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In Vitro *Negative Inotropic Effect of Plasma Collected at the Time of Reperfusion in Humans undergoing Liver Transplantation*

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***Background*: During orthotopic liver transplantation (OLT), acute depression of myocardial contractility has been suspected at the time of the graft reperfusion.**

***Methods*: The authors tested the hypothesis that plasma collected at the time of reperfusion in OLT patients exerted a negative inotropic effect on isolated rat myocardium. Plasma from 13 OLT patients was collected either before surgical incision (group 1, n = 8) or 3–5 min after *vena cava* and portal vein unclamping (group 2, n = 9). Six patients had their pre- and postincision plasma analyzed. A postreperfusion syndrome was observed in 3 of 13 patients. Left ventricular rat papillary muscles were studied at baseline (T0), 30 min after the addition of plasma (T30), and 60 min after the addition of plasma (T60). The authors recorded contraction parameters (maximum unloaded shortening velocity [Vmax], peak extent of systolic shortening at preload [ΔL], maximum active isometric tension [AFi], positive peak tension derivative [+dFi/dt], time-to-peak shortening [TPS], and time-to-peak force [TPF]) and relaxation parameters (maximum lengthening velocity at preload [Vl], negative peak tension derivative [−dFi/dt], index of load sensitivity of relaxation [tRi]).**

***Results*: In group 1, contraction parameters remained unchanged, with the exception of a decreased Vmax at T30 and AFi at T60 (each $P < 0.05$). In group 2, all contraction parameters were significantly decreased at T30 and at T60, with the exception of AFi at T60. Both types of plasma decreased Vl and altered tRi at T30 and T60, whereas only reperfusion plasma decreased −dFi/dt at T30 and T60. At T30, ΔL, −dFi/dt, and tRi were significantly more impaired in group 2 than in group 1. There was no relationship between inotropic changes and mean arterial pressure decrease at the time of reperfusion.**

***Conclusion*: Plasma collected at the time of graft reperfusion in OLT patients exerted negative effects on contraction and relaxation performance in isolated rat left ventricular papillary muscle. (Key words: Heart: diastolic function; myocardial contractility; papillary muscle. Liver: transplantation.)**

DURING orthotopic liver transplantation (OLT), the reperfusion of the graft after unclamping of *vena cava* and portal vein suddenly restores venous return, and this is associated with numerous hemodynamic changes.[1-5] Mean pulmonary artery pressure, pulmonary artery wedge pressure, and superior *vena cava* pressure increase. The sudden increase in preload is responsible for the increased cardiac index *via* the Frank-Starling mechanism. The increased cardiac index together with the early decrease in systemic vascular resistances result in a slight decrease in mean arterial pressure in most patients. Conversely, in fewer than 30% of patients, there is a clinically significant hypotension termed the *postreperfusion syndrome,* as defined by Aggrawal *et al.*,[3] which sometimes requires pharmacologic intervention and is resolved in 10-60 min.[3-5] In OLT patients, Estrin *et al.*[4] have observed that the normally good correlation between pulmonary artery wedge pressure and cardiac index after inferior *vena cava* clamping (*i.e.*, unloading) was not found after unclamping. Thus, despite an improvement in indices of overall left ventricular function when *vena cava* was unclamped, the operation of the Frank-Starling mechanism was abnormal, which argues in favor of a myocardial dysfunction after unclamping,[4] as also suggested by others.[2,3] Intrinsic myocardial depression also has been suspected because the reperfusion of ischemic liver could produce substances eliciting deleterious effects on cardiac contractility.[2-4]

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In OLT patients, systemic vasodilation, decreased heart rate, paradoxical septal motion, and an intrinsic negative inotropic effect could contribute to hemodynamic changes at the time of reperfusion.[1-6] One possibility[4] is that adaptive hemodynamic and neurohumoral mechanisms counterbalance the myocardial depression induced by reperfusion plasma in patients without postreperfusion syndrome. The hypothesis of an intrinsic negative inotropic effect of postreperfusion plasma[2-4] is difficult to confirm *in vivo* for at least two reasons: (1) complex changes of preload, afterload, heart rate, and neurohumoral drive are present during and after reperfusion, and such changes are known to interfere with the indices quantifying cardiac contractility in current clinical practice; and (2) the patients often receive drugs modifying myocardial contractility. By contrast, the effects of a circulating myocardial depressant substance in humans may be more accurately evaluated by *in vitro* studies.[7,8]

In the present study, we tested the hypothesis that the plasma collected at the time of reperfusion in OLT patients exerted a negative inotropic effect *in vitro*. Because plasma from cirrhotic patients has been reported to induce a negative inotropic effect on cultured rat heart cells,[8] we also studied a control group with plasma collected before incision in OLT patients.

