Cardiovascular Research (2009) **81**, 319–327
doi:10.1093/cvr/cvn310

Fish oil, but not flaxseed oil, decreases inflammation and prevents pressure overload-induced cardiac dysfunction

Monika K. Duda[1], Karen M. O'Shea[2], Anselm Tintinu[1], Wenhong Xu[1], Ramzi J. Khairallah[1], Brian R. Barrows[1], David J. Chess[3], Agnes M. Azimzadeh[4], William S. Harris[5], Victor G. Sharov[6], Hani N. Sabbah[6], and William C. Stanley[1,2,3]*

[1]*Division of Cardiology, Department of Medicine, University of Maryland-Baltimore, 20 Penn Street, HSF2, Room S022, Baltimore, MD 21201, USA;* [2]*Department of Nutrition, Case Western Reserve University, Cleveland, OH, USA;* [3]*Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH, USA;* [4]*Department of Surgery, University of Maryland, Baltimore, MD, USA;* [5]*Sanford Research/USD, Sioux Falls, SD, USA; and* [6]*Division of Cardiovascular Medicine, Henry Ford Heart and Vascular Institute, Detroit, MI, USA*

Received 2 September 2008; revised 19 October 2008; accepted 12 November 2008; online publish-ahead-of-print 17 November 2008

Time for primary review: 35 days

KEYWORDS
α-linolenic acid;
Diet;
Docosahexaenoic acid;
Eicosapentaenoic acid;
Heart failure

Aims Clinical studies suggest that intake of ω-3 polyunsaturated fatty acids (ω-3 PUFA) may lower the incidence of heart failure. Dietary supplementation with ω-3 PUFA exerts metabolic and anti-inflammatory effects that could prevent left ventricle (LV) pathology; however, it is unclear whether these effects occur at clinically relevant doses and whether there are differences between ω-3 PUFA from fish [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] and vegetable sources [α-linolenic acid (ALA)].

Methods and results We assessed the development of LV remodelling and pathology in rats subjected to aortic banding treated with ω-3 PUFA over a dose range that spanned the intake of humans taking ω-3 PUFA supplements. Rats were fed a standard food or diets supplemented with EPA+DHA or ALA at 0.7, 2.3, or 7% of energy intake. Without supplementation, aortic banding increased LV mass and end-systolic and -diastolic volumes. ALA supplementation had little effect on LV remodelling and dysfunction. In contrast, EPA+DHA dose-dependently increased EPA and DHA, decreased arachidonic acid in cardiac membrane phospholipids, and prevented the increase in LV end-diastolic and -systolic volumes. EPA+DHA resulted in a dose-dependent increase in the anti-inflammatory adipokine adiponectin, and there was a strong correlation between the prevention of LV chamber enlargement and plasma levels of adiponectin ($r = -0.78$). Supplementation with EPA+DHA had anti-aggregatory and anti-inflammatory effects as evidenced by decreases in urinary thromboxane B_2 and serum tumour necrosis factor-α.

Conclusion Dietary supplementation with ω-3 PUFA derived from fish, but not from vegetable sources, increased plasma adiponectin, suppressed inflammation, and prevented cardiac remodelling and dysfunction under pressure overload conditions.

1. Introduction

Recent epidemiological and animal studies suggest that a high intake of the ω-3 polyunsaturated fatty acids (ω-3 PUFA) — eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) — from fish oil may prevent the development and progression of heart failure.[1,2] Current dietary guidelines recommend a high intake of EPA and DHA to reduce the risk for coronary heart disease,[3] and pharmacological doses (3.4 g/day) are effective in the treatment of hypertriglyceridaemia and may reduce serious arrhythmias and sudden cardiac death.[4,5] The recently reported GISSI heart failure trial found that a low dose of EPA+DHA (0.85 g/day) administered to heart failure patients for 3.9 years significantly decreased mortality by 9% when compared with placebo.[6] The effects of commonly used doses of EPA+DHA (>3 g/day) on cardiac function or mortality in heart failure patients have not been reported. Compared with fish oil, little is known about the effects of α-linolenic acid (ALA), a ω-3 PUFA from vegetable sources such as flaxseed. ALA intake is associated with a reduction in coronary artery disease,[7] although the evidence is considerably less compelling than for EPA+DHA.[3] At present, the ability of EPA+DHA or ALA supplementation to prevent the development of heart failure has not been assessed in prospective clinical or animal studies.

The mechanisms by which ω-3 PUFA could prevent the development and progression of heart failure are

* Corresponding author. Tel: +1 410 706 3585; fax: +1 410 706 3583.
E-mail address: wstanley@medicine.umaryland.edu

unclear,[1,8] but could be linked to their anti-inflammatory effects. ω-3 PUFAs from fish oil activate peroxisome proliferator-activated receptor (PPAR)-γ in adipose tissue and increase expression, secretion, and plasma levels of the anti-inflammatory hormone adiponectin.[1,9,10] Recent studies show that adiponectin limits left ventricular (LV) hypertrophy, remodelling, and contractile dysfunction in response to pressure overload[11] and exerts anti-inflammatory effects, suggesting that high intake of ω-3 PUFA could prevent LV pathology through triggering an increase in adiponectin. At present, it is not known whether ALA also increases adiponectin or whether there is a dose–response relationship between the intake of EPA+DHA or ALA and adiponectin secretion. In addition, fish oil supplementation may decrease the production of the inflammatory mediator tumour necrosis factor (TNF) α,[12] which is increased in heart failure[13] and is implicated in the development of LV remodelling and contractile dysfunction during pressure overload.[14] Cardiac phospholipid composition is altered with fish oil supplementation,[15] with a decreased arachidonic acid,[16] the precursor of prostacyclin and thromboxane A_2, which are elevated during heart failure[17] and exert direct effects on the heart.[18] Taken together, dietary supplementation with EPA+DHA or ALA could prevent the development of heart failure in the chronically stressed heart through increased secretion of adiponectin and suppression of inflammation. Evidence to support this concept is currently lacking, particularly at clinically relevant doses (2–4 g/day).[3]

The goal of the present study was to assess the ability of dietary supplementation with ω-3 PUFA derived from either fish (EPA+DHA) or vegetable sources (ALA) to prevent LV remodelling and pathology in response to pressure overload. We hypothesized that ω-3 PUFA would increase plasma adiponectin in a dose-dependent manner, which would correspond to a decrease in inflammation and prevention of LV chamber expansion. Studies were performed in an established rat model of chronic pressure overload induced by abdominal aortic banding (AAB). This model results in LV hypertrophy and the development of heart failure as evidenced by an increase in LV end-diastolic and -systolic volumes, a decrease in the capacity for adenosine triphosphate (ATP) generation, and expression of foetal genes.[1,19] A wide dose range was used (equivalent to an estimated human dose of 1.6–15 g/day of EPA+DHA or ALA) in order to clearly span human doses. Established molecular markers of heart failure were evaluated, namely, cardiomyocyte apoptosis and the switching in the mRNA expression for myosin heavy chain (MHC) isoforms from MHC-α to MHC-β.

