Inverse shift in serum polyunsaturated and monounsaturated fatty acids is associated with adverse dilatation of the heart

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Inverse shift in serum polyunsaturated and monounsaturated fatty acids is associated with adverse dilatation of the heart

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ABSTRACT

Background Cardiac dilatation is associated with impaired pump function, progression of heart failure and electrical instability. Risk of sudden death is associated with a low blood level of n-3 polyunsaturated fatty acids.

Objective The hypothesis was, therefore, addressed that left ventricular dilatation as assessed by echocardiography is associated with a reduced serum polyunsaturated fatty acid level.

Methods Fatty acids were determined with gas chromatography/mass spectrometry in serum of 308 patients with dilative heart failure unrelated to myocardial infarction (age 48 (SD12) years, NYHA class 2.2 (0.6), ejection fraction 31% (10%)).

Results The extent of left ventricular dilatation as assessed by left ventricular end-diastolic diameter was associated with a reduction of both n-3 and n-6 polyunsaturated fatty acids. The n-3 docosahexaenoic acid (1.0% (0.5%) vs 1.3% (0.6%), $p<0.001$) and the n-6 arachidonic acid (4.6% (1.8%) vs 5.2% (1.9%), $p<0.01$) were reduced in patients with left ventricular dilatation (end-diastolic diameter, 68—90 mm, upper tertile vs 48—61 mm, lower tertile). By contrast, monounsaturated fatty acids were increased (the n-9 oleic acid 26.1% (4.8%) vs 23.9% (4.8%), $p<0.01$). A low docosahexaenoic acid (0.01—0.9%, lower tertile vs 1.4—3.1%, upper tertile) was associated with greater left ventricular dilatation (end-diastolic diameter, 67 (8) vs 63 (7) mm, $p<0.001$). The cut-off for the absence of severe dilatation (end-diastolic diameter >70 mm) was set at >1.24% docosahexaenoic acid. In our sample, the negative-predictive value for severe dilatation was 91% and sensitivity was 84%.

Conclusions Docosahexaenoic acid provides a new sensitive biomarker for monitoring and detecting severe left ventricular dilatation in heart failure patients.

Dilatation of the heart chambers occurs progressively and is associated with impaired pump function, electrical instability and reduced survival. In the GISSI-HF study, left ventricular dilatation was cause of heart failure in about one-third of patients,[1] despite standard heart failure therapy. Dilated hearts of heart failure patients exhibit an increased wall stress,[2] which further compromises an impaired function caused by remodelling of the cardiomyocyte[3] and extracellular matrix.[4] Cardiac dilatation involves disruption and degradation of collagen fibres by activated matrix metalloproteinases. Among factors involved in activation of matrix metalloproteinases, inflammatory mediators derived from mast cells have a major role.[5] A key mechanism of cardiac inflammation involves activation of nuclear transcription factor kappaB (NF-κB)[6] which can partially be counteracted by the n-3 long-chain polyunsaturated fatty acid (PUFA) docosahexaenoic acid and to a lesser extent by eicosapentaenoic acid.[7] In GISSI-HF, administration of n-3 PUFA (46% eicosapentaenoic acid and 38% docosahexaenoic acid ethyl esters) was associated with reductions in the risk of death or hospitalisation for a cardiovascular cause.[1] While possible links with cardiac dilatation remained unresolved in the study,[8] we have previously shown that fish oil (30% n-3 PUFA) can attenuate the dilatation of pressure overloaded rat left ventricle.[9 10] The question arises, therefore, whether ventricular dilatation is linked to levels of n-3 PUFA. We hypothesised that severe cardiac dilatation is associated with a low n-3 PUFA level, which could thus also provide a new biomarker for monitoring progression of dilatation.

