A Diet Rich in Unsaturated Fatty Acids Prevents Progression toward Heart Failure in a Rabbit Model of Pressure and Volume Overload

Den Ruijter et al: Unsaturated Fatty Acids Prevent Heart Failure

Hester M. Den Ruijter#, PhD; Arie O. Verkerk#, PhD;
Cees A. Schumacher, RA; Sander M. Houten, PhD; Charly N.W. Belterman, RA;
Antonius Baartscheer, PhD; Ingeborg A. Brouwer, PhD; Marc van Bilsen, PhD;
Baukje de Roos, PhD; Ruben Coronel, MD, PhD.

From the Experimental Cardiology Group of the Heart Center (H.M.D.R., C.A.S., C.N.W.B., A.B., R.C.) and the department of Anatomy, Embryology and Physiology (A.O.V.), Laboratory Genetic Metabolic Diseases (S.M.H.), Academic Medical Center; Department of Health Sciences and the EMGO Institute for Health Care Research, (I.A.B.) VU University Amsterdam; Department of Physiology (M.V.B.), Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands; and the Rowett Institute of Nutrition and Health, University of Aberdeen, UK. (B de R)

#These authors contributed equally to this study.

Correspondence to:
Ruben Coronel
Experimental Cardiology Group
Academic Medical Center, Room K2-112
Meibergdreef 9, 1105 AZ Amsterdam
The Netherlands
Tel: +31–20–5663267
Fax: +31–20–6975458
E-mail: rubencoronel@gmail.com

Abstract

Background—During heart failure (HF) cardiac metabolic substrate preference changes from fatty acid (FA) towards glucose oxidation. This change may cause progression towards heart failure. We hypothesize that a diet rich in FAs may prevent this process, and that dietary ω3-FAs have an added anti-arrhythmic effect based on action potential (AP) shortening in animals with HF.

Methods and Results—Rabbits were fed a diet containing 1.25% (w/w) high-oleic sunflower oil (HF-ω9, N=11), or 1.25% fish oil (HF-ω3, N=11) or no supplement (HF-control, N=8). Subsequently, HF was induced by volume- and pressure- overload. After 4 months HF-parameters were assessed, electrocardiograms were recorded, blood and ventricular tissue were collected. Myocytes were isolated for patch clamp or intracellular Ca²⁺- recordings to study electrical remodeling and arrhythmogenesis. Both the HF-ω9 and the HF-ω3 groups had larger myocardial FA oxidation capacity than HF-control. The HF-ω3 group had significantly lower mean (±SEM) relative heart and lung weight (3.3±0.13 and 3.2±0.12 g kg⁻¹, respectively) than HF-control (4.8±0.30 and 4.5±0.23) and shorter QTc intervals (167±2.6 vs 182±6.4). The HF-ω9 also displayed a significantly reduced relative heart weight (3.6±0.26), but had similar QTc (179±4.3) compared with HF-control. AP duration in the HF-ω3 group was ~20% shorter due to increased I⁰₁ and I⁰₁, and triggered activity, and Ca²⁺-aftertransients were less than in the HF-ω9 group.

Conclusions—Dietary unsaturated FAs started prior to induction of HF prevent hypertrophy and HF. In addition, fish oil FAs prevent HF-induced electrical remodeling and arrhythmias.

Key Words: heart failure, nutrition, lipids, remodeling heart failure, electrophysiology
In heart failure (HF) metabolic substrate preference shifts from fatty acid oxidation (FAO) towards glucose oxidation.\textsuperscript{1} It has been suggested that this change underlies the progression towards heart failure (HF).\textsuperscript{2} As a consequence, driving the balance towards FAO is expected to prevent or slow down the progression of HF and reduce the associated arrhythmias. We hypothesize that this can be accomplished by a diet rich in fatty acids (FAs).

HF is associated with a prolongation of ventricular action potential (AP), which plays an important role in the genesis of life-threatening ventricular tachyarrhythmias.\textsuperscript{3} Dietary \(\omega_3\) FAs cause a shortening of the AP duration (APD) in healthy animals.\textsuperscript{4} Acute application of \(\omega_3\)-FAs to isolated myocytes from hearts of patients with HF led to APD shortening and reduced incidence of triggered activity.\textsuperscript{5} It is not known whether dietary \(\omega_3\)-FAs exert the same action \textit{in vivo}.

The Diet and Reinfarction and GISSI trials addressed the potential beneficial effects of the Mediterranean diet and showed that increased intake of fish oil reduced cardiovascular death following a myocardial infarction.\textsuperscript{6,7} This was mainly attributed to risk reductions in sudden death in the GISSI-Trial.\textsuperscript{7} Because ventricular tachyarrhythmias often precede sudden death, the effect of fish oil on arrhythmogenesis has been studied intensively in humans and animals.\textsuperscript{5,8-10} These studies show reductions in severity and number of arrhythmias by fish oil. However, the GISSI-HF trial showed that fish oil supplementation for 4 years in patients with severe heart failure (HF) resulted in a relatively small absolute risk reduction in total mortality compared with the original GISSI-trial.\textsuperscript{11}

The difference between the two GISSI trials\textsuperscript{7,11} is that in the latter fish oil was provided to patients that had already developed severe HF\textsuperscript{11}, whereas in the first GISSI trial, patients with a recent myocardial infarction and with developing hypertrophy were included.\textsuperscript{7} The different outcomes between trials may be explained by a diet-induced retardation of the progression of HF in the first but not the second GISSI-trial.
We tested whether long term supplementation of the diet with unsaturated (ω3- or ω9-) FAs prior to the development of HF by combined volume- and pressure overload in rabbits attenuates structural and functional remodeling and leads to less hypertrophy and arrhythmias. Rabbits without dietary supplements were used as control. We measured parameters of HF, electrocardiograms, and markers of FAO, triggered arrhythmias and cardiac electrophysiological parameters 4 months after the induction of HF.

Methods

Design of the study

The investigation was approved by the local ethical committee and conformed to the Guide for the care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

The design of the study is shown in Figure 1. Male New Zealand White rabbits (2.5-3 kg) were allocated to one of four groups. Group one consisted of rabbits on a diet (150 g/day) without added FAs that underwent volume- and pressure overload (HF-control; n=8). Group two and three consisted of rabbits on a diet (150 g/day) supplemented with either 1.25% (w/w) ω9-sunflower oil (HF-ω9; n=11) or ω3-fish oil (HF-ω3; n=11) that underwent volume- and pressure overload. Group four consisted of rabbits on a diet (150 g/day) without added FAs (control; n=5).

HF was induced by combined volume- and pressure overload as described before, typically inducing concentric hypertrophy as well as some left ventricular dilatation of the heart. Volume overload was induced by aortic valve rupture (100% pulse pressure increase). Three weeks later, pressure overload was created by a ~50% suprarenal aortic stenosis. After 4 months, the rabbits were anaesthetized by a combination of ketamine (50 mg im) and xylazine (10 mg im), blood was collected and an ECG was recorded. The rabbits were then heparinized (5000 IU) and killed by intravenous injection of pentobarbital (240 mg). Body weight, heart weight and lung weight were...
measured and the presence of ascites, pericardial and thoracic fluid documented. Left ventricular tissue was collected and stored at -80°C.

