CARDIOVASCULAR DISEASE

High-density lipoprotein delivered after myocardial infarction increases cardiac glucose uptake and function in mice

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Protecting the heart after an acute coronary syndrome is a key therapeutic goal to support cardiac recovery and prevent progression to heart failure. A potential strategy is to target cardiac glucose metabolism at the early stages after ischemia when glycolysis is critical for myocyte survival. Building on our discovery that high-density lipoprotein (HDL) modulates skeletal muscle glucose metabolism, we now demonstrate that a single dose of reconstituted HDL (rHDL) delivered after myocardial ischemia increases cardiac glucose uptake, reduces infarct size, and improves cardiac remodeling in association with enhanced functional recovery in mice. These findings applied equally to metabolically normal and insulin-resistant mice. We further establish direct effects of HDL on cardiomyocyte glucose uptake, glycolysis, and glucose oxidation via the Akt signaling pathway within 15 min of reperfusion. These data support the use of infusible HDL preparations for management of acute coronary syndromes in the setting of primary percutaneous interventions.

INTRODUCTION

To minimize cardiac infarct size, support functional recovery, and prevent progression to heart failure, protecting the myocardium after an acute coronary syndrome is a key therapeutic goal. Under conditions of ischemia and during the early stages of reperfusion, glycolysis is critical for myocyte survival (*1*, *2*). Glycolysis is an important generator of anaerobic adenosine triphosphate (ATP) both acutely during ischemia and during reperfusion of a limited-duration coronary occlusion. This mechanism protects the myocardium against cell damage and subsequent cardiac dysfunction to maintain cardiac contractility. Thus, targeting cardiac glucose metabolism during the acute phase after a cardiac ischemic event is a potential strategy to preserve the myocardium and promote mechanical recovery. Such approaches may be particularly relevant for individuals with type 2 diabetes mellitus where cardiac insulin resistance further impairs glucose uptake and glycolysis, contributing to poorer outcomes after acute coronary syndromes (*3*, *4*).

Previous experimental studies have provided supportive evidence for interventions targeting glucose metabolism during ischemia and reperfusion (*5*–*7*). Specifically, glucose administered either during ischemia (*1*) or early reperfusion (*8*) improved functional recovery. Although clinical studies have delivered mixed results (*9*), in the IMMEDIATE (Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency Care) trial, (*10*) the delivery of an acute glucose-insulin-potassium infusion early (before hospital admission) after an ST elevation myocardial infarction reduced composite cardiovascular end points (*11*). A subgroup analysis also found that at 30 days after infarction, median infarct size was reduced by 80% with glucose-insulin-potassium infusion compared with placebo (*10*). These data support targeting glucose utilization as a viable approach to limit myocardial damage and functional decline resulting from acute coronary syndromes.

High-density lipoprotein (HDL) has been recognized as a novel regulator of glucose metabolism in skeletal muscle (*12*–*18*). These findings raise the possibility that HDL has similar actions in cardiac muscle with the potential for application of infusible HDL therapies, currently in clinical trials, in the setting of acute coronary syndromes (*19*). This indication is completely distinct from the current trials investigating the efficacy of different classes of HDL therapies for chronic HDL-cholesterol elevation, which have varying effects on HDL metabolism and function. With regard to an effect of HDL on glucose metabolism, the major apolipoprotein (apo) of HDL, apoAI, has recently been shown to stimulate cardiac glucose uptake in mice (*20*). Whether HDL modulates cardiac glucose metabolism after myocardial infarction has yet to be examined, despite experimental evidence suggesting beneficial effects of HDL on heart recovery when

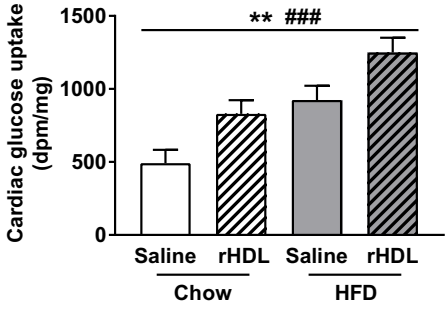

Fig. 1. rHDL increases cardiac glucose uptake after an oral glucose load. Cardiac glucose [[^{3}H]2-deoxyglucose (2-DG)] uptake after an intravenous bolus of saline or rHDL (80 mg/kg) in chow- and HFD-fed mice after an oral glucose load (n = 8 to 10 per group). Data are means ± SEM. Two-way analysis of variance (ANOVA); $^{**}P$ < 0.01, treatment effect; $^{###}P$ < 0.001, diet effect; no treatment-diet interaction.

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delivered before ischemia (*21*–*23*). In particular, it is unknown whether HDL administered after ischemia, in a clinically relevant therapeutic context akin to primary percutaneous coronary intervention, is sufficient to improve cardiac glucose uptake and functional recovery. Furthermore, no study has examined post-ischemic HDL treatment in the setting of insulin resistance, where cardiac glucose metabolism and functional recovery are particularly impaired.

To address this, we investigated whether reconstituted HDL (rHDL; CSL-111) delivered at a clinically relevant dose (*12*) could modulate cardiac glucose metabolism after an oral glucose load in the absence of ischemia in metabolically normal and insulin-resistant mice [high-fat diet (HFD) model]. We examined the effects of a single intravenous injection of rHDL administered after a 30-min occlusion of the left anterior descending coronary artery. Cardiac glucose uptake was assessed after 60 min of reperfusion, and cardiac function, left ventricle infarct size, capillary density, and fibrosis were measured 15 days later. To determine whether HDL directly targeted cardiomyocellular mechanisms, we also examined effects on glucose metabolism and intracellular signaling in a primary cardiomyocyte model and in cardiac tissue early after reperfusion.

RESULTS

rHDL increases cardiac glucose uptake after an oral glucose load

Mice treated with a single dose of rHDL had significantly higher cardiac glucose uptake in both dietary groups after an oral glucose load [chow saline, 492 ± 91 disintegrations per minute (dpm)/mg tissue versus chow rHDL, 829 ± 95 dpm/mg tissue; HFD saline, 925 ± 97 dpm/mg tissue versus HFD rHDL, 1250 ± 101 dpm/mg tissue] (Fig. 1, $P < 0.01$ for treatment and diet). The higher cardiac glucose uptake in the HFD-fed group versus the chow-fed group corresponded with a doubling of plasma insulin ($P < 0.001$) and greater plasma glucose ($P < 0.001$) after the glucose load, but there was no significant effect of treatment (fig. S1).

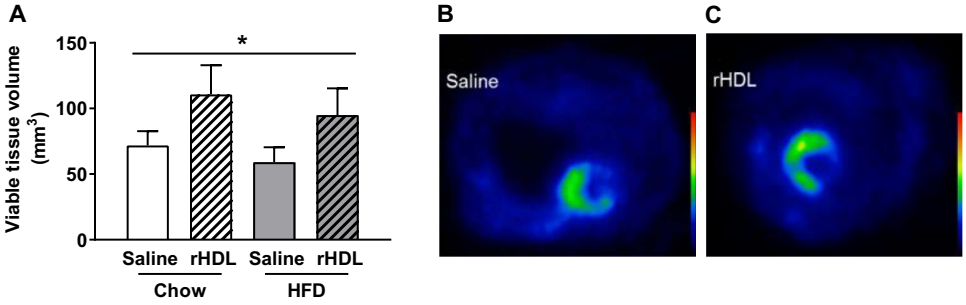

Fig. 2. rHDL increases post-ischemic myocardial glucose uptake. (**A**) Myocardial [^{18}F]-FDG uptake measured as viable tissue volume by PET/CT after myocardial ischemia-reperfusion in chow- and HFD-fed mice treated with either saline or rHDL (80 mg/kg) at the beginning of reperfusion (n = 11 per group). (**B** and **C**) Representative axial views of [^{18}F]-FDG uptake (red, higher) in the thorax, taken at the middle region of PET image slices from the whole heart. This corresponds to the mid ventricle, which is supplied by the left anterior descending coronary artery. (B) Chow saline and (C) chow rHDL. Data are means ± SEM. Two-way ANOVA; *$P < 0.05$, treatment effect, with no diet effect or any treatment-diet interaction.

rHDL delivered post-ischemia increases myocardial glucose uptake

After 30 min of cardiac ischemia, mice were treated with rHDL or saline. After the subsequent 60 min of reperfusion, there was greater myocardial uptake of the [^{18}F]-fluorodeoxyglucose (FDG) tracer on positron emission tomography/computed tomography (PET/CT) imaging in both chow-fed mice (saline, 72 ± 11 mm^3 versus rHDL, 111 ± 22 mm^3; $P < 0.05$) and HFD-fed mice (saline, 59 ± 11 mm^3 versus rHDL, 95 ± 20 mm^3; $P < 0.05$) (Fig. 2). There was no diet effect on [^{18}F]-FDG uptake or any interaction between treatment and diet.

rHDL improves post-ischemic left ventricular function

rHDL treatment increased cardiac output by 11 ± 2% and 23 ± 2% in chow- and HFD-fed mice, respectively (Fig. 3A, $P < 0.05$). Ejection fraction and stroke volume were also increased by rHDL in both diet groups (Fig. 3, B and C; $P < 0.05$), whereas heart rate was reduced (Fig. 3D, $P < 0.05$). rHDL did not modulate any left ventricular (LV) functional parameters in control sham-operated animals (fig. S2, A to D).