METHODS

Serum fatty acids in patients with cardiac dilatation

In a retrospective analysis extending over nine years, 308 patients with suspected non-ischaemic cardiomyopathy[11 12] and a variable degree of left ventricular dilatation were examined. Patients had exertional or non-exertional chest pain or dyspnoea, 12-lead standard ECG alterations or positive exercise testing. In all patients, the presence of coronary artery or heart valve disease has been excluded by heart catheterisation as part of routine diagnostics before the present analysis. Patients with malignancies, severe liver or kidney disease, thyroid disease, diabetes or alcohol abuse were not analysed. Patients were overweight (body mass index 27.1 (SD 4.2) kg/m^2) and did not differ in the stratifications used. When indicated, patients received concomitant standard medication for therapy of heart failure which did not include n-3 PUFA administration. Transthoracic apical, long-axis and short-axis views in B-mode and M-mode echocardiography were used to obtain left ventricular end-diastolic diameter (LVEDD) and ejection fraction. The study was in accordance with the institutional guidelines of the Philipps University Hospital of Marburg. The investigation conforms with the principles outlined in the Declaration of Helsinki.

Blood was drawn under resting conditions in supine position. Lipids were extracted and analysed using a microdetermination method requiring a minimum of 0.01 ml serum.[10] Transesterification of triacylglycerols was performed in alkaline methanol/toluene. An 8610C gas chromatograph and PeakSimple Chromatography Data System

Table 1 Characteristics and serum fatty acids of patients categorised according to tertiles of left ventricular end-diastolic diameter (LVEDD)

	Tertile 1 (T1) LVEDD = 48–61 57.6 (2.6) mm n = 102	Tertile 2 (T2) LVEDD = 61–68 64.5 (1.9) mm n = 103	Tertile 3 (T3) LVEDD = 68–90 73.9 (5.4) mm n = 103	p Value
Age (years)	49.8 (11.7)	48.5 (11.2)	46.5 (12.7)	NS
NYHA class	2.2 (0.6)	2.2 (0.7)	2.4 (0.6)	T1 vs T3<0.01
				T2 vs T3<0.01
Ejection fraction (%)	37.0 (8.3)	33.1 (8.5)	23.8 (8.8)	T1 vs T3<0.001
				T1 vs T2<0.01
				T2 vs T3<0.001
Total saturated FA	38.3 (2.8)	38.2 (2.8)	38.6 (2.8)	NS
Palmitic acid	28.7 (2.3)	29.0 (2.3)	29.2 (2.4)	NS
Stearic acid	8.4 (1.4)	8.1 (1.4)	8.1 (1.5)	NS
Total monounsaturated FA	26.4 (5.3)	28.4 (5.3)	28.9 (5.1)	T1 vs T3<0.001
				T1 vs T2<0.01
Oleic acid	23.9 (4.8)	25.6 (4.7)	26.1 (4.8)	T1 vs T3<0.01
				T1 vs T2<0.05
Total n-6 polyunsaturated FA	27.5 (4.1)	26.2 (4.0)	25.6 (4.3)	T1 vs T3<0.01
				T1 vs T2<0.05
Linoleic acid	20.4 (3.2)	19.4 (3.1)	19.3 (3.3)	T1 vs T3<0.05
				T1 vs T2<0.05
Arachidonic acid	5.2 (1.9)	5.0 (1.6)	4.6 (1.8)	T1 vs T3<0.01
Total long-chain n-3 polyunsaturated FA	1.9 (0.8)	1.7 (0.6)	1.5 (0.7)	T1 vs T3<0.001
				T2 vs T3<0.01
Eicosapentaenoic acid	0.53 (0.33)	0.55 (0.29)	0.45 (0.29)	T2 vs T3<0.05
Docosahexaenoic acid	1.34 (0.57)	1.19 (0.48)	1.01 (0.52)	T1 vs T3<0.001
				T1 vs T2<0.05
				T2 vs T3<0.05
Short-chain n-3 polyunsaturated FA				
α-Linolenic acid	0.25 (0.17)	0.30 (0.19)	0.29 (0.18)	NS
Total trans unsaturated FA	0.18 (0.29)	0.15 (0.16)	0.18 (0.15)	NS
Trans-oleic acid	0.12 (0.13)	0.12 (0.12)	0.16 (0.12)	NS
Trans-linoleic acid	0.05 (0.26)	0.03 (0.12)	0.03 (0.09)	NS

NYHA, New York Heart Association; FA, fatty acids given as percentages.