Diets

The diets (RDS, Wijk bij Duurstede, NL) contained ~3.5% (w/w) fat, mainly linoleic acid. The addition of 1.25% sunflower oil and 1.25% ω3-fish oil increased mono-unsaturated FAs in the ω9-diet (C18:1ω9) and eicosapentaenoic acid and docosahexaenoic acid (EPA-C20:5ω3 and DHA-C22:6ω3) in the ω3-diet (Supplemental Table 1).

Analysis of diet, tissue and plasma

Lipids from the food and tissue were extracted according to Folch et al. Phospholipids were isolated with Bond Elut Aminopropyl solid phase extraction columns. Saponification and methylation of the phospholipids with boron trifluoride (Pierce, IL, USA) was performed and the formed FA methyl esters were subjected to capillary gas chromatography using a Chrompack column (Fused Silica, Chrompack), a flame ionization detector and H2 as carrier gas. FA methyl esters were expressed as fraction of the total amount. To determine the plasma lipid profiles (total, LDL and HDL cholesterol, triglycerides), samples were analyzed with spectrophotometry (enzymatic colour test) using commercial kits (Roche Diagnostics GmbH).

Cell preparation

Midmyocardial myocytes were isolated by enzymatic dissociation from the left ventricular free wall. Aliquots of cells were superfused (37°C) in a recording chamber on the stage of an inverted microscope. Quiescent rod-shaped cross-striated cells with a smooth surface were selected.
Electrophysiology

**ECG.** QT segments were corrected for heart rate using linear regression analysis obtained from 37 healthy control rabbits. Accordingly, the QT intervals were normalized to an RR interval of 4 Hz using the formula QTc=QT−0.26*(RR-250).

**Current-clamp and voltage-clamp.**

See the online supplemental material.

**Ca²⁺ transients**

Intracellular Ca²⁺ (Ca²⁺) was measured in indo-1-am loaded myocytes as described previously. Dual wavelength emission of indo-1 was recorded ((405-440)/(505-540) nm, excitation at 340 nm) and free Ca²⁺ was calculated.

**Gene expression and enzymatic activity determination**

Left ventricular samples were homogenized and total RNA was isolated. An additional wash step of 70% ethanol increased the RNA purity. Integrity of the RNA was checked by means of 260/280 ratio. cDNA synthesis and qPCR assays were performed (primers in Supplemental Table 2). Results were normalized to the geometric mean of two reference genes, i.e., cyclophilin A (CycloA) and hypoxanthine guanine phosphoribosyl transferase (HPRT) using qBase software.

**Carnitine palmitoyltransferase 1 (CPT-I) activity.** Left ventricular tissue was homogenized in cold PBS followed by sonication on ice (twice 40J at ~8W output). Homogenates were centrifuged for 10 minutes at 1000g. CPT-I activity was assessed by measuring the [U-¹³C] palmitoylcarnitine from carnitine and [U-¹³C] palmitoyl-CoA on the tandem-MS as previously
Incubations were performed in the presence and absence of 0.2 mmol/L malonyl-CoA. The activity inhibited by malonyl-CoA was considered to represent CPT1.

**Proteomics**

Cardiac tissue protein homogenates were prepared by grinding frozen tissue from 5 HF-control, 8 HF-α9 and 10 HF-α3 rabbits in liquid nitrogen and homogenizing them in a lysis buffer (7M Urea, 2M Thiourea, 2% CHAPS, 0.06% proteinase inhibitor cocktail (Roche)). Protein concentrations were determined using the RC/DC assay (BioRad), and 300 μg of protein was loaded per gel. BioRad IPG strips (pI 5–8) were used for the separation of proteins in the first dimension, and SDS-PAGE was performed on 18x18 cm acrylamide gradient (8-16%) gels for the separation of proteins in the second dimension. Flamingo (Biorad) fluorescent-stained gels were analyzed using SameSpots (Non-linear Dynamics). Spot density values were exported from the SameSpots software into R 2.11.1. (R Foundation for Statistical Computing, Vienna, Austria) to perform statistical analysis as described below. Spots were excised from the gel using a robotic spot cutter (BioRad), trypsinized using a MassPrep Station (Micromass) and analyzed by LC-ESI-MS/MS.19

**Statistics**

Data are mean±SEM (n,N: number of cells, rabbits). Data were statistically analyzed using one or two-way ANOVA, on ranks (with Kruskal-Wallis test) or repeated measures (RM) ANOVA if appropriate. In RM ANOVA, voltage steps were modelled as a categorical factor, cells as the repeated factor, without interaction. Post-hoc testing for multiple comparisons was done with the Holm-Sidak, Fisher LSD Method (with parametric ANOVA) or Dunn’s test (with non-parametric ANOVA); p<0.05 was considered statistically significant.
Results

Fatty acid oxidation.

Left ventricular activity of CPT-I, a rate-limiting enzyme controlling mitochondrial import of FAs\textsuperscript{20} was significantly increased in the HF-ω9 and HF-ω3 groups compared with HF-control and control (Figure 2A). The cardiac mRNA level of the alpha-subunit of mitochondrial trifunctional protein (HADHA), another FAO enzyme, was markedly decreased in the HF-control group compared with healthy control rabbits. The ω9- and ω3-FA-enriched diets prevented the HF-mediated decline in HADHA mRNA levels and even resulted in significantly higher levels than in healthy control rabbits (Figure 2B, p<0.05 non-parametric ANOVA followed by Dunn’s test). Glutamate dehydrogenase was not different excluding a general proliferation or enlargement of the mitochondria in these groups (data not shown).

Plasma and tissue content.

The diets rich in ω3-FAs resulted in a significant reduction of plasma triglycerides compared with HF-control and HF-ω9. Triglyceride content in the ω3-group was not different from that of control rabbits (Table). There were no differences in total cholesterol or LDL- or HDL-cholesterol between the groups (data not shown).

In the HF-ω3 group, the ω3-FA tissue-content comprised 15.5±0.5% of the total FAs extracted compared with 5.3±0.3% in the HF-control group and 4.7±0.2% in the HF-ω9 group. The incorporation of ω3-FAs was at the expense of ω6-FAs and ω9-FAs. There were no statistical differences in fat composition in the heart between the HF-ω9 and the HF-control groups. Supplemental Table 3 shows the myocardial phospholipid fatty acid composition in the four groups. The percentage of ω9-FA oleic acid in the myocardial phospholipids is highest in the HF-ω9 group and in the control group and lowest in the HF-ω3 group.
Unsaturated fatty acids prevent cardiac hypertrophy during developing HF

Weight gain was similar in the four groups (Table). The increase of the aortic pulse pressure immediately after aortic valve rupture was 102±1.3, 101±0.9 and 100±0.7% for HF-Control, HF-ω9 and HF-ω3, respectively (p>0.05).

The HF-control group had a significantly larger (relative) heart and lung weight than the control rabbits. In addition, the majority of the HF-control rabbits had ascites, thoracic and pericardial fluid (Table). Although the trigger for the development of hypertrophy and HF was the same in all HF-groups, the animals that received diets rich in ω9- and ω3-FAs had a significantly reduced relative heart weight compared with HF-control (p<0.05, ANOVA followed by Fisher’s LSD test - Table). In addition, relative lung weight was lower in the HF-ω3 group than HF-control and did not differ from the control rabbits (p<0.05, ANOVA followed by Fisher’s LSD test - Table). Supplemental Table 4 shows the echocardiographic data that reflect the heart weight data.

 pro-BNP mRNA levels were measured as an indicator of cardiac hypertrophy. Relative myocardial mRNA levels of BNP were significantly lower in the HF-ω3, HF-ω9 and control group than HF-control (Figure 2C, p<0.05, non-parametric ANOVA followed by Dunn’s test).