The cardiac output of HFD-fed mice was lower than that of chow-fed mice measured 15 days after ischemia-reperfusion

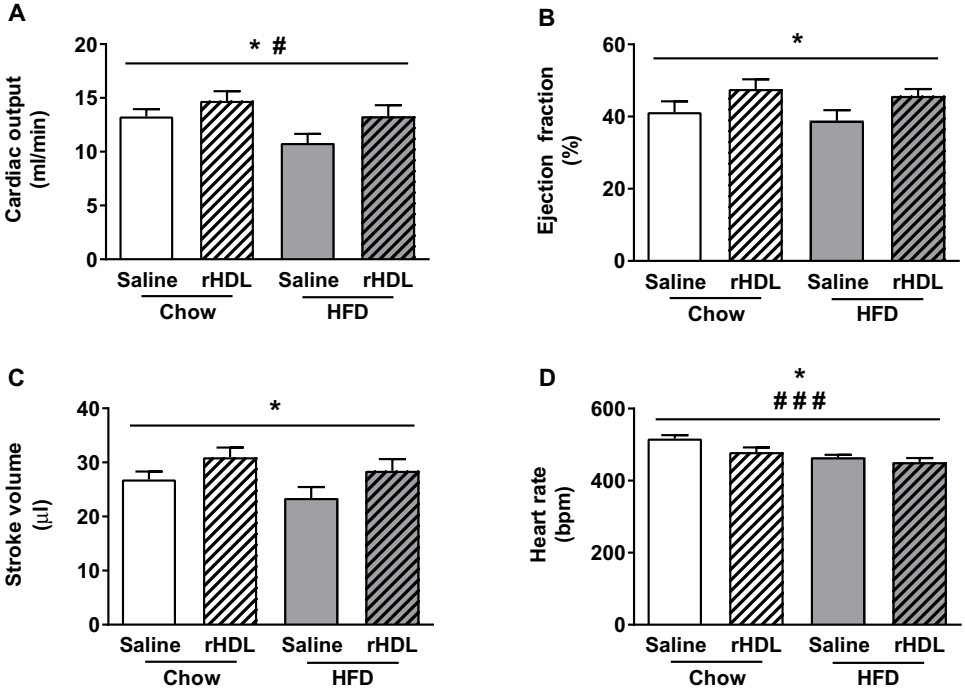

Fig. 3. rHDL increases post-ischemic LV function. Cardiac function measured 15 days after ischemia-reperfusion in chow- and HFD-fed mice treated with either saline or rHDL (80 mg/kg) (n = 14 to 18 per group). (**A**) LV cardiac output, (**B**) ejection fraction, (**C**) stroke volume, and (**D**) heart rate. Data are means ± SEM. Two-way ANOVA; *$P < 0.05$, treatment effect; $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$, diet effect.

(Fig. 3A, $P < 0.05$). This difference appeared to be driven by a lower heart rate because there was no difference in stroke volume between dietary groups (Fig. 3, C and D; $P < 0.001$). There was no difference between dietary groups in any of the cardiac functional parameters before surgery-induced myocardial infarction (fig. S2, E to H).

rHDL reduces LV infarct size and fibrosis and increases capillary density

Both chow- and HFD-fed mice had reduced infarct size when treated with rHDL compared to saline (Fig. 4, A and B; $P < 0.05$). rHDL treatment also reduced interstitial fibrosis and increased capillary density (capillary-to-cardiomyocyte ratio) in the peri-infarct region of both dietary groups (Fig. 4, C to F; $P < 0.01$). The HFD-fed mice had a smaller infarct size compared to chow-fed mice (Fig. 4A, $P < 0.05$) and higher capillary density (Fig. 4E, $P < 0.01$); however, there was no effect of diet on collagen deposition (Fig. 4C). In sham-operated mice, HFD also increased LV capillary density compared to chow mice but had no effect on collagen deposition (fig. S3).

nHDL increases in vitro glucose utilization in cardiomyocytes

To determine whether HDL has direct effects on cardiac glucose metabolism, we treated primary rat neonatal ventricular cardiomyocytes (NVCMs) with lipidated native human HDL (nHDL). Both 50 and 500 μg/ml of nHDL, in the absence of insulin, increased glucose ([^{3}H]2-DG) uptake relative to saline by 53 ± 14% and 89 ± 16%, respectively (Fig. 5A, $P < 0.05$). There was no significant difference in the magnitude of the glucose uptake stimulated by the nHDL treatments and insulin and no additive effect of cotreatment with insulin ($P > 0.05$). nHDL (50 μg/ml) increased basal glycolysis by 38 ± 7%, glycolytic capacity by 44 ± 6%, and glycolytic reserve by 69 ± 13% compared to saline-treated cardiomyocytes (Fig. 5B, $P < 0.05$). These results indicate that nHDL directly increased in vitro glucose uptake and anaerobic glycolysis.

nHDL (50 μg/ml) treatment also increased basal respiration measured by oxygen consumption rate (OCR) and respiration linked to ATP turnover by 59 ± 8% and 59 ± 12%, respectively (Fig. 5C, $P < 0.05$ for both). Moreover, cotreatment with a mitochondrial uncoupler,

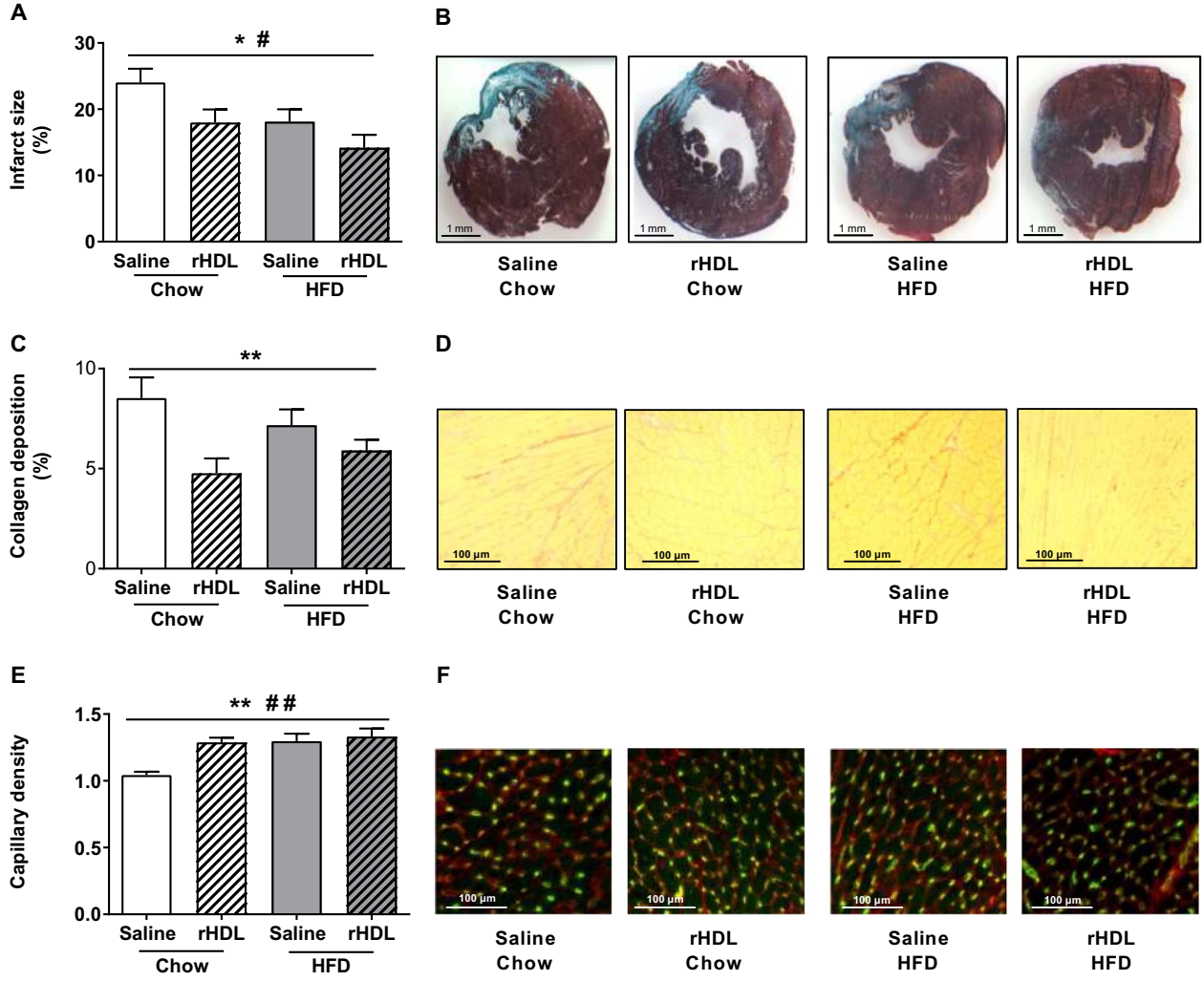

Fig. 4. rHDL reduces LV infarct size and fibrosis and increases capillary density. Cardiac remodeling assessed 15 days after ischemia-reperfusion in chow- and HFD-fed mice treated with either saline or rHDL (80 mg/kg). (**A**) LV infarct size ($n = 11$ to 17 per group), (**C**) collagen deposition (fibrosis, $n = 10$ per group), (**E**) capillary density (number of capillaries per myocyte, $n = 6$ to 12 per group), and corresponding representative sections**:** (**B**) Masson's trichrome, (**D**) Sirius red, (**F**) endothelial cells (BS-1 lectin, green); cardiomyocytes (wheat germ agglutinin, red). Data are means ± SEM. Two-way ANOVA; *$P < 0.05$ and **$P < 0.01$, treatment effect; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, diet effect.