(SRI, Torrance, CA, USA) were used. Fatty acid methyl esters were separated on a SP-256 column of Supelco (Sigma-Aldrich, St Louis, MO, USA) using a 37 fatty acid methyl ester standard (Supelco FAME Mix C4-C24). A CP-3800 gas chromatograph coupled to a Saturn 2200 ion-trap mass spectrometer (Varian Inc, Palo Alto, CA, USA) equipped with chemical (acetonitrile) ionisation was used for identifying minor fatty acids, in particular trans fatty acids.

Statistical analysis

Comparisons between tertiles of LVEDD and docosahexaenoic acid were made by one-way analysis of variance (Statistica of StatSoft, Tulsa, OK, USA). Duncan's multiple range test was used for multiple comparisons. Values are expressed as means (SD). All probability values are two sided. Statistical significance was assumed at $p<0.05$. Multivariate regression models were used to assess the predictive power of various serum fatty acids for LVEDD. Receiver operating characteristic (ROC) analysis was used for selecting an appropriate cut-off for the variables. The cut-off was set at a value of docosahexaenoic acid with the highest accuracy (minimal false-negative and false-positive results corresponding to the highest average of sensitivity and specificity) (MedCalc, MedCalc Software, Mariakerke, Belgium). A 2×2 table was used to calculate sensitivity, specificity and predictive power of serum docosahexaenoic acid level for detecting severe left ventricular dilatation. Means (95% confidence interval) were: sensitivity 84% (73%–92%), specificity 48% (41%–54%), negative-predictive value 91% (85%–96%) and positive-predictive value 31% (25%–38%). For greater sensitivity of 91% (cut-off >1.51% docosahexaenoic acid), specificity was 30%, negative-predictive value 92% and positive-predictive value 27%.

RESULTS

Table 1 shows characteristics of 308 patients with dilative heart failure (age 48 (12) years; New York Heart Association (NYHA) class 2.2 (0.6), ejection fraction 31% (10%)). Patients were categorised according to the extent of dilatation as assessed by LVEDD (tertiles: lower, 48–61 mm; middle, 61–68 mm; upper, 68–90 mm). The serum n-3 docosahexaenoic acid was depressed in patients with moderate dilatation (LVEDD 61–68 mm) and more markedly in severe dilatation (68–90 mm). The n-3 eicosapentaenoic acid was reduced in patients with severe dilatation. Also n-6 PUFA including arachidonic acid were reduced. By contrast, the monounsaturated n-9 oleic acid was increased, while saturated fatty acids and trans fatty acids were not significantly altered.

Also stratifications according to tertiles of serum fatty acids were performed. LVEDD was increased and ejection fraction reduced in the lower (0.01%–0.88%) when compared with the upper (1.38%–3.11%) tertile of docosahexaenoic acid (table 2). For the other fatty acids, no statistically significant influences were observed. In the multiple regression model for LVEDD, docosahexaenoic acid was the only significant variable (regression coefficient $\beta=-0.25$, $p<0.001$).

Table 2 Left ventricular function parameters of patients categorised according to tertiles of serum docosahexaenoic acid (DHA)

	Tertile 1 (T1) DHA =0.01–0.88% 0.63% (0.19%) n=102	Tertile 2 (T2) DHA =0.89–1.37% 1.12% (0.13%) n=103	Tertile 3 (T3) DHA =1.38–3.11% 1.78% (0.36%) n=103	p Value
NYHA class	2.2 (0.7)	2.2 (0.6)	2.3 (0.6)	NS
Ejection fraction (%)	29.1 (9.9)	32.0 (10.7)	32.9 (9.3)	T1 vs T3<0.05 T1 vs T2<0.05
LVEDD (mm)	67.4 (7.5)	65.5 (7.9)	63.2 (6.8)	T1 vs T3<0.001 T2 vs T3<0.05

