Aim: Circulating levels of high-density lipoprotein cholesterol (HDL-C) are decreased in patients with heart failure (HF). We tested whether HDL-C serum levels are associated with cardiac contractile dysfunction in a minipig HF model.

Methods: Blood samples were collected from 13 adult male minipigs: 1) before pacemaker implantation, 2) 10 days after surgery, and 3) 3 weeks after high-rate LV pacing. Serum cholesterol efflux capacity (CEC), an index of HDL functionality, was assessed through four mechanisms: ATP Binding Cassette transporter A1 (ABCA1), ATP Binding Cassette transporter G1 (ABCG1), Scavenger Receptor-Class B Type I (SR-BI) and Passive Diffusion (PD).

Results: HDL-C serum levels significantly decrease in minipigs with HF compared with baseline ($p < 0.0001$). Serum CEC mediated by PD and SR-BI, but not ABCA1 or ABCG1, significantly decrease in animals with HF ($p < 0.05$ and $p < 0.005$, respectively).

Discussion: HDL-C serum levels and partial serum CEC reduction may play a pathophysiological role in the cardiac function decay sustained by high-rate LV pacing, opening new avenues to understand of the pathogenesis of nonischemic myocardial remodeling.

Key words: Cholesterol efflux, HDL, Minipig, Non-ischemic heart failure
development of nonischemic HF is lacking\textsuperscript{12-14}.

**Aim**

To investigate the role of HDL in the progression of cardiac contractile dysfunction in nonischemic cardiomyopathy, in absence of coronary artery disease and without interference of either medications or comorbidities\textsuperscript{15, 16}, we simultaneously assessed changes in circulating lipids and cardiac contractile function in a clinically relevant model of HF, the swine model of pacing-induced HF. Nowadays, it is well known that this animal model shows an excellent similarity with human dyslipidemia.\textsuperscript{17}

We tracked lipid profile and cardiac function in minipigs at three time points: 1) before pacemaker implantation (baseline), 2) 10 days after surgery (before HF), and 3) 3 weeks after high-rate pacing (established HF). In order to better assess serum cholesterol efflux capacity, which depends on the ability of the different HDL subclasses to function as cholesterol acceptors through different efflux mechanisms\textsuperscript{18}, we investigated four main cholesterol efflux pathways: ATP binding cassette transporter A1 (ABCA1), ATP binding cassette transporter G1 (ABCG1), scavenger receptor-class B type I (SR-BI)\textsuperscript{19, 20}, and passive diffusion (PD).

We found that HDL-C serum levels and, in part, serum cholesterol efflux capacity were decreased in minipigs with pacing-induced HF and elevated inflammatory markers, suggesting a putative causal role of HDL in cardiac contractile dysfunction, independent of coronary atherosclerotic stenosis.

**Methods**

**Animal Model of Pacing-Induced HF**

**Surgical Instrumentation:** About 13 male, sexually mature minipigs (35–40 kg) were sedated with a cocktail of tiletamine hydrochloride and zolazepam hydrochloride (8 mg/kg, IM), and atropine sulfate (0.1 mg/kg, IM). General anesthesia was subsequently induced with propofol (2–4 mg/kg, IV) and maintained with 1–2% sevoflurane in 60% air and 40% oxygen. Mechanical ventilation was adjusted based on arterial blood gas values during EKG monitoring.

Subsequently, the heart was exposed through left thoracotomy and the following probes and catheters were placed:

1) a A solid-state pressure gauge (Konigsberg Instruments) to measure LV pressures;

2) a A screw-type unipolar, epicardial pacing lead (5071 IS-1 UNI; Medtronic, Inc.) connected to an implantable pacemaker (PREVAILTM, Medtronic, Inc) to stimulate the LV free wall;

3) a A heparin-coated polyethylene catheter to measure aortic blood pressure and to collect blood samples;

4) a A heparin-coated polyethylene catheter inserted into the coronary sinus to collect blood samples.