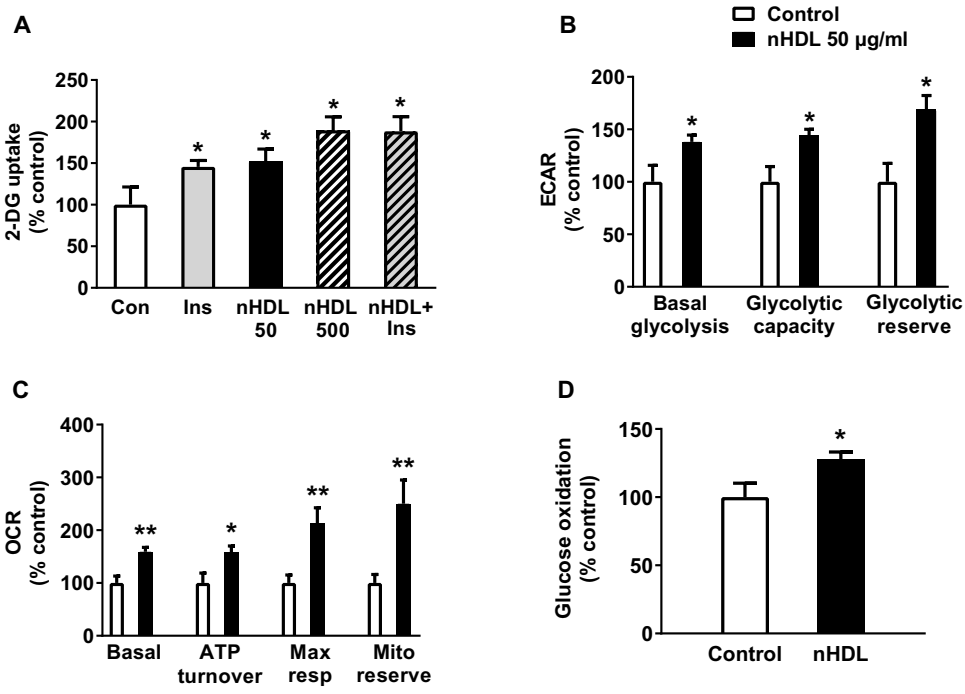

Fig. 5. nHDL increases in vitro glucose utilization in cardiomyocytes. (**A**) Cardiomyocyte 2-DG uptake in response to saline (Con), insulin (Ins; 100 nM), nHDL (50 or 500 μg/ml), or nHDL (500 μg/ml) + insulin (100 nM) cotreatment (n = 5 independent experiments per group). (**B**) Glycolytic parameters included extracellular acidification rate (ECAR): basal glycolysis, glycolytic capacity, and glycolytic reserve after saline (control) or nHDL (50 μg/ml) treatment (n = 10 independent experiments per group). (**C**) Mitochondrial stress test parameters included OCR: basal respiration (basal), ATP turnover, maximum respiratory capacity (max resp), and mitochondrial reserve capacity (mito reserve) after saline (control) or nHDL (50 μg/ml) treatment (n = 7 independent experiments per group). (**D**) Glucose oxidation after saline (control) or nHDL (50 μg/ml) treatment (n = 7 independent experiments per group). Data are means ± SEM. (A) Kruskal-Wallis (K-W) one-way ANOVA followed by Student-Newman-Keuls post hoc test. (B to D) Unpaired Student's t test for each parameter. *P < 0.05 versus control, **P < 0.01 versus control.

carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone, demonstrated that HDL can increase both maximum respiratory capacity and mitochondrial reserve capacity by 113 ± 30% and 150 ± 45%, respectively (Fig. 5C, P < 0.01 for both). These data were substantiated by the observation that nHDL (50 μg/ml) also increased production of [$^{14}CO_2$] and therefore glucose oxidation rate by 28 ± 5% (Fig. 5D, P < 0.05).

nHDL rapidly and transiently activates the Akt signaling pathway

Akt phosphorylation increased within 5 min of treatment with nHDL (50 μg/ml) and returned to basal expression by 30 min after treatment (Fig. 6A, P < 0.05). In parallel, the Akt substrate of 160 kDa (AS160) and the downstream Akt target glycogen synthase kinase-3β (GSK3-β) were phosphorylated within 5 and 10 min of nHDL (50 μg/ml) treatment (Fig. 6, B and C; P < 0.05 for both). These transient increases returned to the basal phosphorylation state by 30 min after treatment. Before treatment of cardiomyocytes with a validated Akt inhibitor (Akti 1/2) for 30 min abolished the nHDL-mediated increase in glucose uptake (Fig. 6D).

rHDL activates the Akt signaling pathway in the early post-ischemic period and stimulates glucose uptake in vivo

Cardiac tissue harvested from chow-fed mice 15 min after treatment with rHDL (80 mg/kg), delivered at the commencement of reperfusion, showed significantly increased phosphorylation of Akt (by 58%) and its downstream target AS160 (by 66%) (Fig. 7, A and B; P < 0.05). There was no change in the corresponding amounts of total Akt or AS160 (Fig. 7, C and D; P > 0.05). In vivo [^{18}F]-FDG PET/CT experiments in chow-fed mice confirmed that rHDL resulted in a greater cardiac glucose uptake in the reperfusion period between 20 and 30 min after ischemia [ratio of standardized uptake value $(SUV)_{mean}$ in cardiac tissue versus blood; saline, 1.30 ± 0.04 versus rHDL, 1.44 ± 0.03; P < 0.05; fig. S4].

DISCUSSION

A single bolus of rHDL delivered after ischemia rapidly increased myocardial glucose uptake, reduced infarct size, and improved structural remodeling in association with enhanced cardiac functional recovery in mice (fig. S5). These effects were evident in both metabolically normal mice and those rendered insulin-resistant by an HFD. Our in vitro studies support a direct modulatory effect of HDL on multiple aspects of cardiomyocyte glucose metabolism. These experimental observations have direct clinical translational relevance to HDL infusion formulations (*19*), which are in clinical development and amenable for delivery in the context of primary percutaneous coronary intervention.

HDL increases myocardial glucose uptake and improves cardiac recovery after ischemia

In the absence of ischemia, we showed that rHDL increased cardiac glucose uptake after an oral glucose load by a similar magnitude in both chow-fed mice and insulin-resistant HFD-fed mice. Translational potential was then demonstrated by our observation that a single bolus of rHDL delivered after ischemia also increased cardiac glucose uptake in the absence of a glucose load. The higher viable tissue volume estimated by [^{18}F]-FDG PET/CT imaging did not differ in magnitude between the chow- and HFD-fed mice. These results are relevant to therapeutic strategies for myocardial salvage after acute coronary syndromes and suggest that benefit may apply equally in the presence and absence of insulin resistance.

Without using metabolizable glucose (1-[^{11}C]-glucose) and lactate ([^{11}C]-lactate) tracers, it can only be speculated that rHDL would additionally increase glucose catabolism. On the basis of our findings in cultured cardiomyocytes, this is a plausible hypothesis because HDL treatment significantly increased key parameters of glycolysis, as well as glucose oxidation and mitochondrial respiration. The coupling of glucose oxidation to glycolysis has emerged as an important consideration for therapeutic approaches that target glucose metabolism to treat myocardial ischemia (*6*, *7*). Our in vitro data suggest that HDL is able to maintain efficient oxidative ATP production, because despite increases in both basal and maximal respiratory capacity, the ratio of

coupled (ATP-producing) to uncoupled respiration was unchanged. These findings suggest that HDL may provide post-ischemic protection of the myocardium by maintaining cardiac efficiency and stimulating ATP turnover (*24*).

The ~20% reduction in infarct size after rHDL treatment in both diet groups is clinically relevant, particularly considering the relatively mild (30 min) ischemic insult. This apparent increase in myocardial salvage after rHDL treatment was associated with improved remodeling and functional recovery in terms of resting LV ejection fraction, stroke volume, and cardiac output. The extent to which these gains relate directly to improvements in cardiac glucose metabolism was not investigated. It is likely that the inflammatory response and oxidative stress associated with ischemia were reduced secondary to HDL-mediated effects on myocardial glucose metabolism. In addition, myocardial salvage may also relate to the direct antioxidative and anti-inflammatory actions of HDL (*25*). Finally, proangiogenic effects of rHDL have been clearly demonstrated both in vitro and in vivo (*26*) and particularly under hypoxic conditions such as ischemia-reperfusion, where HDL activates the hypoxia-inducible factor–1α signaling pathway via SR-B1 with downstream expression of proangiogenic factors such as vascular endothelial growth factor-A and CXCL12 (*27*, *28*).

