

# Enhanced Baroreceptor Control of the Cardiovascular System by Polyunsaturated Fatty Acids in Heart Failure Patients

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<b>OBJECTIVES</b>	The intention of this study was to test the hypothesis that, in heart failure patients, dietary supplementation of polyunsaturated fatty acids (PUFA) enhances arterial baroreceptor control of the cardiovascular system.
<b>BACKGROUND</b>	Administration of PUFA reduces the risk of life-threatening arrhythmias in patients surviving myocardial infarction. This might result from potentiation of arterial baroreflexes, but whether or not PUFA enhance baroreflex function has never been studied in humans.
<b>METHODS</b>	Patients with post-myocardial infarction left ventricular dysfunction underwent beat-to-beat blood pressure (BP) (Finapres, Ohmeda Inc., Englewood, Colorado) and R-R interval (electrocardiogram) recording; baroreceptor reflexes were assessed from the bradycardic and depressor responses to graded neck suction (NS) as well as by computation of the alpha “spontaneous” baroreflex sensitivity index. Assessments were repeated after prolonged treatment with PUFA (2 g/die, n = 15) or placebo (n = 10).
<b>RESULTS</b>	Baseline BP and R-R interval were unaffected by PUFA. Both reflex depressor and bradycardic responses to NS increased after PUFA (respectively from $-0.09 \pm 0.01$ to $-0.16 \pm 0.01$ mm Hg · mm Hg <sup>-1</sup> , p < 0.01, and from $1.25 \pm 0.9$ to $1.76 \pm 1.1$ ms · mm Hg <sup>-1</sup> , p < 0.04) but not after placebo. The spontaneous baroreflex sensitivity increased in the PUFA (from $8.99 \pm 1.4$ to $12.2 \pm 1.2$ ms · mm Hg <sup>-1</sup> , p < 0.02) but not in the placebo group. Polyunsaturated fatty acids (but not placebo) treatment also significantly increased R-R interval total variance and low-frequency and high-frequency spectral powers.
<b>CONCLUSIONS</b>	Dietary PUFA supplementation markedly potentiates baroreflex function and enhances heart rate variability in patients with stable congestive heart failure. (J Am Coll Cardiol 2006;48:1600–6) © 2006 by the American College of Cardiology Foundation

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Dietary supplementation of N-3 polyunsaturated fatty acids (PUFA) has been shown to reduce the risk of life-threatening arrhythmias in post-myocardial infarction (post-MI) patients (1–4). However, the mechanisms underlying such beneficial effect have not been completely clarified. In particular, although PUFA have been shown to

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have direct cardiac effects (5,6), which may play a role in their antiarrhythmic action (e.g., reduction of ventricular automaticity and prolongation of effective refractory period), no evidence exists whether they can also favorably modulate reflex cardiovascular control, whose derangement

was shown to significantly contribute to the high sudden death rate that characterizes the heart failure patient (7).

We therefore set up the present study to test the possibility that in post-MI, clinically stable, mild-to-moderate congestive heart failure patients' dietary PUFA supplementation enhances the control exerted by arterial baroreceptors on the cardiovascular system.

The assessment was based on a 2-fold technical approach: 1) measurement of the bradycardic and depressor responses to selective carotid baroreceptor stimulation by the neck suction technique (8,9); 2) computation of the so-called “spontaneous” baroreceptor control of heart rate by cross-spectral analysis of prolonged beat-to-beat blood pressure recordings (10).