The area under the ROC curve of severe dilatation (LVEDD >70 mm) vs docosahexaenoic acid was 0.69 (SE 0.03, 95% CI 0.64 to 0.74, p<0.001). Based on the ROC curve, the optimal cut-off for detecting the absence of severe dilatation was set at >1.24% docosahexaenoic acid. Figure 1 shows the number of true and false positives and negatives. Sensitivity was 84% and specificity 48%. Based on a 22% prevalence of severe dilatation in our sample, the negative-predictive value for severe dilatation was 91% and the positive-predictive value was 31%. Other fatty acids were associated with lower areas under the ROC curve (arachidonic acid ≤3.96% cut-off, 0.63 area under the ROC curve; eicosapentaenoic acid ≤0.31%, 0.58; oleic acid >27.0%, 0.57; linoleic acid ≤17.4%, 0.55).

DISCUSSION

In view of the ill-defined molecular mechanisms involved in cardiac dilatation, we examined serum fatty acids in patients with dilative heart failure. Among the alterations in fatty acid families, the depression of docosahexaenoic acid was most prominent and was observed already in patients with moderate dilatation. A docosahexaenoic acid level >1.24% as a cut-off was a strong predictor for the absence of severe dilatation. The value of the test comprising a favourable sensitivity and moderate specificity should be seen in the context of clinical tests used to exclude severe, potentially life-threatening diseases such as the d-dimer test with a high negative-predictive value (97%) and sensitivity (92%) to exclude thromboembolism with low specificity (36%) and positive-predictive value (14%).[13]

We propose a diagnostic algorithm for monitoring progression of left ventricular dilatation on the basis of the clinical probability assessment of heart failure, serum docosahexaenoic acid level as new biomarker and echocardiography. The biomarker could rule out the diagnosis of severe dilatation in heart failure patients with ongoing dilatation and could also be used for screening for severe dilatation in cardiovascular risk patients. In case of ongoing n-3 PUFA administration or in patients who might appear as false positive in the present approach, the rate or extent of decline of docosahexaenoic acid level associated with progressive dilatation could be an alternative test parameter.

Further studies are needed to examine potential relations with other biomarkers. Of particular relevance could be brain (B-type) natriuretic peptide (BNP) or N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) which were found to be increased as a result of a high cardiac filling pressure[14] and cardiovascular congestion.[15] Using a thick-walled sphere model and cardiac magnetic resonance imaging, it was shown that left ventricular end-diastolic wall stress was the only independent haemodynamic parameter influencing BNP[11 12] while an echocardiography based approach underestimated wall stress particularly of dilated hearts.[11] Also links with C-reactive protein[15] and hyperglycaemia, which is independent of the diabetic status,[16] should be examined.

Further work is required to examine whether the lower docosahexaenoic acid level in moderate and severe cardiac dilatation results in a diminished anti-arrhythmogenic[17 18] influence. In particular, whether a higher dosage of docosahexaenoic acid is required in patients with cardiac dilatation for reaching a level associated with a reduced risk of sudden cardiac death.[19 20] The apparently lower efficacy of reducing sudden cardiac death by n-3 PUFA in the GISSI-HF,[1] when compared with the GISSI-Prevenzione trial,[21] could arise from lower docosahexaenoic acid levels in dilative heart failure without acute ischaemic release of docosahexaenoic acid from cell membranes.[10]

