Long-chain triglycerides improve recovery from myocardial stunning in conscious dogs

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Abstract

Objectives: Free fatty acid (FFA) oxidation is depressed in the postischaemic stunned myocardium and recovers in parallel with the normalization of contractile performance. Assuming a causal role for this metabolic disturbance in the pathogenesis of stunning, we questioned whether exogenous administration of high dose triglycerides during reperfusion of postischaemic myocardium could improve its functional recovery. Methods: Thirteen dogs were chronically instrumented to measure global and regional haemodynamics and to produce a 10 min episode of regional myocardial ischaemia. In 7 dogs, Intralipid® 20% was administered i.v. during the reperfusion phase. Contractile recovery of stunned myocardium was compared with control saline treatments. The series were repeated in another 6 animals, but oxeficine (CPT I inhibitor) preceeded Intralipid® during reperfusion. Results: Contractile recovery of stunned myocardium was faster and more extensive when Intralipid® was administered during reperfusion than with saline treatment (wall thickening fraction X6 ± 6% of preischaemic controls versus 52 ± 11% at 90 min post-reperfusion; P < 0.05). Oxeficine pretreatment completely abolished this beneficial effect. Conclusions: Exogenous administration of triglycerides during reperfusion of postischaemic myocardium improves functional recovery from stunning. This beneficial effect most likely operates through enhanced FFA availability and/or oxidation since it could be abolished by selective inhibition of the carnitine palmitoyl transferase I enzyme.

Keywords: Ischemia; Stunning; Lipid emulsions; Free fatty acids; Triglycerides; Dog, anesthetized

Introduction

The phenomenon of prolonged but reversible contractile dysfunction after brief myocardial ischaemia and reperfusion ('stunned' myocardium) coincides with significant metabolic abnormalities [1,2]. The time course of recovery of normal oxidative metabolism parallels the functional recovery of stunned myocardium [3]. Metabolic imaging studies, using positron emission tomography (PET), have shown that stunned myocardium is characterised by reduced non-esterified fatty acid oxidation, although overall oxygen consumption remains near normal [4,5]. In addition, the positive inotropic drug dobutamine, while increasing mechanical performance of stunned myocardium, also raises oxygen consumption, although metabolic rate for glucose substrates remains unaltered [6]. In the latter study lipid metabolism was not measured, but it is well known that catecholamines raise free fatty acid (FFA) plasma levels due to an increased lipolysis. Finally, L-propionylcarnitine, a naturally occurring derivative of L-carnitine, stimulates palmitate oxidation and improves functional recovery from global myocardial ischaemia followed by reperfusion in rats [7]. These observations suggest that FFA oxidation is an important determinant of mechanical performance in the postischaemic, reperfused myocardium. Consequently, a postischaemic depression of FFA oxida-
tion could be causative to the reversible contractile impairment of stunned myocardium.

Whether alterations in FFA substrate availability affect the functional performance of stunned myocardium is unclear at present. In most previous studies FFA were administered during both ischaemia and reperfusion. It has been clearly shown that increased levels of FFA during ischaemia aggravate ischaemic damage through increased lipid peroxidation [8–11]. These studies did not discriminate between intraischaemic and postischaemic effects of FFA. In one previous report, however, long-chain triglycerides were administered in the reperfusion phase only, to acutely instrumented dogs following global myocardial ischaemia. Jones et al. demonstrated that a 10% long-chain triglyceride emulsion in these conditions significantly improves contractile recovery as compared to saline controls [12].

The aim of our study was to test the hypothesis that administration, during the reperfusion phase, of long-chain triglycerides, in combination with heparin to increase FFA plasma levels, improves recovery from regional myocardial stunning in conscious chronically instrumented dogs. Functional recovery from postischaemic contracture dysfunction in dogs treated with high doses of Intralipid® 20%, administered 15 min following reperfusion preceded by a 10 min ischaemic episode, was compared to saline-treated controls. Experiments were repeated in the presence of oxfenicine, a CPT I inhibitor, which effectively blocks myocardial FFA metabolism. To avoid the potentially confounding effects of baseline anaesthetics and surgical stress on the cardiovascular system, the study was carried out in conscious, chronically instrumented dogs.