## METHODS

**Study population.** Twenty-five patients (24 men, 1 woman) with chronic post-MI systolic heart failure (ejection fraction lower than 40%) were recruited. In order to avoid that the comparison of baroreflex responses on versus off PUFA supplementation be confounded by modifications in the patient's clinical status in terms of hemodynamic

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**Abbreviations and Acronyms**

DHA	= docosahexaenoic acid
EPA	= eicosapentaenoic acid
HF	= high frequency
LF	= low frequency
NYHA	= New York Heart Association
post-MI	= post-myocardial infarction
PUFA	= polyunsaturated fatty acids

compensation or drug regimen, we restricted recruitment to clinically stable subjects on optimized drug treatment with beta-blockers, angiotensin-converting enzyme inhibitors, and diuretics. Twenty-three patients were also receiving statins. Administration of the above as well as of any other ongoing drug was kept unmodified throughout the study. Exclusion criteria were clinical instability, recent (<3 months) changes in drug regimen, evidence of (inducible) myocardial ischemia, sustained atrial or ventricular arrhythmias, chronic obstructive pulmonary disease, diabetes mellitus, or any other major system disease. All patients were current non-smokers. The protocol was approved by our local ethical review board. All patients gave written consent to participate in the study after being informed about its nature and purpose. Patient characteristics including information on ongoing drug treatment are shown in Table 1.

**Protocol and data collection.** In order to exclude hemodynamic instability, each patient underwent a clinical examination that included history and physical examination with determination of the New York Heart Association (NYHA) heart failure functional class, 12-lead electrocardiogram (ECG), a symptom-limited cardiorespiratory exercise test (ergospirometer model CPX/D, Medical Graphics Italy, Milan, Italy), and a complete echocardiographic evaluation.

Patients were then randomly allocated to treatment for 4 months with PUFA (2 g/day) or with placebo. Polyunsaturated fatty acids were manufactured as soft capsules with a content of eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) of no less than 85% with an EPA/DHA ratio of 0.9 to 1.5. Placebo capsules were provided by the hospital pharmaceutical service. Randomization was performed according to a 3:2 (PUFA/placebo) criterion. The sample size was calculated in order to disclose a 30% to 40% PUFA-related increase in baroreflex sensitivity.

**Table 1.** General Clinical Characteristics of Actively and Placebo-Treated Patient Groups

	Placebo (n = 10)	PUFA (n = 15)
Age (yrs)	60.1 ± 2.7	59.4 ± 2.5
VO <sub>2</sub> peak (ml/kg/min)	18.0 ± 2.08	16.8 ± 1.1
ACE inhibitors	9/10	14/15
Beta-blockers	9/10	14/15
Statins	9/10	14/15
Diuretics	4/10	9/15

Entries preceding and following ± denote mean and SEM.

ACE = angiotensin-converting enzyme; PUFA = polyunsaturated fatty acids; VO<sub>2</sub> = volume of oxygen.

No attempt was made to blind actively treated patients to the taste of PUFA. However, patients were unaware of the taste issue, in that they did not expect to experience any special taste on taking the study pills; thus placebo-treated patients had no elements to suspect that the pills they were taking were pharmacologically inactive.

At the end of the study, blood was drawn to determine high sensitivity C-reactive protein (immunonephelometric method) and plasma concentrations of PFAs, EPA, and DHA (gas chromatography).

Before and during the final week of randomized treatment, each patient underwent a laboratory study for the evaluation of the arterial baroreflex and of cardiovascular variability. Each participant was asked to refrain from drinking coffee and tea in the 24 h preceding the experimental session. All recordings were performed in the early afternoon in a quiet room with a temperature between 20°C and 23°C. The patient lay supine throughout. Recorded signals included: pulsatile finger blood pressure by Finapres (Ohmeda Inc., Englewood, Colorado), ECG and ventilatory activity by a custom-made thoracic impedance device. Signals were fed through an analog-to-digital converter into a personal computer at a sampling rate of 500 Hz, visualized throughout on the computer screen, acquired and stored in the computer memory during the periods of interest (see the following text). The neck suction stimulus was applied by means of a pneumatic chamber fitted around the individual's neck and connected to a commercial vacuum cleaner adapted with a user-controlled leak to obtain the desired degree of suction, monitored via a Statham pressure transducer (Ametek Power Instruments, Rochester, New York). The study started after 5 to 10 min of quiet resting. It consisted of 4 separate and randomly sequenced experimental periods, as follows: period 1: 10-min recording under resting conditions and spontaneous breathing; period 2: 10-min recording under resting conditions and controlled respiration at 15 cycles/min; period 3: 5-min recording under spontaneous breathing during which 2 neck suction stimuli were applied. The first stimulus consisted of a 30-s neck suction at –20 mm Hg after a 120-s control pre-stimulus period. The second stimulus consisted of a 30-s neck suction at –40 mm Hg after a 60-s pre-stimulus period; period 4: same recordings and procedures as period 3. A recovery interval lasting 3 to 5 min was allowed after each experimental period. Univariate and bivariate spectral analysis was only computed on the data collected during period 2, to avoid excess non-stationarities in the analyzed signals.

