Effects of lipids on the functional and metabolic recovery from global myocardial stunning in isolated rabbit hearts

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Abstract

Objectives: High concentrations of free fatty acids may increase myocardial ischaemic damage. However, the administration of lipid emulsions during reperfusion improves the functional recovery of stunned myocardium. From this apparent controversy we hypothesise that the effect of lipids is related to the time of its administration: we compared the effects of pre- and post-ischaemic administration of Intralipid® on stunned myocardium. We also examined the role of fatty acids and phospholipids, respectively, in the effect of lipid emulsions on stunned myocardium. Methods: Myocardial stunning was produced by 15 min of ischaemia and 90 min of reperfusion in isolated blood perfused rabbit hearts. Intralipid® was administered either prior to ischaemia or during reperfusion. Left ventricular pressure (LVP) and its first derivative (LVdP/dt) were measured to assess functional recovery. High energy phosphates were measured with HPLC. The effects of linoleic acid, phosphatidylcholine and their combination were also studied. Results: Only when Intralipid® was administered during reperfusion, it improved recovery from contractile function and increased high energy phosphate content in globally stunned myocardium. Both linoleic acid and phosphatidylcholine significantly improved myocardial function in stunned myocardium. Conclusions: The effect of lipids on the contractile performance and metabolic state of stunned myocardium depends mainly on the timing of its administration with regard to the ischaemia/reperfusion event. Both free fatty acids and phospholipids contribute to the beneficial effect of lipid emulsions on functional recovery of stunned myocardium. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Contractile function; Energy metabolism; Ischaemia; Reperfusion; Stunning

1. Introduction

Myocardial stunning is a clinically important phenomenon that influences ischaemia-related morbidity and mortality significantly [1]. It occurs after a variety of clinical conditions associated with temporary ischaemia and reperfusion including coronary vasospasm, exercise-induced ischaemia, unstable angina, thrombolysis, angioplasty, coronary artery bypass grafting and heart transplantation [1,2]. Despite intensive research the pathophysiology of myocardial stunning remains incompletely understood. The treatment is therefore largely symptomatic and based on the use of positive inotropic agents.

Oxygen free radicals and calcium have been proposed as the main protagonists in causing reversible damage to intracellular proteins and lipid structures during brief myocardial ischaemia and reperfusion [2,3]. However, myocardial stunning also coincides with significant metabolic abnormalities [4,5]. A temporal relationship between the time course of recovery to normal oxidative metabolism and functional recovery of stunned myocardium has been demonstrated [4]. Additional observations suggest that free fatty acid (FFA) oxidation is an important determinant of mechanical performance in the post-ischaemic, reperfused myocardium [6,7]. Recently we demonstrated that the exogenous administration of Intralipid 20%® during the reperfusion phase improves functional recovery of regionally stunned myocardium in conscious dogs [8]. After pharmacological inhibition of myocardial

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FFA oxidation this beneficial effect was eliminated. The findings suggested that the pathophysiological mechanism of myocardial stunning is at least partially related to a deficient turnover of energy substrates, more specifically FFAs.

In the present study we elaborated further on the role of FFAs in the pathophysiology of myocardial stunning. Specific attention was paid to the timing of lipid administration with regard to the sequence of ischaemia and reperfusion. In addition to measurements of cardiac function we also studied the effects of a lipid emulsion on the metabolic state of post-ischaemic myocardium. Finally, different components of lipid emulsions were studied separately in order to identify their relative contribution to the overall effect of Intralipid® on functional recovery of stunned myocardium. An isolated, parabiotic blood perfused rabbit heart model exposed to global myocardial ischaemia and reperfusion was used to complete all experiments.

2. Methods

2.1. Material and methods

Ninety-eight female New Zealand white rabbits (weighing between 2.1 and 3.4 kg) were used for 49 experiments. All animals were treated according to the guidelines of the Animal Care Committee of the Katholieke Universiteit Leuven. The investigation was done according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1985).