Materials and Methods

Patients

Our prospective study included 13 patients who underwent OLT in our institution because of end-stage liver failure, Child C classification. Clinical characteristics of the study population are listed in table 1.

Anesthesia and Surgical Procedure

Anesthesia was maintained with fentanyl and midazolam, and neuromuscular blockade with an infusion of atracrium.[9] Mechanical ventilation was performed with 40% oxygen-air mixture. End-tidal carbon dioxide tension was maintained between 32-36 mmHg. All patients were positioned on a electric blanket set at 39°C. Systemic and pulmonary hemodynamics were monitored as previously described.[9] Arterial pressure was measured by means of a catheter inserted into the radial artery; mean arterial pressure was monitored throughout the procedure (Merlin 1166A & 1094A, M1006 A opt. A68, Hewlett Packard, Les Ullis, France). Continuous calcium chloride was infused to obtain a serum ionized calcium concentration greater than 0.9 mM. Before unclamping the *vena cava* and the portal vein, the liver was flushed with cold 4% albumine *via* the portal vein to reduce the potassium concentration to 10-15 mM in the liver flush solution. Among the study population, 3 of 13 (23%) patients exhibited a postreperfusion syndrome as defined by Aggrawal *et al.* (*i.e.*, patients who experienced a decrease in mean arterial pressure $\geq$ 30% from baseline values shortly after inferior *vena cava* unclamping and graft reperfusion).[3]

Collection of Blood Samples

Blood samples were collected in citrate tubes at the radial artery level before surgical incision (group 1), and 3-5 min after inferior *vena cava* and portal vein unclamping and graft reperfusion (group 2). When collecting blood samples in group 2, we also measured: (1) hemodynamic data, including mean arterial pressure (n = 10); and (2) Pa_{O2} (181 $\pm$ 20 mmHg), K^+ (3.8 $\pm$ 0.2 mEq/l), and Ca^{2+} (1.25 $\pm$ 0.09 mM; n = 7). Samples were centrifuged at 4,000 rpm at 8°C for 5 min, and then plasma was removed and stored at −20°C. Immediately before mechanical analysis, the plasmas were maintained at room temperature for at least 10 min.

Mechanical Protocol

This study was carried out on adult Wistar rats according to our routine protocol.[10,11] Care of the animals conformed to institutional guidelines, and the study was approved by our institution (INSERM). Animals were briefly anesthetized with ether, and hearts were quickly removed and weighed. Left ventricular papillary muscles were carefully excised and vertically suspended in a bathing solution containing (all values, mM): NaCl, 118; KCl, 4.7; $MgSO_4$-$7H_2O$, 1.2; KH_2PO_4, 1.1; $NaHCO_3$, 24; $CaCl_2$-$6H_2O$, 2.5; and glucose, 4.5. The solution was maintained at 29°C and equilibrated with a 95% O_2/5% CO_2 gas mixture, giving a pH of 7.40. The volume of the bathing chamber was 250 ml. Muscle strips were electrically stimulated with rectangular pulses of 5-ms duration just above threshold by means of 2 platinum electrodes. Stimulation frequency was 10 beats/min. After a 30-min stabilization period, preload corresponding to initial length at peak of length-active tension curve (Lmax) was determined. At the end of the study, muscle cross-sectional area (mm^2) was calculated from the length and weight of the muscle, assuming a muscular density of 1. Suitable preparations were selected on the basis of a well-individualized cylindrical shape, whose cross-sectional area did not exceed 1.3 mm^2 and

Table 1. Clinical Characteristics of the Study Population

Patient Number	Sex	Age (yr)	Weight (kg)	Height (cm)	Liver Pathology	Mean Arterial Pressure (mmHg)
1	F	52	53	151	Posthepatitic cirrhosis	81
2	M	36	60	167	Alcoholic cirrhosis	119
3	F	37	70	163	Fulminans hepatitis	85
4	M	36	78	178	Posthepatitic cirrhosis	85
5	F	53	60	154	Fulminans hepatitis	88
6	M	44	72	173	Posthepatitic cirrhosis	85
7	M	54	72	172	Posthepatitic cirrhosis	75
8	F	48	71	160	Chronic hepatitis	80
9	M	51	52	172	Alcoholic cirrhosis	80
10	M	57	74	160	Alcoholic cirrhosis	116
11	F	41	62	152	Primary biliary cirrhosis	84
12	M	65	68	172	Liver malignancy	80
13	M	50	58	174	Sclerosing cholangitis	82

whose ratio of resting force to total isometric force was less than 0.25. Adherent or bifid muscles were excluded from the study.