These findings have potential translational relevance for management of acute coronary syndromes. Primary percutaneous coronary intervention is now the gold standard treatment for acute coronary presentations and provides a practical opportunity for delivery of an intravenous therapy. At the same time, at least two pharmaceutical companies have infusible formulations of reconstituted and recombinant HDLs in clinical trials (CSL Behring and Cerenis). This includes CSL-112, a reformulation of the agent used in the current investigation (modified excipients), which has recently delivered encouraging safety data in a phase 2b trial of patients with acute coronary syndrome (*29*). Translation of our observations with regard to glucose metabolism would require subsequent trials delivering rHDL as early as possible after myocardial infarction and catheter laboratory presentation.

HDL mediates cardiac glucose uptake via the Akt pathway

We, and others, have found that in addition to the direct actions on skeletal muscle (*12*, *15*), HDL may modulate glucose metabolism indirectly via pancreatic insulin secretion (*12*, *15*, *16*, *20*) and blood flow effects. To remove these potential confounders, we undertook in vitro cell culture studies to determine whether HDL can directly stimulate cardiomyocyte glucose metabolism. We showed that HDL treatment potently increased all aspects of glucose breakdown in isolated cardiomyocytes in the absence of insulin. Moreover, a blockade of insulin signaling has previously been shown to have little effect on apoAI-mediated cardiac glucose uptake in HFD-fed mice (*20*). Collectively, these data suggest that HDL has direct glucostimulatory effects on myocardial cells, which are insulin-independent and may be amplified by other extra-muscular actions, including on insulin secretion and blood flow.

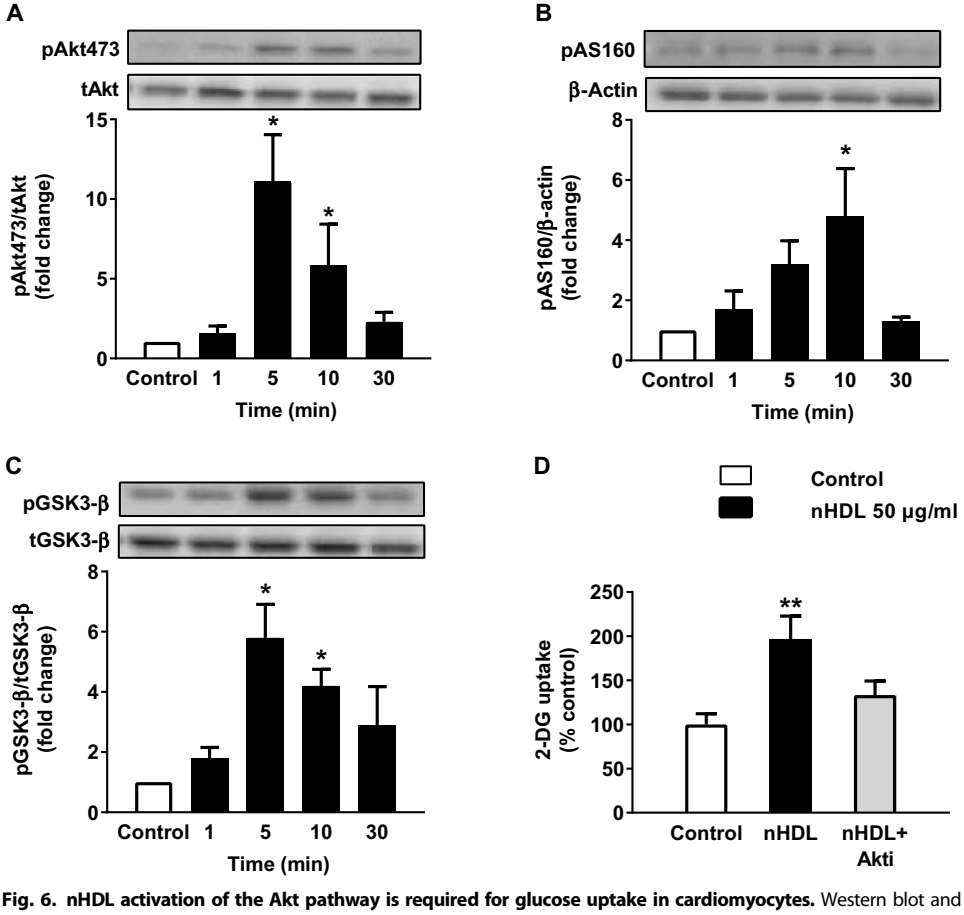

Fig. 6. nHDL activation of the Akt pathway is required for glucose uptake in cardiomyocytes. Western blot and quantitation showing time course of protein phosphorylation of (**A**) Akt [phosphorylated/total (pAkt473/tAkt)], (**B**) AS160 (phosphorylated pAS160/β-actin), and (**C**) GSK3-β [phosphorylated/total (pGSK3-β/tGSKβ)] after saline (control) or nHDL (50 μg/ml) treatment (n = 5 independent experiments per group). (**D**) 2-DG uptake in response to saline (control) and nHDL (50 μg/ml) treatment after pretreatment with Akti (50 nM) for 30 min (n = 9 independent experiments per group). Data are means ± SEM. One-way ANOVA followed by least significant difference (LSD) post hoc test, *P < 0.05 versus control, **P < 0.01 versus control.

Our in vitro cardiomyocyte experiments found that human nHDL-mediated glucose uptake occurred via a mechanism involving Akt signaling. A link between HDL, Akt, and glucose metabolism in cardiomyocytes has not been previously made, but it is aligned with the well-defined role of Akt in insulin-mediated glucose uptake (*30*). HDL-mediated protection against ischemic injury in various animal models of coronary artery disease has been previously attributed to Akt signaling, but the connection to glucose metabolism has not been made (*31*, *32*). Thus, our data support a mechanism by which HDL activates the insulin-mediated glucose uptake pathway in cardiomyocytes, independently of insulin.

The demonstration that rHDL phosphorylates Akt and its downstream target AS160 within 15 min after delivery at the time of reperfusion is consistent with rapid activation of glucose uptake mechanisms in vivo (Fig. 7). Although this finding alone does not prove myocardial salvage through

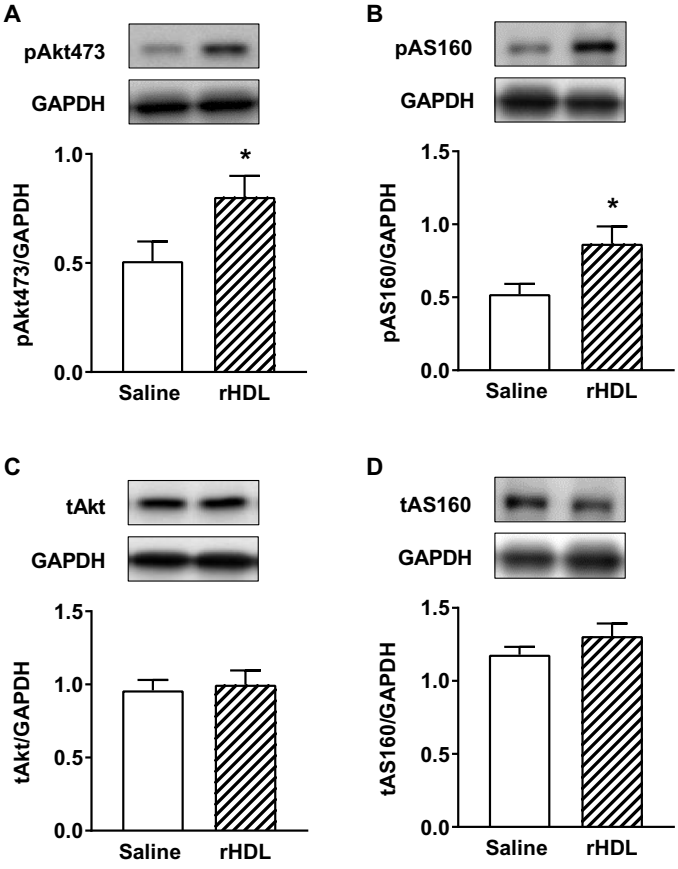

Fig. 7. rHDL activates the Akt pathway in vivo in the early post-ischemic period. Western blot and quantitation of phosphorylation of (**A**) Akt (pAkt473) and its downstream target (**B**) AS160 (pAS160) compared with corresponding (**C**) total Akt (tAkt) and (**D**) total AS160 (tAS160), 15 min after myocardial ischemia-reperfusion and saline (control) or rHDL (80 mg/kg) treatment in the left ventricle of chow-fed mice ($n = 8$ to 9 per group). Data are means ± SEM. Unpaired Student's t test, *$P < 0.05$ versus saline. GAPDH, glyceraldehyde-phosphate dehydrogenase.

effects on glucose metabolism, the evidence for this possibility is strengthened by cardiac glucose uptake measurements made early after reperfusion. Our [^{18}F]-FDG PET/CT findings also confirm that rHDL increases cardiac glucose uptake within 20 to 30 min of delivery (fig. S4). These data suggest that rHDL administration preserves myocardial function through supporting glycolysis and myocyte survival in the early stages of reperfusion. However, it is also possible that other mechanisms downstream of Akt including specific elements of the reperfusion injury salvage kinase (RISK) pathway contribute to myocyte survival and, indirectly, to enhanced glucose uptake (*33*).