Catheters were run subcutaneously to the dorsal region, the chest was closed in layers, and the pneumothorax was reduced, as previously described\textsuperscript{15}. The animals were housed in a single cage to fully recover from surgery and antibiotics and analgesics were provided for 10 days, as previously described\textsuperscript{15, 16}; during the study, animals were fed with standard minipig chow.

**Hemodynamics and Echocardiographic Recordings:** LV pressure was measured in lightly sedated pigs (tiletamine hydrochloride and zolazepam hydrochloride, 8 mg/kg, IM) using the solid-state pressure gauge. The aortic catheter was attached to a strain-gauge transducer to measure blood pressure. All signals were recorded on an eight-channel direct writing polygraph. The LV diastolic performance was calculated by measuring $dP/dt_{\text{min}}$ and tau, the time constant of isovolumetric relaxation. The analog signals were also stored on a computer through an analog–digital converter (National Instruments), at a sampling rate of 250 Hz for later analysis. Analysis was performed using custom software (WinPVAN, v 3.5.8).

Twodimensional and M-mode transthoracic echocardiography was performed using a left parasternal approach at the mid-papillary muscle level. LV ejection fraction (LVEF) was measured as index of global LV function. Measurements were made using a commercially available echocardiography system (MyLab® 30, Esaote, Genoa, Italy) in accordance to the criteria of the American Society of Echocardiography.\textsuperscript{21}

**Experimental Protocol:** The pacemaker was turned on after complete recovery of the animals from surgery (10 days). Pacing the LV at 180 beats/min for 3 weeks induced HF, as previously described\textsuperscript{15}. At the end of the third week of pacing, a LV end-diastolic pressure ≥ 20 mmHg and an ejection fraction < 40% indicated a fully established HF\textsuperscript{15, 16}. This corresponds to a condition of severe, although nonterminal, HF.

Hemodynamic and echocardiographic parameters and blood sampling were performed at baseline, 10 days after surgery and after 3 weeks of LV pacing.

Experimental protocols were approved by the Animal Care Committee of the Italian Ministry of Health and conducted in conformity with the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

**Laboratory Analysis:** Total cholesterol (TC),...
HDL-C, triglycerides (TG), glucose, aspartate aminotransferase (ALT), and alanine aminotransferase (AST) were measured by standard enzymatic techniques (Synchorn CX9 Pro, Beckman Coulter, Inc. Fullerton USA)\(^{17}\); low-density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald formula. Apolipoprotein A-I, complement C3c, ceruloplasmin, and alpha-2 macroglobulin were measured by rate nephelometry (BN-ProSpec, Siemens Healthcare Diagnostics, Italy).

Serum cholesterol efflux capacity (CEC): All serum samples collected from minipigs were stored at -80°C. Serum was slowly thawed on ice just before CEC analysis. We measured four main efflux mechanisms responsible for cholesterol removal from cells, which have been previously characterized\(^{20}\); J774 macrophages in the absence or presence of 8-(4-Chlorophenylthio)adenosine 3',5'-cyclic monophosphate (cpt-cAMP) (Sigma Aldrich, Milano, Italy) at 0.3 mM were used for the evaluation of the passive diffusion (PD) process and total efflux, respectively. In basal condition, most of cholesterol efflux in J774 macrophages is related to PD being the ABCA1 contribution to cholesterol efflux very low. Treatment with cpt-cAMP 0.3 mM dramatically upregulates the ABCA1 protein expression\(^{22}\); the specific ABCA1 contribution was calculated as the difference between cholesterol efflux obtained from cpt-cAMP-stimulated cells and efflux from cells in basal conditions\(^{19,20}\). Rat hepatoma Fu5AH were used for SR-BI-mediated cholesterol efflux\(^{18}\); Chinese hamster ovary (CHO) cells not transfected or transfected with the human Abcag1 gene were used to evaluate ABCG1-mediated efflux\(^{23}\). In all assays, the cells were seeded in 24-well plates in the presence of 10% fetal bovine serum (FBS) (Sigma Aldrich) and labeled with 2µCi/ml of [1,2-3H]-cholesterol (PerkinElmer, Milano, Italy) for 24h and then equilibrated in 0.2% bovine serum albumin (BSA)-containing medium (Sigma Aldrich) for 18h (for total-, PD-, SR-BI-, and ABCA1-dependent efflux) or 90 min (for ABCG1-dependent efflux). After the equilibration time, cells were then exposed to 1% (for ABCG1-dependent efflux) or 2% (for total-, PD-, SR-BI-, and ABCA1-dependent efflux) of whole serum from minipigs collected at the three time points studied (baseline, 10 days after surgery, and 3 weeks after high-rate pacing). Serum CEC was calculated as the percentage radioactivity released to the medium over the total label incorporated by cells. Efflux values were normalized to the CEC obtained with a pool of normolipidemic sera tested during each experiment. Individual CECs were calculated as averages of three replicates. Intra-assay coefficients of variation for the CEC measures were 4.1% for PD, 9.0% for ABCA1, 3.4% for SR-BI, and 5.5% for ABCG1; interassay coefficients were 12.0% for PD, 9.5% for ABCA1, 3.4% for SR-BI, and 5.5% for ABCG1.