Control values of all mechanical parameters were recorded first (T0).Thereafter, 5 ml of either preincision plasma (group 1) or reperfusion plasma (group 2) were added to the medium. Thus, the plasma was diluted 50-fold in the muscle chamber. In cases wherein significant foaming was observed, the experiments were carried out after the foam had been carefully removed. Importantly, the study was blinded in such a way that the investigator was not aware of the nature of the plasma under study (*i.e.*, before surgical incision or reperfusion plasma). On the basis of a preliminary study indicating that the peak mechanical effects of the plasmas were observed 20–30 min after addition, mechanical parameters were measured 30 min (T30) after the addition of plasma in all muscles. To study the potential recovery of mechanical parameters, data were also obtained 60 min after plasma addition (T60). It was not possible to test preincision plasma in five patients and reperfusion plasma in four patients because of abnormalities with regard to the freezing procedure or computer failure during the experimental procedure. Consequently, 17 left ventricular papillary muscles entered the final analysis, corresponding to 8 patients in group 1 and 9 patients in group 2. Six patients had their pre- and postincision plasmas analyzed. It may be argued that a more elegant design would have washed out the plasma, reestablished a baseline, and then introduced the plasma from the other collection of the plasma from the same patient. The reasons for not pursuing this option were as follows: (1) a thorough wash-out cannot be proven; (2) if we admit the possibility that postreperfusion plasma induces a negative inotropic and lusitropic effect, the blind design of our study makes it possible that an irreversible degradation of the preparation can occur in cases where reperfusion plasma is added first. The electromagnetic lever, force transducer, and control device have been previously described.[10,11] All analyses were performed from digital data on a personal computer. Two signals were recorded: force and length. A single recording had 512 points for each signal for a total recording time of 500 ms (sampling rate, 1,024 Hz).

Mechanical Parameters

Mechanical parameters quantifying inotropy, lusitropy, and load dependence of relaxation were measured.[10–13]

Inotropy

Conventional mechanical parameters characterizing contraction phase were calculated from three twitches and were defined as follows (fig. 1):Vmax, maximum unloaded shortening velocity of the preloaded twitch whose load was abruptly clamped to zero load with critical damping just after electrical stimulus (Lmax/s); ΔL, maximum extent of muscle shortening in the twitch with preload only (%Lmax); +dFi/dt, positive peak force derivative of the fully isometric twitch normalized per cross-sectional area ($mN \cdot s^{-1} \cdot mm^{-2}$); AFi, maximum active isometric force normalized per cross-sectional area (mN/mm^2); TPS, time-to-peak shortening

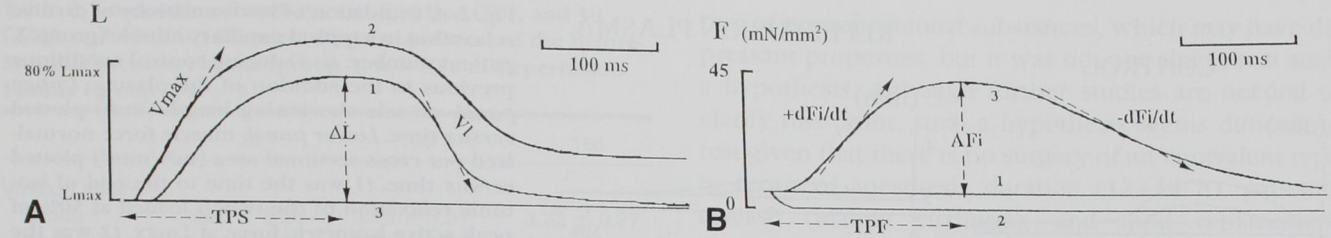

Fig. 1. *A,* **Shortening length (L, %Lmax) plotted *versus* time. Lmax = initial length at peak of length-active tension curve. Twitch 1 was loaded at Lmax with preload only. Twitch 2 was loaded with same preload as twitch 1 and abruptly clamped to zero load with critical damping just after electrical stimulus. Twitch 3 was fully isometric at Lmax. Vl = peak lengthening velocity of twitch 1; TPS = time required to produce maximal shortening in twitch 1; Vmax = maximum unloaded shortening velocity determined from twitch 2; ΔL = maximum extent of shortening of the twitch with preload only.** *B,* **Muscle force normalized per cross-sectional area (F, mN/mm²) plotted *versus* time; +dFi/dt and −dFi/dt = positive and negative peak force derivative of the fully isometric twitch 3 normalized per cross-sectional area; AFi = maximum active force of fully isomteric twitch 3, normalized per cross-sectional area; TPF = time required to produce maximal force in twitch 3.**

of the isotonic twitch with preload only (ms); TPF, time-to-peak force of the fully isometric twitch (ms). Vmax and AFi tested the muscle's inotropic state during low and high loading conditions, respectively. In mechanical studies, the word "tension" can be used to replace "force/cross-sectional area."