The effects of HFD on glucose uptake and cardiac structure and function

Some commentary is required to address the increased resting cardiac glucose uptake after an oral glucose load in HFD-fed mice (Fig. 1) and the fact that viable tissue volume measured by radio-labeled glucose ([^{18}F]-FDG) uptake visualized on PET/CT was not different between diets after ischemia in the absence of a glucose load (Fig. 2). Although lower cardiac glucose uptake might have been expected in an insulin-resistant model, it is important to acknowledge that HFD-fed mice had plasma insulin concentrations about double than those of chow-fed mice. These data are consistent with this model being one of compensated insulin resistance akin to prediabetes (*34*).

The HFD animals exhibited a smaller infarct size and higher LV capillary density relative to chow-fed mice. The insulin-resistant sham-operated mice also had a higher LV capillary density compared to metabolically healthy mice (fig. S3), which again aligns with the HFD being a well-compensated model of insulin resistance, as demonstrated in previous studies (*35*). These counterintuitive cardioprotective effects have been reported previously in different rodent dietary models also incorporating ischemia-reperfusion (*36*, *37*). High-fat feeding has also been shown to promote angiogenesis in skeletal muscle (*38*). However, other studies, particularly those involving diets with a higher fat content than used in the current study (60% versus 43%), have shown a negative impact of HFD on capillary density and cardiac function (*35*, *39*, *40*). Collectively, these studies suggest early compensatory responses to hyperglycemia, which progress to detrimental effects with continued exposure. With regard to our primary aims, HDL treatment was equipotent between diets in increasing post-ischemic myocardial glucose uptake, reducing infarct size and fibrosis, increasing capillary density, and improving cardiac functional recovery.

HDL therapeutics have potential applications in the context of acute coronary syndromes

HDL therapeutics are currently controversial because of the failure of multiple phase 3 trials of therapies to chronically raise HDL-cholesterol concentration, highlighting gaps in our understanding of both HDL biology and therapeutic manipulation. Research focus has now evolved from raising plasma HDL-cholesterol concentration to targeting the function of HDL particles, which are known to be both complex and heterogeneous in composition. The current investigation highlights a new aspect of HDL biology with regard to cardiac glucose metabolism, which is relevant to the management of acute coronary syndromes. HDL formulations delivered via infusion can increase the circulating pool of functional HDL (*19*, *41*) and, as demonstrated in the current study, when delivered after an experimentally induced ischemic insult, represent a potential therapeutic modality to preserve the myocardium and improve functional recovery after an acute coronary syndrome. This window of opportunity for HDL administration is consistent with the timing of primary percutaneous coronary intervention, underpinning a rationale for the use of HDL-based therapies in this context. Further studies will be required to determine the key constituents of HDL, which mediate its beneficial actions, specifically in the context of improving glucose utilization.

Mechanistic links between HDL-mediated increases in myocardial glucose metabolism and reduction in myocardial damage and function are highly plausible, although other elements of the RISK pathway may also contribute (*33*). The current study used an in vitro neonatal cardiomyocyte model to assess the direct actions of HDL on cardiomyocyte glucose metabolism independent of potential in vivo confounders, such as insulin or vasodilation. Clinical studies will be required to determine whether these effects of HDL translate to humans. Furthermore, whether concurrent administration of glucose with rHDL might be of additional clinical benefit is a further area of potential investigation (*10*, *11*).

Our data provide evidence that HDL can modulate cardiac glucose metabolism. rHDL administered at the beginning of reperfusion increased myocardial glucose uptake, improved cardiac function, and reduced infarct size with equal efficacy in both metabolically normal and insulin-resistant mice. Supporting in vitro and in vivo

data suggest that modulation of cardiac glucose metabolism by HDL is mediated through a mechanism involving downstream Akt signaling. These findings provide a rationale for clinical investigations of rHDL delivery in the setting of primary percutaneous coronary intervention for acute coronary syndromes.

MATERIALS AND METHODS

Study design

All studies used metabolically normal and insulin-resistant mice (HFD model). We first examined the effects of rHDL on cardiac glucose metabolism after an oral glucose load in the absence of myocardial ischemia. We then examined the effects of a single intravenous injection of rHDL administered immediately after a 30-min occlusion of the left anterior descending coronary artery on cardiac glucose uptake using [^{18}F]-FDG PET/CT. Cardiac function (echocardiography), left ventricle infarct size, capillary density, and fibrosis (histochemistry) were assessed 15 days later. The effects of HDL on glucose metabolism were further investigated in a primary cardiomyocyte model (Seahorse XF Bioanalyzer). Signaling pathways (Western blot analysis) were also assessed in this primary cardiomyocyte model and in cardiac tissue early after reperfusion. All treatments were randomly allocated, and all analyses were done by investigators blinded to treatment allocation.

Mice

All animal research was conducted in accordance with National Health and Medical Research Council (NHMRC) of Australia guidelines and was approved by the Alfred Medical Research and Education Precinct (AMREP) Animal Ethics committee. Eight- to ten-week-old male C57BL/6 mice bred in-house (AMREP Animal Services) were fed either a standard chow (14 MJ/kg; 8% fat, 21% protein, and 71% carbohydrates; Specialty Feeds) or an HFD [19 MJ/kg; 43% fat (lard), 21% protein, and 36% carbohydrates; SF04-001, Specialty Feeds] for 8 to 10 weeks. Body weight and composition were monitored, and glycemic control was assessed using an oral glucose tolerance test to verify HFD-induced obesity and insulin resistance (*42*).

rHDL preparation

rHDL (CSL-111; CSL Behring) is a complex of human apoAI purified from plasma and soy bean phosphatidylcholine to form particles resembling nascent HDL. Each vial was reconstituted with 50 ml of sterile 0.9% sodium chloride solution to a final concentration of 25 mg apoAI/ml.

In vivo glucose uptake after an oral glucose load

Mice received an intravenous bolus via tail vein injection of either saline or rHDL (80 mg/kg body weight) and [^{3}H]2-DG tracer (10 μCi per mouse; PerkinElmer) immediately before an oral glucose load (2-g glucose/kg lean mass). Hearts were collected 60 min after injection, and 20 to 40 μg of crushed cardiac tissue was homogenized in 1500 μl of dH_2O. Samples were spun at 6500*g* for 10 min at 4°C. To determine total [^{3}H]2-DG radioactivity, we counted 500 μl of the supernatant by liquid scintillation. Free [^{3}H]2-DG was determined by separating 500 μl of supernatant by ion exchange chromatography on Dower I-X8 columns (acetate form). The columns were rinsed twice with dH_2O, and 2 ml of the eluate was counted by liquid scintillation. Phosphorylated [^{3}H]2-DG was calculated as the difference between free and total [^{3}H]2-DG. Tissue uptake was expressed as the amount of phosphorylated [^{3}H]2-DG relative to tissue weight (mg) expressed as (dpm/mg) (*43*).

Coronary artery occlusion and reperfusion surgery

Left anterior descending coronary artery ligation surgery was performed to induce myocardial ischemia in mice (*44*). Briefly, mice received an intraperitoneal injection of anesthetic [ketamine (100 mg/kg; Parnell Laboratories), xylazine (20 mg/kg; Troy Laboratories), atropine (0.96 to 1.2 mg/kg; Pfizer), and carprofen (5 mg/kg; Pfizer)]. A left thoracotomy was made via the third intercostal space, and a releasable 7-0 silk suture was placed around the left anterior descending coronary artery to induce myocardial ischemia. The suture was exteriorized and the thoracic incision was closed. After 30 min of occlusion (ischemia), the ligature was released, and reperfusion was commenced. Sham control animals underwent the same surgical procedure without coronary artery occlusion.

In vivo myocardial glucose uptake after ischemia and reperfusion

To examine the effects of rHDL on cardiac glucose uptake after a 30-min left anterior descending coronary occlusion (ischemia), mice received an intravenous bolus of saline or rHDL (80 mg/kg body weight) and [^{18}F]-FDG (300 μCi per mouse; Cyclotek) at the beginning of reperfusion. PET/CT was used to assess cardiac glucose uptake 60 min after the intravenous rHDL injection. Viable tissue volume was defined as the sum of the voxels with an SUV of more than 50% of SUV_{max} as previously validated (*45*) and expressed as a volume (mm^3).

Cardiac glucose uptake was also measured between 20 and 30 min after reperfusion. Given the presence of radiation in the blood, and so in the LV cavity, at this early time point after [^{18}F]-FDG injection, the data are expressed as the ratio of the SUV_{mean} in the cardiac tissue and the blood (measured in the aorta). PET/CT analyses were performed blinded to treatment group.

LV function

Fifteen days after ischemia-reperfusion surgery, echocardiography was performed to examine LV function. Briefly, mice were anesthetised with isoflurane (usual maintenance dose ~1.7 to 1.8%) and placed in a supine position on a heating pad with body temperature maintained between 35.5° and 36.5°C. Heart rates were maintained at >400 beats/min during all echocardiography recordings (with slight adjustment of amount of anesthesia as necessary). Echocardiograms were performed blinded to treatment group and were then analyzed by another individual also blinded to treatment group. A VisualSonics Vevo 2100 Imaging System with a 40-MHz probe was used to obtain a parasternal long-axis view of the left ventricle in B mode. The following parameters were calculated using Vevo 2100 software (v1.6.0): LV end-systolic and end-diastolic volume, stroke volume, ejection fraction, and cardiac output. All measurements were performed in triplicate by the same individual and averaged.