**Statistical Analyses:** Lipid parameters are expressed as mean ± standard deviation (SD); the mean values of the three experimental time points were compared using ANOVA test for repeated measures; differences with \(p<0.05\) were considered significant. Linear regression analysis with Pearson coefficient (r) was applied to investigate the association between inflammation markers or lipoprotein-related parameters and serum CEC.

**Results**

**Hemodynamics and Echocardiographic Recordings:** As shown in Table 1, spontaneous heart rate and LV end diastolic pressure at the end of the experimental protocol increase significantly in paced minipigs, whereas the \(dP/dt_{\text{max}}\) and \(dP/dt_{\text{min}}\) significantly decrease, although tau values significantly increase. LV ejection fraction was significantly decreased after sustained high-rate LV pacing, compared with baseline.
before surgery and after recovery from surgery.

**Circulating Lipids in a Model of Non-Ischemic Heart Disease:** After 3 weeks of high-rate LV pacing, serum LDL concentration increase compared with baseline values (26.1 ± 11.2 vs. 36.3 ± 12.6 mg/dl, \(p < 0.05\)), yet TC and TG levels were similar. Compared with baseline, serum HDL-C levels decreased significantly 10 days after surgery (24.5 ± 4.8 vs. 19.0 ± 4.8 mg/dl, \(p < 0.05\)) and after established HF (24.5 ± 8.0 vs. 12.4 ± 5.7 mg/dl, \(p < 0.0001\)), with a significant 35% reduction in animals with HF compared with blood obtained 10 days after surgery (12.4 ± 5.7 vs. 19.0 ± 4.8 mg/dl, \(p < 0.005\); Table 2). The TC/HDL-C ratio significantly increased after established HF compared with both baseline (5.2 ± 2.4 vs. 2.5 ± 0.5, \(p < 0.0001\)) and 10 days after surgery (5.2 ± 2.4 vs. 3.0 ± 0.6, \(p < 0.0005\)). Serum apoA-I levels decrease by 32% (20.9 ± 9.3 vs. 14.2 ± 4.5 mg/dl, \(p < 0.005\)) and 44% (20.9 ± 9.3 vs. 11.8 ± 5.5 mg/dl, \(p < 0.005\)) 10 days after surgery and after HF was established, respectively, compared with baseline values, in accordance with previous studies\(^ {22}\). Ceruloplasmin serum concentrations increased 10 days after surgery (24.7 ± 20.8 vs. 60.7 ± 43.2 mg/dl, \(p < 0.05\)) and, even further, after established HF (24.7 ± 20.8 vs. 83.9 ± 54.6 mg/dl, \(p < 0.005\)). Even though there was no significant change in the C3 levels, \(a_2\)-macroglobulin levels decrease at borderline significance after surgery (Table 2).