**Data analysis.** Systolic and diastolic blood pressure, mean arterial pressure, and R-R interval were derived beat-by-beat from the recorded blood pressure and ECG signals.

**CAROTID SINUS BARORECEPTOR STIMULATION.** The peak bradycardic and depressor responses observed within 15 s of the onset of neck suction were calculated as the average value of the 2 consecutive beats showing the greatest and smallest value of R-R interval and mean arterial pressure,

respectively. The regression of the peak reflex responses to the graded negative neck chamber pressures was calculated, the slope of the regression being taken as the measure of baroreflex sensitivity. The magnitude of the observed responses in periods 3 and 4 were averaged.

**UNIVARIATE SPECTRAL ANALYSIS.** A detailed description of the spectral analysis technique used in this study has been reported previously (11,12). In brief, time series of blood pressure and pulse interval values obtained during controlled breathing were analyzed by a parametric spectral estimation method based on an autoregressive model. The method allows the spectral power to be quantified in terms of central frequency (in Hz) and amplitude (in  $\text{ms}^2$  or  $\text{mm Hg}^2$ ) of the different variability components. Spectral powers of blood pressure and R-R interval time series were calculated in pre-set bands, termed low frequency (LF) (0.04 to 0.15 Hz) and high frequency (HF) (0.15 to 0.50 Hz) bands (13).

**BIVARIATE CROSS-SPECTRAL ANALYSIS.** The cross-spectral analysis used to calculate the spontaneous baroreflex sensitivity has also been previously described (10). In brief, the coherence between the changes in systolic blood pressure and R-R interval was calculated during controlled breathing and considered to be significant when the squared coherence modulus was above 0.5 (11,12). The root-squared ratio between the spectral powers of coherent R-R interval and systolic blood pressure variations, called alpha index, which quantifies the sensitivity of baroreflex control of heart rate under closed-loop conditions, was separately calculated for the LF and HF bands (10).

**Statistical comparisons.** Statistical significance of the differences in baseline parameters and in alpha indexes after versus before treatment was assessed by the non-parametric Wilcoxon rank test.

Significance of the differences of the slopes calculated by analyzing the responses to the neck suction stimulus after versus before treatment was assessed by an analysis of covariance generalized linear model with a single-factor repeated measures design. The factors considered by the model included: 1) a between subjects' independent factor

(i.e., PUFA vs. placebo group); 2) a within subjects' repeated factor (i.e., after vs. before treatment); and 3) a covariate (i.e., the neck suction stimulus) (14). Moreover, we also tested whether the increments of slopes after versus before treatment were significantly different between the 2 groups (unpaired *t* test).

Significance of the changes in spectral powers after versus before treatment were evaluated by the paired *t* test after logarithmic transformation or by the Wilcoxon non-parametric rank test as appropriate.

For each comparison, the level of statistical significance was set at a value of  $p < 0.05$ .

## RESULTS

**Blood chemistry.** At the final evaluation, the plasma concentrations of polyunsaturated acids were significantly higher in the treated compared with the placebo group (placebo group: EPA  $4.8 \pm 1.3$ , DHA  $10.7 \pm 1.1 \mu\text{g} \cdot \text{mg}^{-1} \cdot \text{l}^{-1}$ ; PUFA group: EPA  $10.5 \pm 1.1$ , DHA  $15.8 \pm 0.9 \mu\text{g} \cdot \text{mg}^{-1} \cdot \text{l}^{-1}$ , both  $p < 0.01$ ). High sensitivity C-reactive protein was similar in the 2 groups and was unaffected by PUFA treatment (placebo group:  $2.6 \pm 1.5$ ; PUFA group:  $2.5 \pm 0.7 \text{mg} \cdot \text{l}^{-1}$ ,  $p = \text{NS}$ ).

