}, possibly because the importance of autophagy in mitigation of I/R injury, the benefits of inhibiting palmitate and stimulating glucose oxidation in other cardiac disease settings, such as failing or insulin-resistant hearts by targeting endothelial lipid handling through autophagosomes or FABP4/5 may still exist and require further investigation.

Using the isolated heart system holds the inherent limitation that the ex vivo hearts are no longer exposed to the conditions they were experiencing in vivo. This implies that direct translation into clinical conditions should be carefully practiced. In addition, the parameters of ex vivo cardiac function, although revealing, are not the same as usually investigated functional parameter for in situ hearts, such as in echocardiography. However, the working heart system has its advantages as a research tool because it, for instance, allows chronic changes in cardiac metabolism to be evaluated in isolation from extra-cardiac physiologic/pathologic factors while direct effects of acute treatments on cardiac metabolism and function can also be assessed. Because energy metabolism is directly related to cardiac work, the simultaneous assessment of metabolic rates and cardiac work represent another advantage of this system over other available techniques, such as muscle cell homogenates or mitochondrial preparations, which suffer

serious technical issues. Using a mouse model, in addition to offering the advantage of genetic manipulation that was used to address the research goals of our study, it provides metabolic responses to insulin that are similar to the human heart, allowing easier assessment of changes in cardiac energy metabolism.

CONCLUSIONS

The results reported demonstrate that fatty acid metabolism within the heart is reliant on an intact endothelial autophagy network. To the best of our knowledge, this is the first demonstration linking disrupted endothelial autophagy with dysregulated cardiac energy metabolism via altered FABP expression. Our findings support the notion that the endothelial autophagy network in conjunction with FABPs may offer novel therapeutic targets for normalizing deranged myocardial bioenergetics.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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