The isolated blood-perfused rabbit heart model was described previously by Chen et al. [9]. Briefly, two rabbits are required for each experiment. One rabbit serves as a support animal, whereas from the second animal the heart is isolated and mounted on a Langendorff perfusion system. Following premedication with piritramide 6 mg i.m. (dipidolor®, Janssen Pharmaceutics, Beerse, Belgium), animals were anaesthetised with 20 mg kg⁻¹ pentobarbital (Nembutal®, Sano®, Brussels, Belgium). A tracheotomy was performed and a 3.5-mm tracheal tube was inserted. The lungs were ventilated with a mixture of oxygen in air (50% oxygen) using a Mark 7 (Bird®) respirator. In support animals, anaesthesia was maintained throughout the study with a continuous infusion of 4 mg kg⁻¹ h⁻¹ pentobarbital (Nembutal®) and 2 mg kg⁻¹ h⁻¹ piritramide (dipidolor®). Blood gases were kept within physiological range by adjustment of ventilation. Following administration of 500 IU kg⁻¹ of heparin i.v., the right femoral artery was cannulated with a 20-gauge catheter for monitoring of blood pressure and control of blood gases. The left carotid artery was cannulated with a 16-gauge catheter for the delivery of arterial blood to the perfusion system and a 14-gauge catheter in the right jugular vein returned the venous blood. Heparin was continued at a rate of 300 IU kg⁻¹ h⁻¹.

Arterial blood was pumped through a roller pump into an overflow blood reservoir located at a fixed distance above the isolated heart preparation, allowing for a constant perfusion pressure of 60 mmHg. The overflowed blood and coronary venous effluent from the isolated heart were collected into the heart chamber, passed through a blood transfusion filter (Baxter Healthcare Corporation) and returned to the support animal. The temperature of the isolated heart was maintained at 37 ± 0.5°C. This was achieved by warming the tubing, overflow reservoir, heart chamber and support rabbit using a water-heating system. The perfusion system was primed with 30 ml of Ringer’s solution and 20 ml of Geloplasma® (Mérieux Benelux, Brussels, Belgium), 3 ml of sodium bicarbonate 0.8 M and 5000 IU of heparin. Previous experiments performed at our research laboratory demonstrated that myocardial function of the isolated organ and the support rabbit could be maintained in stable conditions for 4–5 h [9].

Isolated hearts were harvested through a median sternotomy after 1000 IU kg⁻¹ of heparin had been given. Hearts were submerged into ice cold Ringer’s solution (0–4°C) and mounted to the perfusion system via the aorta within 90 s. Immediately following aortic cannulation, Langendorff perfusion was initiated at a constant perfusion pressure of 60 mmHg. A silicon catheter was inserted through the pulmonary artery into the right ventricle for drainage of the coronary venous effluent. An 18-gauge catheter was positioned through the apex of the left ventricle for venting of intraventricular blood. A pair of pacing needles was positioned through the free wall of the right ventricle and a thermistor needle was placed through the ventricular septum for measurement of myocardial temperature. Finally, a fluid-filled latex balloon was placed into the left ventricle through the left atrium.

The fluid-filled balloon was connected to a pressure transducer (Model 1280C, Hewlett-Packard, MA, USA) allowing for measurement of left ventricular pressure (LVP), left ventricular end-diastolic pressure (LVEDP) and maximal rate of left ventricular pressure rise (LVDp/dt). Prior to initiation of the experimental protocol the latex balloon was filled to a LVEDP of 12 mmHg. The perfusion pressure (PP) of the system was monitored through a side arm of the aortic cannula using a disposable pressure transducer (Baxter Healthcare). A flow probe (T206 small animal flow meter, Transonic Systems, Ithaca, NY, USA) was fixed just proximal of the aortic cannula. This allowed for measurement of coronary blood flow (CBF). Hearts were paced throughout the experiments at a constant rate of 180 bpm. All parameters were recorded on a heat writing polygraph (WT-655G, Nihon Kohden, Tokyo, Japan).

2.2. Part one: timing of Intralipid® administration

Twenty-one isolated hearts and 21 support rabbits were
instrumented for the first part of our two-step study. The isolated hearts were randomly allocated to three different treatment groups of seven hearts each. Group a (Control group) received 10 ml of NaCl 0.9% prior and following ischaemia; Group b (IL-pre group) received 10 ml Intralipid® 20% prior to ischaemia. Saline (10 ml) was infused during reperfusion. Group c (IL-post group) received 10 ml of Intralipid® 20% during reperfusion. Saline was administered prior to the period of ischaemia. The substrate was administered through a connection line adjacent to the aortic root directly into the coronary perfusate.