In the HF-ω3 and HF-ω9 group, SERCa2a mRNA levels were significantly higher than HF-control (Figure 2D, p<0.05, non-parametric ANOVA followed by Dunn’s test). In the HF-ω3 group, SERCa2a mRNA levels were even higher than in healthy control rabbits.

Fish oil fatty acids prevent electrophysiological remodeling

In line with the increased heart weight in the HF-control group, the QRS duration was longer in HF-control than control (p<0.05, ANOVA followed by Fisher’s LSD test). The HF-ω9 and HF-ω3 groups also had longer QRS durations than the control rabbits, but this was significantly less than the HF-control group.
QTc was shorter in the HF-ω3 rabbits than HF-control and HF-ω9, and similar to that of healthy controls (Table). The heart rate did not differ between the groups. Figure 3A displays typical APs recorded from isolated myocytes of the four groups at 1Hz pacing frequency. AP duration at 90% repolarization (APD₉₀) is significantly prolonged in the HF-control group compared with control at pacing frequencies below 3 Hz (Figure 3B). Whereas APD₉₀ in the HF-ω9 group did not differ from HF-control, it was significantly shorter in the HF-ω3 group than in the HF-control group and not different from control (Figure 3B). Similarly, the AP at 50% repolarization (APD₅₀, 2 Hz) in HF-control and HF-ω9 group was significantly longer (202±8 and 197±16 ms, respectively) than control (174±6 ms). The APD₅₀ in the HF-ω3 group (172±10 ms) was similar to that of healthy control rabbits. Both HF-ω3 and control were significantly different from HF-control and HF-ω9 (all p<0.05, Repeated Measures ANOVA). Cell capacitance data reflected heart weight (Supplemental Table 5).

Figure 3C shows representative examples of Ca²⁺ transients in 2Hz stimulated myocytes of all groups. Myocytes of the HF group have increased diastolic [Ca²⁺]ᵢ, decreased Ca²⁺ transient amplitude and slowing of relaxation compared to Ctrl and in HF-ω3 groups. In the HF-ω9 group diastolic Ca²⁺ was not different from the HF group and thus increased compared to Control. In the HF-ω3 groups, however, Ca²⁺ transient amplitude and relaxation time were not different compared to Control and HF-ω3 groups. Data are summarized in Figure 3D. Diastolic Ca²⁺ levels were significantly lower in the HF-ω3 (98±7.9 nM, n=19, p<0.05 ANOVA followed by Holm-Sidak test) than in HF-control (128±8.4 nM, n=26) and HF-ω9 (120±6.8 nM, n=32). Diastolic Ca²⁺ levels were similar to those of healthy control rabbits (94±5.7 nM, n=18).

*Dietary fish oil increases Iₒ and Iᵥ in heart failure*

To investigate the mechanism by which dietary fish oil prevents AP prolongation, we studied the major ion currents in the four groups (shown in Figure 4A).
**L-type Ca\(^{2+}\)** current. Figure 4B shows typical examples and current-voltage (I-V) relationships of the L-type Ca\(^{2+}\) current (I\(_{Ca,L}\)). Mean I\(_{Ca,L}\) densities were similar in the control, HF-control and HF-\(\omega 3\) group (Figure 4B). In the HF-\(\omega 9\) group, I\(_{Ca,L}\) was significantly larger at plateau potentials (-5 to +5 mV) compared with all other groups (p<0.05 Repeated Measures ANOVA). Voltage-dependency of I\(_{Ca,L}\) (in)activation did not differ significantly between the four groups (data not shown).

**Na\(^{+}\)-Ca\(^{2+}\)** exchange current. Figure 4C shows typical examples and I-V relationships of the Na\(^{+}\)-Ca\(^{2+}\) exchange current (I\(_{NCX}\)). There were no differences in the reverse (outward) mode and in the forward (inward) mode of the I\(_{NCX}\) between the four groups (Figure 4C).

**K\(^{+}\)** currents. Figure 5A shows typical examples and I-V relationships of the transient outward K\(^{+}\) current (I\(_{to1}\)). Mean I\(_{to1}\) densities were significantly larger in the HF-\(\omega 3\) group than HF-control (p<0.05 at +20 and +30 mV, Repeated Measures ANOVA, Figure 5A). In the HF-\(\omega 9\) group, I\(_{to1}\) was larger at +30 mV than HF-control. Voltage-dependency of I\(_{to}\) activation and inactivation did not differ significantly between the four groups (data not shown). Figure 5B shows typical examples and I-V relationships of the inward rectifier K\(^{+}\) current (I\(_{K1}\)). Mean I\(_{K1}\) densities were similar in the HF-control group compared with control and were significantly larger at -110 and -100 mV in the HF-\(\omega 3\) group than in the HF-\(\omega 9\) group (Figure 5B, p<0.05 Repeated Measures ANOVA). In addition, at -100 and -90 mV, I\(_{K1}\) densities of the HF-control and HF-\(\omega 9\) group were significantly smaller than control. Figure 5C shows typical examples and I-V relationships of the delayed rectifier K\(^{+}\) current (I\(_{Kr}\)). Neither the I\(_{Kr}\) tail densities nor the activation properties differed significantly between the four groups.

*Fish oil inhibits triggered arrhythmias*

Representative examples of transmembrane potentials in the four groups in the presence of 1 \(\mu M\) noradrenalin after rapid pacing are shown in Figure 6A. In the HF-control and the HF-\(\omega 9\)
myocytes the last stimulated AP (arrow) is followed by delayed afterdepolarizations (DADs) and a triggered AP. In the HF-ω3 myocyte, a DAD was present, but triggered APs did not occur. In isolated myocyte of the control group no arrhythmias occurred.

Triggered APs and DADs were abundantly present in the HF-control and in the HF-ω9 group compared with control (both p<0.05- non-parametric ANOVA followed by Dunn’s test - Figure 6B). There was a significant reduction in both triggered APs and DADs in the HF-ω3 group compared with both HF-control and HF-ω9. The incidence of triggered APs was 17% and 14% in the HF-control and HF-ω9 group respectively and significantly less in the control (0%) and HF-ω3 myocytes (7%).

Proteomics.

A hypothesis free proteomic approach was followed to identify potential protein expression changes that could underlie the electrophysiological differences between the HF-ω9 and HF-ω3 groups. In general, few significant differences were detected between HF-ω9, HF-ω3 and control groups. The main changes were represented by an increase of levels of cardiac albumin and very long-chain specific acyl-CoA dehydrogenase by HF-ω9, and an increase of levels of NADH dehydrogenase flavoprotein 1 and ATP synthase subunit by HF-ω9 and HF-ω3, compared with control (Supplemental Figure and Supplemental Table 6).

Discussion

In this study we showed that long-term dietary supplementation with unsaturated FAs started before the induction of HF attenuated the development of cardiac hypertrophy and prevented progression toward HF. Supplementation with fish oil but not sunflower oil also prevented electrical remodeling associated with HF, leading to reduced susceptibility of
arrhythmias. The data support the notion that FA-enriched diets maintain myocardial FAO capacity and are associated with reduced progression of HF and arrhythmogenesis.