2. Methods

The experimental protocol was approved by 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 methods used here have been described in detail previously [13]. Briefly, after overnight fasting a total of 13 mongrel dogs (either sex, weighing between 18 and 24 kg) were premedicated intramuscularly with piritramide 1 mg · kg⁻¹ (Dipidolor®, Janssen Pharmaceutics) and ketamine 5 mg · kg⁻¹ (Ketalar®, Parke-Davis). Animals were anaesthetized with intravenous pentobarbital, 15 mg · kg⁻¹ (Nembutal®, Sanofi). The trachea was intubated and the lungs were mechanically ventilated. Anaesthesia was maintained with halothane 0.8–1.2% in oxygen-enriched air.

Under aseptic conditions, a left thoracotomy was performed through the fifth intercostal space. A Tygon® catheter was inserted into the descending thoracic aorta for the measurement of aortic blood pressure (ABP) and for withdrawal of arterial blood samples. The heart was suspended in a pericardial cradle and a second Tygon® catheter was positioned in the left atrium for measurement of left atrial pressure (LAP). Through an apical stab wound, a microtransducer (Janssen Pharmaceutics, Beerse, Belgium) was introduced into the left ventricle. A 20 MHz pulsed Doppler blood flow velocity probe (Baylor College of Medicine, Houston, TX) was fitted around the left anterior descending (LAD) and left circumflex (Cx) coronary arteries. 10 MHz pulsed Doppler wall thickening probes (Baylor College of Medicine, Houston, TX) were sutured to the epicardium of the LAD and Cx coronary artery perfused areas. Finally, a hydraulic occluder (Dimed, Deurne, Belgium) was positioned around the LAD coronary artery proximal to the LAD flow probe. The thorax was closed in layers and all leads were tunneled subcutaneously to the dorsum of the neck and exteriorized. A jacket was fitted to the dogs to protect the probes from damage.

Postoperative analgesia consisted of piritramide 1 mg · kg⁻¹ (Dipidolor®, Janssen Pharmaceutics) every 6 h for 24 h. All animals received i.m. ampicillin anhydricum (400 mg · kg⁻¹) (Albipen®) for 7 days. Animals were trained for at least 10 days to get accustomed to the laboratory environment and to lie quietly in a cage when connected to the monitoring system.

Aortic and left atrial pressures were measured using disposable pressure transducers (Baxter Healthcare Co.). Pressure-, flow- and wall thickening signals were processed through a 6-channel pulsed Doppler system (Baylor College of Medicine, Houston, TX). The left ventricular micromanometer was calibrated on-line to aortic and left atrial pressures. The signal was electronically differentiated to obtain LV dP/dt (Gould Inc., Cleveland, OH). All signals were recorded on an 8-channel thermal writing polygraph (Gould Inc., Cleveland, OH).

Animals (n = 13) were allocated to two study groups (Group 1, n = 7; Group 2, n = 6). In each group, prior to the ischaemic studies, the effects of Intralipid® 20% (Group 1) or oxfenicine (Group 2) in normal myocardium were evaluated. Subsequently, all animals within each group, were subjected to two experiments, separated by 7 days, to test our hypothesis. A cross-over design was chosen to avoid any temporal or sequence bias within one group. The experiments were coded as follows.

Group 1: (a) Intralipid®/non-ischaemic hearts. Intralipid® (20%, 7 ml · kg⁻¹) was administered intravenously as a continuous infusion over 30 min. Haemodynamic parameters were recorded on preset moments in time for 1 h after the start of infusion. (b) Stunned myocardium/Intralipid®. A 10 min LAD coronary artery occlusion followed by complete reperfusion was performed. An infusion of 7 ml · kg⁻¹ Intralipid® 20% was started 15 min following reperfusion and completed over a period of 30 min. Measurements of regional and global haemodynamics were repeated up to 4 days following reperfusion. (c) Stunned myocardium/control. A 10 min LAD coronary
artery occlusion was followed by complete reperfusion. Animals received only saline during reperfusion. Measurements of regional and global haemodynamics were repeated up to 4 days following reperfusion.