Following a 20-min period of stabilisation, 10 ml of saline or 10 ml of Intralipid® 20% were administered over 30 min. The substrate infusion was immediately followed by a 15-min period of normothermic, global myocardial ischaemia. Following reperfusion, haemodynamics were followed for 90 min. During reperfusion, 10 ml of saline or 10 ml of Intralipid® 20% were administered over 30 min. The post-ischaemic infusion of substrate was initiated 15 min following reperfusion and stopped at 45 min reperfusion.

At the end of the reperfusion period, a myocardial biopsy was taken from the anterior free wall of the left ventricle. The myocardial muscle was freeze clamped and stored at −80°C until HPLC analysis of high-energy phosphate content was performed. Adenine nucleotides and metabolites were determined as described by Wynants and Van Belle [10]. Energy Charge (EC) was defined as (ATP+0.5ADP)/(ATP+ADP+AMP).

Intralipid® 20% is a long-chain triglyceride-based lipid emulsion derived from soybean oil. Triglycerides (200 g l⁻¹) are emulsified in 1 l of distilled water, using 12 g l⁻¹ of a phospholipid emulsifier derived from egg yolk. This emulsifier contains primarily phosphatidylcholine. The solution also contains 25 g l⁻¹ of glycerol. The long-chain triglycerides include five different long-chain free fatty acids: linoleic acid (C₁₈:₂ω₆), linolenic acid (C₁₈:₃ω₆), oleic acid (C₁₈:₁ω₉), palmitic acid (C₁₆:₀) and stearic acid (C₁₈:₀).

2.3. Part two: effects of Intralipid®’s subcomponents, administered during reperfusion from ischaemia, on recovery from myocardial stunning

To investigate the effects of Intralipid®’s subcomponents on recovery from myocardial stunning, 28 isolated hearts and 28 support rabbits were instrumented as previously described. Following preparation, the isolated hearts were allowed to stabilise for 20 min. Subsequently, two baseline measurements were recorded within a 10-min interval. Then a 15-min period of global myocardial ischaemia was induced. Following reperfusion, haemodynamic variables were recorded for 90 min.

The isolated hearts were randomly allocated to four different study groups receiving one of four substrates. The substrates were administered over 30 min during reperfusion. Substrate administration was started after 15 min of reperfusion. Group d (SOPP group) received 10 ml of SOPP, a 4% solution of human albumin (CAF-DCF, Brussels, Belgium) that served as the vehicle for the other treatment groups since neither fatty acids nor phospholipids could be dissolved in saline; Group e (LIN group) received 10 mg of linoleic acid (Sigma, St Louis, MO, USA) dissolved in 10 ml SOPP; Group f (PC group) received 10 mg of phosphatidylcholine (Sigma) in 10 ml SOPP; Group g (LIN-PC group) received 10 mg linoleic acid and 10 mg phosphatidylcholine dissolved in 10 ml SOPP. The substrates were administered through a connection line adjacent to the aortic root directly in the coronary perfusate. The solutions were prepared by adding the substrate powder to warmed SOPP (36°C) while gently stirring. Sodium hydroxide (Janssen Pharmaceutica) pellets were added until a clear solution was obtained. Hydroxychloride (VEL, Leuven, Belgium) was used to backtitrate the solution to a pH of approximately 7.2.

At the end of the protocol (after 90 min of reperfusion) the isolated hearts were perfused with a solution containing 0.1 M Na₂HPO₄, 0.1 M NaH₂PO₄ and 2% glutaraldehyde for preservation and storage until histological examination was performed. When the experiments were finalised the support rabbit was killed with an overdose of potassium chloride, following intravenous injection of piritramide 10 mg kg⁻¹ (dipidolor®) and 30 mg kg⁻¹ pentobarbital (Nembutal®).

