J Mol Cell Cardiol 27, 1745–1755 (1995)

Influence of Dietary Polyunsaturated Fatty Acids on Contractility, Lusitropy and Compliance of Isolated Rat Myocardium

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(Received 16 February 1993, accepted in revised form 23 January 1995)

D. CHEMLA, A. JAVOUHEY-DONZEL, I. SUARD, V. MAUPOIL, Y. LECARPENTIER, J-C. POURNY, G. ROCQUELIN AND L. ROCHETTE. Influence of Dietary Polyunsaturated Fatty Acids on Contractility, Lusitropy and Compliance of Isolated Rat Myocardium. *Journal of Molecular and Cellular Cardiology* (1995) 27, 1745–1755. Two groups of 15 rats were fed for 4 weeks with diets containing 15% by weight of fat varying in polyunsaturated fatty acids (PUFA) content and type. Diet C18:2 (n-6) contained 20% of total fatty acids as linoleic acid and small amount of (n-3) PUFA (0.4% of the total fatty acids). Diet LC (n-3) contained the same amount of 18:2 (n-6) and of long chain (n-3) C20 and C22 PUFA (10% of the total fatty acids). Contents of both saturated fatty acids and amount of total PUFA were kept constant in the two diets. Left ventricular papillary muscle mechanics were studied blind at L_{max} and over the entire load-continuum, in terms of inotropy, characteristics of the force-velocity relationship, relaxation and compliance. Inotropy, force-velocity relationships and muscle compliance were similar in both groups. There was a trend towards a lower peak lengthening velocity at preload in the LC (n-3) group ($P=0.10$) together with an unchanged peak rate of isometric force decline. This resulted in a significant impairment of the two mechanical indexes testing the load dependence of myocardial relaxation ($P=0.019$ and $P=0.002$). In conclusion, short-term differences in PUFA regimen were associated with an unchanged myocardial contractility and economy of force generation. The decreased load dependence of relaxation together with unchanged myocardial compliance strongly favored a physiological relevance of the previously reported modifications of sarcoplasmic reticulum phospholipid composition and calcium transport under (n-3) PUFA regimen.

KEY WORDS: Polyunsaturated fatty acids; Myocardial contraction; Diastolic function; Load dependence of relaxation.

Introduction

Dietary polyunsaturated fatty acids (PUFA) have been shown to markedly influence the development of atherosclerotic lesions, particularly in the coronary vascular bed (Simopoulos, 1991). Besides their beneficial role in the pathogenesis of myocardial ischemia, PUFA may also directly influence cardiac function by inducing changes in membrane phospholipid composition and membrane function

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Part of this work was presented at the GRRC Congress, Montpellier, France, April 6–8, 1994, and appears in *J Mol Cell Cardiol* (1994) 26, 3: XXX, 11.18 (abstract)

at the level of the sarcolemma, sarcoplasmic reticulum or mitochondria (Lamers *et al.*, 1987). This results in modifications of calcium handling under various PUFA regimens, and may lead to modulation of both myocardial sensitivity to arrhythmias (Lepran *et al.*, 1981; McLennan *et al.*, 1985, 1987) and cardiac adrenergic receptor system (Wince and Rutledge 1981; Courtois *et al.*, 1992), particularly with respect to ageing (Charnock *et al.*, 1985).

The influence of PUFA regimen on cardiac inotropic state is more complex. The effects of (n-6)-rich PUFA diets on myocardial contractility have led to conflicting results. Enhanced contractility has been observed in Langendorff-perfused rat hearts under such diets (Hoffmann 1986). Improved left ventricular ejection fraction has been also reported in intact mammals fed with a linoleic acid rich polyunsaturated vegetable oil supplement (Charnock *et al.*, 1987). Conversely, this was not observed in *in situ* heart of pig (Hartog *et al.*, 1987) or rat (Magnuson *et al.*, 1988). Studying rat cardiac muscle cells in culture incubated for 24 h in a media containing either (n-6) or (n-3) as the sole source of PUFA, Courtois *et al.* recently observed that the main parameters of cell contraction were unchanged (Courtois *et al.*, 1992). Similarly, changes in (n-6) and (n-3) fatty acids did not modify the myocardial performance in previous papillary muscle experiments (McLennan *et al.*, 1987). As previously noted by Lamers *et al.*, these results do not permit relevant comparison due to the variety of diets used, experimental settings, and mechanical indices studied (Lamers *et al.*, 1987).