2. Methods

2.1 Experimental design

Measurements were performed with investigators blinded to treatment. The animal protocol was conducted according to the Guideline for the Care and Use of Laboratory Animals (NIH publication No. 85-23) and was approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee.

Five-week-old male Wistar rats were fed a standard chow or modified standard chows containing ω-3 PUFA composed mainly of EPA+DHA or ALA. After 1 week on the assigned diet, rats were randomly assigned to either sham surgery or AAB ($n = 9$–11/group), and dietary treatment was continued for 12 weeks. Systolic arterial pressure was measured at 5 weeks using the tail cuff method, and echocardiographic assessment of LV function was performed at 11 weeks post-surgery. Twelve weeks after surgery, rats were anaesthetized with isoflurane, blood and urine were drawn, and the heart was harvested for biochemical analysis (see Supplementary material).

2.2 Diets

All food was custom-manufactured (Research Diets Inc., New Brunswick, NJ, USA). The standard food was similar to typical commercial rodent food, with 65% of total energy from carbohydrate (75% from cornstarch, 15% maltodextrin, and 10% from sucrose by energy), 15% energy from fat (78% from cocoa butter and 22% soybean oil), and 20% protein (casein supplemented with L-cysteine). The ω-3 PUFA diets had identical protein and carbohydrate composition as the standard food and had 15% of the total energy from fat. The EPA+DHA diets derived 2.3% of the total energy from soybean oil and 0.7, 2.3, or 7% of the total energy as EPA+DHA from fish oil (Ocean Nutrition, Dartmouth, Nova Scotia, Canada). Fish oil was 21% EPA and 49% DHA by mass. The ALA diets derived 2.2% of the total energy from soybean oil and 0.7, 2.3, or 6.6% as ALA from flaxseed oil (55% ALA by mass). Both EPA+DHA and ALA diets derived the balance of fat from cocoa butter. These doses correspond to a human intake of approximately 1.6, 5.1, and 15.5 g/day of either EPA+DHA or ALA (calculated assuming an energy intake of 2000 kcal/d), which is in the range of the currently approved dose of EPA+DHA for the treatment of hypertriglyceridaemia (3.6 g/day).

2.3 Echocardiography

LV function was evaluated using a Vevo 770 High-Resolution Imaging Systems (Visual Sonics) with a 30 MHz linear array transducer (model 716). Briefly, rats were anaesthetized with 1.5–2.0% isoflurane by mask, the chest was shaved, the animal situated in the supine position on a warming pad, and electrocardiogram limb electrodes were placed. Two-dimensional cine loops and guided M-mode frames were recorded from the parasternal short and long axis. All data were analysed offline at the end of the study with software resident on the ultrasound system.

2.4 Metabolic and biochemical variables

Plasma was analysed for free fatty acids, triglycerides, and glucose using enzymatic spectrophotometric methods. TNFα, adiponectin, leptin, and insulin were measured by enzyme-linked immunosorbent assay (ELISA). Urine was analysed for thromboxane B_2 and 6-keto prostaglandin F1α by ELISA. LV tissue was analysed for triglyceride contents using enzymatic spectrophotometric methods and for phospholipid fatty acid composition by gas chromatography, as described previously.[15] mRNA expression for genes encoding atrial natriuretic peptide (ANP), MHC-α and -β, and medium chain acyl-CoA dehydrogenase (MCAD) were measured by real-time reverse transcriptase–polymerase chain reaction, as previously described.[1] Activities of the mitochondrial marker enzymes citrate synthase, MCAD, aconitase, and isocitrate dehydrogenase were measured spectrophotometrically. Western blot analysis was performed for total and phosphorylated Akt and AMP-activated protein kinase (AMPK). Histological analysis was performed for apoptosis, cardiomyocyte cross-sectional area, and capillary density (see Supplementary material).

2.5 Statistical analysis

The effects of AAB on cardiac structure and function and biochemical variables with the standard chow diet were examined using an unpaired *t*-test. The effects of EPA+DHA and ALA supplementation were evaluated separately within the AAB and sham groups using one-way analysis of variance (ANOVA) for normal distribution or

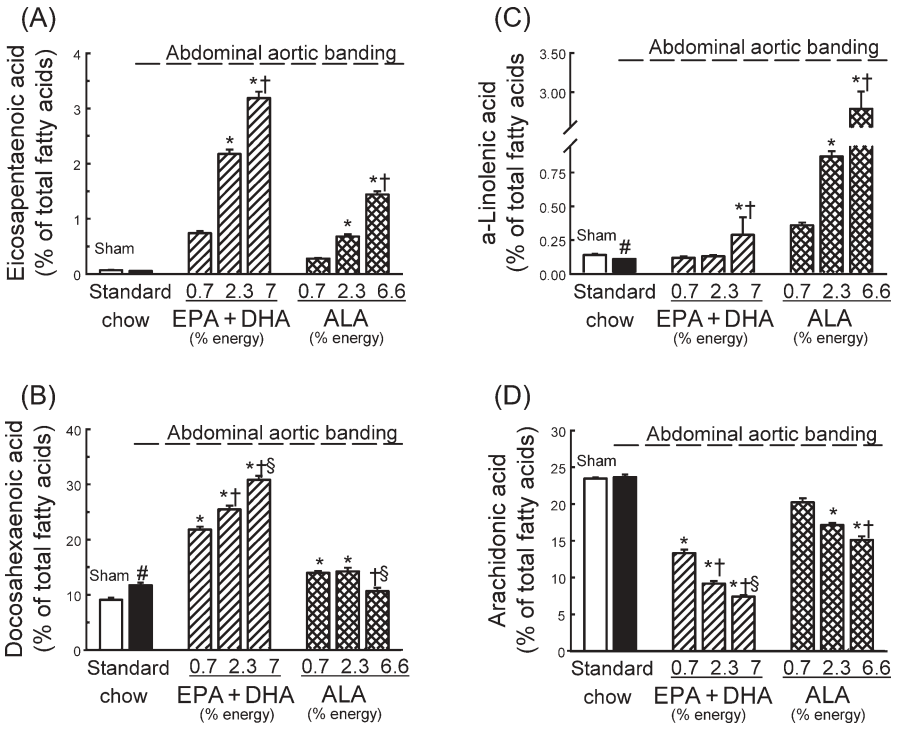

Figure 1 Effect of abdominal aortic banding and dietary manipulation on cardiac content of eicosapentaenoic acid (EPA) (*A*), docosahexaenoic acids (DHA) (*B*), α-linolenic acid (ALA) (*C*), and arachidonic acid (*D*). Data are mean ± SEM; $n = 8$. $^{\#}P < 0.05$ vs. sham; $^{*}P < 0.05$ vs. standard chow diet; $^{\dagger}P < 0.05$ vs. 0.7 EPA+DHA or ALA diet, respectively; $^{\S}P < 0.05$ vs. 2.3 EPA+DHA or ALA, respectively.

one-way ANOVA on ranks for non-normal distribution. *Post hoc* comparisons were made using the Bonferroni *t*-test for multiple comparisons. Mean values are presented ± SEM, and a $P < 0.05$ level of significance was used.