**Cardiovascular parameters.** Echocardiographically determined left ventricular function as well as NYHA functional class and body weight were all unchanged at the final compared with the initial evaluation in both the PUFA- and the placebo-treated groups (Table 2).

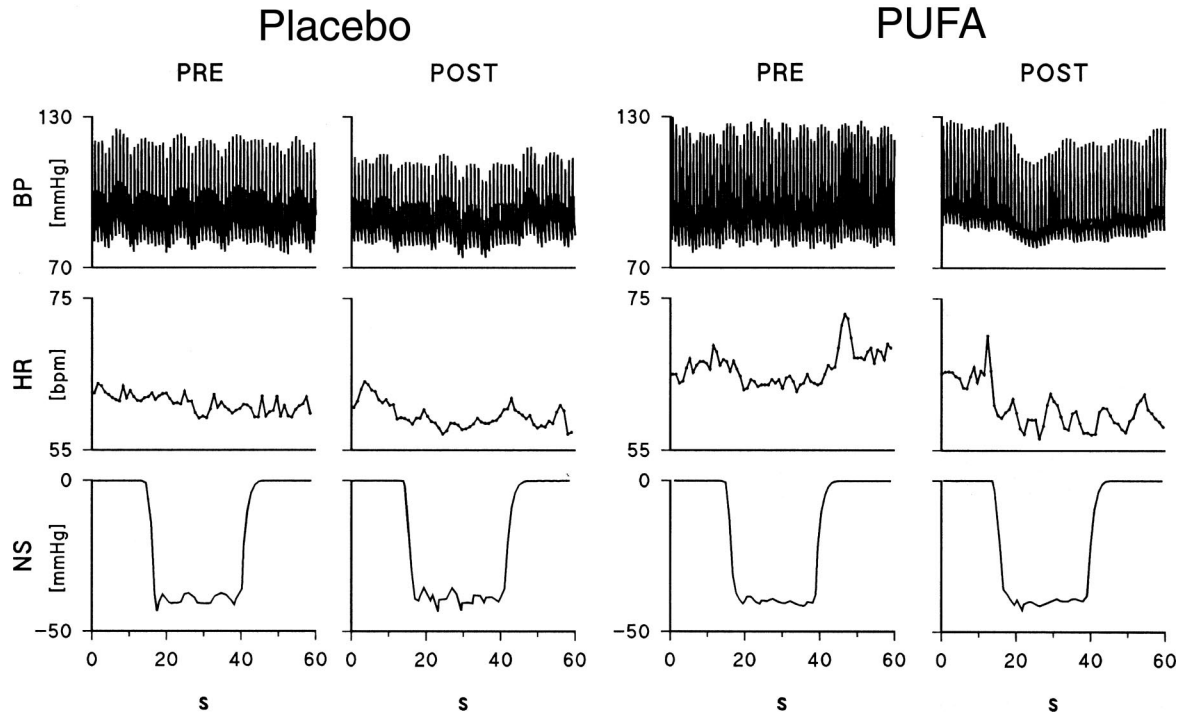
Baseline blood pressure was similar in the 2 groups and was unaffected by either PUFA or placebo treatment. Baseline R-R interval was also similar in the 2 groups and was unchanged after placebo treatment whereas after PUFA treatment it showed a trend to a lengthening (amounting to about 60 to 70 ms or 4 to 5 beats/min), which, however, did not reach statistical significance (Table 2). In line with previous findings, R-R interval variability, computed as either total variance or spectral power, was significantly and markedly enhanced after PUFA treatment, whereas it was unchanged after placebo treatment (Table 2).