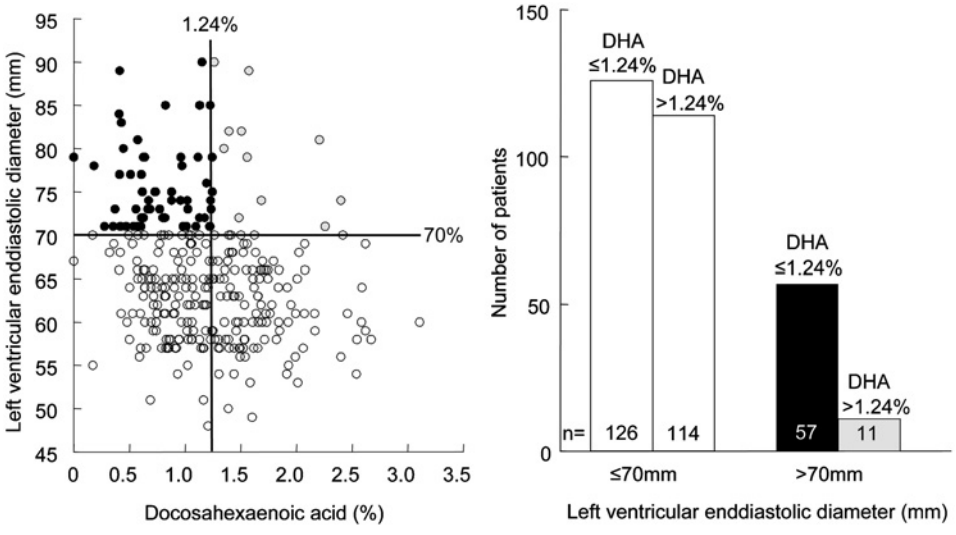

Figure 1 (Left) Plot of serum docosahexaenoic acid versus left ventricular end-diastolic diameter (LVEDD). The lines depict the optimal cut-off for detecting the absence of severe dilatation (LVEDD >70 mm, solid circles) set at >1.24% docosahexaenoic acid. (Right) Number of patients with LVEDD ≤70 mm or LVEDD >70 mm categorised according to docosahexaenoic acid (DHA) ≤1.24% or >1.24%. When docosahexaenoic acid was >1.24%, only 11 out of 68 patients (308 patients in total) exhibited severe dilatation. 1.24% docosahexaenoic acid was used as cut-off for maximising the sums of sensitivity and specificity.

Apparently small reductions in n-3 PUFA are associated with an increased risk of sudden death. In the Physicians' Health Study, whole blood n-3 PUFA exhibited an inverse relation with risk of sudden death in men without evidence of previous cardiovascular disease. In those who died suddenly, docosahexaenoic acid was significantly reduced from 2.38% (0.78%) to 2.12% (0.65%) and eicosapentaenoic acid was not significantly reduced.[19] For a comparison of these values with the present data, one has to take into account that the docosahexaenoic level is higher in whole blood than in serum.

Whether the reduction in unsaturated fatty acids, in particular docosahexaenoic acid, promotes dilatation or is also a consequence of dilatation[10] remains an important issue. Since administration of eicosapentaenoic acid and docosahexaenoic acid attenuated ventricular dilatation,[9 10] one could deduce that a low docosahexaenoic acid level predisposes to cardiac dilatation. In accordance, only a few patients with >1.24% docosahexaenoic acid exhibited severe left ventricular dilatation. A low docosahexaenoic acid value could be associated with enhanced pro-inflammatory mechanisms. Since n-3 PUFA suppresses the production of pro-inflammatory cytokines such as tumour necrosis factor α,[22] it could interfere with matrix metalloproteinase activation.[23] A low level of n-3 PUFA could also be associated with reduced anti-inflammatory mechanisms of peroxisome proliferator-activated receptors (PPARs).[24] Although PPARα binds oleic acid and eicosapentaenoic acid with nearly equal affinity, only eicosapentaenoic acid activated PPARα in hepatocytes.[25] Reduced anti-inflammatory actions[26] could be of particular relevance in hypertrophied hearts which exhibit a reduced PPARα expression.[27]

In conclusion, docosahexaenoic acid provides a new biomarker for monitoring and detecting severe left ventricular dilatation in heart failure patients. Further work is required to assess whether the reduced n-3 PUFA and associated diminished anti-inflammatory actions promote dilatation.

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Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

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