2.4. Statistical analysis

The haemodynamic effects of Intralipid® prior or following ischaemia were compared to the haemodynamic effects of saline (control) administration. The haemodynamic effects of phosphatidylcholine, linoleic acid and both combined were compared to the haemodynamic effects of SOPP (solvents). Two-way ANOVA (repeated measures ANOVA for time and factorial ANOVA for treatment) was done to statistically analyse the haemodynamic data. When appropriate, post hoc testing consisted of Student’s t-test with Bonferroni modification. Biochemical data were compared using the unpaired t-test. A P value below 0.05 was considered statistically significant. Data are presented as a mean±S.E.M.

3. Results

Histological examination showed no evidence of myocardial infarction or irreversible cellular damage in any of the isolated hearts studied. Throughout all experiments the haemodynamic condition of the support animals remained stable as shown by the continuous recordings of arterial blood pressure. No differences in arterial blood pressure of support animals were observed between the groups (Table 1).
Table 1
Mean arterial blood pressure (mABP) of the support animals in all experimental conditions prior, during and following global myocardial ischaemia

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>Ischaemia</th>
<th>Reperfusion (min)</th>
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<tr>
<td></td>
<td>15°</td>
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<td>90°</td>
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<tr>
<td>mABP</td>
<td>(mmHg)</td>
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<tr>
<td>IL-pre</td>
<td>54±5</td>
<td>57±2</td>
<td>53±5</td>
</tr>
<tr>
<td>Control</td>
<td>52±6</td>
<td>58±4</td>
<td>50±4</td>
</tr>
<tr>
<td>IL-post</td>
<td>55±2</td>
<td>57±4</td>
<td>54±1</td>
</tr>
<tr>
<td>LIN-10</td>
<td>56±2</td>
<td>58±4</td>
<td>56±5</td>
</tr>
<tr>
<td>PC-10</td>
<td>53±3</td>
<td>57±3</td>
<td>52±2</td>
</tr>
<tr>
<td>LIN-PC</td>
<td>51±3</td>
<td>56±6</td>
<td>51±5</td>
</tr>
<tr>
<td>SOPP</td>
<td>55±6</td>
<td>57±5</td>
<td>49±6</td>
</tr>
</tbody>
</table>

* Data are presented as a mean±S.E.M.

30 min of substrate infusion, see text for details on the different study groups.

3.1. Part one: timing of Intralipid® administration (Figs. 1 and 2 and Table 2)

Prior to ischaemia, i.e., in baseline conditions, there were no differences in LVEDP, LVDP, LdP/dt nor CBF between the three groups (groups a–c). The pre-ischaemic infusion of saline or Intralipid did not influence baseline haemodynamic measurements. Upon the start of normothermic ischaemia, contractile function of the left ventricle ceased within 5 min in all three groups. On no occasion did left ventricular contracture occur.

Following reperfusion, after an initial hyperaemic phase, coronary blood flow returned to baseline values within 10 min of reperfusion. Contractile function of the isolated hearts resumed following reperfusion but was severely

Fig. 1. Maximal rate of left ventricular pressure rise presented as % of baseline value prior, during and following 15 min of global myocardial ischaemia in isolated blood perfused rabbit hearts. Twenty-one hearts were randomly allocated to three different study groups of seven hearts each. The control group received 10 ml of NaCl 0.9% prior to and following ischaemia. Intralipid 20% pre-ischaemia received 10 ml Intralipid® 20% prior to ischaemia. Saline (10 ml) was infused during reperfusion. Intralipid 20% post-ischaemia received 10 ml of Intralipid® 20% during reperfusion. Saline was administered prior to the period of ischaemia. *P<0.05 saline versus treated.
to ischaemia. Energy Charge was also higher in the IL-post group.

3.2. Part two: effects of Intralipid®'s subcomponents, administered during reperfusion from ischaemia, on recovery from myocardial stunning (Fig. 3 and Table 3)

Prior to ischaemia (baseline), no differences in LVEDP, LVDP, LdP/dt nor CBF were observed between the four groups (groups d–g). As in part one, contractile failure was complete within 5 min in all four groups. Immediately prior to substrate treatment, all groups displayed a similar degree of contractile dysfunction. During substrate infusion linoleic acid, phosphatidylcholine and a combination of both substances significantly improved myocardial contractile function of globally stunned isolated rabbit hearts as compared to SOPP controls. Only the combination of linoleic acid and phosphatidylcholine produced a sustained amelioration in contractile function after the cessation of treatment as compared to controls.