**Table 2.** Mean Initial and Final Values of Ejection Fraction, Body Weight, Blood Pressure, R-R Interval, and R-R Interval Variability Parameters

	Placebo		PUFA	
	Initial	Final	Initial	Final
Ejection fraction (%)	$36.6 \pm 2.5$	$35.8 \pm 2.1$	$34.7 \pm 1.5$	$34.5 \pm 1.8$
Weight (kg)	$77.9 \pm 4.5$	$78.1 \pm 3.2$	$77.3 \pm 2.0$	$78.1 \pm 2.4$
NYHA functional class	$2.44 \pm 0.3$	$2.39 \pm 0.3$	$2.34 \pm 0.2$	$2.36 \pm 0.3$
SBP (mm Hg)	$124 \pm 3$	$120 \pm 4$	$119 \pm 5$	$119 \pm 4$
DBP (mm Hg)	$81 \pm 3$	$78 \pm 3$	$78 \pm 3$	$79 \pm 3$
R-R interval (ms)	$954 \pm 37$	$953 \pm 34$	$921 \pm 29$	$987 \pm 39$
R-R variance ( $\text{ms}^2$ )	$814 \pm 112$	$814 \pm 92$	$845 \pm 124$	$1,265 \pm 220^*$
R-R-LF ( $\text{ms}^2$ )	$101 \pm 34$	$96 \pm 39$	$50 \pm 21$	$190 \pm 51^\dagger$
R-R-HF ( $\text{ms}^2$ )	$113 \pm 39$	$138 \pm 47$	$210 \pm 69$	$370 \pm 106^*$

Entries are mean values  $\pm$  SEM. \* $p < 0.05$  final versus initial evaluation;  $^\dagger p < 0.01$  versus initial evaluation.

DBP = diastolic blood pressure; HF = high frequency; LF = low frequency; NYHA = New York Heart Association; PUFA = polyunsaturated fatty acids; SBP = systolic blood pressure.



**Figure 1.** Carotid baroreceptor stimulation. Examples showing the effects of neck suction (NS) (–40 mm Hg, **bottom**) on blood pressure (BP) (**top**), and heart rate (HR) (**center**) in a placebo- (**left**) and in a polyunsaturated fatty acid (PUFA)-treated (**right**) patient both before (pre) and after (post) treatment. Note the modest responses to carotid baroreceptor stimulation observed in both patients at the initial evaluation and the clearcut depressor and bradycardic responses after 4 months of treatment with PUFA but not with placebo.

**Responses to carotid baroreceptor stimulation.** As shown in the examples of [Figure 1](#), the bradycardic responses to neck suction were larger after versus before PUFA treatment, whereas they showed no consistent modifications after versus before placebo treatment (center panels); the same applied to the depressor responses to neck suction (top panels). As shown in [Figure 2](#), this pattern was confirmed in the group analysis in which the average slopes of the bradycardic (or depressor) responses to neck suction were significantly steeper after versus before PUFA treatment ( $p < 0.005$  for the R-R interval slopes and  $< 0.00013$  for the mean arterial pressure slopes) but not after versus before placebo treatment. In fact, the change in the bradycardic slopes was significantly greater in the PUFA- than in the placebo-treated group ( $+0.51 \pm 0.16$  vs.  $-0.11 \pm 0.26$   $\text{ms} \cdot \text{mm Hg}^{-1}$ ,  $p < 0.04$ ), and the same was observed for the depressor slopes ( $-0.06 \pm 0.01$  vs.  $+0.02 \pm 0.01$   $\text{mm Hg} \cdot \text{mm Hg}^{-1}$ ,  $p < 0.006$ ).

**“Spontaneous” baroreceptor control of heart rate.** As shown in [Figure 3](#), the alpha index (computed in either the LF or HF spectral band) was similar in the 2 groups at baseline, whereas at the final evaluation its values were significantly increased in the group treated with PUFA but were unchanged in the placebo-treated group.