Lusitropy

Conventional mechanical parameters characterizing the relaxation phase were defined as follows (fig. 1): maximum lengthening velocity of the twitch with preload only (Vl); and the negative peak of the force derivative of the fully isometric twitch normalized per cross-sectional area ($-dFi/dt$; $mN \cdot s^{-1} \cdot mm^{-2}$). These two indices tested changes in the muscle's lusitropy, *i.e.*, changes in its relaxation rate.[14,15]

Load Sensitivity of Relaxation

The property of load sensitivity of relaxation reflects the capacity of the myocardium to regulate the time course of relaxation according to loading conditions.[10,16] In a typical load-sensitive papillary muscle, isometric relaxation of a moderately loaded twitch (*i.e.*, a twitch during which the muscle shortens) occurs earlier than the superimposed relaxing phase of the fully isometric twitch (*i.e.*, a twitch during which no shortening occurs). This is typically observed in the myocardium from healthy adult mammals. Conversely, in load-insensitive muscle, superimposed isometric relaxation phases almost coincide in time, irrespective of the level of afterload. The load sensitivity of relaxation was quantified by the ratio of two times (tRi = t1/t2; fig. 2A).[10-13,16] This ratio ranges from approximately 0.70-0.85 in a typical load-sensitive relaxation to 1 for a typical load-insensitive relaxation and provides a precise scale of measurement of load sensitivity. Impaired load sensitivity of relaxation has been mainly attributed to impaired calcium reuptake by the sequestering systems, especially the sarcoplasmic reticulum.[10]

Statistical Analysis

Data were expressed as means ± SEM. Within each group, we compared paired samples from the same patient studied on the same rat heart at different time points. Comparisons between T30 and T60 values with the reference value at T0 were thus performed using the Wilcoxon paired signed-rank test. Further, comparisons between group 1 and group 2 at each timepoint were also performed using the Mann-Whitney U test. Correlations were performed by using the Spearman test. All P values were Bonferroni corrected. A P value < 0.05 was required to rule out the null hypothesis.

Results

Contraction and relaxation parameters are presented in Tables 2 and 3. Group 1 and group 2 were homogeneous with regard to baseline (T0) values of all the mechanical indices studied.

Inotropic Effects

At T30, preincision plasma (group 1) did not modify ΔL, TPS, TPF, or AFi, whereas there was a 17% decrease in Vmax (table 2). At T60, Vmax recovered, whereas AFi was significantly decreased. After addition of the reperfusion plasma (group 2), all contraction parameters significantly decreased at T30. In particular, there was a 36% decrease in Vmax, and a 53% decrease in