**Serum Cholesterol Efflux Capacity:** We first analyzed total serum CEC in macrophages stimulated to express ABCA1 by treatment with cpt-cAMP at 0.3 mM. Compared with baseline, total CEC decrease by 20% after surgery and after established HF (from 12.73 ± 0.29% to 10.25 ± 2.88% and to 10.42 ± 3.27%, respectively; Fig. 1), albeit not statistically significant.

No significant change in ABCA1- and ABCG1-mediated serum CEC in animals with pacing-induced HF was observed (Fig. 2, Panel A and B). Conversely, passive diffusion (PD)-mediated serum CEC decrease in animals with HF compared with baseline (from 8.27 ± 1.19% to 5.97 ± 2.12%, \(p < 0.05\)), with no changes between baseline and 10 days after surgery (Fig. 2, Panel C). SR-BI-mediated serum CEC significantly decrease in heart failing animals compared with baseline (3.03 ± 1.17% vs. 4.88 ± 0.56%, \(p < 0.005\)) or 10 days after surgery (3.03 ± 1.17% vs. 3.95 ± 0.91%, \(p < 0.05\); Fig. 2, Panel D). In addition, in all samples a positive correlation was found between SR-BI-CEC values and serum HDL-C levels (r = 0.872, \(p < 0.0001\)), and between PD-CEC values and HDL-C levels (r = 0.601, \(p < 0.005\), which appear to be driven by the animals with HF and not by animals at basal or at 10 days after surgery (Fig. 3, Panel A and B). We also found a negative correlation between both SR-BI- and PD-mediated serum CEC values and total serum levels of ceruloplasmin in animals with HF (r = −0.917, \(p < 0.005\) and r = −0.886, \(p < 0.05\), respectively; Fig. 3, Panel C and D).

**Discussion**

The major findings of this study are: 1) circulating HDL-C and apoA-I significantly decrease in healthy minipigs 10 days after surgical implantation of

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**Table 2.** Baseline biohumoral data before device implantation, ten days after surgery and after established HF.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Before HF (10 days after surgery)</th>
<th>Established HF (3 weeks of LV pacing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>57.7 ± 16.0</td>
<td>55.2 ± 9.8</td>
<td>54.2 ± 13.0</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>24.5 ± 8.0</td>
<td>19.0 ± 4.8(^$)</td>
<td>12.4 ± 5.7(^**)</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>26.1 ± 11.2</td>
<td>31.6 ± 9.2</td>
<td>36.3 ± 12.6(^$)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>31.7 ± 7.6</td>
<td>25.9 ± 14.7</td>
<td>31.4 ± 14.8</td>
</tr>
<tr>
<td>Total Chol/ HDL-cholesterol</td>
<td>2.5 ± 0.5</td>
<td>3.0 ± 0.6</td>
<td>5.2 ± 2.4(^**)</td>
</tr>
<tr>
<td>Apoprotein AI (mg/dL)</td>
<td>20.9 ± 9.3</td>
<td>14.2 ± 4.5(^$)</td>
<td>11.8 ± 5.5(^$)</td>
</tr>
<tr>
<td>Fraction Complement C3 (mg/dL)</td>
<td>12.9 ± 14.0</td>
<td>12.1 ± 4.9</td>
<td>10.3 ± 6.4</td>
</tr>
<tr>
<td>Ceruloplasmin (mg/dL)</td>
<td>24.7 ± 20.8</td>
<td>60.7 ± 43.2(^$)</td>
<td>83.9 ± 54.6(^$)</td>
</tr>
<tr>
<td>Alpha 2 Macroglobulin (mg/dL)</td>
<td>119.8 ± 30.2</td>
<td>79.6 ± 56.6</td>
<td>80.7 ± 49.0</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>74.6 ± 14.6</td>
<td>73.5 ± 30.0</td>
<td>77 ± 15.7</td>
</tr>
<tr>
<td>AST (UI/L)</td>
<td>27.3 ± 9.2</td>
<td>35.1 ± 42.5</td>
<td>35.4 ± 34.7</td>
</tr>
<tr>
<td>ALT (UI/L)</td>
<td>39.1 ± 10.7</td>
<td>30.7 ± 5.4</td>
<td>23.7 ± 6.8</td>
</tr>
<tr>
<td>GGT(UI/L)</td>
<td>74.4 ± 40.1</td>
<td>50.1 ± 32.5</td>
<td>74.1 ± 41.6</td>
</tr>
</tbody>
</table>