## DISCUSSION

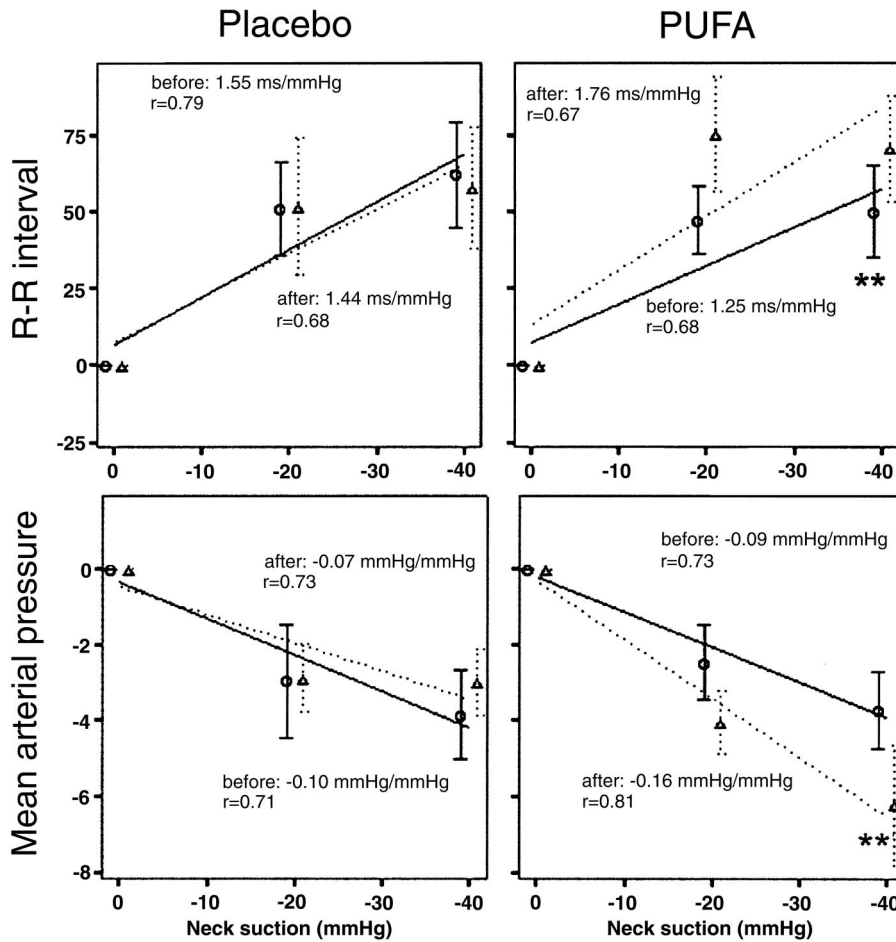
Our study provides the first demonstration that a 4-month dietary supplementation of polyunsaturated fatty acids is accompanied by a partial restoration of several indices of

cardiovascular homeostatic control that are typically depressed in the heart failure patient, namely: 1) baroreceptor control of heart rate; 2) baroreceptor control of the peripheral vasculature; and 3) heart rate variability. It also shows that the extent of the baroreflex improvement was far from trivial: in the neck suction experiments, the calculated slopes for the bradycardic and depressor responses were respectively 40% and 75% steeper during PUFA treatment than at baseline. This made the slopes only slightly less steep in PUFA-treated heart failure patients compared with age-matched healthy patients studied by the same technique (14).

Before discussing the mechanisms underlying these results and their possible pathophysiological and clinical implications, some methodological strengths of our study have to be emphasized.