The reduced hypertrophy in the HF-ω9 group in itself makes the heart less vulnerable for re-entrant arrhythmias. However, the relatively long action potential, and increased diastolic Ca²⁺ and I_{Ca,L} resulted in a similar number of arrhythmogenic triggers as in the HF-control group. Thus, ω3-FAs should be preferred over ω9-FAs because these reduce both hypertrophy and triggered arrhythmias.

Unsaturated fatty acids maintain myocardial fatty acid oxidation capacity.

HF leads to a reduction in cardiac FAO capacity.¹ The increased CPT-1 activity and the increased mRNA levels of the mitochondrial trifunctional protein in the HF-ω3 and HF-ω9 groups indicate that increased FA intake may counteract this. The hypothesis that FAs maintain myocardial FAO should be evaluated further by measuring the change in myocardial metabolism. Expression of both genes as well as many other genes involved in FAO are regulated by peroxisome proliferator-activated receptor (PPAR), a transcription factor activated by FAs (including ω3- and ω9-FAs).²² Thus, our data confirm other that unsaturated (fish oil) FAs suppress cardiomyocyte hypertrophy in vitro and in vivo, and we speculate that this was effected by activating PPARs.²²

The cardioprotective mechanisms of fish oils are diverse, and include anti-arrhythmic, anti-atherosclerotic and anti-inflammatory effects.²³,²⁴ In addition, ω3-FAs incorporate in the mitochondrial membranes of cardiac myocytes and change FAO and mitochondrial respiration.²⁵

Omega-3 and ω9 fatty acids prevent cardiac hypertrophy.

We found that both dietary ω3- and ω9-FAs prevented cardiac hypertrophy following combined volume- and pressure overload. Epidemiological studies show that increased intake of
ω3-FAs is associated with reduced incidence of HF.26 Similarly, fish oil diets in rats with pressure-overload also prevented hypertrophy.27 Although there is limited experimental and clinical evidence of the role of high fat diets in the pathogenesis of HF, both animal studies and human studies show that high unsaturated fat diets are not harmful for the heart and may even improve biomarkers of cardiovascular disease (for review, see28).

**Omega-3 fatty acids are increased in cardiac tissue.**

In the HF-ω3 group, this diet increased myocardial levels of ω3-FAs by ~10% at the expense of ω6-FAs. In patients, cardiac ω3-FAs may increase up to ~6% after fish oil supplementation for one month.29 The similarity between our data and the levels obtained in man suggests that the effects observed in this study are relevant in human HF.

Note that the vaccenic acid (18:1n7) percentage is low, but highest in the HF-ω3 group suggesting that this transFA did not have any detrimental effect in our study as suggested recently.30

**Unsaturated fatty acids lower triglycerides during developing HF**

Our data show that the HF-ω3 and HF-ω9 groups both had lower plasma triglycerides levels than the HF-control group. Omega-3 FAs appear to be superior in lowering plasma triglycerides as these were lower in the HF-ω3 group than in the HF-ω9 group. Also in humans, the Mediterranean diet, rich in monounsaturated fatty acids primarily from olives and olive oil, and fish oils markedly decreased triglyceride levels.31 Therefore, monounsaturated fatty acids not only have anti-hypertrophic effects, but also have favorable metabolic effects.
Dietary fish oil but not sunflower oil prevents electrical remodeling in HF.

In addition to a slowing effect on the progression of HF, the fish oil diet resulted in a reduced QTc interval on the surface electrocardiogram. This observation was supported in isolated myocytes of the HF-ω3 group that displayed shorter APs. In all cardiomyopathy models, I_{to} density is reduced. This was confirmed in our study in which we observed a ~50% decrease in I_{to} in the HF-control group. The HF-ω3 and the HF-ω9 group did not display remodeling of I_{to} which explains the shorter AP in the HF-ω3 group. In the HF-ω9 group, however, I_{Ca,L} was larger than in the other groups, explaining the longer APs, and the higher diastolic Ca^{2+} levels compared to the HF-ω3 group. In addition, increased Ca^{2+} influx through L-type Ca^{2+} channels by necessity leads - under steady-state conditions- to increased efflux of Ca^{2+} through the Na^{+}-Ca^{2+} exchanger and to increased depolarizing I_{NCX} and AP prolongation. Because calcium handling was impaired in the HF-ω9 group despite the normalization of SERCA2a mRNA we cannot exclude that SERCA function was nevertheless compromised.

Fish oil has disparate effects on ion currents, and both an increase and decrease in K^{+} currents have been described. This appeared to depend on whether the FAs were applied to the cells or incorporated in the membranes. Our study supports the idea that the effect of fish oil on cardiac arrhythmias not only depends on the mode of application, but also on the underlying cardiac pathology.

We followed a proteomics approach to identify potential protein expression changes between the HF-ω9, HF-ω3 and control groups. This failed to show major significant differences between groups, apart from an indication that oxidation of very long chain FAs, and oxidative phosphorylation may be increased in HF-ω9 and to a lesser extend HF-ω3. Therefore, the observed electrophysiological differences between the HF-ω9 and HF-ω3 groups cannot be explained by regulation of visualized proteins in the 2D gel, albeit that our approach may not have
been sufficiently sensitive to detect regulation of pore forming and accessory subunits for each individual ion channel which may have been affected by the dietary interventions.

**Potential significance and limitations.**

Dietary fish oil supplements are recommended for patients after a myocardial infarction, as their cardioprotective efficacy has been demonstrated in various clinical trials on the Mediterranean diet. The result of the recent GISSI-HF, however, showed a modest protection against cardiovascular death and hospitalization in patients with established HF.

We studied the effect of fish oil during the development of HF without interference of medical therapies. In this setting, fish oil supplementation prevented both structural and electrical remodeling associated with HF. This may explain why fish oil supplementation causes a larger reduction in sudden death in patients at risk for HF (after acute myocardial infarction) than in patients with established HF, even in the presence of optimal medical therapy. Indeed, increased fish oil consumption is reported to be associated with a reduced incidence of HF. We would argue that the traditional diet-heart hypothesis may need to be adjusted after confirmation of our findings in man. The molecular mechanisms underlying these effects remain uncertain, although our data suggest that an increased capacity for myocardial FAO plays a role as well as the alteration of ion channel kinetics.

We were unable to specify the phospholipid content of the sarcolemma in the four groups, and were limited by the paucity of suitable antibodies for validation of protein changes.

**Summary and conclusion.**

Dietary unsaturated FAs supplementation initiated before the development HF reduces hypertrophy. In addition, fish oil FAs prevent HF-induced electrical remodeling and triggered
arrhythmias. Our study may explain why fish oil FAs are powerful anti-arrhythmic drugs during the development of HF.

Acknowledgements

The authors thank Betty van der Struijs, Chantal Munts and Jan Ruijter for excellent support.

Sources of Funding

This work was supported by the Netherlands Heart Foundation (grants 2003B079 and 2007B018), the SEAFOODplus program of the European Union (grant 506359), EU FP6 grant LSHM-CT-2005-018833/EUGeneHeart to M.v.B and the Netherlands Organisation for Scientific Research (VIDI-grant No. 016.086.336 to SMH).

Disclosures

None.

References


Table. Characteristics (end of the study).