The aim of our study was to carry out a blinded comparison of the mechanical effects induced on rat heart by two regimens containing the same amount of saturated fatty acids and total amount of PUFA, but with differences in (n-6) and (n-3) content and type (Javouhey *et al.*, 1990). Both (n-3) and (n-6) fatty acids are not interconvertible in the human body and are important components of all cell membranes. Whereas membrane proteins are genetically determined, PUFA composition of membranes is to a great extent dependent on the dietary intake (Simopoulos, 1991). Papillary muscle experiments allowed the characterization of intrinsic properties of the myocardium in terms of contraction (inotropy) and economy of force generation, as derived from the force-velocity curve characteristics (Hill, 1938; Alpert and Mulieri, 1982; Lecarpentier *et al.*, 1987a). Furthermore, particular attention was paid to the study of relaxation phase (lusitropy) (Katz, 1983) and muscle compliance (Glantz and Parmley, 1978) for two reasons. First, diastolic abnormalities play a contributory and sometimes predominant role in most states of cardiac alterations, even at a stage when contractility is not yet depressed (Grossman, 1991). Second, relaxation rate mainly depends upon an efficient sequestration of calcium by the sarcoplasmic reticulum (Tada *et al.*, 1978; Katz, 1988), and dietary PUFA regimens modify both the sarcoplasmic reticulum (SR) phospholipid composition and calcium transport (Croset *et al.*, 1989; Swanson *et al.*, 1989). Our initial hypothesis was that the changes in membrane phospholipid composition and calcium transport previously reported in the SR under (n-3) diet (Croset *et al.*, 1989; Swanson *et al.*, 1989) would be moderate in our 4-week study and would initially affect the contractile cycle at the level of the relaxation phase only.

Materials and Methods

Animals

Thirty male Sprague–Dawley strain SPF rats were used. The animals weighed 80–100 g at the start of the experiment and were housed in individual stainless steel cages in a room of controlled temperature (21 ± 1°C), humidity (55–60%) and lighting (12 h dark–light cycle) and were allowed free access to food and sterile water. The rats were maintained on a commercial diet (UAR 113) for five days before transfer on to the experimental diets (Rocquelin *et al.*, 1986).

Diets

Two groups of 15 animals each were formed and fed with test diets containing 15% by weight of fat varying in the (n-6) and (n-3) PUFA content and type (Javouhey *et al.*, 1990). Diet "18:2" contained 20% of total fatty acids as linoleic acid and small amounts of (n-3) PUFA (0.4% of total fatty acids) ("C18:2 (n-6) group", $n=15$). Diet "LC (n-3)" contained the same amount of C18:2 (n-6) and of long chain (n-3) C20 and C22 PUFA (10% of the total fatty acids) ["LC (n-3) group", $n=15$]. Content of saturated fatty acids was kept constant in all diets (20% of total fatty acids) and amount of total PUFA was also constant (22% of total fatty acids). Various mixtures of refined vegetable oils (olive, palm, sunflower) and a fish oil (menhaden) were used to obtain the appropriate fatty acid composition of the

Table 1 Composition of dietary fats and their fatty acids profile (weight % of total)

Oil	Diet C18:2 n-6	Diet LC(n-3)
Olive	63.7	63.5
Palm	15.7	—
Sunflower	20.6	8.0
Menhaden	—	28.5
Fatty Acid		
14:0	0.2	2.1
16:0	15.1	12.5
16:1 n-7	0.6	3.2
18:0	3.9	3.4
18:1 n-9	56.9	51.1
18:1 n-7	1.2	2.8
18:2 n-6	20.7	10.9
18:3 n-3	0.5	0.8
18:4 n-3	—	1.2
20:0	0.4	0.4
20:1 n-9	0.2	0.6
20:4 n-3	—	0.5
20:5 n-3	—	4.3
21:5 n-3	—	0.2
22:5 n-3	—	0.6
22:6 n-3	—	4.1
Others	0.6*	2.3†
Saturated	19.8	20.0
(n-6) PUFA	20.7	11.4
(n-3) PUFA	0.5	11.9
PUFA/saturated	1.07	1.16

*15:0, 16:1 n-9, 17:0, 22:0. Each one is less than 0.2%.
†15:0, 16:1 n-9, 16:2 n-4, 16:3 n-4, 16:3 n-3, 16:4 n-1, 17:0, 18:2 n-4, 18:3 n-6, 18:3 n-4, 18:4 n-1, 20:1 n-7, 20:2 n-6, 20:3 n-6, 20:4 n-6, 20:3 n-3, 22:0, 22:1 n-11, 22:1 n-9, 22:5 n-6, 24:0, 24:1 n-9. Each one is less than 0.3%.

dietary fats (Table 1). The overall amount of vitamin E was equivalent in the two diets, 137 and 127 mg/kg for the C18:2 (n-6) and LC (n-3) diets respectively. This was above that normally recommended for the rat diets used. Animals were fed the test diets *ad libitum* for 4 weeks. Diets were prepared and changed every 2 days. The study was approved by our Institutions (INRA and INSERM).