3. Results

3.1 Left ventricular phospholipid composition

As shown in *Figure 1* (Supplementary material, *Table S1*), AAB had modest but statistically significant effect on cardiac membrane phospholipid composition in rats fed with standard chow, as seen in the increased DHA and DPA (docosapentaenoic acid) n3 and in the decreased oleic, linolenic, and ALA acids contents. Dietary supplementation with both sources of ω-3 PUFA caused dramatic changes in phospholipid fatty acid composition in both AAB and sham rats (*Figure 1*) (Supplementary material, *Table S1*). In the animals fed with EPA+DHA, the content of EPA and DHA increased in a dose-dependent manner, with a larger percentage increase in EPA than DHA (*Figure 1A* and *B*). With ALA supplementation, there was a large increase in the content of ALA and EPA, but only a small increase in DHA at the two lower doses, with no increase at the high dose (*Figure 1A–C*). Both EPA+DHA and ALA supplementation reduced the levels of arachidonic and oleic acids, although this effect was more pronounced with EPA+DHA than ALA (*Figure 1D*) (Supplementary material, *Table S1*).

3.2 Left ventricular mass, remodelling, and contractile function

Body mass was not different between groups before surgery or at the end of the study in both AAB and sham rats (*Table 1*) (Supplementary material, *Table S2*). In sham groups, dietary supplementation with either EPA+DHA or ALA did not affect LV or RV mass (Supplementary material, *Table S2*). Neither ω-3 PUFA supplementation nor AAB affected tail artery systolic blood pressure measured at 5 weeks (*Table 1*) (Supplementary material, *Table S3*). AAB caused a 37% increase in LV mass normalized to tibia length on the standard chow diet. LV mass was not significantly different between standard chow AAB rats and those treated with either EPA+DHA or ALA (*Table 1*). There were no differences between groups in tibia length or right ventricular mass (*Table 1*) (Supplementary material, *Table S2*).

Treatment of sham animals with EPA+DHA did not affect echocardiographic parameters. However, ALA decreased LV ejection fraction and velocity of circumferential shortening in sham animals at the high dose, but had no effects at the two lower doses or on any other parameters at the high dose (Supplementary material, *Table S3*). With the standard chow diet, there was significant LV remodelling and systolic dysfunction with AAB compared with sham, as seen in the increase in end-diastolic and -systolic volumes and reduction in the velocity of circumferential shortening and ejection fraction (*Figure 2A* and *B* and *Table 1*) (Supplementary material, *Table S3*). These detrimental effects were attenuated by EPA+DHA supplementation in a dose-dependent fashion, but were unaffected by ALA supplementation, except for the attenuation of the increase in the end-systolic volume and reduction in the velocity of circumferential shortening with 2.3% ALA diet.

3.3 mRNA expression

An up-regulation in the mRNA expression for ANP and MHC-β and a decrease in MHC-α are established molecular markers

Table 1 Body and heart masses, echocardiography, and blood pressure results

Treatment:	Standard chow		Abdominal aortic banding					
			EPA+DHA (% energy)			ALA (% energy)		
Diet:	Sham	Abdominal aortic banding	0.7	2.3	7	0.7	2.3	6.6
Pre-surgery body mass (g)	212 ± 3	210 ± 5	204 ± 4	211 ± 5	206 ± 7	214 ± 5	199 ± 2	215 ± 3
Terminal body mass (g)	533 ± 18	507 ± 17	509 ± 13	510 ± 11	494 ± 21	528 ± 16	492 ± 7	542 ± 23
Tibia length (cm)	4.50 ± 0.06	4.53 ± 0.03	4.47 ± 0.03	4.59 ± 0.06	4.64 ± 0.06	4.57 ± 0.03	4.51 ± 0.03	4.57 ± 0.06
LV mass/tibia length (g/cm)	0.21 ± 0.01[a]	0.29 ± 0.01	0.28 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.29 ± 0.01	0.27 ± 0.01	0.30 ± 0.01
RV mass/tibia length (g/cm)	0.053 ± 0.004	0.055 ± 0.002	0.052 ± 0.002	0.058 ± 0.002	0.059 ± 0.003	0.053 ± 0.004	0.055 ± 0.003	0.071 ± 0.008
Biventricular mass/tibia length (g/cm)	0.27 ± 0.01	0.35 ± 0.01[a]	0.33 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.34 ± 0.02	0.32 ± 0.01	0.37 ± 0.01
Velocity of circumferential shortening (1/s)	8.75 ± 0.32	6.85 ± 0.32[a]	7.94 ± 0.26	8.93 ± 0.38[b]	9.83 ± 0.54[b,c]	7.02 ± 0.25	7.85 ± 0.19[b]	6.31 ± 0.25[d]
Ejection fraction (%)	87.8 ± 0.8	80.0 ± 1.9[a]	85.7 ± 1.6	88.9 ± 1.3[b]	91.1 ± 1.7[b]	80.9 ± 1.0	84.9 ± 0.8	78.6 ± 1.7[d]
Heart rate (bpm)	381 ± 10	362 ± 13	377 ± 17	390 ± 13	386 ± 15	340 ± 10	374 ± 16	362 ± 13
Anterior wall thickness (mm)	2.04 ± 0.07	2.47 ± 0.06[a]	2.43 ± 0.06	2.27 ± 0.05	2.39 ± 0.06	2.31 ± 0.05	2.42 ± 0.05	2.42 ± 0.05
Posterior wall thickness (mm)	2.16 ± 0.07	2.71 ± 0.10[a]	2.49 ± 0.06	2.34 ± 0.04[b]	2.49 ± 0.06	2.54 ± 0.09	2.55 ± 0.07	2.54 ± 0.07
Relative wall thickness (mm)	0.54 ± 0.03	0.60 ± 0.02[a]	0.61 ± 0.02	0.60 ± 0.01	0.68 ± 0.02[d]	0.56 ± 0.01	0.61 ± 0.03	0.58 ± 0.02
Systolic blood pressure (mmHg)	119 ± 2	114 ± 2	115 ± 1	117 ± 3	115 ± 2	117 ± 1	115 ± 4	116 ± 3

Data are mean ± SEM; $n = 9$–11.
[a] $P < 0.05$ vs. sham.
[b] $P < 0.05$ vs. standard chow diet.
[c] $P < 0.05$ vs. 0.7 EPA+DHA diet.
[d] $P < 0.05$ vs. 2.3 EPA+DHA or ALA diet.