Group 2: (a) Oxfenicine/normal hearts. Oxfenicine (L-(+)-p-hydroxyphenylglycine), a carnitine palmitoyl transferase I inhibitor (33 mg · kg⁻¹) was administered i.v. over 5 min. Haemodynamics were recorded for 60 min after the start of oxfenicine. (b) Oxfenicine followed by Intralipid®/stunned myocardium: a 10 min LAD coronary artery occlusion followed by complete reperfusion was performed. An infusion of 7 ml · kg⁻¹ Intralipid® 20% was started 15 min following reperfusion and completed over a period of 30 min. Measurements of regional and global haemodynamics were repeated up to 4 days following reperfusion. Following reperfusion, but prior to Intralipid® 20% treatment, oxfenicine was administered intravenously over 5 min (33 mg · kg⁻¹). (c) Stunned myocardium/control. A 10 min LAD coronary artery occlusion was followed by complete reperfusion. Animals received saline instead of Intralipid® 20% during reperfusion. No oxfenicine was infused. Measurements of regional and global haemodynamics were repeated up to 4 days following reperfusion.

In both groups, each dog was given i.v. heparin 200 IU · kg⁻¹ prior to all study conditions.

Intralipid® 20% (Kabi Pharmacia, Sweden) is a long-chain triglyceride based lipid emulsion derived from soy bean oil. Triglycerides (200 g · l⁻¹) are emulsified in 1 litre of distilled water, using 12 g · l⁻¹ of a phospholipid emulsifier derived from egg yolk. The solution also contains 25 g · l⁻¹ of glycerol. The long-chain triglycerides include 5 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).

Blood samples were withdrawn at baseline, after the Intralipid® infusion and 30 min after the end of the lipid infusion to determine arterial pH, pO₂, haemoglobin, triglycerides, total protein content and glucose. The same determinations were also performed at baseline, after oxfenicine infusion and at the end of the study period in oxfenicine-treated/normal hearts (2c).

The effects of Intralipid® and Intralipid® + oxfenicine respectively versus saline were analysed using two-way repeated measures ANOVA, including factors time and treatment condition. When appropriate post hoc testing consisted of Student’s t-test with Bonferonni modification. Data on the effects of Intralipid® and oxfenicine in normal myocardium were analysed using one-way repeated measures ANOVA. P < 0.05 was considered statistically significant. Data are presented as a mean ± s.e.m.

3. Results

3.1. Effects of Intralipid® on the normal myocardium (Group 1a)

Intralipid® 20%, 7 ml · kg⁻¹, administered as a continuous infusion over 30 min, increased LV dP/dtₘₐₓ from 2092 ± 180 to 2371 ± 203 mmHg · s⁻¹ at 1 h after the start of the Intralipid® infusion (P < 0.05 versus baseline). Heart rate increased moderately from 90 ± 3 to 103 ± 5 bpm (P < 0.05 versus baseline). No other haemodynamic changes were observed throughout the study period.