Experimental protocol

All animals completed the study. The animals were coded due to the blinded design of the mechanical study. After a brief anesthesia with ether left thoracotomy was performed. Hearts were quickly removed and weighed, and left ventricular papillary muscles were carefully excised (Lecarpentier *et al.*, 1979; Chemla *et al.*, 1986). Cardiac preparations were suspended vertically in a tissue chamber containing the following Krebs-Ringer solution (in mM): 118 NaCl, 4.7 KCl, 1.2 Mg SO_4-$_7H_2O$, 1.1 KH_2PO_4, 24 $NaHCO_3$, 2.5 $CaCl_2$-$_6H_2O$, 4.5 glucose. The solution was bubbled with a gas mixture of 95%O_2/5%CO_2 and maintained at pH 7.4 and 29°C. Preparations were stimulated by means of two platinum electrodes positioned parallel to the muscle and delivering rectangular pulses of 5 ms slightly above threshold. Muscles were preloaded at the initial muscle length corresponding to the apex of the length-active isometric tension curve i.e. L_{max}, thus enabling the preparations to recover during a 45 min period. Meanwhile, muscles were stimulated at 12/min. After the stabilization period, muscles were studied at this stimulation frequency. The study was blinded in such a way that the experimenter was not aware of the nature of the dietary fatty acid regimen given to the animal. At the end of the study, the cross-sectional area (mm^2) was calculated from the length at L_{max} and weight of the muscle, assuming a muscular density of 1. Suitable papillary muscle specimens were selected on the basis of well-individualized cylindrical shape, with a resting tension to total tension ratio <0.20. So as to ensure that the preparations were not hypoxic at the level of central muscle core, only muscles whose cross-sectional area did not exceed 1.25 mm^2, and with a load-dependent relaxation, as previously defined (Lecarpentier *et al.*, 1979; Brutsaert *et al.*, 1980; Sys et al., 1984; Lecarpentier *et al.*, 1987b), were selected for analysis. Adherent or bifide muscles were also excluded from the study. On the basis of these criteria, 13 and 11 papillary muscles were studied in C18:2 (n-6) and LC (n-3) groups, respectively.

Electromagnetic lever system and recording

The electromagnetic lever system has been previously described (Lecarpentier *et al.*, 1979; Chemla *et al.*, 1986). Briefly, this lever system allows simultaneous monitoring of muscle force and length. The load applied to the muscle is determined by a servo-controlled current through the coil of an electromagnetic lever system. The equivalent moving mass of the whole system is 155 mg, and its compliance is 0.2 μm/mN.

The displacement of the lever is measured by means of a photoelectric transducer consisting of an incandescent lamp, a miniature photodiode and a preamplifier. Muscular shortening induces a displacement of the lever, which modulates the light emitted by the lamp. The photodiode receptor converts the variations in light intensity into voltage alterations. The system has a linear range of up to

2.5 mm of muscle shortening. The force measurement amplitude ranges from 0 to 140 mN. During contraction, the load applied on the muscle may be abruptly altered (load-clamp) by stepwise change in the current. For the purposes of this study, all analyses were made from digital recordings obtained by means of a computer (IPC). A single recording had 512 points for each signal for a total recording time of 500 ms.

Mechanics of LV papillary muscles

Inotropy

Conventional mechanical parameters characterizing contraction phase are defined as follows (Fig. 1): maximum unloaded shortening velocity (V_{max}) determined by means of the zero-load clamp technique, in which the twitch loaded with the preload corresponding to L_{max} was abruptly clamped to zero-load just after the electrical stimulus; maximum extent of muscle shortening (ΔL) in the twitch with preload only; maximum isometric force normalized per cross-sectional area ($TFmax/mm^2$); positive peak of the force derivative per mm^2 of the fully isometric twitch ($+dF/dt_{max}/mm^2$); time-to-peak shortening (TPS) of the isotonic twitch with preload only; time-to-peak force (TPF) of the fully isometric twitch. V_{max} and $+dF/dt_{max}/mm^2$ tested the muscle's inotropic state under low and heavy loading conditions, respectively.

Determination of the Hill equation and economy of force generation

The force–velocity relationship was derived from the peak shortening velocity (V) plotted against the isotonic force normalized per cross-sectional area (TF/mm^2), both of which were measured in ten twitches, the load being increased regularly from zero up to the fully isometric twitch (Fig. 2). Data were fitted by means of multiple linear regression and the least squares method, following Hill's equation (Hill, 1938; Alpert and Mulieri, 1982; Lecarpentier *et al.*, 1987a; Chemla *et al.*, 1992):

$$(TF/mm^2 + a)\,(V + b) = (cTF\ max/mm^2 + a)\,b$$

where TF/mm^2 is the normalized isotonic force, $cTF\ max/mm^2$ the calculated maximal value of TF/mm^2 when the twitch is fully isometric, V the peak shortening velocity of the isotonic twitch, and $-a$ and $-b$ the asymptotes of the hyperbola (Fig. 2).