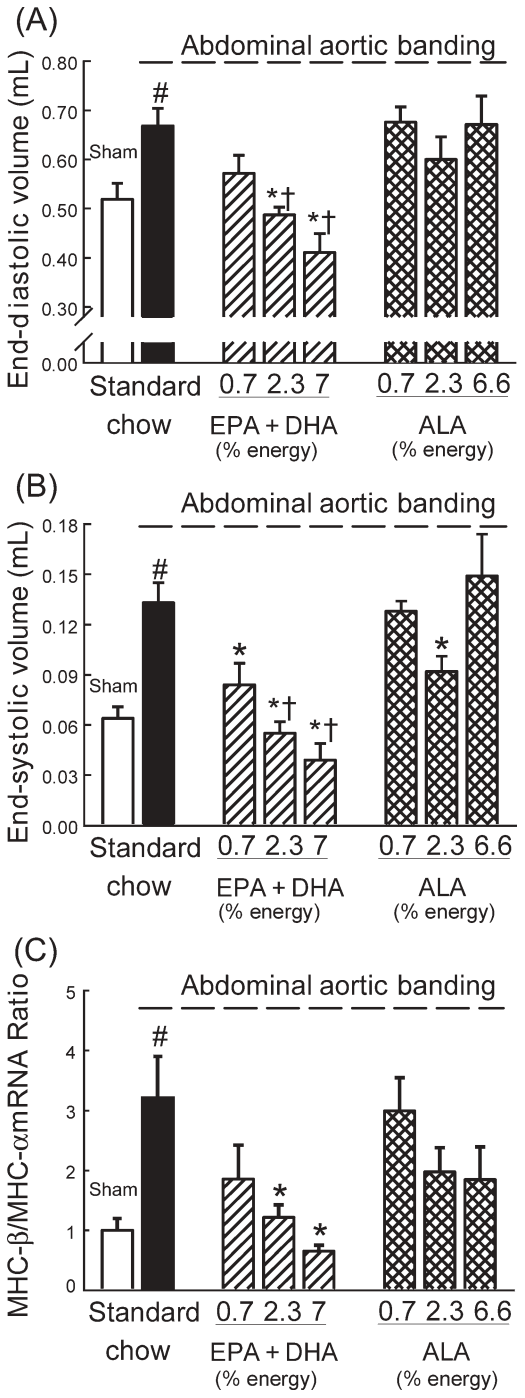

Figure 2 Echocardiographic assessment of left ventricular volume (*A*) and end-systolic volume (*B*), and the mRNA expression of the ratio of myosin heavy chain (MHC)-β to MHC-α (*C*) expressed as a fraction of the sham standard chow diet. Data are mean ± SEM; $n = 9$–11. $^{\#}P < 0.05$ vs. sham; $^{*}P < 0.05$ vs. standard chow diet; $^{\dagger}P < 0.05$ vs. 0.7 EPA+DHA diet.

of the heart failure and LV hypertrophy.[20,21] In sham animals, treatment with 2.3 and 7% EPA+DHA and 2.3 and 6.6% ALA significantly decreased MHC-β mRNA compared with standard chow (Supplementary material, *Table S4*). In addition, 2.3% ALA also decreased MHC-α. AAB caused a switch in the mRNA for MHC from the α- to β-isoform with the standard chow diet, as seen in the increased ratio of MHC-β to MHC-α (*Figure 2C*). The effect was attenuated by the EPA+DHA diets, with the significant decrease with 2.3 and 7% EPA+DHA diets. The mRNA for ANP was increased

three-fold by AAB on the standard food. Although there was a strong trend for a decrease in ANP with treatment with EPA+DHA, the difference was not statistically significant (Supplementary material, *Table S4*). The ALA diets did not prevent the increase in mRNA expression for ANP or the ratio of MHC-β to MHC-α (Supplementary material, *Table S4* and *Figure 2C*).

3.4 Histology

Cardiomyocyte cross-sectional area was increased in the AAB compared with sham animals fed with the standard chow (*Table 2*). The effect was not altered by ω-3 PUFA diets, except for a significantly greater cardiomyocyte cross-sectional area with 7% EPA+DHA diet. There were no differences in capillary density, except for a decrease with 7% EPA+DHA diet compared with standard chow in AAB animals. There was an insignificant trend for greater cardiomyocyte apoptosis with AAB on the standard chow (25%) and a significant decrease in apoptosis with 7% EPA+DHA and 6.6% ALA diets with AAB (*Table 2*).

3.5 Adipokines and fat mass

Plasma adiponectin concentration was not affected by AAB in animals fed the standard chow, but was elevated by EPA+DHA supplementation in a dose-dependent manner in both sham and ABB groups (*Figure 3A*) (Supplementary material, *Table S5*). In contrast, the ALA diets had no effect on plasma adiponectin concentration, regardless of surgical treatment (*Figure 3A*) (Supplementary material, *Table S5*). In the AAB animals, the LV end-systolic volumes were negatively correlated with plasma concentration of adiponectin ($r = -0.781$; $P < 0.001$) (*Figure 3C*), and a strong relationship was found between LV end-diastolic volume and adiponectin ($r = -0.706$; $P < 0.001$) (*Figure 3B*). Serum leptin was significantly decreased in the AAB group compared with the sham group on the standard chow diet, but was unaffected by either EPA+DHA or ALA diets (Supplementary material, *Table S5*). Retroperitoneal adipose mass was reduced by 40% in the AAB group compared with the sham group on the standard chow, but epididymal and mesenteric adipose masses were unchanged. The ω-3 PUFA diets did not affect adipose tissue mass, except for a decrease in the retroperitoneal adipose with 7% EPA+DHA compared with 2.3% EPA+DHA diet (Supplementary material, *Table S2*).

3.6 Akt and AMPK

The mechanisms for the beneficial effects of adiponectin on cardiac structure and function have been linked to the activation of AMPK[11] and to the inhibition of the pro-growth serine–threonine kinase Akt.[22] However, neither EPA+DHA or ALA affected the ratio of phospho-AMPK to total AMPK, or the ratio of phospho-Akt to total Akt in either sham of AAB groups, as assessed by western blot (Supplementary material, *Table S6*). The total amounts of AMPK or Akt were also unaffected.

3.7 Metabolic parameters

With the standard chow, there was no difference in circulating levels of free fatty acids and triglycerides in the AAB group compared with the sham group (*Figure 4*). The

Table 2 Left ventricular histological data

Treatment:	Standard chow		Abdominal aortic banding					
Diet:	Sham	Abdominal aortic banding	EPA+DHA (% energy)			ALA (% energy)		
			0.7	2.3	7	0.7	2.3	6.6
Myocyte cross-sectional area (μm^2)	497 ± 18	654 ± 23[a]	651 ± 18	681 ± 54	848 ± 35[b–d]	583 ± 14	652 ± 31	609 ± 33
Capillary density (capillary/mm^2)	2300 ± 100	2308 ± 89	2580 ± 103	2206 ± 127	1866 ± 70[b,c]	2268 ± 83	2399 ± 108	2252 ± 156
Apoptosis (DNAfr/1000 myocytes)	0.14 ± 0.02	0.17 ± 0.02	0.13 ± 0.01	0.12 ± 0.02	0.09 ± 0.01[b]	0.14 ± 0.01	0.13 ± 0.01	0.11 ± 0.01[b]

Data are mean ± SEM; $n = 8$.
[a] $P < 0.05$ vs. sham.
[b] $P < 0.05$ vs. standard chow diet with ABB.
[c] $P < 0.05$ vs. 0.7 EPA+DHA diet.
[d] $P < 0.05$ vs. 2.3 EPA+DHA or ALA.