### Table 1

Effect of Intralipid® on general and regional haemodynamics in conscious dogs subjected to a 10 min coronary artery occlusion of the LAD coronary artery, followed by reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ischaemia</th>
<th>Reperfusion</th>
<th>15 min</th>
<th>45 min</th>
<th>60 min</th>
<th>120 min</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>IL 88 ± 6</td>
<td>118 ± 11</td>
<td>97 ± 3</td>
<td>92 ± 6</td>
<td>89 ± 6</td>
<td>94 ± 7</td>
<td>104 ± 7</td>
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<tr>
<td></td>
<td>C 95 ± 4</td>
<td>116 ± 6</td>
<td>116 ± 8</td>
<td>98 ± 3</td>
<td>98 ± 3</td>
<td>102 ± 4</td>
<td>99 ± 8</td>
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<tr>
<td>Mean aortic blood pressure (mmHg)</td>
<td>IL 87 ± 6</td>
<td>90 ± 5</td>
<td>87 ± 4</td>
<td>92 ± 3</td>
<td>90 ± 3</td>
<td>87 ± 3</td>
<td>86 ± 5</td>
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<tr>
<td></td>
<td>C 96 ± 4</td>
<td>95 ± 3</td>
<td>96 ± 4</td>
<td>95 ± 5</td>
<td>97 ± 5</td>
<td>93 ± 4</td>
<td>90 ± 4</td>
<td></td>
</tr>
<tr>
<td>Left atrial pressure (mmHg)</td>
<td>IL 5.0 ± 0.7</td>
<td>7.8 ± 1.4</td>
<td>5.5 ± 0.6</td>
<td>7.0 ± 0.9</td>
<td>5.8 ± 0.9</td>
<td>4.3 ± 0.1</td>
<td>4.8 ± 0.8</td>
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<tr>
<td></td>
<td>C 6.5 ± 0.5</td>
<td>6.5 ± 0.5</td>
<td>5.0 ± 0.4</td>
<td>4.5 ± 1.5</td>
<td>5.5 ± 0.5</td>
<td>5.0 ± 1.0</td>
<td>6.0 ± 0.4</td>
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<tr>
<td>Left ventricular dP/dtₘₐₓ (mmHg/s)</td>
<td>IL 2175 ± 139</td>
<td>2325 ± 89</td>
<td>2258 ± 141</td>
<td>2392 ± 196</td>
<td>2433 ± 188</td>
<td>2375 ± 200</td>
<td>2192 ± 62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 2383 ± 164</td>
<td>2242 ± 107</td>
<td>2167 ± 170</td>
<td>2192 ± 174</td>
<td>2141 ± 128</td>
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<td>2033 ± 146</td>
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<td>Coronary blood flow velocity LAD (kHz)</td>
<td>IL 5.5 ± 0.9</td>
<td>0.0 ± 0.0</td>
<td>6.0 ± 1.0</td>
<td>4.0 ± 0.5</td>
<td>3.8 ± 0.5</td>
<td>4.2 ± 0.7</td>
<td>5.3 ± 0.9</td>
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<tr>
<td></td>
<td>C 5.7 ± 0.9</td>
<td>0.0 ± 0.0</td>
<td>7.7 ± 2.0</td>
<td>7.3 ± 1.7</td>
<td>7.2 ± 1.7</td>
<td>6.8 ± 1.4</td>
<td>7.0 ± 1.4</td>
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<tr>
<td>Coronary blood flow velocity Cx (kHz)</td>
<td>IL 5.5 ± 0.6</td>
<td>7.9 ± 0.9</td>
<td>5.8 ± 0.3</td>
<td>5.1 ± 0.5</td>
<td>5.4 ± 0.5</td>
<td>5.4 ± 0.5</td>
<td>6.0 ± 1.0</td>
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<tr>
<td></td>
<td>C 7.5 ± 2.1</td>
<td>9.5 ± 2.3</td>
<td>7.5 ± 1.4</td>
<td>7.1 ± 1.6</td>
<td>7.2 ± 1.6</td>
<td>6.6 ± 1.4</td>
<td>7.4 ± 1.4</td>
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<tr>
<td>Wall thickening fraction Cx (% of control)</td>
<td>IL 100 ± 0</td>
<td>82 ± 13</td>
<td>90 ± 9</td>
<td>94 ± 14</td>
<td>95 ± 9</td>
<td>98 ± 8</td>
<td>91 ± 8</td>
<td></td>
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<tr>
<td></td>
<td>C 100 ± 0</td>
<td>102 ± 5</td>
<td>106 ± 7</td>
<td>91 ± 6</td>
<td>91 ± 4</td>
<td>95 ± 7</td>
<td>103 ± 16</td>
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LAD = left anterior descending coronary artery; Cx = left circumflex coronary artery; LVdP/dtₘₐₓ = maximal rate of left ventricular pressure rise; IL = Intralipid®-treated group, Intralipid® was started 15 min following reperfusion; C = saline-treated group. * P < 0.05 vs. baseline. No intergroup differences were observed.
Plasma levels of triglycerides increased from $66 \pm 5$ mg · dl⁻¹ prior to the infusion to $1762 \pm 127$ mg · dl⁻¹ at the end of the Intralipid® infusion. Thirty minutes after the Intralipid® infusion was stopped, triglycerides were still significantly elevated ($782 \pm 137$ mg · dl⁻¹). Arterial pH decreased from $7.384 \pm 0.005$ at baseline to $7.326 \pm 0.013$ at the end of the lipid infusion ($P < 0.05$ versus baseline). Total protein content, glucose and pO₂ remained unchanged throughout the study. Haemoglobin increased following Intralipid® infusion from $11.3 \pm 0.4$ mg · dl⁻¹ at baseline to $13.5 \pm 0.2$ mg · dl⁻¹ at the end of the Intralipid® infusion ($P < 0.05$).