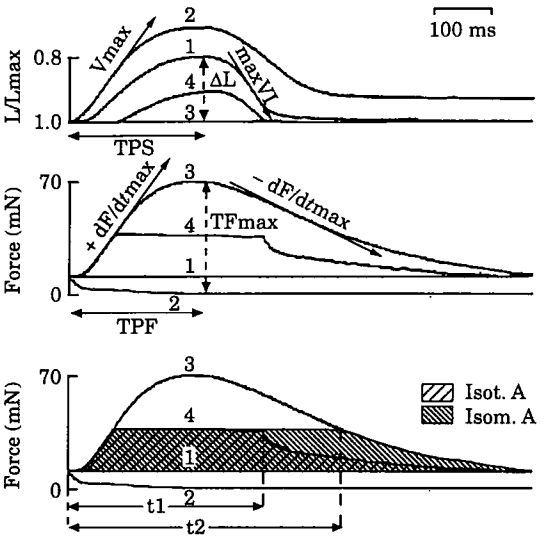

Figure 1 Mechanical parameters of contraction and relaxation. Upper trace: shortening length (L/L_{max}) plotted *v.* time. Middle trace: muscle force (F) plotted *v.* time. Twitch 1 was loaded at L_{max} with preload only. Twitch 2 was loaded with the same preload as that of twitch 1 and was abruptly clamped to zero-load with critical damping just after the stimulus. Twitch 3 was totally isometric at L_{max}. Twitch 4 was afterloaded with half the value of the isometric active force at L_{max}. V_{max}, maximum unloaded shortening velocity of twitch 2; ΔL: maximum extent of muscle shortening of twitch 1; max VI, peak lengthening velocity of twitch 1; $+dF/dt_{max}$ and $-dF/dt_{max}$, positive and negative peaks of isometric force derivative of twitch 3, respectively; TPF, time-to-peak force of the twitch 3. Lower trace: muscle force (F) plotted *v.* time. Two indices were used to quantify the load sensitivity of relaxation. The first one is the ratio of two areas: isotA/isomA and the second one is the ratio of two times (tRi = t1/t2). IsotA was limited by the isotonic force *v.* time curve loaded at 50% of peak active isometric force at L_{max}. IsomA was limited by the isometric force *v.* time curve below the same level of load. t1 was the time to the end of the isotonic relaxation of the twitch loaded at 50% of peak active isometric force at L_{max}. t2 was the time at which the isometric twitch relaxes to the same level of load. The values of the two indices of load-sensitive relaxation were about 0.70 in typical load-sensitive relaxation. These values approach 1 in a typical load-insensitive relaxation. Muscle n°5. C18:2 (n-6) group. $L_{max} = 6$ mm; cross-sectional area (csa) = 0.75 mm^2.

This makes it possible to calculate the G curvature of the force-velocity relationship:

$$G = (cTF\ max/mm^2)/a = (cV_{max})/_b,$$

where cV_{max} is the calculated maximal unloaded shortening velocity. In terms of energy, the G curvature of the force–velocity relationship has been proposed as reflecting the efficiency of muscle contraction (Alpert and Mulieri, 1982; Woledge *et al.*, 1985; Lecarpentier *et al.*, 1987a). The maximum

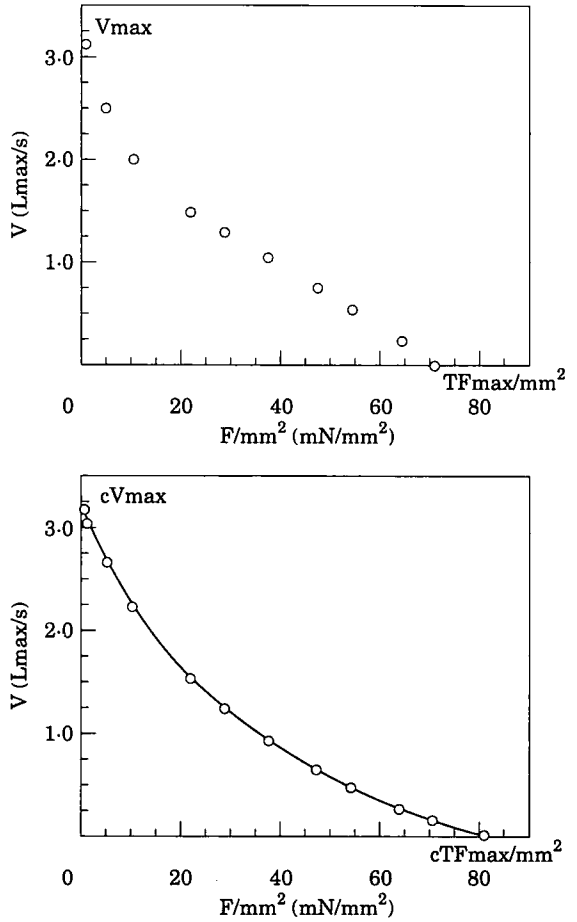

Figure 2 Upper panel: Typical force-velocity relationship. Muscle's velocity of shortening was measured at 10 levels of total load ranging from zero up to fully isometric load (TF/mm^2). The maximum unloaded shortening velocity (V_{max}) was measured by means of the zero-load clamp technique. Lower panel: The experimental data were fitted by means of the Hill equation (see methods). The intercepts of the fitting curve with the force and velocity axis are cFmax/mm^2 and cV_{max}, respectively. Muscle n° 1; C18:2 (n-6) group. L_{max} = 5.0 mm; csa = 0.7 mm^2; V_{max} = 3.13 L_{max}/s; TF/mm^2 = 71.2 mN/mm^2; cV_{max} = 3.16 L_{max}/s; $cF_{max}{}^2$ = 81.7 mN/mm^2; characteristics of Hill hyperbola are as follows: a = 44.4 mN/mm^2; b = 1.71 L_{max}/s; G = 1.84; E_{max} = 35.79 L_{max}/s.mN/mm^2. The correlation coefficient between experimental values and the model estimate was r = 0.993. V_{max} and cV_{max} were similar, while TF/mm^2 was lower than cF_{max}/mm^2 due to a physiological, disproportionate decrease of V at heavy load.

power output E_{max} was also calculated, as previously described (Woledge *et al.*, 1985; Lecarpentier *et al.*, 1987a).