<table>
<thead>
<tr>
<th></th>
<th>Control (N=5)</th>
<th>HF-control (N=8)</th>
<th>HF-ω9 (N=11)</th>
<th>HF-ω3 (N=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>3410(80)</td>
<td>3430(67)</td>
<td>3470(44)</td>
<td>3590(41)</td>
</tr>
<tr>
<td><strong>Heart weight (g)</strong></td>
<td>7.8(0.37)*</td>
<td>16.7(1.29)</td>
<td>12.7(1.04)*</td>
<td>11.7(0.84)*</td>
</tr>
<tr>
<td><strong>Relative heart weight (g kg⁻¹)</strong></td>
<td>2.3(0.10)*</td>
<td>4.8(0.3)</td>
<td>3.6(0.26)*⁺</td>
<td>3.3(0.13)*⁺</td>
</tr>
<tr>
<td><strong>Lung weight (g)</strong></td>
<td>11.6(0.94)</td>
<td>15.5(1.05)</td>
<td>13.0(1.49)</td>
<td>11.6(1.25)</td>
</tr>
<tr>
<td><strong>Relative lung weight (g kg⁻¹)</strong></td>
<td>3.4(0.28)*</td>
<td>4.5(0.23)</td>
<td>3.7(0.39)</td>
<td>3.2(0.12)*</td>
</tr>
<tr>
<td><strong>Presence of ascites (%)</strong></td>
<td>0</td>
<td>75</td>
<td>36</td>
<td>18</td>
</tr>
<tr>
<td><strong>Presence of thoracic fluid (%)</strong></td>
<td>0</td>
<td>88</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td><strong>Presence of pericardial fluid (%)</strong></td>
<td>0</td>
<td>63</td>
<td>64</td>
<td>36</td>
</tr>
</tbody>
</table>

**Plasma lipids**

<table>
<thead>
<tr>
<th></th>
<th>Control (N=5)</th>
<th>HF-control (N=4)</th>
<th>HF-ω9 (N=5)</th>
<th>HF-ω3 (N=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td>0.55(0.03)*</td>
<td>0.90(0.06)</td>
<td>0.61(0.03)*</td>
<td>0.45(0.05)*⁺⁺</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td>0.51(0.07)</td>
<td>0.49(0.10)</td>
<td>0.43(0.02)</td>
<td>0.43(0.02)</td>
</tr>
</tbody>
</table>

**ECG parameters**

<table>
<thead>
<tr>
<th></th>
<th>Control (N=5)</th>
<th>HF-control (N=8)</th>
<th>HF-ω9 (N=11)</th>
<th>HF-ω3 (N=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>QRS duration (ms)</strong></td>
<td>48(1.1)*</td>
<td>61(0.9)</td>
<td>55(1.1) *⁺</td>
<td>55(0.9) *⁺</td>
</tr>
<tr>
<td><strong>Heart rate (Hz)</strong></td>
<td>2.8(0.18)</td>
<td>3.1(0.15)</td>
<td>3.0(0.16)</td>
<td>2.8(0.11)</td>
</tr>
<tr>
<td><strong>QTc (ms)</strong></td>
<td>143(3.0)</td>
<td>161(10.4)</td>
<td>155(5.8)</td>
<td>137(2.5)*⁺⁺</td>
</tr>
</tbody>
</table>

Data are presented as mean(sem). ANOVA. *p<0.05 vs HF-control; †p<0.05 vs control and ⁺p<0.05 vs HF-ω9.
Figure Legends

**Figure 1.** Study outline.

**Figure 2.** Fatty acid oxidation capacity. A. CPT-1 activity in the ventricular myocardium of the four groups. Data are obtained from 5 to 6 animals per group. B. mRNA expression of hadha relative to control. Data are obtained from at least 3 animals per group. C. Myocardial mRNA levels of markers of hypertrophy. pro-BNP. D. SERCa2a. All data are obtained from at least 5 rabbits per group and are relative to control. * p<0.05 vs HF-control; † p<0.05 vs control and ‡ p<0.05 vs HF-ω9 (One-way ANOVA).

**Figure 3.** Fish oil prevents action potential (AP) prolongation. A. Representative APs (1 Hz pacing frequency). B. Averaged APD90 as a function of pacing frequency. * p<0.05 vs HF-control; † p<0.05 vs control and ‡ p<0.05 vs HF-ω9 (Repeated Measures ANOVA). C. Representative examples of Ca²⁺ transient in 2-Hz stimulated myocytes. D. Average data of diastolic Ca²⁺, Ca²⁺ transients amplitude and 80% recovery time of the Ca²⁺ transients in myocytes from the HF-control (n=26 cells), HF-ω9 (n=32), HF- ω3 (n=19) and Ctrl (n=18) groups. *p<0.05 vs HF-control; †p<0.05 vs control and ‡p<0.05 vs HF-ω9 (One-way ANOVA).

**Figure 4.** A. Schematic representation of the AP and its major ion currents. B. Top: two-step protocol for L-type Ca²⁺ current (I_{Ca,L}) measurement. Middle: typical examples of I_{Ca,L} during voltage steps to 0 mV. Bottom: Average current–voltage (I–V) relationship of I_{Ca,L}. *p<0.05 vs HF-control; †p<0.05 vs control and ‡p<0.05 vs HF-ω3 (Repeated Measures ANOVA) C. Top: the Na⁺-Ca²⁺ exchange current (I_{NCX}) was measured in Ca²⁺-buffered conditions as the Ni²⁺-sensitive
current during a descending voltage ramp protocol. Middle: typical examples of $I_{\text{NCX}}$. Bottom: Average $I-V$ relationship of $I_{\text{NCX}}$.

**Figure 5.** A. Top: two-step protocol for transient outward $K^+$ current ($I_{\text{to}1}$) measurement. Middle: typical examples of $I_{\text{to}1}$ during voltage steps to 40 mV. Bottom: Average $I-V$ relationship of $I_{\text{to}1}$.

B. Top: protocol for inward rectifier $K^+$ current ($I_{\text{K}1}$) measurement. Middle: typical examples of $I_{\text{K}1}$ during voltage steps to -110 mV. Bottom: Average $I-V$ relationship of $I_{\text{K}1}$. *p<0.05 vs HF-control; †p<0.05 vs control and ‡p<0.05 vs HF-ω9 (Repeated Measures ANOVA)

C. Top: protocol for delayed rectifier $K^+$ current ($I_{\text{Kr}}$) measurement. Middle: typical examples of $I_{\text{Kr}}$ during voltage steps to 10 mV. $I_{\text{Kr}}$ was defined as the tail current upon stepping back to the holding potential. Bottom: Average $I-V$ relationship of $I_{\text{Kr}}$.