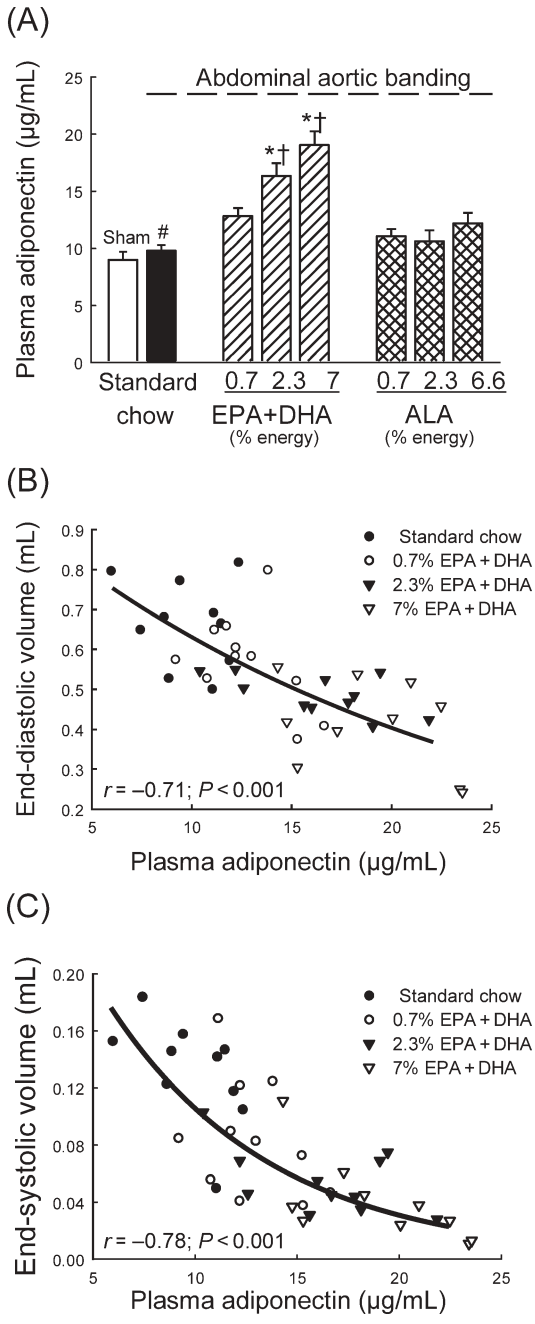

Figure 3 Plasma concentration of adiponectin (*A*). Data are mean ± SEM; $n = 9$–11. $^{\#}P < 0.05$ vs. sham; $^{*}P < 0.05$ vs. standard chow diet; $^{\dagger}P < 0.05$ vs. 0.7 EPA+DHA-diet. Left ventricular end-diastolic (*B*) and-systolic volumes (*C*) plotted as a function of plasma adiponectin levels.

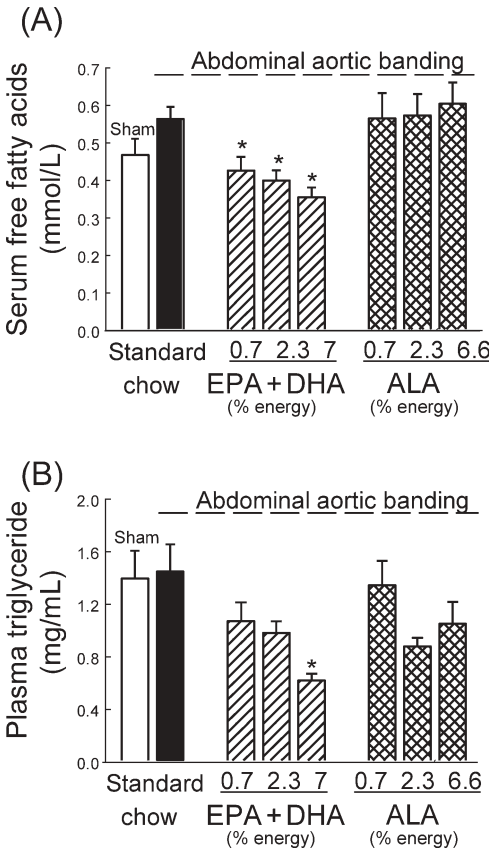

Figure 4 Serum concentration of free fatty acids (*A*) and plasma level of triglycerides (*B*). Data are mean ± SEM; $n = 9$–11. $^{*}P < 0.05$ vs. standard chow diet.

EPA+DHA diets significantly decreased the serum free fatty acid level, and there was a trend for a decrease in plasma triglyceride concentration, with a significant decrease in the 7% EPA+DHA diet with AAB (*Figure 4*). Similar results were observed with sham animals, except that triglyceride levels were decreased at all doses of EPA+DHA (Supplementary material, *Table S5*). Treatment with ALA had no effect on free fatty acids or triglycerides in either sham or AAB group. Plasma glucose concentration was unaffected by AAB or dietary modifications. A similar lack of effect was observed on plasma insulin concentration, except for a significant decrease in the 7% EPA+DHA diet (Supplementary material, *Table S5*). AAB with the standard diet decreased the cardiac content of triglycerides, which was unaffected by ω-3 PUFA, except for an increase with the 7% EPA+DHA diet (Supplementary material, *Table S5*).

ω-3 PUFAs are ligands of PPAR-α,[23] which stimulate the expression of gene-encoding proteins involved in cardiac fatty acid metabolism in mitochondria.[24] Cardiac hypertrophy and failure decrease the fatty acid oxidation pathway and impair mitochondrial function,[24] thus supplementation with ω-3 PUFA could preserve the expression and activity of mitochondrial enzymes and thus prevent the deterioration in myocardial energy metabolism that occurs with heart failure.[8,24] The cardiac mRNA expression and the activity for the mitochondrial fatty acid oxidation enzyme MCAD were reduced by ∼20% ($P < 0.05$ for MCAD activity) in AAB compared with sham rats fed with the standard chow. There was a non-significant trend for the EPA+DHA diets to restore the mRNA expression of MCAD, which was not mirrored by changes in the enzyme activity (*Figure 5*). AAB with the standard chow diet also decreased the activities of citrate synthase, aconitase, and isocitrate dehydrogenase (Supplementary material, *Table S7*). There were no differences between standard chow and EPA+DHA or ALA supplementation in the activities of citrate synthase, aconitase, and isocitrate dehydrogenase.