Lusitropy and load sensitivity of relaxation

Conventional mechanical parameters characterizing the relaxation phase are defined as follows

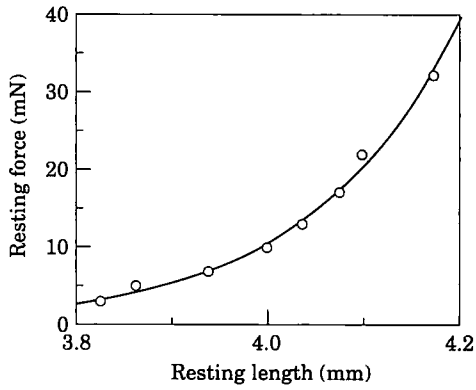

Figure 3 A typical experimental relationship between resting muscle length and resting force. The elastic properties of the papillary muscle were computed using the following equation: $\sigma = \alpha(e^{\beta\varepsilon} - 1)$, in which σ = Langrangian stress (resting force/undeformed area); ε = Langrangian strain, (L–Lo)/Lo, with L = resting muscle length and Lo = resting equilibrium length (where σ and ε equal zero); and α and β are elastic constants, which describe muscle's exponential stress–strain property (Glanz and Parmley, 1978). Muscle n° 3; C18:2 (n-6) group; L_{max} = 4 mm; csa = 0.95 mm^2; α = 1.8 mN/mm^2; β = 17.4; Lo = 3.6 mm; Sum of squares residual SSQR = 0.018.

(Fig 1): maximum lengthening velocity of the twitch with preload only (max VI) and the negative peak of the force derivative of the fully isometric twitch normalized per cross-sectional area ($-dF/dt_{max}$/mm^2). These two indices tested changes in muscle's lusitropy i.e. changes in relaxation rate (Katz, 1983). The property of load sensitivity of relaxation was also studied. This concept reflects the capacity of the myocardium to regulate the time course of relaxation according to the loading conditions (Brutsaert *et al.*, 1980; Lecarpentier *et al.*, 1979). The load sensitivity of relaxation was quantified by computer-assisted calculation of both the ratio of two areas [(isotonic A/isometric A) (isot A/isom A)], and the ratio of two times (tRi = t1/t2) (Fig. 1). These ratios range from approximately 0.70–0.85 in a typical load-sensitive relaxation, to 1 for a typical load-insensitive relaxation (Lecarpentier *et al.*, 1979; Chemla *et al.*, 1986; Antony *et al.*, 1992) (Fig. 1). In a typical load-sensitive papillary muscle, isometric relaxation of an afterloaded twitch occurs earlier than in the superimposed relaxing phase of the fully isometric twitch; consequently, t1 is markedly shorter than t2, resulting in both tRi and isot A/isom A ratio $<<1$. This is typically observed in the myocardium from healthy adult mammals. Conversely, in a load-insensitive muscle, superimposed isometric relaxation phases almost coincide in time, irrespective of the level of afterload. Consequently, both tRi and isot A/isom

A ratio are then close to 1. Isot A/isom A ratio and tRi provide a precise scale of measurement of load sensitivity (Lecarpentier *et al.*, 1979; Chemla *et al.*, 1986; Cory *et al.*, 1994). Load sensitivity of mammalian cardiac relaxation is thought to depend mainly upon the presence of an effective calcium sequestering system, and in particular the SR (Lecarpentier *et al.*, 1979; Cory *et al.*, 1994).

Myocardial compliance

The elastic properties of the papillary muscle were computed using an exponential relation between stress (σ) and strain (ε), as previously recommended (Glanz and Parmley, 1978):

$$\sigma = \alpha(e^{\beta\varepsilon} - 1),$$

in which σ = Lagrangian stress (resting force/undeformed area); ε = Lagrangian strain = (L–Lo)/Lo, with L = resting muscle length and Lo = resting equilibrium length (where σ and ε equal zero); and α and β are elastic constants, which describe muscle's exponential stress–strain properties (Glanz and Parmley, 1978) (Fig. 3). Five to eight different preloads were applied to the muscle (corresponding to five to eight initial muscle lengths), by means of loading steps ranging from preload = 4–5 mN to 20–35 mN. Computer-assisted calculation of α, β and Lo was obtained in the two groups. Data were fitted on a IPC using TransEra HTBasic Advanced Math Library (TransEra Corporation, Provo, Utah, USA). In cases where a stress–strain mono-exponential function was obtained, the accuracy between the experimental results and the model estimate was tested by computing the sum of squared residuals (SSQR).

Statistical analysis

Data were expressed as means ± S.E.M. Comparison between groups was performed using the unpaired Student's *t*-test after analysis of variance. A *P* value <0.05 was considered statistically significant.

Results

At the time of the study, there were no significant differences between the two dietary groups as regards body and heart weights, heart to body weight ratio, papillary muscle length at L_{max}, muscle cross-sectional area, and resting force to total force ratio (Table 2).