Data are reported as means ± SD. AST, aspartate amino transferase; ALT, alanine amino transferase; GGT, gamma-glutamyltransferase. *\(p < 0.05\) Ten days after surgery vs established HF; \(^\$\)\(p < 0.05\) Baseline vs established HF; \(^\$\)\(p < 0.05\) baseline vs ten days after surgery.
a pacing device (before pacing initiation), when cardiac function is fully recovered and similar to baseline; and 2) HDL-C and apoA-I further decrease when animals develop heart failure, however, TC and TG levels remain unchanged by chronic stressors, such as surgery and high-rate pacing.

A previous study suggested that during acute-phase stress processes, HDL-C and apoA-I levels decrease within 12h and rebound to the baseline values within 2 weeks\textsuperscript{24}. In our study, circulating levels of HDL-C and apoA-I remained significantly lower than baseline 10 days after surgery, although animals appeared fully recovered and healthy. HDL-C and apoA-I were further reduced after 3 weeks of high-rate LV pacing. It is plausible that the pro-inflammatory state induced by surgery leads to a progressive HDL-C and apoA-I depletion, which could further impair cardiac contractile function during the development of heart failure. To demonstrate this hypothesis, we first excluded alterations in the pathways responsible for intracellular cholesterol homeostasis, a well-established risk factor for atherosclerotic cardiac dysfunction\textsuperscript{25}.

The amount of apoA-I in HDL-C can differ among species\textsuperscript{26}; apoA-I and HDL-C levels may fluctuate within the same individual. ApoA-I deficiency results in a marked, but not full, reduction in HDL-C in mice\textsuperscript{27, 28} and in humans, where it is associated with premature coronary artery disease\textsuperscript{29}. Therefore, we report for the first time that in a minipig model of nonischemic HF, apoA-I level is associated with a decrease in HDL-C, as observed in other species.

To investigate if the serum ability to promote RCT could be altered in our HF model, we measured serum capacity to promote cholesterol efflux (CEC) from macrophages. Despite that cholesterol efflux from macrophages represents only a fraction of overall flux through the RCT pathway; it plays a critical and major role in RCT and in protection against atherosclerotic dysfunction. In particular, it has been recently identified as a functional cardiovascular risk biomarker and a promising target for prevention and therapies of cardiovascular diseases, independently from HDL serum concentration\textsuperscript{19, 30-34}. In heart failing minipigs, we found a modest (20%), nonsignificant change in total serum cholesterol efflux capacity compared to baseline, even if myocardial perfusion was globally decreased\textsuperscript{15, 16}.

Atheroprotective properties of serum HDL-C are mediated by several mechanisms, including anti-inflammatory and anti-oxidant effects; however, the main atheroprotective function is related to their capacity to act as cell cholesterol acceptors and promote cholesterol efflux from cells. This activity depends not only on the circulating levels of HDL-C, but also primarily on their composition and function. Serum cholesterol efflux capacity (CEC) has been recently identified as a potential new biomarker of cardiovascular risk as it negatively correlates to the incidence
Moreover, free apoA-I active as ABCA1 acceptor constitutes only about 5% of circulating apoA-I\textsuperscript{20, 38}. Furthermore, we previously observed that the correlation between ABCG1-mediated CEC and HDL-C level is weak as well, explaining, at least in part, the lack of effect of the decrease in HDL-C level on ABCAG1-CEC\textsuperscript{20}.