Infarct size, fibrosis, and capillary density

After echocardiography, left ventricles were excised, frozen in liquid nitrogen, and sectioned at a thickness of 7 μm. Masson's trichrome was used to assess infarct size as a percentage of LV area, and Sirius red was used to measure myocardial interstitial fibrosis at the infarct border zone (peri-infarct region). Capillary density in the same region was calculated as the number of capillaries per cardiomyocyte. Capillaries were visualized and counted by endothelial cell BS-1 lectin staining (1:100, fluorescein isothiocyanate–conjugated Griffonia simplicifolia, Sigma-Aldrich) and cardiomyocytes with wheat germ agglutinin (1:200,

Texas Red–conjugated, Thermo Fisher Scientific). For each heart, five different sections from the point of left anterior descending artery occlusion to the apex were quantified and averaged. Image analysis and measurements were performed using ImageJ software (National Institutes of Health Image).

Cardiomyocyte studies

NVCMs were isolated from 1- to 2-day-old Sprague Dawley rats (30 isolations with an average of six pups per isolation). Ventricles were digested with trypsin (1 mg/ml, Sigma-Aldrich) and heart collagenase type 2 (2.5 mg; Worthington). Cardiomyocytes were cultured in 5 mM glucose Dulbecco's modified Eagle's medium (Gibco Life Technologies) with 10% fetal calf serum (Thermo Fisher Scientific).

Native HDL isolated from healthy human plasma (eight batches supplied by the Australian Red Cross Blood Service) using ultracentrifuge purification was used for in vitro studies. rHDL is a delipidated form of HDL, which is lipidated immediately upon entry into the circulation where it integrates into the native HDL pool (*46*). Delivery of rHDL in vivo therefore provides a mechanism to boost the circulating functional nHDL pool. Therefore, we used functional native (lipidated) HDL for our in vitro experiments.

Glucose uptake was measured using [^{3}H]2-DG and a Seahorse XF Bioanalyzer to assess glycolytic function and mitochondrial respiration (*47*). Briefly, NVCMs were preincubated with nHDL (50 μg/ml) for 1 hour. Basal glycolysis was determined through fluorescent sensor detection of protons (H^+) after addition of 10 mM glucose (Novachem), maximal glycolytic capacity was determined after addition of the ATP synthase inhibitor oligomycin (1 μM, Sigma-Aldrich), and nonglycolytic proton production was determined after addition of 50 mM 2-DG (Sigma-Aldrich). Nonglycolytic proton production was subtracted from basal and maximal glycolysis measures. Glycolytic reserve was calculated as the difference between basal glycolysis and maximal glycolytic capacity. For assessment of mitochondrial respiration after nHDL treatment (50 μg/ml), the following drugs were injected in order: 1 μM oligomycin (Sigma-Aldrich) for measurement of ATP turnover, 1 μM carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (Sigma-Aldrich) to depolarize the mitochondrial membrane for a measure of maximal respiratory capacity, and 1 μM rotenone (Sigma-Aldrich) in combination with 1 μM antimycin A (Sigma-Aldrich) to inhibit complex 1 and complex 3 of the electron transport chain for a measure of nonmitochondrial respiration. Nonmitochondrial respiration rate was considered background and subtracted from all data. Glucose oxidation was quantitated by measuring captured CO_2 production after cells underwent [^{14}C]-glucose incubation (*44*). Protein expression was assessed by Western blotting. All primary antibodies [pSer^{473}Akt (1:2000 dilution), tAkt (1:2000), pGSK3-αβ (1:1000), tGSK3-β (1:1000), and β-actin (1:2000)] were obtained from Cell Signaling except for pThr^{642}AS160 (1:1000, Biosource). The well-validated Akt inhibitor (Akti 1/2, Sigma-Aldrich) was added 30 min before relevant treatments.

In vivo Akt signaling

Immediately after myocardial ischemia-reperfusion, mice received an intravenous bolus of saline or rHDL (80 mg/kg). Fifteen minutes after injection of treatment, left ventricles were excised, frozen in liquid nitrogen, and then homogenized for protein expression analysis via Western blot. All primary antibodies [pSer^{473}Akt (1:2000 dilution), tAkt (1:2000), pThr^{642}AS160 (1:1300), and GAPDH (1:2,000)] were obtained from Cell Signaling except for tAS160 (1:1000, Upstate Biotechnology).

Statistical analysis

Logarithmic transformation was applied to nonnormally distributed data. Mouse data were compared using ANOVA to determine the effects of treatment (rHDL, saline), diet (chow, HFD), and their interaction. For cell culture, unpaired *t* tests, ANOVA, or K-W tests were applied as appropriate, and Fisher's LSD (ANOVA) or Student-Newman-Keuls (K-W) post hoc tests were used for comparison of individual means when ANOVAs or K-W were significant. Results are expressed as means ± SEM, and $P < 0.05$ was deemed significant. Statistical analyses were conducted using SigmaStat (v3.5). Individual subject-level data are presented in table S1.

SUPPLEMENTARY MATERIALS

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Fig. S1. HFD-fed mice have higher plasma glucose levels than chow mice.

Fig. S2. rHDL does not modulate LV functional parameters in control sham-operated mice, and HFD does not affect LV function.

Fig. S3. rHDL does not modulate capillary density or fibrosis.

Fig. S4. rHDL increases cardiac glucose uptake in the early post-ischemic period.

Fig. S5. Schematic overview of proposed mechanisms.

Table S1. Individual subject-level data.

REFERENCES AND NOTES

1. F. R. Eberli, E. O. Weinberg, W. N. Grice, G. L. Horowitz, C. S. Apstein, Protective effect of increased glycolytic substrate against systolic and diastolic dysfunction and increased coronary resistance from prolonged global underperfusion and reperfusion in isolated rabbit hearts perfused with erythrocyte suspensions. *Circ. Res.* **68**, 466–481 (1991).
2. P. H. McNulty, D. Jagasia, G. W. Cline, C. K. Ng, J. M. Whiting, P. Garg, G. I. Shulman, R. Soufer, Persistent changes in myocardial glucose metabolism in vivo during reperfusion of a limited-duration coronary occlusion. *Circulation* **101**, 917–922 (2000).
3. A. Icks, H. Claessen, I. Kirchberger, M. Heier, A. Peters, I. Trentinaglia, G. Giani, W. von Scheidt, C. Meisinger, Mortality after first myocardial infarction in diabetic and non-diabetic people between 1985 and 2009. The MONICA/KORA registry. *Eur. J. Epidemiol.* **29**, 899–909 (2014).
4. K. J. Harjai, G. W. Stone, J. Boura, L. Mattos, H. Chandra, D. Cox, L. Grines, W. O'Neill, C. Grines, Comparison of outcomes of diabetic and nondiabetic patients undergoing primary angioplasty for acute myocardial infarction. *Am. J. Cardiol.* **91**, 1041–1045 (2003).
5. R. W. Jeremy, G. Ambrosio, M. M. Pike, W. E. Jacobus, L. C. Becker, The functional recovery of post-ischemic myocardium requires glycolysis during early reperfusion. *J. Mol. Cell. Cardiol.* **25**, 261–276 (1993).
6. W. G. T. Masoud, J. R. Ussher, W. Wang, J. S. Jaswal, C. S. Wagg, J. R. Dyck, C. A. Lygate, S. Neubauer, A. S. Clanachan, G. D. Lopaschuk, Failing mouse hearts utilize energy inefficiently and benefit from improved coupling of glycolysis and glucose oxidation. *Cardiovasc. Res.* **101**, 30–38 (2014).
7. J. R. Ussher, W. Wang, M. Gandhi, W. Keung, V. Samokhvalov, T. Oka, C. S. Wagg, J. S. Jaswal, R. A. Harris, A. S. Clanachan, J. R. B. Dyck, G. D. Lopaschuk, Stimulation of glucose oxidation protects against acute myocardial infarction and reperfusion injury. *Cardiovasc. Res.* **94**, 359–369 (2012).
8. D. L. Johnston, E. D. Lewandowski, Fatty acid metabolism and contractile function in the reperfused myocardium. Multinuclear NMR studies of isolated rabbit hearts. *Circ. Res.* **68**, 714–725 (1991).
9. Y.-T. Zhao, C.-L. Weng, M.-L. Chen, K.-B. Li, Y.-G. Ge, X.-M. Lin, W.-S. Zhao, J. Chen, L. Zhang, J.-X. Yin, X.-C. Yang, Comparison of glucose-insulin-potassium and insulin-glucose as adjunctive therapy in acute myocardial infarction: A contemporary meta-analysis of randomised controlled trials. *Heart* **96**, 1622–1626 (2010).
10. H. P. Selker, J. R. Beshansky, P. R. Sheehan, J. M. Massaro, J. L. Griffith, R. B. D'Agostino, R. Ruthazer, J. M. Atkins, A. J. Sayah, M. K. Levy, M. E. Richards, T. P. Aufderheide, D. A. Braude, R. G. Pirrallo, D. D. Doyle, R. J. Frascone, D. J. Kosiak, J. M. Leaming, C. M. Van Gelder, G.-P. Walter, M. A. Wayne, R. H. Woolard, L. H. Opie, C. E. Rackley, C. S. Apstein, J. E. Udelson, Out-of-hospital administration of intravenous glucose-insulin-potassium in patients with suspected acute coronary syndromes: The immediate randomized controlled trial. *JAMA* **307**, 1925–1933 (2012).
11. H. P. Selker, J. E. Udelson, J. M. Massaro, R. Ruthazer, R. B. D'Agostino, J. L. Griffith, P. R. Sheehan, P. Desvigne-Nickens, Y. Rosenberg, X. Tian, E. M. Vickery, J. M. Atkins, T. P. Aufderheide, A. J. Sayah, R. G. Pirrallo, M. K. Levy, M. E. Richards, D. A. Braude, D. D. Doyle, R. J. Frascone, D. J. Kosiak, J. M. Leaming, C. M. Van Gelder, G.-P. Walter, M. A. Wayne, R. H. Woolard, J. R. Beshansky, One-year outcomes of out-of-hospital