4. Discussion

This study shows that a long-chain triglyceride emulsion, when administered during the reperfusion phase, improves the functional recovery and metabolic state of globally stunned myocardium in isolated rabbit hearts. The data confirm our previous findings in dogs subjected to regional myocardial ischaemia and reperfusion [8] but, in addition, show that the treatment window is limited to the post-ischaemic phase only. When the lipid emulsion was administered prior to the ischaemic event, the consequences of brief ischaemia and reperfusion were not different from those observed in untreated animals. Furthermore, our data show that both linoleic acid, as the predominant source of free fatty acids, and phosphatidylcholine, a major source of phospholipids in Intralipid, contribute to the beneficial effect of the lipid emulsion on stunned myocardium. However, the combination of these two substances had an additive action that matched the effect of Intralipid® both in the extent and the duration of action on functional improvement of stunned myocardium.

Biochemical analysis showed that hearts treated with Intralipid® following ischaemia had significantly higher levels of adenosine triphosphate (ATP) as compared to SAL-treated hearts or hearts treated with Intralipid® prior depressed in all treatment groups. Prior to substrate treatment, the extent of functional impairment was similar in the three groups.

Soon after the start of post-ischaemic substrate infusion, contractile function significantly improved in the IL-post group when compared to the SAL and IL-pre groups. This difference persisted beyond the cessation of treatment. At 45 min of reperfusion LVDP had increased to 82±8 mmHg in the IL-post group, while LVDP was only 54±4 mmHg in the SAL group and 57±6 in the IL-pre group (P<0.05).

Biochemical analysis showed that hearts treated with Intralipid® following ischaemia had significantly higher levels of adenosine triphosphate (ATP) as compared to SAL-treated hearts or hearts treated with Intralipid® prior
**Table 2**

Left ventricular end diastolic pressure (LVEDP, mmHg), left ventricular developed pressure (LVDP, mmHg), maximal rate of left ventricular pressure rise (LVDP/dt, mmHg/s) and coronary blood flow (CBF, ml/min) in isolated rabbit hearts prior, during and following ischaemia.*

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>30 min</th>
<th>Ischaemia</th>
<th>Reperfusion (min)</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDP (mmHg)</td>
<td>IL-pre</td>
<td>12±0</td>
<td>11±1</td>
<td>15±2</td>
<td>12±1</td>
<td>10±1</td>
<td>10±1</td>
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</tr>
<tr>
<td></td>
<td>Control</td>
<td>12±0</td>
<td>12±0</td>
<td>12±1</td>
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<td>12±0</td>
<td>12±1</td>
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<td>12±1</td>
<td>10±1</td>
<td>10±1</td>
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<tr>
<td>LVDP (mmHg)</td>
<td>IL-pre</td>
<td>111±9</td>
<td>108±8</td>
<td>36±2</td>
<td>54±5</td>
<td>57±6</td>
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<td>Control</td>
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<td>39±5</td>
<td>48±5</td>
<td>54±4</td>
<td>59±4</td>
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<tr>
<td></td>
<td>IL-post</td>
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<td>101±5</td>
<td>38±3</td>
<td>71±7</td>
<td>82±8</td>
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<tr>
<td>LVDP/dt (mmHg/s)</td>
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<td>1153±130</td>
<td>1181±76</td>
<td>467±43</td>
<td>645±67</td>
<td>701±95</td>
<td>620±71</td>
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<td>521±38</td>
<td>589±35</td>
<td>653±34</td>
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<tr>
<td></td>
<td>IL-post</td>
<td>1029±73</td>
<td>1028±70</td>
<td>406±34</td>
<td>800±103*</td>
<td>901±108*</td>
<td>886±75*</td>
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<tr>
<td>CBF (ml/min)</td>
<td>IL-pre</td>
<td>11±1</td>
<td>10±1</td>
<td>11±2</td>
<td>11±2</td>
<td>11±2</td>
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<td></td>
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<tr>
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<td>Control</td>
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<tr>
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<td>11±1</td>
<td>11±1</td>
<td>11±2</td>
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</table>

*IL-pre, Intralipid was administered prior to ischaemia; IL-post, Intralipid was administered following ischaemia; control, no Intralipid was administered. Data are presented as a mean±S.E.M.

b Thirty minutes of substrate infusion.