Table 2 Clinical characteristics of the studied population, and physical characteristics of the left ventricular papillary muscles

	C18:2 n-6	LC (n-3)	*P*
BW (g)	332 ± 3	330 ± 5	0.69
HW (g)	0.99 ± 0.03	0.96 ± 0.03	0.54
HW/BW $.10^{-3}$	3.01 ± 0.09	2.90 ± 0.08	0.39
L_{max} (mm)	4.9 ± 003	5.2 ± 0.4	0.58
csa (mm^2)	0.94 ± 0.04	0.91 ± 0.04	0.52
RF/TF (%)	5.0 ± 1.1	6.9 ± 1.5	0.31

Values are mean ± S.E.M. BW: body weight; HW: heart weight; L_{max}: initial muscle length corresponding to the apex of the length-active isometric tension curve; csa: muscle cross-sectional area; RF: muscle resting force; TF: muscle total isometric force.

Inotropy

There were no significant differences between the two groups in parameters of inotropy studied under both low and heavy loading conditions. At low load, maximum unloaded shortening velocity, time-to-peak shortening of the twitch with preload only and maximum extent of shortening of the twitch with preload only were similar in the two diet groups (Table 3). Under heavy loading conditions, time-to-peak force was also similar in the two groups (Table 3). There was a trend towards a decreased peak rate of force rise and total force of the fully isometric twitch in the LC (n-3) group, as compared with C18:2 (n-6) group, but it did not reach statistical significance ($P = 0.14$ and $P = 0.13$ respectively).

Force–velocity relationships

The analysis of the force–velocity (F–V) relationships allowed the study of muscle performance over the whole continuum of load borne by the myocardium, from zero-load up to the full isometric twitch (Figure 2). The force–velocity relationships were similar in both groups. The curvature G of the F–V relationship was similar in the two groups (Table 3), and this indicated a similar economy of force generation under (n-6) and (n-3) regimens. Peak power output was also similar in the two groups, with a non significant trend towards a decrease in E_{max} in the LC (n-3) group, as compared with C18:2 (n-6) group ($P = 0.15$) (Table 3). In each group, the V_{max} and cV_{max} values (i.e. the

Table 3 Mechanical parameters of the contraction phase (inotropy), and force-velocity relationship characteristics

	C18:2 n-6	LC (n-3)	*P*
V_{max} (L_{max}/s)	3.78±0.19	3.65±0.28	0.69
ΔL at preload (L/L_{max})	0.21±0.01	0.20±001	0.31
TPS (ms)	181±3	182±6	0.89
$+dF/dt_{max}/mm^2$ ($mN/s/mm^2$)	795±71	651±60	0.14
TF/mm^2 (mN/mm^2)	77.1±5.6	64.1±5.8	0.13
TPF (ms)	165±3	164±6	0.78
cV_{max} (L_{max}/s)	3.77±0.18	3.72±0.32	0.87
$cFmax/mm^2$	91.0±5.0	73.8±8.3	0.08
G	2.47±0.15	2.60±0.20	0.59
Emax (L_{max}/s) × (mN/mm^2)	43.5±3.8	35.3±3.9	0.15

Values are mean ± S.E.M. V_{max}: maximum unloaded shortening velocity; L_{max}: initial muscle length corresponding to the apex of the length-active isometric tension curve; ΔL: maximum extent of muscle shortening in the twitch with pre-load only; TPS: time-to-peak shortening; $+dF/dt_{max}/mm^2$: positive peak of the force derivative of the fully isometric twitch normalized per muscle cross-sectional area; TF/mm^2: total isometric force normalized per cross-sectional area; TPF: time-to-peak force. Characteristics of the force-velocity relationship: cV_{max}: calculated maximal unloaded shortening velocity; $cFmax/mm^2$: calculated maximal value of TF/mm^2; G: curvature of the force–velocity relationship; E_{max}: peak power output.

measured and calculated maximum shortening velocity) were not significantly different. The cFmax/mm^2 was higher than TF/mm^2 in the C 18:2 (n-6) group ($P<0.01$) but not in the LC (n-3) group ($P=0.15$).

Lusitropy

Parameters of the relaxation phase are summarized in Table 4. Under heavy loading conditions, peak rate of isometric force decline was similar in the two groups. Under low loading conditions, peak lengthening velocity of the twitch with preload only tended to be lower in the LC (n-3) group (3.12±0.26 L_{max}/s) than in the C18:2 (n-6) group (3.71±0.23 L_{max}/s, $P=0.10$). This trend was associated with a significant impairment of the load sensitivity of relaxation in the LC (n-3) group compared with the C18:2 (n-6) group, as attested by the significant increase in both isotA/isomA ($P=0.019$) and tRi ratios ($P=0.002$). This indicated that the physiological influence of loading conditions on relaxation time course was less pronounced in LC (n-3) group than in C18:2 (n-6) group.