administration of intravenous glucose, insulin, and potassium (GIK) in patients with suspected acute coronary syndromes (from the IMMEDIATE [Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency Care] Trial). *Am. J. Cardiol.* **113**, 1599–1605 (2014).
12. B. G. Drew, S. J. Duffy, M. F. Formosa, A. K. Natoli, D. C. Henstridge, S. A. Penfold, W. G. Thomas, N. Mukhamedova, B. de Courten, J. M. Forbes, F. Y. Yap, D. M. Kaye, G. van Hall, M. A. Febbraio, B. E. Kemp, D. Sviridov, G. R. Steinberg, B. A. Kingwell, High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. *Circulation* **119**, 2103–2111 (2009).
13. A. L. Siebel, A. K. Natoli, F. Y. T. Yap, A. L. Carey, M. Reddy-Luthmoodoo, D. Sviridov, C. I. K. Weber, G. Meneses-Lorente, C. Maugeais, J. M. Forbes, B. A. Kingwell, Effects of high-density lipoprotein elevation with cholesteryl ester transfer protein inhibition on insulin secretion. *Circ. Res.* **113**, 167–175 (2013).
14. M. Lehti, E. Donelan, W. Abplanalp, O. Al-Massadi, K. M. Habegger, J. Weber, C. Ress, J. Mansfeld, S. Somvanshi, C. Trivedi, M. Keuper, T. Ograjsek, C. Striese, S. Cucuruz, P. T. Pfluger, R. Krishna, S. M. Gordon, R. A. Silva, S. Luquet, J. Castel, S. Martinez, D. D'Alessio, W. S. Davidson, S. M. Hofmann, High-density lipoprotein maintains skeletal muscle function by modulating cellular respiration in mice. *Circulation* **128**, 2364–2371 (2013).
15. B. J. Cochran, W. J. Ryder, A. Parmar, S. Tang, A. Reilhac, A. Arthur, A. Charil, H. Hamze, P. J. Barter, L. Kritharides, S. R. Meikle, M.-C. Gregoire, K.-A. Rye, In vivo PET imaging with [^{18}F]FDG to explain improved glucose uptake in an apolipoprotein A-I treated mouse model of diabetes. *Diabetologia* **59**, 1977–1984 (2016).
16. M. A. Fryirs, P. J. Barter, M. Appavoo, B. E. Tuch, F. Tabet, A. K. Heather, K.-A. Rye, Effects of high-density lipoproteins on pancreatic β-cell insulin secretion. *Arterioscler. Thromb. Vasc. Biol.* **30**, 1642–1648 (2010).
17. P. J. Barter, K.-A. Rye, J.-C. Tardif, D. D. Waters, S. M. Boekholdt, A. Breazna, J. J. P. Kastelein, Effect of torcetrapib on glucose, insulin, and hemoglobin A_{1c} in subjects in the investigation of lipid level management to understand its impact in atherosclerotic events (ILLUMINATE) trial. *Circulation* **124**, 555–562 (2011).
18. K. G. Stenkula, M. Lindahl, J. Petrlova, J. Dalla-Riva, O. Göransson, S. W. Cushman, E. Krupinska, H. A. Jones, J. O. Lagerstedt, Single injections of apoA-I acutely improve in vivo glucose tolerance in insulin-resistant mice. *Diabetologia* **57**, 797–800 (2014).
19. B. A. Kingwell, M. J. Chapman, Future of high-density lipoprotein infusion therapies: Potential for clinical management of vascular disease. *Circulation* **128**, 1112–1121 (2013).
20. J. Domingo-Espin, M. Lindahl, O. Nilsson-Wolanin, S. W. Cushman, K. G. Stenkula, J. O. Lagerstedt, Dual actions of apolipoprotein A-I on glucose-stimulated insulin secretion and insulin-independent peripheral tissue glucose uptake lead to increased heart and skeletal muscle glucose disposal. *Diabetes* **65**, 1838–1848 (2016).
21. L. Calabresi, G. Rossoni, M. Gomaraschi, F. Sisto, F. Berti, G. Franceschini, High-density lipoproteins protect isolated rat hearts from ischemia-reperfusion injury by reducing cardiac tumor necrosis factor-α content and enhancing prostaglandin release. *Circ. Res.* **92**, 330–337 (2003).
22. Y. Kiya, S.-i. Miura, S. Imaizumi, Y. Uehara, Y. Matsuo, S. Abe, S. Jimi, H. Urata, K.-A. Rye, K. Saku, Reconstituted high-density lipoprotein attenuates postinfarction left ventricular remodeling in rats. *Atherosclerosis* **203**, 137–144 (2009).
23. G. Theilmeier, C. Schmidt, J. Herrmann, P. Keul, M. Schäfers, I. Herrgott, J. Mersmann, J. Larmann, S. Hermann, J. Stypmann, O. Schober, R. Hildebrand, R. Schulz, G. Heusch, M. Haude, K. von Wnuck Lipinski, C. Herzog, M. Schmitz, R. Erbel, J. Chun, B. Levkau, High-density lipoproteins and their constituent, sphingosine-1-phosphate, directly protect the heart against ischemia/reperfusion injury in vivo via the $S1P_3$ lysophospholipid receptor. *Circulation* **114**, 1403–1409 (2006).
24. M. A. Frias, S. Pedretti, D. Hacking, S. Somers, L. Lacerda, L. H. Opie, R. W. James, S. Lecour, HDL protects against ischemia reperfusion injury by preserving mitochondrial integrity. *Atherosclerosis* **228**, 110–116 (2013).
25. L. Camont, M. Lhomme, F. Rached, W. Le Goff, A. Negre-Salvayre, R. Salvayre, C. Calzada, M. Lagarde, M. J. Chapman, A. Kontush, Small, dense high-density lipoprotein-3 particles are enriched in negatively charged phospholipids: Relevance to cellular cholesterol efflux, antioxidative, antithrombotic, anti-inflammatory, and antiapoptotic functionalities. *Arterioscler. Thromb. Vasc. Biol.* **33**, 2715–2723 (2013).
26. J. T. M. Tan, M. K. C. Ng, C. A. Bursill, The role of high-density lipoproteins in the regulation of angiogenesis. *Cardiovasc. Res.* **106**, 184–193 (2015).
27. H. C. G. Prosser, J. T. M. Tan, L. L. Dunn, S. Patel, L. Z. Vanags, S. Bao, M. K. C. Ng, C. A. Bursill, Multifunctional regulation of angiogenesis by high-density lipoproteins. *Cardiovasc. Res.* **101**, 145–154 (2014).
28. J. T. M. Tan, H. C. G. Prosser, L. L. Dunn, L. Z. Vanags, A. Ridiandries, T. Tsatralis, L. Leece, Z. E. Clayton, S. C. G. Yuen, S. Robertson, Y. T. Lam, D. S. Celermajer, M. K. C. Ng, C. A. Bursill, High-density lipoproteins rescue diabetes-impaired angiogenesis via scavenger receptor class B type I. *Diabetes* **65**, 3091–3103 (2016).
29. C. M. Gibson, S. Korjian, P. Tricoci, Y. Daaboul, M. Yee, P. Jain, J. H. Alexander, P. G. Steg, A. M. Lincoff, J. J. P. Kastelein, R. Mehran, D. M. D'Andrea, L. I. Deckelbaum, B. Merkely, M. Zarebinski, T. O. Ophuis, R. A. Harrington, Safety and tolerability of CSL112, a reconstituted, infusible, plasma-derived apolipoprotein A-I, after acute myocardial infarction: The AEGIS-I trial (ApoA-I Event Reducing in Ischemic Syndromes I). *Circulation* **134**, 1918–1930 (2016).
30. L. Bertrand, S. Horman, C. Beauloye, J.-L. Vanoverschelde, Insulin signalling in the heart. *Cardiovasc. Res.* **79**, 238–248 (2008).
31. S. Imaizumi, S.-i. Miura, K. Nakamura, Y. Kiya, Y. Uehara, B. Zhang, Y. Matsuo, H. Urata, M. Ideishi, K.-A. Rye, M. Sata, K. Saku, Antiarrhythmogenic effect of reconstituted high-density lipoprotein against ischemia/reperfusion in rats. *J. Am. Coll. Cardiol.* **51**, 1604–1612 (2008).
32. M.-C. Brulhart-Meynet, V. Braunersreuther, J. Brinck, F. Montecucco, J.-C. Prost, A. Thomas, K. Galan, G. Pelli, S. Pedretti, N. Vuilleumier, F. Mach, S. Lecour, R. W. James, M. A. Frias, Improving reconstituted HDL composition for efficient post-ischemic reduction of ischemia reperfusion injury. *PLOS ONE* **10**, e0119664 (2015).
33. D. J. Hausenloy, D. M. Yellon, Reperfusion injury salvage kinase signalling: Taking a RISK for cardioprotection. *Heart Fail. Rev.* **12**, 217–234 (2007).
34. A. A. Gupte, L. J. Minze, M. Reyes, Y. Ren, X. Wang, G. Brunner, M. Ghosn, A. M. Cordero-Reyes, K. Ding, D. Pratico, J. Morrisett, Z.-Z. Shi, D. J. Hamilton, C. J. Lyon, W. A. Hsueh, High-fat feeding-induced hyperinsulinemia increases cardiac glucose uptake and mitochondrial function despite peripheral insulin resistance. *Endocrinology* **154**, 2650–2662 (2013).
35. L. Haar, X. Ren, Y. Liu, S. E. Koch, J. Goines, M. Tranter, M. A. Engevik, M. Nieman, J. Rubinstein, W. K. Jones, Acute consumption of a high-fat diet prior to ischemia-reperfusion results in cardioprotection through NF-κB-dependent regulation of autophagic pathways. *Am. J. Physiol. Heart Circ. Physiol.* **307**, H1705–H1713 (2014).
36. J. E. Jordan, S. A. Simandle, C. D. Tulbert, D. W. Busija, A. W. Miller, Fructose-fed rats are protected against ischemia/reperfusion injury. *J. Pharmacol. Exp. Ther.* **307**, 1007–1011 (2003).
37. D. Donner, J. P. Headrick, J. N. Peart, E. F. du Toit, Obesity improves myocardial ischaemic tolerance and RISK signalling in insulin-insensitive rats. *Dis. Model. Mech.* **6**, 457–466 (2013).
38. M. Silvennoinen, R. Rinnankoski-Tuikka, M. Vuento, J. J. Hulmi, S. Torvinen, M. Lehti, R. Kivelä, H. Kainulainen, High-fat feeding induces angiogenesis in skeletal muscle and activates angiogenic pathways in capillaries. *Angiogenesis* **16**, 297–307 (2013).
39. M. J. Raher, H. B. Thibault, E. S. Buys, D. Kuruppu, N. Shimizu, A.-L. Brownell, S. L. Blake, J. Rieusset, M. Kaneki, G. Derumeaux, M. H. Picard, K. D. Bloch, M. Scherrer-Crosbie, A short duration of high-fat diet induces insulin resistance and predisposes to adverse left ventricular remodeling after pressure overload. *Am. J. Physiol. Heart Circ. Physiol.* **295**, H2495–H2502 (2008).
40. H. Zeng, V. R. Vaka, X. He, G. W. Booz, J.-X. Chen, High-fat diet induces cardiac remodelling and dysfunction: Assessment of the role played by SIRT3 loss. *J. Cell. Mol. Med.* **19**, 1847–1856 (2015).
41. B. A. Kingwell, M. J. Chapman, A. Kontush, N. E. Miller, HDL-targeted therapies: Progress, failures and future. *Nat. Rev. Drug Discov.* **13**, 445–464 (2014).
42. N. Turner, G. M. Kowalski, S. J. Leslie, S. Risis, C. Yang, R. S. Lee-Young, J. R. Babb, P. J. Meikle, G. I. Lancaster, D. C. Henstridge, P. J. White, E. W. Kraegen, A. Marette, G. J. Cooney, M. A. Febbraio, C. R. Bruce, Distinct patterns of tissue-specific lipid accumulation during the induction of insulin resistance in mice by high-fat feeding. *Diabetologia* **56**, 1638–1648 (2013).
43. J. H. Kim, T. P. Stewart, M. Soltani-Bejnood, L. Wang, J. M. Fortuna, O. A. Mostafa, N. Moustaid-Moussa, A. M. Shoieb, M. F. McEntee, Y. Wang, L. Bechtel, J. K. Naggert, Phenotypic characterization of polygenic type 2 diabetes in TALLYHO/JngJ mice. *J. Endocrinol.* **191**, 437–446 (2006).
44. X.-M. Gao, Y. Liu, D. White, Y. Su, B. G. Drew, C. R. Bruce, H. Kiriazis, Q. Xu, N. Jennings, A. Bobik, M. A. Febbraio, B. A. Kingwell, R. Bucala, G. Fingerle-Rowson, A. M. Dart, E. F. Morand, X.-J. Du, Deletion of macrophage migration inhibitory factor protects the heart from severe ischemia–reperfusion injury: A predominant role of anti-inflammation. *J. Mol. Cell. Cardiol.* **50**, 991–999 (2011).
45. M. K. O'Connor, T. Hammell, R. J. Gibbons, In vitro validation of a simple tomographic technique for estimation of percentage myocardium at risk using methoxyisobutyl isonitrile technetium 99m (sestamibi). *Eur. J. Nucl. Med.* **17**, 69–76 (1990).
46. S. Patel, B. G. Drew, S. Nakhla, S. J. Duffy, A. J. Murphy, P. J. Barter, K.-A. Rye, J. Chin-Dusting, A. Hoang, D. Sviridov, D. S. Celermajer, B. A. Kingwell, Reconstituted high-density lipoprotein increases plasma high-density lipoprotein anti-inflammatory properties and cholesterol efflux capacity in patients with type 2 diabetes. *J. Am. Coll. Cardiol.* **53**, 962–971 (2009).
47. D. A. Ferrick, A. Neilson, C. Beeson, Advances in measuring cellular bioenergetics using extracellular flux. *Drug Discov. Today* **13**, 268–274 (2008).