*p<0.05 versus control.

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**Fig. 3. Maximal rate of left ventricular pressure rise presented as percent of baseline value prior, during and following 15 min of global myocardial ischaemia in isolated blood perfused rabbit hearts.** Twenty-eight hearts were randomly allocated to four different study groups of seven hearts each, receiving one of four substrates. The substrates were administered over 30 min during reperfusion. Substrate administration was started after 15 min reperfusion. SOPP group received 10 ml of SOPP; LIN-10 group received 10 mg of linoleic acid dissolved in a volume of 10 ml; PC-10 group received 10 mg of phosphatidylcholine dissolved in a volume of 10 ml; LIN-PC group received 10 mg linoleic acid and 10 mg phosphatidylcholine dissolved in a volume of 10 ml. *p<0.05 versus saline treated.
that different pathophysiological mechanisms are at play. In our experimental model, histological examination of the rabbit hearts showed no evidence of myocardial infarction. Consequently, we attribute the observed post-ischaemic dysfunction entirely to the phenomenon of myocardial stunning.

4.1. Mechanism of action

The precise mechanism for the treatment of myocardial stunning with lipids during reperfusion is not known. In a previous report we showed that selective inhibition of myocardial free fatty acid metabolism, using a carnitine palmitoyl transferase I inhibitor (oxfenicine), completely blocked the beneficial effects of lipids on regional stunning. We suggested that the beneficial effects of lipids on recovery from stunning were due to a correction of free fatty acid substrate metabolism, which is depressed during early reperfusion [8]. The present study provides further arguments in favour of this hypothesis since hearts treated with Intralipid also had higher ATP contents and higher energy charge. Several other observations suggest that a deficient FFA metabolism may have an important role in the pathogenesis of myocardial stunning. Energy production is decreased [13] in stunned myocardium and recovery of FFA oxidation parallels functional recovery [6]. Stimulation of FFA oxidation by L-propionylcarnitine has been demonstrated to improve cardiac performance [7]. Furthermore, the administration of oleic acid stimulates phospholipase D activity and the production of phosphatidic acid, which improves functional recovery in stunned myocardium [14].

We administered linoleic acid, the main FFA component in Intralipid®, separately and also observed an improved functional recovery. It is very unlikely that long-chain FFAs have direct effects on myocardial contractility. Firstly, we have shown previously that long-chain triglycerides have no direct inotropic effects in the normal canine heart [15]. Secondly, in a model of regional myocardial stunning Intralipid selectively improved the function of stunned myocardium but did not affect the inotropic state of normal myocardium [8].

Phosphatidylcholine also improved functional recovery from stunning in our experimental model. Endogenous phosphatidylcholine is hydrolysed in myocytes by phospholipase D to form phosphatidic acid [16]. Both exogenous phospholipase D and phosphatidic acid have been shown to augment contractility in rabbit and rat myocardium [14,17]. This results from an increase in intracellular calcium through sarcolemmal influx and release from the sarcoplasmatic reticulum. It has been suggested that phosphatidic acid plays a key role in the regulation of intracellular calcium concentrations and myocardial function [18]. However, reduced calcium transients are probably not the key deficit responsible for myocardial stunning [19]. Phosphatidic acid also plays a crucial role in the regulation of cellular oxidative metabolism since the inhibition of its production with ethanol inhibits oxidative metabolism in neutrophils [16].