3.8 Tumour necrosis factor-α and eicosanoids

Serum TNF-α concentration was significantly elevated in the standard chow diet in AAB animals compared with sham

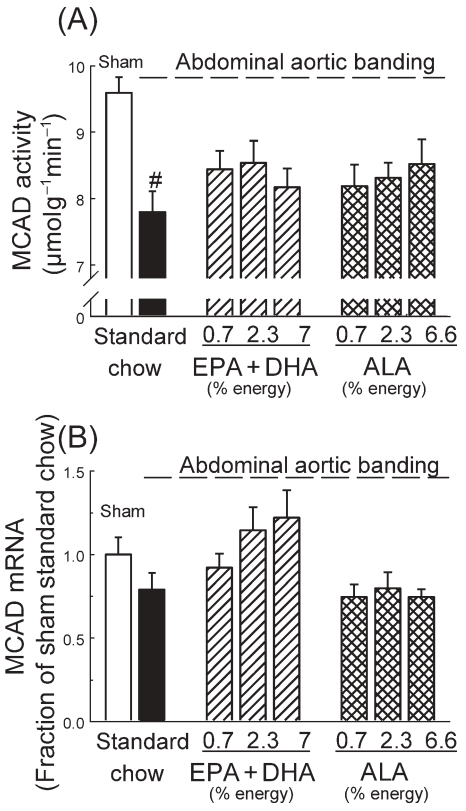

Figure 5 Cardiac medium chain acyl-CoA dehydrogenase (MCAD) activity (*A*) and mRNA expressed as a fraction of the sham standard chow diet (*B*). Data are mean ± SEM; $n = 9$–11. $^{\#}P < 0.05$ vs. sham.

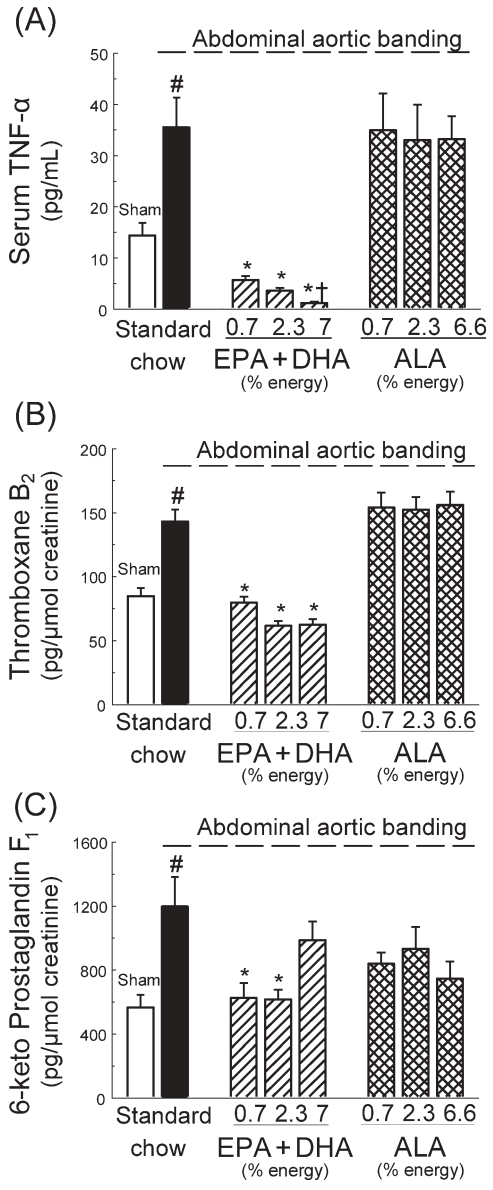

Figure 6 Serum levels of tumour necrosis factor (TNF)-α. (*A*) Urinary levels of thromboxane B_2 (*B*) and 6-keto prostaglandin F_1 (*C*), the primary breakdown products from thromboxane A_2 and prostaglandin PGI_2, respectively. Data are mean ± SEM; $n = 9$–11. $^{\#}P < 0.05$ vs. sham; $^{*}P < 0.05$ vs. standard chow diet; $^{\dagger}P < 0.05$ vs. 0.7 EPA+DHA-diet.

animals, which was blunted by EPA+DHA supplementation, but not by ALA (*Figure 6A*). Similarly, in sham animals, EPA+DHA decreases TNF-α concentration to undetectable levels, but ALA had no effect. With the standard chow, AAB elevated urinary thromboxane B_2 and 6-keto prostaglandin F_1, the stable breakdown products of thromboxane A_2 and prostacyclin, respectively (*Figure 6B* and *C*). The EPA+DHA diet significantly decreased urinary thromboxane B_2 compared with the standard diet AAB animals. The 6-keto prostaglandin F_1 levels were decreased in the 0.7 and 2.3% EPA+DHA groups, but not in the 7% EPA+DHA group (*Figure 6*). The EPA+DHA diets more effectively decreased urine levels of thromboxane B_2 than 6-keto prostaglandin F_1, as seen in the greater ratio of 6-keto prostaglandin F_1 to thromboxane B_2 (Supplementary material, *Table S8*). There were no effects of ALA diet on urine levels of thromboxane B_2 and 6-keto prostaglandin F_1 (*Figure 6B* and *C*) (Supplementary material, *Table S8*).

4. Discussion

The novel findings of the present investigation are that dietary supplementation with EPA+DHA (i) causes a dose-dependent increase in plasma adiponectin and prevention of LV chamber enlargement; (ii) prevents the switch in MHC isoform from MHC-α to MHC-β; and (iii) prevents the increase in urinary thromboxane B_2 and sharply decreases serum TNF-α. In contrast, ALA supplementation had minimal anti-inflammatory and cardioprotective effects, despite robust incorporation into cardiac membrane phospholipids. Taken together, these findings provide the first clear evidence that dietary supplementation with ω3 PUFA derived from fish, but not from vegetable sources, can prevent cardiac remodelling and dysfunction under pressure overload conditions.

The changes in myocardial phospholipid fatty acid composition observed here, although quantitatively different from what is seen in humans, were nevertheless qualitatively similar. For example, giving ∼2.9% energy as EPA+DHA for 1 month to patients awaiting cardiac surgery raised the EPA+DHA content of right atrial appendage from 5.3 to 11.5% and lowered arachidonic acid from 21 to 16%.[25] In rats given EPA+DHA at 2.3% energy, cardiac tissue EPA+DHA levels increased from 12 to 27%, whereas arachidonic acid fell from 24 to 9%. Thus the doses of fish oil employed in the present study resulted in changes in cardiac phospholipid composition similar to those observed

in clinical studies. In addition, the changes in membrane phospholipid composition was similar between sham and AAB animals, as was the increase in adiponectin and the decrease in TNF-α, suggesting that these effects occur independent of pressure overload.