The cholesterol mass efflux from macrophages occurs also through a PD along a concentration gradient between the membrane and HDL maintained by LCAT-mediated esterification of cholesterol molecules in the HDL particles. Cholesterol efflux through PD may be facilitated by the presence of the membrane of the SR-BI receptor\textsuperscript{39}. Interestingly, we found a significant decrease in PD- and SR-BI-mediated serum CEC values in animals with heart failure compared to base-

**Fig. 2.** Serum cholesterol efflux capacity (CEC) from minipigs at baseline, 10 days after pacing implantation, and after established HF. Data are reported as mean ± SD. Panel A, B, C, and D represent measurement of specific CEC pathways (ABCA1-mediated; ABCG1-mediated, passive diffusion (PD); SR-BI-mediated).

*\( p < 0.05 \); **\( p < 0.005 \)

and prevalence of cardiovascular events\textsuperscript{5, 19, 30, 31, 33-35}. Cholesterol efflux to extracellular acceptors, via several different pathways, represents a response to increasing intracellular load of lipids and exerts an antiatherosclerotic activity by both passive and receptor-mediated mechanisms\textsuperscript{20}.

In this study we did not detect any alteration in serum ABCA1- and ABCG1-mediated CEC, two active pathways of CEC, after surgery or after high-rate cardiac pacing. Although both levels of ApoA1 and HDL-C decrease after surgery or high-rate cardiac pacing, ABCA1- and ABCG1-mediated cholesterol efflux capacity were unchanged. Our finding can be explained because neither apoA-I nor HDL-C serum concentration values reflect actual pre-beta HDL serum content, specific acceptor for ABCA1\textsuperscript{36, 37}. Moreover, free apoA-I active as ABCA1 acceptor constitutes only about 5% of circulating apoA-I\textsuperscript{20, 38}. Furthermore, we previously observed that the correlation between ABCG1-mediated CEC and HDL-C level is weak as well, explaining, at least in part, the lack of effect of the decrease in HDL-C level on ABCAG1-CEC\textsuperscript{20}.

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cardiomyopathy due to both HDL low circulating levels and impaired HDL efficiency as cholesterol acceptor from macrophages. Furthermore, studies are warranted to better investigate the mechanisms underlying the relationship between reduced serum cholesterol efflux, mediated by PD and SR-BI, and the progressive decay of cardiac contractile function in the absence of coronary artery disease.

We cannot exclude that lipid alterations in our animals were worsened by an inflammatory response due to sustained LV pacing-induced dyssynchrony\textsuperscript{15, 16), rather than myocardial blood hypo-perfusion. Indeed, it is known that serum HDL levels are decreased during acute inflammatory responses; serum amyloid A (SAA) accumulates on HDL and HDL remodels via line. The reduction observed in PD- and SR-BI-mediated serum CEC in animals with heart failure may be more strictly related to changes in total HDL serum levels. As we previously highlighted\textsuperscript{40), we found a strong positive correlation between HDL levels and both SRBI-mediated and PD-mediated CEC. These data are consistent with the well-established concept about the close functional relation between the two efflux mechanisms. In particular, the SR-BI cholesterol efflux process facilitates the passive exchange of cholesterol between cell and cholesterol acceptors in the extracellular space\textsuperscript{41).}

Our data are also in accordance with Patel at al.\textsuperscript{42), which showed an impairment of HDL cholesterol efflux capacity in humans affected by ischemic cardiomyopathy due to both HDL low circulating levels and impaired HDL efficiency as cholesterol acceptor from macrophages. Furthermore, studies are warranted to better investigate the mechanisms underlying the relationship between reduced serum cholesterol efflux, mediated by PD and SR-BI, and the progressive decay of cardiac contractile function in the absence of coronary artery disease.