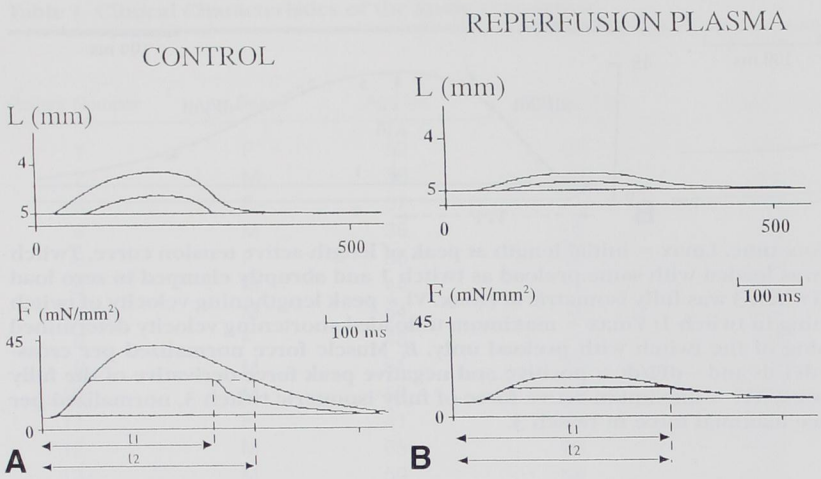

Fig. 2. ***A,*** **Evaluation of load sensitivity of cardiac relaxation in a typical papillary muscle (group 2, patient number: n · 4) during control conditions previous to the addition of the plasma.** ***Upper panel,*** **muscle shortening length (mm) plotted** ***versus*** **time.** ***Lower panel,*** **muscle force normalized per cross-sectional area (mN/mm²) plotted** ***versus*** **time.** ***t1*** **was the time to the end of isotonic relaxation of the twitch loaded at 50% of peak active isometric force at Lmax.** ***t2*** **was the time at which the fully isometric twitch relaxes to the same level of load. Load sensitivity of relaxation was quantified by measuring tRi,** ***i.e.,*** **the t1:t2 ratio. The relaxation of rat myocardium was typically load-sensitive with t1 < t2, thus leading to a tRi value = 0.83. The onset of isometric relaxation occurred sooner when the muscle had shortened (t1) than in the fully isometric twitch (t2), and the corresponding force traces during relaxation were clearly dissociated. This illustrated the influence of load/shortening on relaxation time course.** ***B,*** **Evaluation of load sensitivity of cardiac relaxation 30 min after the addition of reperfusion plasma (T30) in same muscle as** ***A.*** ***Upper panel,*** **muscle shortening length (mm) plotted** ***versus*** **time.** ***Lower panel,*** **muscle force normalized per cross-sectional area (mN/mm²) plotted** ***versus*** **time. At T30, t1 = t2, thus leading to a tRi value of 1. The corresponding force traces were superimposed, indicating that load/length no longer modulated relaxation time course. A marked negative inotropic effect was also observed.**

AFi. The 43% decrease in ΔL contrasted with the unchanged ΔL in group 1. At T60, all parameters were still significantly modified compared with their reference value at T0, with the exception of AFi.

At T30, ΔL and AFi were significantly lower in group 2 than in group 1 (table 2). Mechanical parameters recovered at T60 with the exception of TPF, which was significantly lower in group 2 than in group 1.

The individual responses in the six patients in which both plasmas were tested are indicated for Vmax (fig. 3A), ΔL (fig. 3B), and TPF (fig. 4A).

Lusitropic Effects and Load Dependence of Relaxation

Compared with baseline values, the preincision plasma (group 1) induced a 29% decrease in Vl and impaired the load dependence of relaxation (*i.e.*, tRi increased) at T30; conversely, −dFi/dt was unchanged (table 3). At T60, Vl and tRi were still lower than at T0, with no changes in −dFi/dt. After the addition of reperfusion plasma (group 2), Vl (−59%) and −dFi/dt (−41%) significantly decreased at T30 compared with baseline values, and there was a major increase of tRi. Figure 2B illustrates the load independence of relaxation in a typical muscle from group 2. At T60, Vl and tRi, but not −dFi/dt, were still significantly modified compared with their reference value at T0.

At T30, −dFi/dt was significantly lower, and tRi was higher in group 2 than in group 1 (table 3), whereas Vl was not significantly different in the two groups ($P = 0.09$).

Figure 4B indicates the responses for Vl in the six patients in whom both plasmas were tested.

Relationship Between Mechanical Changes and Hemodynamic and Biological Data

Compared with baseline values (89 ± 5 mmHg; range, from 75 to 119 mmHg), mean arterial pressure decreased during graft reperfusion (64 ± 5 mmHg; range, 30–88 mmHg; $P < 0.05$). There was no relationship between %ΔL at peak effect of the reperfusion plasma and percentage changes in mean arterial pressure during the reperfusion period ($r = 0.30$; $P = 0.47$). Similarly, there was no relationship between %AFi at peak effect of the reperfusion plasma and mean arterial pressure changes during the reperfusion period ($r = 0.10$; $P = 0.80$). Lastly, there was no relationship between ΔL at peak effect of the reperfusion plasma on the one hand and blood K^+ ($r = 0.49$ $P = 0.28$) and Ca^{2+} ($r = 0.41$; $P = 0.35$; $n = 6$) on the other.

Discussion

The present study indicates that plasma collected at the time of graft reperfusion in patients undergoing

Table 2. Contraction Parameters at Baseline (T0), and 30 (T30), and 60 (T60) Minutes after the Addition of the Before-Surgical Incision Plasma (group 1, n = 8) and Reperfusion Plasma (group 2, n = 9)