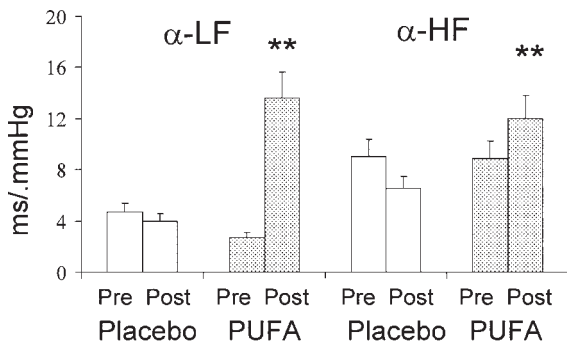
The most important one is that we assessed the baroreflex by a 2-fold approach: 1) the neck suction technique (8,9), which allows one to grade the stimulus applied to carotid baroreceptors and thus to characterize the baroreflex stimulus-response relationship both for the cardiac and the blood pressure component of the baroreflex (15); 2) computation of the alpha index, which cross-spectrally estimates the ability of the baroreflex to modulate heart rate under “spontaneous” conditions (i.e., in absence of any laboratory stimuli) (10,11).

A second technical feature relates to inclusion in the protocol of blood pressure recordings sessions that were conducted during controlled breathing and were used to



**Figure 2.** R-R interval (top) and mean arterial blood pressure (bottom) responses to carotid baroreceptor stimulation by graded neck suction in resting supine individuals before (solid lines, circles) and after (dotted lines, triangles) 4 months of dietary polyunsaturated fatty acid (PUFA) supplementation (right) or placebo (left). Mean values  $\pm$  SEM of the responses to  $-20$  mm Hg and  $-40$  mm Hg neck suction and the derived regression lines relating the reflex response to the stimulus are shown. Slopes and correlation coefficients ( $r$ ) are indicated for each regression line. \*\* $p < 0.01$  versus before supplementation.

perform univariate and bivariate spectral analysis: the purpose was to avoid a serious pitfall of frequency domain techniques (i.e., the occurrence of erratic modifications of cardiovascular variability related to episodes of irregular breathing), which is a quite common event in heart failure patients (16,17).



**Figure 3.** Alpha baroreflex index before and after placebo (white bars) and polyunsaturated fatty acid (PUFA) (shaded bars) treatment. Note that in the PUFA-treated group the alpha index increased significantly both in the low-frequency (LF) and high-frequency (HF) bands. \*\* $p < 0.01$  versus baseline.

Although addressing the mechanisms underlying PUFA-induced modifications of baroreflex function was not among the goals of our study, our results prompt several considerations on this issue. One, the baroreflex potentiation cannot be specifically ascribed to a treatment-related attenuation of the severity of heart failure, because the functional echocardiographic parameters as well as the patients' clinical status and NYHA functional class were superimposable at baseline and after PUFA treatment. Two, the evidence that the potentiation involved not only the heart rate but also the blood pressure control exerted by arterial baroreceptors suggests that the influence of PUFA involves both the vagal (which largely accounts for the reflex heart rate changes) and the sympathetic component of the baroreflex and is therefore presumably expressed at sites located in the afferent and/or central portion of the reflex arch. We can speculate that afferent mechanisms may include an attenuation (18) of the arterial stiffening brought about by heart failure (19), although a direct effect of PUFA on baroreceptor firing in response to physiological stimuli or on its central neural processing might also be involved (20-22). Three, wherever

the site of the action of PUFA, the observation that serum high sensitive C-reactive protein concentration was superimposable in the actively and placebo-treated groups suggests that no anti-inflammatory effect of PUFA was involved. This is not surprising because C-reactive protein levels were low even in the placebo-treated group, probably due to the fact that most recruited patients were under statin treatment (Table 1), which by its own exerts an anti-inflammatory influence (23).