**Figure 6.** Fish oil prevents triggered activity. A. Representative examples of the tracing periods. Arrows indicate last paced AP. B. Data summary of the number of triggered APs and delayed afterdepolarizations (DADs). Symbols as in Figure 2. *p<0.05 vs HF-control and ‡p<0.05 vs HF-ω9 (One-way ANOVA)
Development of heart failure

Volume overload (week 3)
Pressure overload (week 5)
Animal sacrifice (week 16)

Group 1: HF rabbits with control diet (HF-control)
Group 2: HF rabbits with ω-9 sunflower diet (HF-ω9)
Group 3: HF rabbits with ω-3 fish oil diet (HF-ω3)
Group 4: Control rabbits with control diet (control)

Rabbits enter the study (week 1)
A

B

C

-80 mV

-40 mV

-20 mV

-110 mV

500 ms, ΔV=10 mV

1000 ms, ΔV=10 mV

40 mV

100 ms

10 pA/pF

0 pA/pF

4 pA/pF

0 pA/pF

1 pA/pF

4 s, ΔV=10 mV

-60 mV

Membrane potential (mV)

Membrane potential (mV)

Membrane potential (mV)

-120

-100

-80

-60

-40

-20

0

20

40

60

-120

-100

-80

-60

-40

-20

0

20

40

60

0

2

4

6

8

10

12

0

2

4

6

8

10

0

2

4

6

8

10

0

2

4

6

8

10

0

2

4

6

8

10

Control (n=19)

HF-control (n=12)

HF-ω9 (n=18)

HF-ω3 (n=11)

Control (n=7)

HF-control (n=10)

HF-ω9 (n=10)

HF-ω3 (n=11)

Control (n=11)

HF-control (n=10)

HF-ω9 (n=10)

HF-ω3 (n=13)

Control (n=11)

HF-control (n=10)

HF-ω9 (n=10)

HF-ω3 (n=13)

Control (n=7)

HF-control (n=10)

HF-ω9 (n=10)

HF-ω3 (n=13)
A Diet Rich in Unsaturated Fatty Acids Prevents Progression toward Heart Failure in a Rabbit Model of Pressure and Volume Overload

Hester M. Den Ruijter, Arie O. Verkerk, Cees A. Schumacher, Sander M. Houten, Charly N.W. Belterman, Antonius Baartscheer, Ingeborg A. Brouwer, Marc van Bilsen, Baukje de Roos and Ruben Coronel

_Circ Heart Fail._ published online April 2, 2012;
_Circulation: Heart Failure_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/early/2012/04/02/CIRCHEARTFAILURE.111.963116

Data Supplement (unedited) at:
http://circheartfailure.ahajournals.org/content/suppl/2012/04/02/CIRCHEARTFAILURE.111.963116.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation: Heart Failure* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation: Heart Failure* is online at:
http://circheartfailure.ahajournals.org/subscriptions/
SUPPLEMENTAL MATERIAL

Supplemental methods:

**Cellular electrophysiology.** APs and ion currents were recorded with the amphotericin-B-perforated and ruptured patch-clamp technique, respectively. Voltage control, data acquisition, and analysis were accomplished using custom software. Potentials were corrected for the estimated change in liquid junction potential. Adequate voltage control was achieved with low-resistance pipettes (1.5-2.5 MΩ), and series resistance of the patch pipettes and membrane capacitance compensation of at least 80%. Membrane currents and potentials were filtered (low-pass) and digitized (1-kHz and 2-kHz), AP measurements at 5-kHz and 20-kHz, respectively.

**Current-clamp experiment.**

APs were measured using a modified Tyrode’s solution containing (mmol/L): NaCl 140, KCl 5.4, CaCl$_2$ 1.8, MgCl$_2$ 1.0, glucose 5.5, HEPES 5.0; pH 7.4 (NaOH). Pipette solution contained: K-gluconate 125, KCl 20, NaCl 10, amphotericin-B 0.22, HEPES 10; pH 7.2 (KOH). APs were elicited at 0.5 to 4-Hz (3-ms, 1.5× threshold current pulses through the pipette). We analyzed APD at 20, 50 and 90% repolarization (APD$_{20}$, APD$_{50}$, and APD$_{90}$, respectively) of myocytes with resting membrane potential of >-80 mV. Data from 10 consecutive APs were averaged. Susceptibility to triggered APs and delayed afterdepolarizations (DADs) was tested by applying a 3-Hz pacing episode (10-s) followed by an 8-s pause in presence of 1 µM noradrenaline. The average amount of triggered APs and DADs of 5 consecutive 8-second pauses was calculated.

**Voltage-clamp experiments.**

The L-type Ca$^{2+}$ current (I$_{Ca,L}$), inward rectifier K$^+$ current (I$_{K1}$), transient outward K$^+$ current (I$_{to1}$), rapid component of the delayed rectifier K$^+$ current (I$_{Kr}$), and Na$^+$-Ca$^{2+}$ exchange current (I$_{NCX}$) were measured with specific bath and pipette solutions as described previously in detail (see 1, 2 and with voltage-clamp protocols shown in the corresponding figures. I$_{Ca,L}$ was measured in the presence of 0.25 mmol/L DIDS (Sigma-Aldrich, MO, USA) to block the Ca$^{2+}$-activated Cl$^-$ current (I$_{Cl,Ca}$). I$_{to1}$ was measured in the presence of
5 µmol/L E-4031 (Eisai Inc., NJ, USA) to block I_{Kr} and of 1 mmol/L CdCl_{2}, which blocks inward Ca^{2+} currents and prevents activation of I_{Cl, Ca}^{−}. CdCl_{2} also strongly inhibits inward Na^{+} currents. Suppression of these inward and outward currents allows accurate determination of I_{o1}. I_{NCX} was measured as 10 mmol/L Ni^{2+}-sensitive current. I_{Kr}, and I_{K1} were measured in the presence of 5 µM nifedipine (Sigma-Aldrich, MO, USA) to block I_{Ca,L}.

The presence of the slow component of the delayed rectifier K^{+} current (I_{Ks}) in rabbit ventricular myocytes is debated (see ref and primary references cited therein). In a subset of experiments, we therefore have studied the contribution of I_{Kr} and I_{Ks} in myocytes. While a clear tail current was present in control conditions after 4-s depolarizing pulses from −50 to 40 mV, tail currents were not observed in the presence of the I_{Kr} blocker E4031 (Supplemental figure 1A). This indicates that the tail current is due to I_{Kr} rather than I_{Ks}. To further substantiate this finding, we have subsequently added the I_{Ks} blocker chromanol-293B (90 µM, Tocris Cookson Inc., MO, USA). Currents sensitive to the I_{Ks} blocker were virtually absent (figure in ref). As we discussed previously, the absence of I_{Ks} in our cell preparations is likely related to differentially expressed I_{Ks} in rabbit ventricles with a markedly smaller I_{Ks} in the apex than the base. In addition, I_{Ks} is significantly smaller in midmyocardial than in subepi- and subendocardial myocytes. In our cell preparation method, we have mainly isolated midmyocardial myocytes from the apex of the heart.

In our experiments, therefore, tail currents after depolarizing voltage-clamp steps in our experiments were attributed to I_{Kr}. Current densities were calculated by dividing current amplitudes by cell capacitance.
Supplemental Table 1. Fatty acid composition of the diet (% of total fat content).

<table>
<thead>
<tr>
<th>Group</th>
<th>Control diet</th>
<th>ω9-sunflower oil diet</th>
<th>ω3-fish oil diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated fatty acids</td>
<td>19.6</td>
<td>16.2</td>
<td>21.8</td>
</tr>
<tr>
<td>MUFAs</td>
<td>21.3</td>
<td>44.0</td>
<td>17.9</td>
</tr>
<tr>
<td>C18:1ω9</td>
<td>17.4</td>
<td>42.4</td>
<td>12.2</td>
</tr>
<tr>
<td>PUFAs</td>
<td>56.1</td>
<td>37.6</td>
<td>56.6</td>
</tr>
<tr>
<td>ω3 fatty acids</td>
<td>9.9</td>
<td>7.1</td>
<td>26.3</td>
</tr>
<tr>
<td>C20:5ω3</td>
<td>0</td>
<td>0.1</td>
<td>9.2</td>
</tr>
<tr>
<td>C22:6ω3</td>
<td>0</td>
<td>0.1</td>
<td>6.3</td>
</tr>
<tr>
<td>ω6 fatty acids</td>
<td>46.1</td>
<td>30.5</td>
<td>27.8</td>
</tr>
<tr>
<td>C18:2ω6</td>
<td>46.1</td>
<td>30.5</td>
<td>26.8</td>
</tr>
<tr>
<td>Unknown fatty acids</td>
<td>3.0</td>
<td>2.2</td>
<td>3.6</td>
</tr>
</tbody>
</table>

MUFAs: monounsaturated fatty acids, PUFAs: polyunsaturated fatty acids.
Supplemental Table 2. Primers used for qPCR.