	T0	T30	T60
Vmax, Lmax/s			
Group 1	3.80 ± 0.26	3.15 ± 0.31*	3.32 ± 0.27
Group 2	3.93 ± 0.30	2.51 ± 0.39*	2.69 ± 0.40*
ΔL, %Lmax			
Group 1	21 ± 1	21 ± 2	20 ± 4
Group 2	21 ± 1	12 ± 2*†	15 ± 3*
TPS, ms			
Group 1	182 ± 10	179 ± 8	173 ± 5
Group 2	182 ± 3	165 ± 7*	158 ± 5*
TPF, ms			
Group 1	161 ± 9	159 ± 4	154 ± 3
Group 2	165 ± 3	146 ± 6*	140 ± 5*†
AFi, mN/mm^2			
Group 1	47.8 ± 5.9	42.3 ± 5.6	42.0 ± 4.8*
Group 2	29.7 ± 4.2	14.0 ± 2.7*†	21.4 ± 5.1

Values are means ± SEM. Vmax = maximum unloaded shortening velocity measured by the zero-load clamp technique; Lmax = initial muscle length corresponding to the apex of the length-active tension curve; ΔL = extent of muscle shortening in isotonic twitch with preload only; TPS = time-to-peak shortening of the isotonic twitch with preload only; TPF = time-to-peak force of the fully isometric twitch; AFi = active isometric force normalized per cross-sectional area. * $P < 0.05$ *vs.* T0 value; † $P < 0.05$ *vs.* group 1 value.

OLT exerted a transient, negative effect on myocardial performance in rat left ventricular papillary muscle. An impairment of the muscle's lusitropic state (*i.e.*, relaxation phase) was also observed.

The limitations of our study need to be discussed. Each of the 13 patients donated plasma before incision and after graft reperfusion. Plasma specimen underwent testing in a randomized, blind manner. Unfortunately, given technical difficulties, pre- and postincision plasma were tested in only eight and nine patients, respectively; matched analyses of initial postreperfusion specimens were performed in six patients. It is important to note that the design chosen for our study makes the matched analyses questionable because the same rat heart muscle preparation was not used for each patient. We cannot exclude the possibility that the lack of statistical significance with respect to several responses also relates to sample size. One other limitation is the absence of an additional group with plasma obtained from patients having procedures of equal duration of liver transplantation, so as to assess the effects of time or other factors for the myocardial depressant effects of the plasma. We cannot exclude the possibility that prolonged anesthesia or surgery does not induce production of neurohormonal substances, which may have depressant properties, but it was not our aim to test such a hypothesis. Although further studies are needed to clarify this point, such a hypothesis seems difficult to test given that there is no surgery of an equivalent type in terms of anesthesia duration (12–14 h), patient's disease (mainly cirrhosis), and fluid replacement (mainly fresh plasma in our study).

The relevance of the experimental model needs to be discussed. Previous work has used mammalian myocardial cells or papillary muscle specimen to show the negative inotropic effects of plasma from either patients with septic shock and cirrhosis[5,6] or animals with various causes of shock.[17,18] Papillary muscle experiments have been shown to be physiologically relevant during various conditions modifying the muscle's inotropic state.[19] Further, it has recently been shown that papillary muscle dynamics closely follow the dynamics of the left ventricle as a whole.[20] The characteristics of rat myocardium, which differs from human myocardium, and the use of a low bath temperature (29°C), also should be considered when discussing our results. Finally, our results raise the possibility of a myocardial depressant substance in reperfusion plasma, but it was not in the scope of our study to determine the characteristics and nature of this substance, and this point deserves further studies.

We observed a moderate impairment of Vmax at T30 and AFi at T60 in the preincision group 1 (table 2), and this is in keeping with the negative effect on cultured rat heart cells observed after the addition of plasma

Table 3. Relaxation Parameters at Baseline (T0), 30 (T30), and 60 (T60) Minutes after the Addition of the Before-Surgical Incision Plasma (group 1, n = 8) and Reperfusion Plasma (group 2, n = 9)

	T0	T30	T60
VI, Lmax/s			
Group 1	3.04 ± 0.25	2.16 ± 0.28*	2.13 ± 0.18*
Group 2	2.86 ± 0.25	1.17 ± 0.25*	1.49 ± 0.84*
−dFi/dt, mN/s/mm^2			
Group 1	237 ± 37	224 ± 30	258 ± 30
Group 2	146 ± 23	86 ± 19*†	128 ± 25†
tRi			
Group 1	0.81 ± 0.01	0.87 ± 0.02*	0.89 ± 0.04*
Group 2	0.83 ± 0.02	0.94 ± 0.01*†	0.93 ± 0.02*

Values are means ± SEM. VI = maximum lengthening velocity of the twitch with preload only; −dFi/dt = negative peak of force derivative of the fully isometric twitch normalized per cross-sectional area; tRi = index of load sensitivity of relaxation. * $P < 0.05$ *vs.* T0 value; † $P < 0.05$ *vs.* group 1 value.