When linoleic acid and phosphatidylcholine were administered simultaneously, recovery of contractile function

<table>
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<th>Ischaemia</th>
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<tr>
<td></td>
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<td>15s</td>
<td>45s</td>
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<tr>
<td>LVEDP (mmHg)</td>
<td>LIN</td>
<td>12±0</td>
<td>17±2</td>
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<tr>
<td></td>
<td>PC</td>
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<td>SOPP</td>
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<td>LVDP (mmHg)</td>
<td>LIN</td>
<td>124±4</td>
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<td>PC</td>
<td>119±6</td>
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<tr>
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<td>LIN-PC</td>
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<td>SOPP</td>
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<td>LVEDP (mmHg/s)</td>
<td>LIN</td>
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<td>PC</td>
<td>2157±169</td>
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<td>1964±130*</td>
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* LVEDP, left ventricular diastolic pressure; LVDP, left ventricular developed pressure; LVEDP/dt, maximal rate of left ventricular pressure rise; LIN, linoleic acid group; PC, phosphatidylcholine group; LIN-PC, linoleic acid and phosphatidylcholine group; SOPP, control group. Data are presented as a mean±S.E.M.

* Thirty minutes of substrate infusion.

*P<0.05 versus SOPP treated control hearts.
was sustained for a longer period of time as compared to the separate effect of either component alone. This observation suggests that the mechanisms of action of FFA and phospholipids are complementary. Since only single doses of both agents were used in our study, further work is required to confirm this statement.

4.2. Pathophysiology of stunning

Our data suggest that disturbances in myocardial energy metabolism play a role in the pathophysiology of myocardial stunning but they do not provide a clue to the cause of these disturbances. When reconciling the current scientific evidence on the pathogenesis of myocardial stunning it is clear that the generation of oxygen free radicals plays a key role in ischaemia/reperfusion-induced myocardial damage. Membrane lipids and intracellular proteins are the main target of oxidative stress [3] and this may include enzymes involved in oxidative metabolism. Goldhaber et al. demonstrated that oxygen radicals inhibit glycolysis and oxidative metabolism [20]. Recently it was shown that creatine kinase is a main target of reactive oxygen species in cardiac tissue [21]. Others have shown that mitochondrial function is impaired due to free radical damage [22].

The exogenous administration of lipid emulsions seems to partially restore the alterations in myocardial metabolism and function of the stunned myocardium. However, none of the substances used in our study could restore contractile performance to the level of pre-ischaemic conditions. The pathogenesis of myocardial stunning is multifactorial and some of the target sites involved may not be affected by exogenous lipid components. Gao et al. [19] and Matsumura et al. [23] showed that oxygen free radicals, generated during reperfusion degrade troponin I. It is very unlikely that any type of metabolic support enhances the repair of myofilaments given the time frame in which the effects of lipids were observed in our study.

4.3. Limitations of the model

The isolated heart model was used to measure the functional effects of different substrates (Intralipid® or subcomponents) on global myocardial stunning without interference of potentially confounding systemic (e.g., vascular) effects. We chose to perfuse the isolated heart with blood substrates from a support animal, since this allows for a more physiological experimental set-up as compared to crystalloid perfused isolated hearts. It has been shown that anaesthesia, heparinisation and insertion of the perfusion system significantly alters the fatty acid and norepinephrine concentration of the blood donor [24]. Despite these changes, the haemodynamics of the blood donor, and the contractile function and metabolism of the isolated heart remained unchanged. Glucose and fatty acid uptake of the isolated hearts remained constant during aerobic perfusion and fatty acids were the preferred myocardial substrate [24]. Others have provided evidence that the stunned myocardium preferentially metabolises fatty acids following reversible ischaemia [25].

Although we did not measure fatty acid or norepinephrine concentrations in the blood donor animal, we observed that the support animal remained haemodynamically stable throughout the experimental protocol in all groups. We assume that if the above-mentioned changes in blood donor metabolism were present in our experiments, they were similar for all treatment groups. Therefore the reported differences in myocardial function and high energy content of the isolated stunned hearts should merely reflect a treatment effect of the different substances used.

In summary, our data show that a long-chain triglyceride emulsion and the specific subcomponents of such an emulsion improve the functional recovery and metabolic state of stunned myocardium. We emphasise that this effect is present only when the substances are administered during reperfusion. Both FFAs and phospholipids contribute to improve the performance of stunned myocardium but their mechanism of action remains to be investigated. These observations may open new perspectives to the treatment of ischaemia/reperfusion damage. Furthermore they should encourage research with regard to the role of FFA oxidation in the pathophysiology of myocardial stunning.

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