Diet high in N6 PUFA lowers cardiac membrane N3:N6 fatty acid ratio and increases atrial mass and cardiomyocyte size

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Cardiac hypertrophy is an independent risk factor for cardiac morbidity and mortality (Levy *et al.*, 1990). Human trials have shown that omega-3 (N3) polyunsaturated fatty acids (PUFA) used in secondary prevention post myocardial infarction, are associated with a reduction in cardiovascular events (Marchioli *et al.*, 2005). Experimental evidence suggests that increasing myocardial phospholipid omega3:omega6 (N3:N6) PUFA ratio is cardioprotective (McLennan & Abeywardena, 2005). It is not known whether dietary N3 PUFA can protect against pathological cardiac growth. The aim of this study was to determine the effects of high PUFA diet (N3 & N6) on cardiac membrane fatty acid composition, growth and cardiomyocyte size.

Male Sprague-Dawley rats aged 8 weeks, were fed a fabricated diet high in either N3 (N3D, Nu-Mega fish oil), N6 (N6D, sunflower oil) or saturated fatty acids (SFD, cocoa butter) for 4 weeks. Other PUFA levels were low and caloric intake controlled among diet groups (16.8 MJ/ Kg). At feeding completion, rats were anaesthetized using halothane and killed by decapitation. Hearts were removed and dissected to measure tissue mass (atria, right ventricle, left ventricle) and tissues were snap frozen in liquid nitrogen for biochemical analysis (n = 8/ diet group). Membrane phospholipid fatty acid (FA) composition was determined in left ventricle (LV) tissue by gas chromatography. In another group of diet treated rats (n = 4/ diet group) hearts were excised and perfused in Langendorff mode for cardiomyocyte isolation by collagenase digestion for measurement of cardiomyocyte size. Length and width measurement were made for 100 LV myocytes per heart. All data are presented as mean ± SEM and analyzed by one-way ANOVA.

Somatic growth over the dietary treatment period was equivalent for the diet groups and at feeding completion there were no significant differences in body mass (N3D 485 ± 10 g, N6D 491 ± 9 g, SFD 492 ± 10 g). No significant differences were detected for whole heart mass (N3D 1338 ± 34 mg, N6D 1360 ± 45 mg, SFD 1394 ± 33 mg) or LV mass (N3D 958 ± 28 mg, N6D 957 ± 33 mg, SFD 980 ± 25 mg) between the three dietary groups. Atrial mass was greater in the N6D group, relative to N3D and SFD (N6D 121 ± 5 mg *vs.* N3D 102 ± 4 mg, SFD 114 ± 4 mg, p = 0.046), and was also greater when normalized atria to body mass (N6D 0.25 mg/g ± 0.1 *vs.* N3D 0.2 mg/g ± 0.01, SFD 0.2 ± 0.01 mg/g, p = 0.031). Relative to N3D and SFD groups, the N6D group had a significantly increased LV cardiomyocyte width (N6D 12.9 ± 0.5 μm *vs.* N3D 12.0 ± 0.4 μm, SFD 12.6 ± 0.3 μm, p = 0.031). LV cardiomyocyte length was not significantly different between treatment groups.

Long chain PUFA membrane incorporation reflected the differences in diet composition. Hearts of PUFA fed rats (N3D & N6D) showed significantly higher levels of membrane total PUFA compared to hearts of SFD rats. N6D rats had a significantly greater level of membrane N6 compared to N3D and SFD rats. The N3:N6 LV membrane ratio in the N6D and SFD rats was one-fifth the level measured in N3D rats (Table).

	% total membrane fatty acids		
Fatty Acid Class	**SF Diet**	**N6 Diet**	**N3 Diet**
Total Saturated FA	34.7 ± 0 .1	34.4 ± 0.1	34.3 ± 0.3
Total PUFA	53.8 ± 0.2	57.2 ± 0.2$^{\#}$	54.9 ± 0.2*
Total N6	43.7 ± 0.2	48.5 ± 0.2$^{\#}$	27.5 ± 0.5*
Total N3	10.6 ± 0.3	9.1 ± 0.2	27.7 ± 0.5*
N3:N6	0.2 ± 0.1	0. 2± 0.1	1.0 ± 0.1*

*p < 0.05 *vs* N6D and SFD; $^{\#}p$ < 0.05 *vs* SFD

These findings demonstrate that an N6 diet promotes atrial and LV cardiomyocyte growth. While no change was observed in LV mass, the increase in cell width suggests that dietary treatment may result in LV hypertrophy with a prolonged feeding period. Dietary N3 FA incorporation into the membranes may act as a protective stimulus supressing whole heart and cardiomyocyte hypertrophy.

Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP (1990) *New England Journal of Medicine*, **322:**1561-6.

Marchioli R, Levantesi G, Macchia A, Magginoi AP, Masrfisi RM, SIlletta MG, Tavazzi L, Tognoni G, Valagussa F on behalf of the GISSI-Prevenzione Investigators (2005) *The Journal of Membrane Biology*, **206:**117-28.

McLennan PL & Abeywardena MY (2005) *The Journal of Membrane Biology,* **206:** 85-102.