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\begin{figure}
\centering
\begin{subfigure}{0.45\textwidth}
\centering
\includegraphics[width=\textwidth]{A.png}
\caption{Pearson $r = 0.872, p<0.0001$}
\end{subfigure}
\begin{subfigure}{0.45\textwidth}
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\includegraphics[width=\textwidth]{B.png}
\caption{Pearson $r = 0.601, p<0.005$}
\end{subfigure}
\begin{subfigure}{0.45\textwidth}
\centering
\includegraphics[width=\textwidth]{C.png}
\caption{Pearson $r = -0.917, p<0.005$}
\end{subfigure}
\begin{subfigure}{0.45\textwidth}
\centering
\includegraphics[width=\textwidth]{D.png}
\caption{Pearson $r = 0.886, p<0.05$}
\end{subfigure}
\caption{Correlation between SR-BI- and PD-mediated cholesterol efflux capacity (CEC) and HDL-C level (panel A and B, respectively); correlation between SR-BI- and PD-mediated CEC and ceruloplasmin plasma level in serum samples after established HF (panel C and D, respectively). Correlation is expressed using Pearson coefficient ($r$).}
\end{figure}
acute-phase group II phospholipases. In addition, SAA-containing HDL has been shown to have reduced ability to facilitate cholesterol efflux from macrophages. In our study, circulating levels of ceruloplasmin, an established proinflammatory mediator, were still significantly elevated 10 days after surgery, indicating a residual inflammatory response to surgery, associated with HDL reduction. Moreover, the levels of ceruloplasmin further increased in pigs with dysynchronous heart failure and were associated with even lower HDL-C levels. It is well known that the myocardial synthesis of ceruloplasmin depends on mononuclear cells adherent to coronary vessels and is triggered by several inflammatory mediators, such as TNF-α. We have previously demonstrated a rise in myocardial TNF-α expression in minipigs with similar decay of cardiac function, which could explain the observed increase in ceruloplasmin. In addition, we found that ceruloplasmin levels inversely correlate with SR-BI- and PD-CEC values during sustained LV dyssynchrony, suggesting a synergistic role of inflammation and impaired HDL/apoA-I levels in the onset and progression of HF, as already observed in other studies.

Assuming that inflammation, myocardial blood flow redistribution, and cardiomyocytes hypertrophy during ventricular remodeling play an adaptive role in response to decreased cardiac output, our study suggests that decreased HDL/apoA-I and partially impaired cholesterol efflux capacity, mediated by PD and SR-BI, are negative acute-phase mediators of HF progression. In accordance with our hypothesis, lower apoA-I serum levels were detected in adult pigs during the acute-phase inflammatory response induced by infection with Actinobacillus pleuropneumoniae or Streptococcus suis. Conversely, it has been suggested that elevated circulating HDL levels may exert a protective effect against ischemia/reperfusion injury in an isolated rat heart. Our findings are clinically relevant as our observations are made in a reliable, large animal model of nonischemic HF. Pacing-induced HF in large animals is considered the gold standard preclinical model for its similarities with human dilated cardiomyopathy. Our experimental observations support the hypothesis that during sustained high-rate LV dyssynchrony, a progressive decrease in HDL-C serum levels and functionality, as measured by cholesterol efflux capacity, may contribute to worsen the cardiac contractile function in the absence of detectable coronary flow-limiting stenosis. Unraveling the mechanisms behind the direct effects of HDL dysfunction on cardiomyocyte function in settings of a proinflammatory microenvironment, may be useful to clarify the pathophysiology of nonischemic HF and the management of patients with dilated cardiomyopathy.

Study Limitations
First, we did not assess the composition of HDL individual proteins, other than apoA-I, which could be present during the acute phase after surgical stress and pacing and might have a role in the pathogenesis of nonischemic heart failure. Second, intermediate blood samples, during the development of heart failure, would have allowed a time-course evaluation of the decay of cardiac contractile function and the impairment of HDL concentration/functionality.

Conclusions
In our model of minipig pacing-induced HF, a progressive decrease in HDL-C serum levels and functionality, measured by cholesterol efflux capacity, appear to be associated with a worsening in cardiac function without evidence of coronary stenosis.

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This study has been realized with public funds.

Disclosures
The authors declare no conflict of interest.

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