3.2. Effects of oxfenicine on the normal myocardium (Group 2a)

Throughout the study period, no haemodynamic changes were observed. Blood gas analysis as well as concentrations of haemoglobin, triglycerides, total protein and glucose remained identical to baseline values.

### Table 2
Effect of Intralipid® and oxfenicine on general and regional haemodynamics in conscious dogs subjected to a 10 min coronary artery occlusion of the LAD coronary artery, followed by reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ischaemia</th>
<th>Reperfusion</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>15 min</td>
<td>45 min</td>
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<tr>
<td>Heart rate (bpm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL + OX</td>
<td>85 ± 2</td>
<td>111 ± 1</td>
<td>97 ± 11</td>
</tr>
<tr>
<td>C</td>
<td>82 ± 5</td>
<td>119 ± 11</td>
<td>96 ± 9</td>
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<tr>
<td>Mean aortic blood pressure (mmHg)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IL + OX</td>
<td>97 ± 1</td>
<td>98 ± 6</td>
<td>96 ± 5</td>
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<tr>
<td>C</td>
<td>94 ± 6</td>
<td>98 ± 11</td>
<td>95 ± 9</td>
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<tr>
<td>Left atrial pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IL + OX</td>
<td>4.0 ± 0.6</td>
<td>6.0 ± 0.6</td>
<td>6.0 ± 0.9</td>
</tr>
<tr>
<td>C</td>
<td>3.0 ± 0.6</td>
<td>4.0 ± 0.7</td>
<td>5.0 ± 1.2</td>
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<tr>
<td>Left ventricular dP/dtₘₚₚₚ (mmHg/s)</td>
<td></td>
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<tr>
<td>IL + OX</td>
<td>2417 ± 56</td>
<td>2400 ± 37</td>
<td>2400 ± 75</td>
</tr>
<tr>
<td>C</td>
<td>2325 ± 114</td>
<td>2283 ± 117</td>
<td>2292 ± 116</td>
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<tr>
<td>Coronary blood flow velocity LAD (kHz)</td>
<td></td>
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<tr>
<td>IL + OX</td>
<td>4.4 ± 0.7</td>
<td>6.0 ± 0.6</td>
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<td>C</td>
<td>4.4 ± 0.4</td>
<td>6.0 ± 0.6</td>
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<td>Coronary blood flow velocity Cx (kHz)</td>
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<tr>
<td>IL + OX</td>
<td>4.9 ± 0.4</td>
<td>6.8 ± 0.4</td>
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<tr>
<td>C</td>
<td>5.7 ± 0.6</td>
<td>7.6 ± 0.7</td>
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<tr>
<td>Wall thickening fraction Cx (% of control)</td>
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<tr>
<td>IL + OX</td>
<td>100 ± 0</td>
<td>109 ± 18</td>
<td>107 ± 7</td>
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<tr>
<td>C</td>
<td>100 ± 0</td>
<td>110 ± 15</td>
<td>98 ± 8</td>
</tr>
</tbody>
</table>

LAD = left anterior descending coronary artery; Cx = left circumflex coronary artery; LVdP/dtₘₚₚₚ = maximal rate of left ventricular pressure rise; IL + OX: = oxfenicine- and Intralipid®-treated group, Intralipid® was started 15 min following reperfusion; C = saline-treated control group. * P < 0.05 vs. baseline. No intergroup differences were observed.
3.3. Effects of Intralipid on regional contractile performance of ‘stunned’ myocardium (Group 1b versus 1c) (see Fig. 1 and Table 1)

LAD coronary artery occlusion resulted in an immediate decline of systolic wall thickening fraction which evolved into systolic wall thinning. Prior to reperfusion no differences were observed between the saline- and Intralipid-treated groups. Following reperfusion but prior to the start of Intralipid infusion, systolic wall thickening improved transiently but remained equally depressed in both groups.