Our results have clinical implications. One, our dietary treatment ameliorated 3 parameters whose depression correlates with an adverse prognosis in heart failure patients, namely baroreflex sensitivity (7), overall heart rate variability, and LF spectral power of R-R interval (24,25). Two, restoration of the depressor responses to neck suction by PUFA may favorably affect cardiovascular homeostasis, which means better cardiovascular adjustments to exercise, digestion, orthostatic stimuli, and other daily life behaviors. Three, the favorable effect of PUFA on the baroreflex occurred in heart failure patients that were all under treatment with both beta-blockers and angiotensin-converting enzyme inhibitors (i.e., with drugs that are known to potentiate the vagal and sympathetic components of the baroreflex in heart failure) (26,27). This means that the baroreflex effect of these substances is additive to that of other treatments. Four, the trend we observed to a reduction in baseline heart rate with PUFA is in line with a recently reported meta-analysis according to which these substances are accompanied by a 2 to 3 beat/min reduction in heart rate (28); on the other hand, it is not surprising that we failed to observe any trend to a reduction in baseline blood pressure because in virtually all patients the ongoing drug regimen administered for the treatment of heart failure was already exerting a significant blood pressure-lowering influence.

Five, the PUFA-related changes in LF spectral power of heart rate variability suggest that this treatment evoked an attenuation of sympathetic overactivity; this is because, at variance with healthy subjects, in heart failure patients exaggerated sympathetic tone translates into blunted rather than enhanced LF oscillations of heart rate (29). Likewise, the PUFA-related increase in HF power likely reflects restoration of the blunted cardiac parasympathetic influences. As to this interpretation, one might argue that estimation of sympathetic function by a spectral approach is inferential in nature and must be interpreted with caution, and that our baroreflex study tested the sympatho-inhibitory but not the sympatho-excitatory limb of the reflex; this would mean that, in all, our findings do not exclude that while able to moderate tonic sympathetic outflow and to potentiate phasic sympatho-inhibitory responses, PUFA treatment may fail to restrain or might even enhance phasic sympatho-excitatory responses. No matter how likely it may be, this possibility will merit direct experimental testing, especially considering the provocative suggestion (30) that PUFA may not lower, or may even raise, the propensity to ventricular arrhythmias; it must at any rate be emphasized

that this relates to patients with a primary arrhythmogenic substrate and is unlikely to apply to the large population of post-MI heart failure patients (31), such as our own or those in the GISSI Study (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico acuto-1) (1), in whom PUFA proved to be anti- rather than pro-arrhythmic.

Our study also has some potential limitations. First, we could only determine fatty acid concentrations at the end of the experimental treatment period. This was because we were supplied with a limited number of plasma sampling kits, which made it impossible for us to sample both pre- and post-treatment, as well as to perform determinations other than in plasma (e.g., in red cell membranes). It is however to be considered that: 1) we collected dietary information in all patients; in particular, we ascertained that none of the recruited patients ate seafood more often than once a week (which is quite normal for Northern Italian dietary habits), whereas the 2 g daily dosage administered to the PUFA group subjects determines an intake of PUFA corresponding to that of individuals eating large amounts of seafood (at least 200 g of fatty fish per day); 2) compared with the placebo-treated subjects, the PUFA-treated ones displayed plasma PUFA concentrations 100% and 50% larger for EPA and DHA, respectively. We can thus most reasonably exclude that the observed differences in plasma concentrations of PUFA between the 2 groups were pre-existent to our randomized treatment, so that this issue should not have acted as a confounder in the interpretation of the baroreflex findings.

Second, although PUFA treatment enhanced patients' baroreflex function, this was not paralleled by significant improvements in clinical status as inferred by body weight, NYHA functional class, and echocardiographic parameters. Although they should be interpreted with caution because of the limited size of our population, these clinical data suggest that in the heart failure syndrome PUFA administration has little symptomatic relevance. This does not detract, however, from the clinical relevance of the baroreflex potentiation, which one would expect to have more bearing with a reduction in the risk of sudden and all-cause death than with an improvement in the symptomatic picture.

In conclusion, we demonstrated that administration to heart failure patients of a diet enriched in PUFAs is associated with a substantial improvement of arterial baroreflex function (in terms of both cardiac and peripheral vascular control) and of heart rate variability (total variance as well as power of its spectral components). Notably, alterations in most of the cardiovascular homeostatic parameters examined in our experiments are known to predict an adverse prognosis in the heart failure patient, and PUFA treatment proved to be able to improve each of them.

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