We observed a strong negative correlation between plasma adiponectin concentration with LV remodelling and contractile dysfunction, suggesting a causal role for EPA+DHA-induced elevation in adiponectin in the prevention of LV chamber enlargement. Previous studies showed that the elevation in plasma adiponectin with EPA+DHA is due to the activation of PPAR-γ in adipose tissue and up-regulation of expression and secretion of adiponectin.[1,9] Adiponectin-deficient mice have enhanced LV hypertrophy and dysfunction in response to pressure overload, which can be rescued by adenovirus-mediated delivery of adiponectin.[11] In the present study, EPA+DHA supplementation elevated plasma adiponectin in a dose-dependent manner. These protective effects of adiponectin have been attributed to the activation of AMPK[11] and the inhibition of Akt;[22] however, in the present study and our previously investigation,[1] ω-3 PUFA supplementation did not affect the activation of AMPK or Akt, suggesting that any protective effect of adiponectin is mediated by other mechanisms.

It is well established that the inflammatory response contributes to the development of heart failure,[26,27] and elevated adiponectin is associated with reduced inflammation,[10] which could prevent LV remodelling and pathology. Adiponectin inhibits TNF-α-induced inflammation in human aortic endothelial cells.[28] In the present study, pressure overload was associated with elevated serum TNF-α levels, and the attenuation of LV remodelling and cardiac dysfunction by EPA+DHA supplementation was accompanied by a decline in serum TNF-α concentration. This observation is consistent with previous studies in TNF-knockout mice that showed reduced inflammatory and fibrogenic responses, apoptosis, and cardiac remodelling in response to pressure overload.[29] The suppression of serum TNF-α and the prevention of LV structural and molecular remodelling in the present study suggest a causal role for the TNF-α lowering effects of EPA+DHA in the prevention of heart failure. Additional studies in adiponectin-knockout mice are required to determine whether EPA+DHA-induced up-regulation of adiponectin is an essential component of the suppression of serum TNF-α and/or the prevention of LV dysfunction.

We found that pressure overload elevated urinary levels of thromboxane B_2 and 6-keto prostaglandin F_1, which could be linked to shear stress-induced production of thromboxane A_2 by platelets and prostacyclin by endothelial cells. In addition, cardiomyocytes can produce eicosanoids in response to inflammatory mediators such as TNF-α.[30] Fish oil supplementation has previously been show to decrease myocardial thromboxane and prostaglandins,[31] and we showed here that EPA+DHA supplementation decreases urinary levels of both thromboxane B_2 and 6-keto prostaglandin F_1. In addition, this decrease corresponds with a parallel decline in the content of arachidonic acid in phospholipids, suggesting that decreased availability of the precursor could be responsible for this effect.

Supplementation with EPA+DHA could prevent LV remodelling and dysfunction through modification of mitochondrial membrane properties and ligand activation of PPARs and increased expression of key metabolic enzymes. LV hypertrophy and chamber enlargement induced by pressure overload are associated with a decrease in the activity of mitochondrial enzymes involved in the fatty acid oxidation and energy transduction.[24] Both EPA and DHA are activators of PPAR-α *in vitro*;[23] however, we did not observe a significant increase in the mRNA or activity of the PPAR-α-regulated fatty acid oxidation enzyme MCAD. In addition, there were no effects on the activities of the mitochondrial enzymes citrate synthase, isocitrate dehydrogenase, or aconitase, suggesting that supplementation with EPA+DHA did not increase the capacity of mitochondrial carbon substrate oxidation and NADH generation. Alternatively, supplementation with EPA+DHA could exert a protective effect through improvement in mitochondrial function and the efficiency of ATP generation. Pepe and McLennan[32] showed that isolated perfused hearts from rats with fed fish oil had reduced myocardial oxygen consumption without a decrease in LV power generation, at low or high workload, and during ischaemia or reperfusion, resulting in greater LV mechanic efficiency. The mechanism for this effect is not clear, but could be due to improved mitochondrial coupling and/or a decrease in ATP hydrolysis by processes not directly related to force generation. Fish oil supplementation can alter the function of cardiac mitochondria by decreasing matrix Ca^{2+},[33] and increasing state III respiration with succinate as the substrate.[34] Additional studies are required to elucidate the diverse effects of EPA and DHA in the improved cardiac response to pressure overload. Specifically, proteomic analysis of plasma, tissue, and isolated cardiac mitochondria may prove fruitful in identifying novel effects of ω-3 PUFA, as recently suggested by the proteomic analysis of serum lipoproteins in healthy volunteers treated with fish oil.[35]

4.1 Conclusions and clinical implications

Patients with hypertension are at risk for developing heart failure[36] and thus are treated with drugs that lower afterload and suppress the neurohormonal over-activation that drives the progression to failure. Nevertheless, many optimally treated patients develop heart failure, thus new cardioprotective therapies are required that act independent of mechanisms addressed by currently used drugs. Anti-inflammatory agents, such as pentoxifylline,[37] TNF-α antibodies,[38] or adiponectin mimetic agents,[39] or drugs that modify myocardial energy metabolism, such as perhexiline,[40,41] are promising therapeutic approaches for the prevention and treatment of heart failure. The results of the present study show that dietary supplementation with EPA+DHA, but not ALA, exerts similar effects, as seen in the prevention of LV remodelling and dysfunction, associated with elevated plasma adiponectin and reduced inflammation, as evidenced by decreases in urinary thromboxane B_2 metabolites and serum TNF-α. Thus dietary supplementation with EPA+DHA acts on established therapeutic targets that are not addressed with currently approved medications and may be effective adjunctive therapy for the prevention and treatment of heart failure.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Acknowledgements

We are grateful to Willem J. Kop for statistical guidance and Keyur Shah and Biao Lei for analytical assistance.

Conflict of interest: W.S.H. is a scientific advisor to Monsanto Co and Glaxo Smith Klein Inc. The other authors have no disclosures.

Funding

This work was supported by NIH grants HL074237and HL091307 to W.C.S.