Almost instantly after the start of the Intralipid infusion and for the first 12 h thereafter, mechanical performance of the postischaemic myocardium was significantly better in the Intralipid-treated animals when compared to saline-treated controls (Fig. 1). Recovery of regional myocardial function was complete within 12 h in the Intralipid-treated group, but dysfunction persisted for 24 h in saline-treated controls.

Plasma triglycerides increased to 1739 ± 253 mg·dl⁻¹ at the end of the Intralipid infusion while arterial pH decreased from 7.36 ± 0.02 to 7.33 ± 0.02 (P < 0.05). Haemoglobin increased following Intralipid infusion from 11.9 ± 0.5 mg·dl⁻¹ at baseline to 14.1 ± 0.7 mg·dl⁻¹ at the end of the Intralipid infusion (P < 0.05).

3.4. Effects of oxfenicine combined with Intralipid on regional contractile performance of ‘stunned’ myocardium (Group 2b versus 2c) (see Fig. 2 and Table 2)

Regional and global haemodynamic effects prior to the start of treatment were similar to those observed in Groups 1b and 1c.

In oxfenicine-pretreated animals, Intralipid infusion did not affect recovery of wall thickening fraction when compared to saline-treated controls (Fig. 2). Complete functional recovery from stunning was reached 24 h following myocardial ischaemia in both groups (Fig. 2).

Plasma triglycerides increased to 2048 ± 205 mg·dl⁻¹ at the end of the Intralipid infusion (P < 0.05). Haemoglobin increased following Intralipid infusion from 12.0 ± 0.5 mg·dl⁻¹ at baseline to 15.0 ± 0.3 mg·dl⁻¹ at the end of the Intralipid infusion (P < 0.05).

4. Discussion

This study shows that the elevation of plasma triglycerides with high doses of Intralipid 20%, administered during postischaemic reperfusion only, improves functional recovery in regionally stunned myocardium of conscious dogs. Our data are consistent with previous observations made by Jones et al. in acutely instrumented dogs following global ischaemia and reperfusion [12]. The effects of increased lipid-substrate availability in the stunned, reperfused myocardium must be clearly distinguished from their influence exerted during acute myocardial ischaemia. Oliver et al. [14] reported an increased incidence of severe arrhythmias in patients suffering a myocardial infarction when plasma FFA concentrations were high. However, in another clinical trial Gupta et al. [15] failed to confirm this finding. Experimental studies have been less controversial. In isolated muscle preparations [16] and isolated hearts [17,18] as well as in acutely instrumented animals [9,10] it was shown that elevated FFA levels during ischaemia result in an increased incidence of arrhythmias and impaired cardiac pump performance. Several explanations...
have been proposed to explain the untoward effects of lipids including the augmentation of free radicals generated by lipid peroxidation and the intracellular accumulation of lipid intermediates.

These effects, however, cannot be invoked after normalization of coronary blood flow. In contrast, the reversible contractile dysfunction that occurs after brief ischaemia and reperfusion may result from a relative deficiency in energy consumption [19] and paradoxically the elevation of high energy substrates during reperfusion could, at least theoretically, restore this abnormality. Pioneering studies on the phenomenon of myocardial stunning showed that intracellular concentrations of ATP remained significantly depressed after reperfusion and that the time course of recovery of high-energy phosphates occurred parallel with the recovery of contractile performance [3]. In addition, creatine kinase activity is decreased and ADP levels are diminished in the stunned myocardium [20]. While these data indeed suggest that myocardial stunning results from inefficient energy production, other studies have challenged this hypothesis [19].