<table>
<thead>
<tr>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>pro-BNP</td>
<td>GTCTCCGAACAGTCTCC</td>
</tr>
<tr>
<td>Serca2a2</td>
<td>GAACCTCCCAAGTCCAA</td>
</tr>
<tr>
<td>hadha</td>
<td>ATTCCATTTGTCAGGCAACAG</td>
</tr>
<tr>
<td>HPRT</td>
<td>GACCAGTCAACAGGGGACAT</td>
</tr>
<tr>
<td>Cyclo A</td>
<td>AAGAAGATCCACATTGCAACT</td>
</tr>
</tbody>
</table>

BNP: B-type natriuretic peptide, hadha: hydroxyacyl-CoA dehydrogenase, HPRT: hypoxanthine guanine phosphoribosyl transferase, cyclo A: cyclophilin A.
Supplemental Table 3. Myocardial phospholipids composition (\%).

<table>
<thead>
<tr>
<th></th>
<th>HF-control</th>
<th>HF-ω9</th>
<th>HF-ω3</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>c8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c10</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c12</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>c14</td>
<td>1.4</td>
<td>0.9</td>
<td>1.3</td>
<td>0.7</td>
</tr>
<tr>
<td>c14:1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c15</td>
<td>0.5</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>c16</td>
<td>15.9</td>
<td>13.0</td>
<td>14.6</td>
<td>12.0</td>
</tr>
<tr>
<td>c16:1</td>
<td>1.4</td>
<td>0.9</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>c16:2n-4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c17</td>
<td>0.6</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>c16:3n-4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c18</td>
<td>7.4</td>
<td>8.6</td>
<td>8.0</td>
<td>10.7</td>
</tr>
<tr>
<td>c18:1n-9</td>
<td>16.4</td>
<td>19.5</td>
<td>13.4</td>
<td>19.1</td>
</tr>
<tr>
<td>c18:1n-7</td>
<td>1.9</td>
<td>1.9</td>
<td>2.2</td>
<td>1.6</td>
</tr>
<tr>
<td>c18:2n-6</td>
<td>36.4</td>
<td>32.9</td>
<td>29.7</td>
<td>30.8</td>
</tr>
<tr>
<td>c18:3n-6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c19</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>c18:3n-3</td>
<td>4.0</td>
<td>2.8</td>
<td>3.2</td>
<td>0.0</td>
</tr>
<tr>
<td>c18:3n-4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>c18:4n-3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>c20</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c20:1n-12</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>cis</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c20:1n-9 cis</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>c20:1n-7 cis</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c20:2n-6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c20:3n-6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c20:4n-6</td>
<td>6.4</td>
<td>8.9</td>
<td>5.6</td>
<td>11.7</td>
</tr>
<tr>
<td>c20:3n-3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c20:4n-3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c20:5n-3</td>
<td>0.0</td>
<td>0.1</td>
<td>3.9</td>
<td>0.0</td>
</tr>
<tr>
<td>c22</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c22:1n-11</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c22:1n-9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c22:1n-7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c22:2n-6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c22:4n-6</td>
<td>0.3</td>
<td>0.2</td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td>c22:3n-3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c22:5n-6</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c22:5n-3</td>
<td>0.8</td>
<td>1.1</td>
<td>1.9</td>
<td>1.2</td>
</tr>
<tr>
<td>c24</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>c22:6n-3</td>
<td>0.4</td>
<td>0.8</td>
<td>6.4</td>
<td>0.5</td>
</tr>
<tr>
<td>c24:1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Supplemental table 4
Echocardiographic data.

<table>
<thead>
<tr>
<th></th>
<th>Septum dia</th>
<th>Septum sys</th>
<th>LV dia</th>
<th>LV sys</th>
<th>LV diameter dia</th>
<th>LV diameter sys</th>
<th>FS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ctrl (n=19)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>0.25</td>
<td>0.41</td>
<td>0.28</td>
<td>0.37</td>
<td>1.55</td>
<td>0.86</td>
<td>44.56</td>
</tr>
<tr>
<td>SEM</td>
<td>0.00</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>1.27</td>
</tr>
<tr>
<td><strong>HF ctrl (n=20)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>0.35</td>
<td>0.44</td>
<td>0.38</td>
<td>0.43</td>
<td>2.22</td>
<td>1.70</td>
<td>23.32</td>
</tr>
<tr>
<td>SEM</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.07</td>
<td>0.07</td>
<td>1.08</td>
</tr>
<tr>
<td><strong>HF-ω9 (n=8)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>0.34</td>
<td>0.45</td>
<td>0.34</td>
<td>0.44</td>
<td>2.14</td>
<td>1.52</td>
<td>29.15</td>
</tr>
<tr>
<td>sem</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.07</td>
<td>0.07</td>
<td>1.49</td>
</tr>
<tr>
<td><strong>HF-ω3 (n=10)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>0.32</td>
<td>0.42</td>
<td>0.35</td>
<td>0.47</td>
<td>2.08</td>
<td>1.48</td>
<td>29.69</td>
</tr>
<tr>
<td>sem</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.05</td>
<td>0.06</td>
<td>1.36</td>
</tr>
</tbody>
</table>

Ctrl and HF ctrl: historical control data. HF induces a concentric hypertrophy as well as a dilatation and a decrease in fractional shortening (FS). In a small number of HF-control and control rabbits from our study the measurements were repeated (data not shown) in view of the abundance of echocardiographic data from healthy control rabbits. These data did not differ from the historical control data.

Supplemental table 5
Cell capacitance (pF).

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>HF-C</th>
<th>HF-ω9</th>
<th>HF-ω3</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>140.2</td>
<td>276.5</td>
<td>240.1</td>
<td>246.0</td>
</tr>
<tr>
<td>sem</td>
<td>5.2</td>
<td>8.5</td>
<td>9.4</td>
<td>8.8</td>
</tr>
<tr>
<td>n=(cells)</td>
<td>57</td>
<td>54</td>
<td>63</td>
<td>50</td>
</tr>
</tbody>
</table>
Supplemental Table 6. Measures of confidence for protein identification and characterization by LC-MS/MS analysis of heart proteins which were significantly increased or decreased upon intervention with a diet containing high-oleic sunflower oil (ω9), fish oil (ω3) or no supplement (c).