References

1. Duda MK, O'Shea KM, Lei B, Barrows BR, Azimzadeh AM, McElfresh TE *et al*. Dietary supplementation with omega-3 PUFA increases adiponectin and attenuates ventricular remodeling and dysfunction with pressure overload. *Cardiovasc Res* 2007;**76**:303–310.
2. Mozaffarian D, Bryson CL, Lemaitre RN, Burke GL, Siscovick DS. Fish intake and risk of incident heart failure. *J Am Coll Cardiol* 2005;**45**: 2015–2021.
3. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA *et al*. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation* 2006;**114**:82–96.
4. Schacky C, Harris WS. Cardiovascular benefits of omega-3 fatty acids. *Cardiovasc Res* 2007;**73**:310–315.
5. Leaf A, Kang JX, Xiao YF, Billman GE. Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. *Circulation* 2003;**107**:2646–2652.
6. GISSI-HF Investigators. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. *Lancet* 2008;**372**:1223–1230.
7. Gebauer SK, Psota TL, Harris WS, Kris-Etherton PM. n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *Am J Clin Nutr* 2006;**83**(Suppl. 6):1526S–1535S.
8. Stanley WC, Recchia FA, Okere IC. Metabolic therapies for heart disease: fish for prevention and treatment of cardiac failure? *Cardiovasc Res* 2005; **68**:175–177.
9. Neschen S, Morino K, Rossbacher JC, Pongratz RL, Cline GW, Sono S *et al*. Fish oil regulates adiponectin secretion by a peroxisome proliferator-activated receptor-gamma-dependent mechanism in mice. *Diabetes* 2006;**55**:924–928.
10. Engeli S, Feldpausch M, Gorzelniak K, Hartwig F, Heintze U, Janke J *et al*. Association between adiponectin and mediators of inflammation in obese women. *Diabetes* 2003;**52**:942–947.
11. Shibata R, Ouchi N, Ito M, Kihara S, Shiojima I, Pimentel DR *et al*. Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nat Med* 2004;**10**:1384–1389.
12. Mehra MR, Lavie CJ, Ventura HO, Milani RV. Fish oils produce anti-inflammatory effects and improve body weight in severe heart failure. *J Heart Lung Transplant* 2006;**25**:834–838.
13. Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *N Engl J Med* 1990;**323**:236–241.
14. Sun M, Chen M, Dawood F, Zurawska U, Li JY, Parker T *et al*. Tumor necrosis factor-alpha mediates cardiac remodeling and ventricular dysfunction after pressure overload state. *Circulation* 2007;**115**:1398–1407.
15. Harris WS, Sands SA, Windsor SL, Ali HA, Stevens TL, Magalski A *et al*. Omega-3 fatty acids in cardiac biopsies from heart transplantation patients: correlation with erythrocytes and response to supplementation. *Circulation* 2004;**110**:1645–1649.
16. Metcalf RG, James MJ, Gibson RA, Edwards JR, Stubberfield J, Stuklis R *et al*. Effects of fish-oil supplementation on myocardial fatty acids in humans. *Am J Clin Nutr* 2007;**85**:1222–1228.
17. Castellani S, Paladini B, Paniccia R, Di SC, Vallotti B, Ungar A *et al*. Increased renal formation of thromboxane A2 and prostaglandin F2 alpha in heart failure. *Am Heart J* 1997;**133**:94–100.
18. Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: structures, properties, and functions. *Physiol Rev* 1999;**79**:1193–1226.
19. Garnier A, Fortin D, Delomenie C, Momken I, Veksler V, Ventura-Clapier R. Depressed mitochondrial transcription factors and oxidative capacity in rat failing cardiac and skeletal muscles. *J Physiol* 2003;**551**:491–501.
20. Nakao K, Minobe W, Roden R, Bristow MR, Leinwand LA. Myosin heavy chain gene expression in human heart failure. *J Clin Invest* 1997;**100**: 2362–2370.
21. Okere IC, Young ME, McElfresh TA, Chess DJ, Sharov VG, Sabbah HN *et al*. Low carbohydrate/high-fat diet attenuates cardiac hypertrophy, remodeling, and altered gene expression in hypertension. *Hypertension* 2006;**48**:1116–1123.
22. Wang Y, Lam JB, Lam KS, Liu J, Lam MC, Hoo RL *et al*. Adiponectin modulates the glycogen synthase kinase-3beta/beta-catenin signaling pathway and attenuates mammary tumorigenesis of MDA-MB-231 cells in nude mice. *Cancer Res* 2006;**66**:11462–11470.
23. Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS *et al*. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc Natl Acad Sci USA* 1997;**94**:4318–4323.
24. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev* 2005;**85**:1093–1129.
25. Metcalf RG, James MJ, Gibson RA, Edwards JR, Stubberfield J, Stuklis R *et al*. Effects of fish-oil supplementation on myocardial fatty acids in humans. *Am J Clin Nutr* 2007;**85**:1222–1228.
26. Haudek SB, Taffet GE, Schneider MD, Mann DL. TNF provokes cardiomyocyte apoptosis and cardiac remodeling through activation of multiple cell death pathways. *J Clin Invest* 2007;**117**:2692–7201.
27. Mann DL. Stress-activated cytokines and the heart: from adaptation to maladaptation. *Annu Rev Physiol* 2003;**65**:81–101.
28. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H *et al*. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation* 2000;**102**:1296–1301.
29. Sun M, Chen M, Dawood F, Zurawska U, Li JY, Parker T *et al*. Tumor necrosis factor-alpha mediates cardiac remodeling and ventricular dysfunction after pressure overload state. *Circulation* 2007;**115**:1398–1407.
30. Titheradge MA. Nitric oxide in septic shock. *Biochim Biophys Acta* 1999; **1411**:437–455.
31. Abeywardena MY, McLennan PL, Charnock JS. Differential effects of dietary fish oil on myocardial prostaglandin I2 and thromboxane A2 production. *Am J Physiol* 1991;**260**:H379–H385.
32. Pepe S, McLennan PL. Cardiac membrane fatty acid composition modulates myocardial oxygen consumption and postischemic recovery of contractile function. *Circulation* 2002;**105**:2303–2308.
33. Pepe S, Tsuchiya N, Lakatta EG, Hansford RG. PUFA and aging modulate cardiac mitochondrial membrane lipid composition and Ca2+ activation of PDH. *Am J Physiol* 1999;**276**:H149–H158.
34. McMillin JB, Bick RJ, Benedict CR. Influence of dietary fish oil on mitochondrial function and response to ischemia. *Am J Physiol* 1992;**263**: H1479–H1485.
35. de Roos B, Geelen A, Ross K, Rucklidge G, Reid M *et al*. Identification of potential serum biomarkers of inflammation lipid modulation that are altered by fish oil supplementation in healthy volunteers. *Proteomics* 2008;**8**:1965–1974.
36. Vasan RS, Larson MG, Leip EP, Evans JC, O'Donnell CJ, Kannel WB *et al*. Impact of high-normal blood pressure on the risk of cardiovascular disease. *N Engl J Med* 2001;**345**:1291–1297.
37. Sliwa K, Woodiwiss A, Kone VN, Candy G, Badenhorst D, Norton G *et al*. Therapy of ischemic cardiomyopathy with the immunomodulating agent pentoxifylline: results of a randomized study. *Circulation* 2004;**109**: 750–755.
38. Bozkurt B, Torre-Amione G, Warren MS, Whitmore J, Soran OZ, Feldman AM *et al*. Results of targeted anti-tumor necrosis factor therapy with etanercept (ENBREL) in patients with advanced heart failure. *Circulation* 2001;**103**:1044–1047.
39. Ouchi N, Shibata R, Walsh K. Targeting adiponectin for cardioprotection. *Expert Opin Ther Targets* 2006;**10**:573–581.
40. Lee L, Campbell R, Scheuermann-Freestone M, Taylor R, Gunaruwan P, Williams L *et al*. Metabolic modulation with perhexiline in chronic heart failure: a randomized, controlled trial of short-term use of a novel treatment. *Circulation* 2005;**112**:3280–3288.
41. Ashrafian H, Frenneaux MP, Opie LH. Metabolic mechanisms in heart failure. *Circulation* 2007;**116**:434–448.