However, metabolic imaging studies using positron emission tomography have shown that metabolic substrate preference in stunned myocardium is altered when compared to non-ischaemic conditions. There is an enhanced preference for glucose derivatives [21] while oxidative phosphorylation of FFA is decreased. While positive inotropic drugs improve contractile performance in stunned myocardium, they simultaneously increase FFA phosphorylation and improve myocardial efficiency at higher doses [6]. In contrast, pharmacological blockade of FFA oxidation with oxfenicine, a CPT I blocking agent, impairs functional recovery from stunning in pigs which received Intralipid® during both ischaemia and reperfusion [22]. It appears that substrate preference for FFA over glucose derivatives coincides with improved mechanical performance. Evidence along the same lines comes from observations made on hibernating myocardium, where a sustained reduction of myocardial perfusion, contractile function and oxidative phosphorylation of FFA are found in the presence of a sustained glycolytic activity (perfusion–metabolism mismatch) [23].

Consequently it may be suggested that oxidative phosphorylation of FFA may serve predominantly for the maintenance of contractile performance while energy production via glucose derivatives is directed preferentially to the preservation of cellular viability. A substrate ‘switch’ from FFA to anaerobic glycolysis is known to occur during severe myocardial ischaemia, when contractile activity ceases almost instantly. The persistence of such a response throughout reperfusion may be part of the cause of contractile failure in myocardial stunning, and perhaps myocardial hibernation. Although numerous investigators support the hypothesis that stunning is caused primarily by free oxygen radical generation, this does not exclude our metabolic substrate theory. The sustained metabolic abnormalities during reperfusion and recovery from stunning, may be the, or one of many consequences of free oxygen radical generation during ischaemia.

Our metabolic substrate hypothesis can only partially explain the contractile abnormalities in stunned myocardium. In fact, we only demonstrated an improved recovery profile but not a complete reversal of function using high doses of exogenously administered lipids. Previous studies have indicated that excitation–contraction uncoupling and abnormalities of the contractile apparatus play an important role in the pathophysiological process of stunning [24]. In this respect, Gao et al. suggested that structural damage of the myofilaments was responsible for the decreased myofilament Ca²⁺ responsiveness [25]. Since increased plasma FFA concentrations and myocardial FFA uptake increase the myocardial phosphocreatine to ATP ratio, and this effect is blocked by oxfenicine [26], it is tentative to speculate that they would accelerate the repair of subcellular damage, by increased protein resynthesis [27], in the postischaemic reperfused myocardium. However, the time frame in which protein synthesis occurs does not entirely match the immediate effects of FFA as observed in our study.

The beneficial effects of Intralipid® on functional recovery in stunned myocardium as shown in our study cannot be explained by differences in the severity of ischaemia since regional myocardial function was depressed to the same levels as in saline-treated controls during ischaemia and early reperfusion (i.e., prior to the start of the Intralipid® infusion). We have shown previously that residual flow in the ischaemic area through innate coronary collaterals is an important determinant of the extent of ischaemic injury [28]. However, our experimental design was developed to control for this independent variable by using the same animal for control and treatment experiments. Although repetitive coronary artery occlusions can promote collateral growth in dogs, this requires multiple coronary artery occlusions over a prolonged period of time to occur [29]. In our study, animals underwent a maximum of two ischaemic events, separated by 7 days, and the order of treatment conditions was randomized to eliminate a temporal or sequence bias.

In addition, there were no differences in heart rate, arterial blood pressure or left atrial filling pressures between Groups 1b and 1c during recovery from stunning. It is also unlikely that the beneficial effects of Intralipid® were due to a modification of reperfusion injury. Although extracellular acidosis attenuates calcium influx during reperfusion, lipids were administered only 15 min after reperfusion in our study. Previously, it was established in clinical studies that parenteral fat emulsions produce a metabolic acidosis [30], without disturbance of intracellular pH [31]. Intralipid® emulsion also contains very low doses of α- and γ-tocopherol. Since oxygen radicals are mainly produced during ischaemia and early reperfusion, the delayed administration of anti-oxidants with Intralipid® prob-
ably had no effect on the formation of free oxygen radicals. Finally, Intralipid treatment resulted in an increase of plasma haemoglobin. Although no direct explanation is available, this may be related to an α₁-adrenergic mediated splenic contraction as previously observed in dogs by Sato et al. [32]. More importantly, however, oxifenicine blocked the functional effects of Intralipid treatment, but did not prevent the rise in haemoglobin. Consequently, increased haemoglobin levels do not play a pivotal role in the Intralipid-mediated improvement of stunning.