<table>
<thead>
<tr>
<th>SSP</th>
<th>Accession</th>
<th>Protein</th>
<th>Pathway</th>
<th>Mr&lt;sub&gt;exp&lt;/sub&gt;</th>
<th>Mr&lt;sub&gt;theor&lt;/sub&gt;</th>
<th>Score*</th>
<th>Matched peptide sequences</th>
<th>Change</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>240</td>
<td>P49065</td>
<td>Albumin</td>
<td>Transport</td>
<td>71</td>
<td>70.9</td>
<td>8</td>
<td>K.FLYEYSR.R&lt;br&gt; K.QTALVELVK.H&lt;br&gt; K.SLHDFGDK.I&lt;br&gt; K.EACFAVEGPK.L&lt;br&gt; K.CCSESLVD.R&lt;br&gt; K.TVVGEFTALLD.K.C&lt;br&gt; K.AILTECEAADK.G&lt;br&gt; K.ACVADSAANCDK.S&lt;br&gt; K.AFFGHYLYEVAR.R&lt;br&gt; K.AFFGHYLYEVAR.R&lt;br&gt; K.VPQVSTPTLVEISR.S&lt;br&gt; K.VLDEFQPLDEPK.N&lt;br&gt; K.KVPQVSTPTLVEISR.S&lt;br&gt; R.RPCFSALGDPETYVPK.E</td>
<td>+2</td>
<td>100,000</td>
</tr>
<tr>
<td>244</td>
<td>P49065</td>
<td>Albumin</td>
<td>Transport</td>
<td>71</td>
<td>70.9</td>
<td>4</td>
<td>K.FLYEYSR.R&lt;br&gt; K.SLHDFGDK.I&lt;br&gt; K.EACFAVEGPK.L&lt;br&gt; K.TVVGEFTALLD.K.C&lt;br&gt; R.HPDYSVLLLR.L&lt;br&gt; K.ACVADSAANCDK.S&lt;br&gt; R.DTYGDVADCEK.K&lt;br&gt; R.RHPDYSVLLLR.L&lt;br&gt; K.AFFGHYLYEVAR.R&lt;br&gt; K.AFFGHYLYEVAR.R&lt;br&gt; K.VPQVSTPTLVEISR.S&lt;br&gt; K.VLDEFQPLDEPK.N&lt;br&gt; K.KVPQVSTPTLVEISR.S&lt;br&gt; R.LPCVEDYLSVNL.R&lt;br&gt; K.ECCHGDLLECADDR.A&lt;br&gt; R.RPCFSALGDPETYVPK.E&lt;br&gt; R.RPCFSALGDPETYVPK.E</td>
<td>+2</td>
<td>30,000</td>
</tr>
<tr>
<td>246</td>
<td>P49065</td>
<td>Albumin</td>
<td>Transport</td>
<td>71</td>
<td>70.9</td>
<td>3</td>
<td>K.QTALVELVK.H&lt;br&gt; K.EACFAVEGPK.L&lt;br&gt; K.CCSESLVD.R&lt;br&gt; K.ECCDKPILEK.A&lt;br&gt; K.TVVGEFTALLD.K.C&lt;br&gt; R.HPDYSVLLLR.L&lt;br&gt; K.AILTECEAADK.G&lt;br&gt; K.NYEEAKDLFL.GK.F&lt;br&gt; R.DTYGDVADCEK.K&lt;br&gt; K.AFFGHYLYEVAR.R&lt;br&gt; K.VPQVSTPTLVEISR.S&lt;br&gt; K.VLDEFQPLDEPK.N&lt;br&gt; K.VLDEFQPLDEPK.N&lt;br&gt; K.KVPQVSTPTLVEISR.S&lt;br&gt; K.KVPQVSTPTLVEISR.S&lt;br&gt; K.YMCEHQETISSHLK.E + Ox (M)</td>
<td>+2</td>
<td>20,000</td>
</tr>
<tr>
<td>246</td>
<td>P49065</td>
<td>Albumin</td>
<td>Transport</td>
<td>71</td>
<td>70.8</td>
<td>1</td>
<td>K.FLYEYSR.R&lt;br&gt; K.QTALVELVK.H&lt;br&gt; K.TVVGEFTALLD.K.C&lt;br&gt; K.AFFGHYLYEVAR.R</td>
<td>+2</td>
<td>10,000</td>
</tr>
<tr>
<td>P49065</td>
<td>Albumin Transport</td>
<td>70</td>
<td>70.8</td>
<td>+2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------------------</td>
<td>----</td>
<td>-------</td>
<td>----</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P48818</td>
<td>Very long-chain specific acyl-CoA dehydrogenase, mitochondrial - based on similarity to bos taurus (bovine)</td>
<td>65</td>
<td>70.9</td>
<td>+2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q9BW16</td>
<td>Malic enzyme – chain A (methionine replaced by selenomethionine) - based on similarity to homo sapiens</td>
<td>64</td>
<td>62.5</td>
<td>+2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P10809</td>
<td>Heat shock protein 60 - based on similarity to homo sapiens</td>
<td>59</td>
<td>61.3</td>
<td>+2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q8HXQ9</td>
<td>NADH dehydrogenase ubiquinone] flavoprotein 1, mitochondrial - based on similarity to macaca fascicularis</td>
<td>47</td>
<td>51.3</td>
<td>+2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P06576</td>
<td>ATP synthase subunit beta, mitochondrial - based on similarity to homo sapiens</td>
<td>Oxidative phosphorylation, Metabolic pathways</td>
<td>47</td>
<td>56.5</td>
<td>1.0</td>
<td>K.VLDSGAPIK.I +2</td>
<td>2.0</td>
<td>K.IGLFGGAGVGK.T +2</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R.TIAMDTGEILVR.G + Ox (M) +2</td>
<td>2.0</td>
<td>R.IMINIGEPIDER.G + Ox (M) +3</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R.VALTGTVAEYFR.D +2</td>
<td>2.0</td>
<td>K.TVLMELINNVAK.A +2</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R.IMDPNIVGEHYDVAR.G + Ox (M) +2</td>
<td>2.0</td>
<td>K.VLDSGAPIKIPVGPETLGR.I +3</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R.EGNDLYHEMIESGVIKL.D + Ox (M) +3</td>
<td>2.0</td>
<td>K.SLQDIIAILGMDELSEEDK.I + Ox (M) +3</td>
<td>3.0</td>
</tr>
<tr>
<td>P13800</td>
<td>Cytochrome bc1 complex iii chain A - based on similarity to bovine</td>
<td>Oxidative phosphorylation, Metabolic pathways, Cardiac muscle contraction</td>
<td>44</td>
<td>49.8</td>
<td>1.0</td>
<td>R.SGMFWLR.F + Ox (M) +2</td>
<td>2.0</td>
<td>R.EHTAYYIK.A +2</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R.EHTAYYIK.A +2</td>
<td>2.0</td>
<td>K.NNGAGYFVEHLAFK.G +2</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K.NNGAGYFVEHLAFK.G +3</td>
<td>2.0</td>
<td>R.RIPLAEWESR.I +3</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R.DVVFNYLHATAFQGTPLAQSVEG +2</td>
<td>2.0</td>
<td>R.PSENVR.K +3</td>
<td>3.0</td>
</tr>
<tr>
<td>Q35946</td>
<td>Protein DJ-1 - based on similarity to bos taurus (bovine)</td>
<td></td>
<td>23</td>
<td>19.8</td>
<td>1.0</td>
<td>R.DVVICPDASLEDAK.E +3</td>
<td>2.0</td>
<td>K.GAEEMETVIPVDVMR.R + 2 Ox (M) +3</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K.GAEEMETVIPVDVMR.R + 2 Ox (M) +3</td>
<td>2.0</td>
<td>K.GAEEMETVIPVDVMR.R + 2 Ox (M) +3</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Probability Based Mowse Score for MS/MS based identifications: individual ions scores > 44 indicate identity or extensive homology (p<0.05). Ions score is –10*Log(P), where P is the probability that the observed match is a random event.
Supplemental Figure.

Example of gel.
Supplemental references