Table 4 Mechanical parameters of the relaxation phase (lusitropy), and of load sensitivity of the relaxation

	C18:2 n-6	LC (n-3)	*P*
max VI (L_{max}/s)	3.71±0.23	3.12±0.26	0.100
$-dF/dt_{max}/mm^2$ ($mN/s/mm^2$)	226±15	206±20	0.419
isot A/isom A	0.74±0.01	0.77±0.01	0.019
tRi	0.72±0.01	0.76±0.01	0.002

Values are mean ± S.E.M. max VI: peak lengthening velocity of the twitch with preload only; L_{max}: initial muscle length corresponding to the apex of the length-active isometric tension curve; $-dF/dt_{max}/mm^2$: negative peak of the force derivative of the fully isometric twitch normalized per cross-sectional area; isot A/isom A and tRi: indices of load sensitivity of the relaxation phase, see Figure 1.

Compliance

The analysis of stress–strain relationship was not available in 1/13 muscle from the C18:2 (n-6) group and in 1/11 muscle from the LC (n-3) group because it was not possible to obtain steady, reproducible recordings at different initial muscle lengths in these two muscles. Nine out of 12 muscles (75%) and 8/10 muscles (80%) exhibited a monoexponential stress–strain relationship in the C18:2 (n-6) and LC (n-3) groups respectively. The calculated values of elastic constants α and β, and the resting equilibrium length Lo are summarized in Table 5A and B. There was no difference between the two groups as regards α, β, Lo and SSQR. There was no monoexponential relationship between stress and strain in three muscles from the C18:2 (n-6) group and two muscles from the LC (n-3) group.

Discussion

In the present study, short-term (i.e. 4 weeks) differences in PUFA regimen did not modify contractile performance whatever the level of load in rat myocardium. The lack of intrinsic inotropic effects induced by PUFA regimen has been previously reported in fully isometric twitch (Charnock *et al.*, 1985; McLennan *et al.*, 1987). Our results extended this result over the entire load-continuum, and this point seems worth noting, given that heart contracts and ejects against various levels of afterload. PUFA may influence myocardial function by inducing changes in membrane phospholipid composition and in membrane function, especially at the mitochondrial level (Lamers *et al.*, 1987). The

Table 5A Characteristics of myocardial compliance in the C18:2 n-6 group ($n=9$).

Group	Section (mm^2)	L_{max} (mm)	Lo (mm)	α (mN/mm^2)	β	SSQR
n-6	0.70	5.0	4.9	8.7	27.6	0.015
n-6	0.95	4.0	3.6	1.8	17.4	0.018
n-6	0.96	5.0	4.8	8.4	17.5	0.048
n-6	0.95	4.0	3.9	13.4	16.7	0.030
n-6	1.15	6.0	5.7	2.4	31.4	0.033
n-6	0.93	4.0	3.9	5.7	46.7	0.003
n-6	0.77	6.5	5.9	7.7	10.7	0.004
n-6	1.22	6.5	5.9	0.7	29.0	0.044
n-6	0.97	6.5	5.8	6.1	9.5	0.042
Mean	0.96	5.3	4.9	6.1	22.9	0.026
S.E.	0.05	0.4	0.3	1.3	3.9	0.004

Table 5B Characteristics of myocardial compliance in the LC (n-3) group ($n=8$).

Group	Section (mm^2)	L_{max} (mm)	Lo (mm)	α (mN/mm^2)	β	SSQR
n-3	1.05	6.0	5.5	1.9	18.3	0.015
n-3	0.75	4.0	3.5	4.3	9.5	0.009
n-3	0.64	7.0	6.5	4.7	16.5	0.008
n-3	0.91	3.5	3.2	4.0	13.0	0.001
n-3	0.90	5.5	5.2	1.9	29.9	0.004
n-3	1.04	5.5	5.3	9.5	22.8	0.044
n-3	0.86	3.5	3.4	8.8	13.7	0.004
n-3	0.89	3.5	2.9	2.1	7.8	0.053
Mean	0.88	5.0	4.6	4.7	16.6	0.020
S.E.	0.06	0.5	0.5	1.1	2.9	0.008
p value (n-6) v (n-3)	0.32	0.45	0.38	0.42	0.19	0.38

Values are mean ± S.E.M. The stress–strain relationships were studied according to the following relationship: $\sigma=\alpha\ (e^{\beta\varepsilon}-1)$. σ = Lagrangian stress (resting force/undeformed area); ε = Lagrangian strain, (L–Lo)/Lo; L = resting muscle length; Lo = resting equilibrium length (where σ and ε equal zero); L_{max}: L corresponding to the apex of the length-active isometric tension curve; α and β: elastic constants; SSQR: the sum of squared residuals. Only the muscles accurately fitted by means of the equation are presented.

similar curvature of the F-V relationship furnishes an indirect argument in favor of a similar economy of force generation and unchanged energy balance within the myocyte in the two groups (Hill, 1938; Alpert and Mulieri, 1982; Woledge *et al.*, 1985; Lecarpentier *et al.*, 1987a). Lastly, the similar value of V_{max} in the two groups is consistent with the unchanged ATPase activity previously reported in rat myocardium under different dietary fats (de Deckere and Ten Hoor 1980; Schwartz *et al.*, 1981).