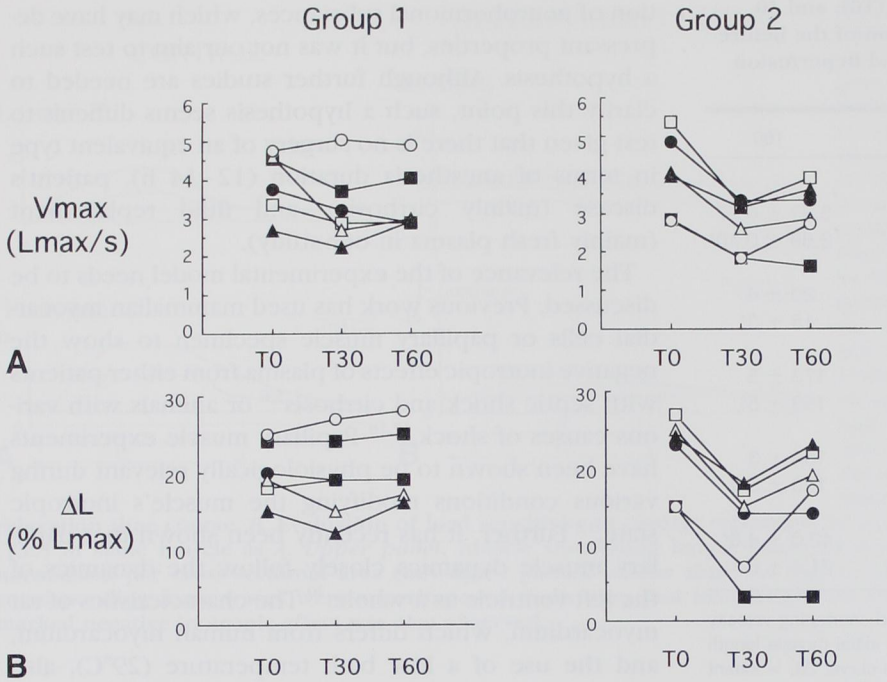

Fig. 3. *A,* **Maximum unloaded shortening velocity (Vmax) before (T0) and 30 min (T30) and 60 min (T60) after the addition of preincision plasma (group 1) and postreperfusion plasma (group 2) in the six patients in whom both plasmas were tested. Each symbol represents a different patient.** *B,* **Maximum extent of shortening of the twitch with preload only (ΔL) before (T0) and 30 min (T30) and 60 min (T60) after the addition of preincision plasma (group 1) and postreperfusion plasma (group 2) in the six patients in whom both plasmas were tested. Each symbol represents a different patient.**

from cirrhotic patients.[8] Plasma collected at the time of graft reperfusion in patients undergoing OLT exerted a negative effect on myocardial performance in rat left ventricular papillary muscle. All contraction parameters were decreased at T30 and at T60, with the exception of AFi at T60. An impairment of muscle's lusitropic state (*i.e.*, relaxation phase) was also observed in both groups. Mechanical effects were significantly more pro-