Oxifenicine blocks carnitine palmitoyl transferase I and subsequently inhibits the entry of FFA into the mitochondria of the myocyte [33]. Blocking of FFA metabolism by i.v. administration of oxifenicine had no significant effects on global haemodynamics. Regional myocardial wall thickening of the normally perfused myocardium was unaffected by oxifenicine treatment. Nevertheless, oxifenicine specifically abolished the beneficial effects of Intralipid on recovery from regional stunning. The results suggest that improved recovery from stunning, in animals treated with Intralipid, is mediated by increased substrate (FFA) availability and possibly by an improved FFA oxidation. Noteworthy is a study performed by Cook and Housmans, who showed that Intralipid 10% had significant positive inotropic properties in isolated ferret myocardium, which were not related to increased intracellular Ca²⁺ concentrations. The authors speculated that Intralipid served as a metabolic substrate for the isolated cardiac muscle [34].

Although FFA concentrations were not determined in our study, previous reports have clearly demonstrated that administration of heparin results in increased lipoprotein lipase activity, causing an increased breakdown of triglycerides into FFA [35]. Previous reports also clearly demonstrate that increased circulating FFA concentrations resulted in an increased uptake of FFA by the myocytes [36].

Our observation of improved functional recovery in stunned myocardium due to the presence of a lipid emulsion is contradictory to a study by Lopaschuk et al. The authors described an impaired recovery of function of postischaemic isolated hearts of diabetic rats reperfused with excessive palmitate [37]. This was attributed to the depressant effect of increased FFA on glucose oxidation. However, other studies showed that, particularly during the reperfusion phase, such a depressant effect does not occur [21]. Furthermore, isolated hearts from diabetic rats might react differently to ischaemia, stunning and metabolic treatment of stunning as do in vivo hearts from non-diabetic dogs. Experimentally induced diabetes has species-dependent effects on myocardial ischaemic damage [38,39]. In a similar experimental model Tamm et al. reported a decreased recovery of function when palmitate was the only substrate added to a perfusion medium of reperfused isovolumically beating hearts [21]. When glucose was added to the perfusion medium, contractile performance in postischaemic myocardium was significantly improved. Whereas these studies emphasize the importance of glucose substrate oxidation during reperfusion, neither one addressed the question whether increased FFA substrate availability affected recovery from stunning. In our study, blood glucose levels remained stable throughout all experimental protocols at physiological levels. Further studies are required to determine the effects of abnormal blood glucose levels.

In summary, we have shown that the administration of high-dose LCT emulsions during reperfusion improves contractile recovery from regional myocardial stunning in conscious dogs. We hypothesise that high plasma levels of triglycerides stimulate oxidative phosphorylation of FFA and correct contractile abnormalities either through an unspecified increase of metabolic substrate availability or through a specific correction of FFA substrate preference, which is depressed in stunned myocardium. This hypothesis is supported by our observation that oxifenicine, a CPT I blocker, completely eliminates the beneficial effects of Intralipid on regional myocardial stunning.

Our data do not support the indiscriminate use of parenterally administered lipids in patients with ischaemic heart disease. In view of the current evidence that lipids are potentially harmful during ischaemia, this practice cannot be justified. We emphasize that in our study high-dose parenteral lipids were targeted to the reperfusion phase of postischaemic myocardium in order to elucidate the mechanism(s) involved in the pathogenesis of myocardial stunning. It is hoped that a metabolic hypothesis may eventually generate new therapeutic approaches to the clinically relevant phenomenon of stunning.

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