The main result of the present study is that short-term differences in PUFA regimen were sufficient to modify relaxation properties of the rat myocardium. Compared with the C18:2 (n-6) group, a moderate impairment of the load sensitivity of relaxation was observed in the LC (n-3) group. This property reflects the capacity of the myocardium to regulate the time course of relaxation according to the loading conditions (Brutsaert *et al.*, 1980). In LC (n-3) group, the two ratios quantifying the load sensitivity of relaxation (i.e. isot A/isom A and tRi) remained less than 1. This indicates that load sensitivity of relaxation was impaired, but not abolished as observed in amphibian heart or after chemical destruction of the SR, where these ratios reach 1 (Lecarpentier *et al.*, 1979). Similarly, the non significant trend in peak lengthening velocity decrease argues in favor of only a slight impairment of the SR function (Tada *et al.*, 1978; Katz 1988). However, the short duration of PUFA regimen needs to be taken into account. The impaired load sensitivity of relaxation together with a decrease in lengthening velocity has been mainly attributed to a less efficient SR (Lecarpentier *et al.*, 1979; Brutsaert *et al.*, 1980; Cory *et al.*, 1994). Changes in SR composition and calcium transport have been consistently reported under (n-3) PUFA regimen (Croset *et al.*, 1989; Swanson *et al.*, 1989). These authors have reported that a reduction in the (n-6)/(n-3) fatty acid ratio was associated with a lower

relative activity of Ca^{2+}–Mg^{2+}-ATPase and a lower initial rate of calcium transport and maximum calcium uptake in SR vesicles from mouse heart.

We observed a similar compliance of papillary muscles in the two groups, with similar values of the elastic constants α and β, as derived from the stress–strain relationships, thus reflecting similar intrinsic elastic properties of the myocardium. It was important to test this point because imbalance in dietary fatty acids can produce myocardial lesions (Lamers *et al.*, 1987) which in turn can modify myocardial stiffness. Furthermore, compliance of the myocardial unit is an important determinant of the compliance of the left ventricular chamber (Gilbert and Glantz 1989). Unchanged filling pressure has been reported in *in situ* hearts of pigs submitted to different dietary fat regimens (Hartog *et al.*, 1987), as well as in isolated rat hearts (de Deckere and Ten Hoor 1980), but the compliance of the cardiac chamber has been poorly investigated in such experimental models. An increased muscle stiffness would have tended to decrease early lengthening rate (Gilbert and Glantz 1989), and thus would have modified the load sensitivity of relaxation; this hypothesis was clearly ruled out in our study. Differences in relaxation performance observed between groups could not be explained by differences in muscle stiffness and were due to intrinsic alterations of the processes leading to inactivation within cell.

Limitations of our study need to be discussed. First, the results obtained can be strictly attributed to the experimental conditions under study, particularly in terms of bath temperature. Membrane function is very much dependent on the fluidity of the bilayer which is determined by, for example, cholesterol content, temperature and fatty acid composition. We cannot exclude the possibility that the (29°C→37°C)-induced increase of the bilayer fluidity could modify the results reported in the present study. The experimental conditions presented here, however, are those commonly used in papillary muscles experiments (Lecarpentier *et al.*, 1979; Brutsaert *et al.*, 1980; Chemla *et al.*, 1986), which have previously been demonstrated to be physiologically relevant (Brutsaert *et al.*, 1980; Suga *et al.*, 1983). Another limitation is that muscles were analysed in terms of mechanical properties only. Diets containing the same amount of saturated fatty acids and total amount of PUFA, but differing in (n-6) and (n-3) content and type are part of our routine protocol (Javouhey *et al.*, 1990). Such diets have been shown to modify the heart fatty acid composition in a very reproducible way; it is therefore unlikely that the diets used in the present study could have induced different modifications of myocardial fatty acid composition than those previously described (Javouhey *et al.*, 1990). The dead ends of the muscle might well have modified the coupling between sarcomere length and muscle length (Kentish *et al.*, 1986). These small non-contractile areas of the muscle are part of the elastic element in series, which plays a role in isometric twitch; conversely, the stress–strain relationships depend mainly upon elastic elements in parallel. Lastly, the similar inotropic level of the two groups of diets was measured under steady-state conditions. This does not preclude different contractile responses to various experimental stresses, especially hypoxia (Grynberg *et al.*, 1988) or beta-adrenoceptor stimulation, but it was not in the scope of the present study to test such conditions.

In conclusion, using a blind study design, we observed that short-term (i.e. four weeks) differences in PUFA regimen were associated with a moderate impairment of relaxation properties in the LC (n-3) group, as compared to the C18:2 (n-6) group. Our results also extended over the entire continuum of loads the lack of inotropic effects of PUFA regimen at baseline previously reported in the fully isometric twitch only, and indicated an unchanged economy of force generation in the myocardium. Lastly, an unchanged myocardial compliance was demonstrated at the papillary muscle level under two different diets.

Acknowledgements

The study was supported by grants from INSERM no 900911, and the Conseil Régional de Bourgogne. The authors gratefully acknowledge the expert assistance of Catherine Coirault, Nathalie Pery and Ken Hylton, and thank Lucien Guenot for his technical assistance.

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