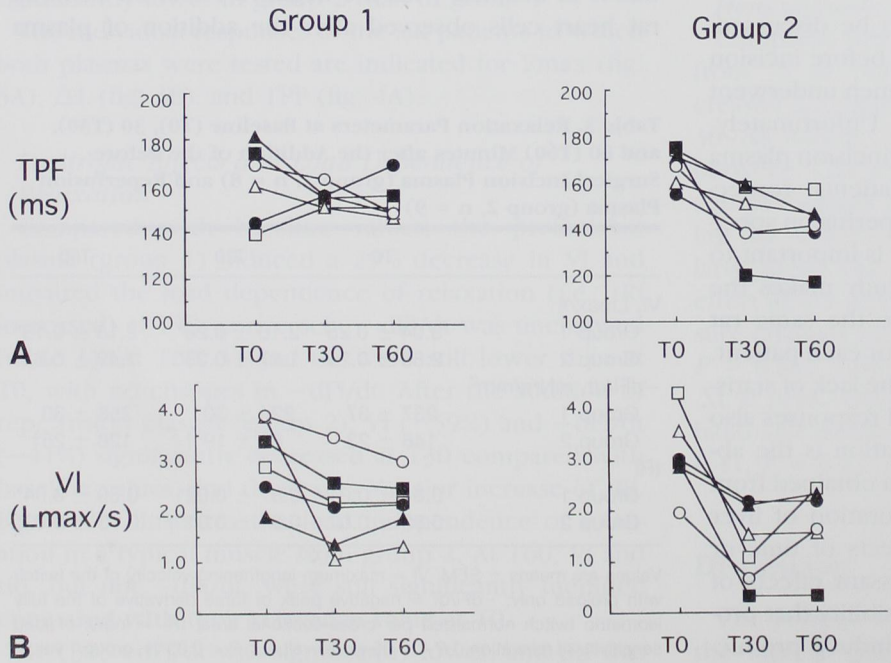

Fig. 4. *A,* **Time-to-peak force (TPF) before (T0) and 30 min (T30) and 60 min (T60) after the addition of preincision plasma (group 1) and postreperfusion plasma (group 2) in the six patients in which both plasmas were tested. Each symbol represents a different patient.** *B,* **Maximum lengthening velocity of the twitch with preload only (Vl) before (T0) and 30 min (T30) and 60 min (T60) after the addition of preincision plasma (group 1) and postreperfusion plasma (group 2) in the six patients in which both plasmas were tested. Each symbol represents a different patient.**

nounced in group 2 than in group 1 when ΔL, AFi, −dFi/dt, and tRi were considered. The mechanical pattern observed in the postreperfusion group 2 is consistent with reversible impairment of excitation–contraction coupling and of at least one of the key steps leading to an increase in activating calcium in muscle cell.[21] This could include plasma-induced modifications of calcium entry, calcium-induced calcium-release from the sarcoplasmic reticulum, calcium binding to myofilaments, myosin adenosine triphosphatase activity, and acto-myosin cross-bridge interactions.[21–23] After intracellular calcium release, activation, and muscle contraction, the relaxation phase is linked to the decrease in intracellular calcium concentration, which is mainly determined by calcium uptake by the sarcoplasmic reticulum and by the affinity of the myofilaments to calcium.[14,19] The effects of the plasma on lightly loaded contraction (ΔL) and heavily loaded contraction (AFi) are consistent with impaired calcium uptake by the sarcoplasmic reticulum and altered affinity of the myofilaments to calcium.[24–26] Impaired load sensitivity of relaxation has been mainly attributed to impaired calcium reuptake by the sequestering systems, especially the sarcoplasmic reticulum.[10,27,28] Thus, the greater impairment of the load sensitivity of relaxation in group 2 than in group 1 (Table 3) was also consistent with a significant alteration of effective calcium sequestration by the sarcoplasmic reticulum, induced by postreperfusion plasma. Because the plasma was diluted 50-fold in the muscle chamber, *in vivo* negative inotropic and lusitropic effects of the postreperfusion plasma could have been underestimated in our study.

The reperfusion of ischemic liver causes an efflux of cold, acidic, hyperkalemic blood from the graft liver into the left ventricle and systemic circulation.[1–5] However, temperature was controlled in our study, and there was no relationship between mechanical changes and biological data. Similarly, hyperkalemia, hypothermia, and acidosis do not appear to play a major role in reperfusion hypotension.[4,5] After reperfusion, the graft could also release vasoactive substances responsible, at least in part, for the peripheral vasodilation.[5,17,29,30] Several natural compounds inducing blood vessel relaxation may also modify the contraction–relaxation sequence of the myocardium.[31] If we accept the possibility that OLT patients had in their blood vasoactive substances at the time of reperfusion,[5,17,29,30] it is therefore possible that these substances also modified the contraction–relaxation sequence of the myocardium, possibly *via* a common pathway for endothelial control of vascular and myocardial function.[31] However, numerous vasoactive substances have a very short half-time and are therefore unlikely to be present in the sample at the time of the study. Further, recent studies have shown that circulating prostanoids[29] or endotoxins[30] could not fully explain the hemodynamic changes associated with reperfusion.

No relationship was found between the extent of plasma negative inotropic effect at peak on the one hand and percentage changes in mean arterial pressure during reperfusion period on the other. The mean decrease in mean arterial pressure during graft reperfusion was 27%, and 23% patients exhibited a postreperfusion syndrome (as previously defined in reference 3), a proportion close to that of 30% reported by Aggrawal *et al.*[3] Although preliminary, our results thus indicate that the negative inotropic effect induced by reperfusion plasma does not fully account for mean arterial pressure changes at the time of reperfusion. However, we cannot exclude other possibilities: (1) the decrease in mean arterial pressure might mainly reflect a vasodilator effect and not a negative inotropic effect, and the diagnosis of negative inotropic effect as afterload decreases is difficult *in vivo*; and (2) the relationship exists, but the relatively few number of patients studied did not enable us to show it (beta risk).

In conclusion, results from our study indicate that plasma collected at the time of graft reperfusion in OLT patients exerted negative effects on contraction and relaxation performance in isolated rat left ventricular papillary muscle.

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