Acknowledgments: We thank M. de Veer and P. Ward from Monash Biomedical Imaging for assistance with the PET/CT studies. We also thank CSL Ltd. for providing the rHDL (CSL-111). **Funding:** This work was supported by the National Heart Foundation (NHF) of Australia (G 11M 5914, A.L.S. and B.A.K.), NHMRC of Australia (APP103652, B.A.K.), and the Operational Infrastructure Support scheme of the Victorian State Government. B.A.K. and X.-J.D. are NHMRC research fellows. D.C.H. was supported by an Australian Diabetes Society Skip Martin

Fellowship. C.E.H. is an NHF Career Development Fellow (CR11M6066). The remaining authors have no funding disclosures. **Author contributions:** S.E.H., A.L.R., A.L.S., and B.A.K. conceived the study. S.E.H., A.L.R., D.C.H., K.A., H.K., C.Z., A.L.C., H.L.K, M.R., and Y.-C.C. collected the data. S.E.H., A.L.R., A.L.S., and B.A.K. drafted the manuscript. L.M.D., X.-J.D., C.E.H., M.A.F., A.L.S., and B.A.K. supervised the study. D.C.H., K.A., H.K., A.L.C., X.-J.D., C.E.H., M.A.F., A.L.S., and B.A.K. revised the manuscript. A.L.S. and B.A.K. acquired the funding. **Competing interests:** CSL Ltd. provided the rHDL (CSL-111) but played no role in study design, data collection, analysis, or interpretation. CSL has provided travel support to the Baker Heart and Diabetes Institute. There are no other disclosures. **Data and materials availability:** All relevant data are available in the manuscript.

Submitted 20 December 2016
Resubmitted 30 May 2017
Accepted 22 August 2017
Published 11 October 2017
10.1126/scitranslmed.aam6084

Citation: S. E. Heywood, A. L. Richart, D. C. Henstridge, K. Alt, H. Kiriazis, C. Zammit, A. L. Carey, H. L. Kammoun, L. M. Delbridge, M. Reddy, Y.-C. Chen, X.-J. Du, C. E. Hagemeyer, M. A. Febbraio, A. L. Siebel, B. A. Kingwell, High-density lipoprotein delivered after myocardial infarction increases cardiac glucose uptake and function in mice. *Sci. Transl. Med.* **9**, eaam6084 (2017).

Science Translational Medicine

High-density lipoprotein delivered after myocardial infarction increases cardiac glucose uptake and function in mice

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Sci Transl Med **9**, eaam6084.
DOI: 10.1126/scitranslmed.aam6084

Lipoprotein lends a hand for heart attacks

Preventing myocyte damage after myocardial infarction could help stop the development of heart failure. Heywood *et al*. administered reconstituted high-density lipoprotein (rHDL) after inducing cardiac ischemia in mice and showed that treatment caused increased glucose uptake in myocytes, reduced infarct size, and improved ventricle function. rHDL was effective in prediabetic and healthy mice, suggesting that it may be a promising treatment for acute coronary syndrome.