Comparison of the cardiac-specific effects of dietary omega-3 and omega-6 polyunsaturated fatty acids in male and female rats

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Clinical trials have demonstrated that omega-3 polyunsaturated fatty acids (PUFA) reduce sudden death in patients with recent myocardial infarction (Burr *et al.,* 1989). Experimental studies provide further evidence of a cardioprotective role of omega-3 PUFA mediated through antiarrhythmic actions and altered Ca^{2+} handling. Whether these effects may be attributed to membrane-incorporated and/or free diffusible PUFA is controversial (Den Ruijter *et al.,* 2008). Furthermore, epidemiologic, clinical and experimental studies have almost exclusively focused on males. Given there are reported sex-related differences in cardiac function, and that these differences involve altered Ca^{2+} handling, it is surprising that the question of whether significant cardioprotection in the female heart can be achieved through dietary PUFA has not yet been explored. This experimental study investigated the sex-specific cardiac effects of dietary omega-3 (ω3) and omega-6 (ω6) PUFA.

Six-week old male and female Sprague Dawley rats were fed a fully fabricated isoenergetic diet high in either ω3 (N3D, Nu-Mega fish oil) or ω6 (N6D, sunflower oil) PUFA for eight weeks. At feeding completion, rats were anaesthetised with pentobarbitone sodium (20 mg/kg, IP), hearts rapidly excised and assigned to one of two experimental protocols. In an *ex vivo* study, hearts were perfused in Langendorff mode with oxygenated (95% O_2, 5% CO_2) bicarbonate buffer (37°C) at a constant pressure. Left ventricular pressure was measured continuously with an isovolumetric, intraventricular balloon inflated to produce an end diastolic pressure of 4 mmHg. *Ex vivo* functional parameters were determined following 30 minutes aerobic perfusion. In an *in vitro* study, hearts were perfused in Langendorff mode with 50 mg/ml collagenase to enzymatically disperse cardiomyocytes. Isolated myocytes were loaded with the Ca^{2+} indicator Fura-2 and placed in a superfusion chamber on the stage of an inverted fluorescence microscope. Fura-2 fluorescence (ratio 380/365nm) and cell shortening (edge detection) were monitored using an IonOptix fluorescence and contractility system (IonOptix Corporation, Maryland, USA). Cells were then superfused with 2mM Ca^{2+} HEPES-solution and paced at 4 Hz to examine basal functional parameters. All data are presented as mean ± SEM and analysed by two-way ANOVA.

We have previously shown that with these dietary interventions ω3 and ω6 PUFA enrichment of cardiac membranes is achieved. Somatic growth over the dietary period was equal for N3D and N6D groups and at feeding completion there were no significant differences in body mass between dietary groups within each sex. Body mass of female rats was significantly lower (male: N3D: 573 ± 14 g, N6D: 582 ±15 g; female: N3D: 303 ± 9 g, N6D: 327 ± 12 g; diet $p > 0.05$; sex $p < 0.05$, n = 9-12/diet group). There was no effect of diet on systolic blood pressure measured using tail cuff plethysmography, although pressure observed in female rats was significantly lower. No significant sex or diet differences were observed in basal left-ventricular developed pressure, heart rate, dP/dt max or min. A significant reduction in rate-pressure product was observed in N3D for both sexes (male: N3D: 30983 ± 2315 $mmHg.min^{-1}$, N6D: 37439 ± 897 $mmHg.min^{-1}$; female: N3D: 32513 ± 1815$mmHg.min^{-1}$, N6D: 35056 ± 1677 $mmHg.min^{-1}$; diet $p < 0.05$; sex p>0.05, n = 5-7/diet group). Myocyte diastolic Ca^{2+} was significantly lower in N3D groups (Ca^{2+} ratio; male: N3D: 1.79 ± 0.04, N6D: 2.00 ± 0.08; female: N3D: 1.73 ± 0.06, N6D: 1.86 ± 0.07; diet $p < 0.05$; sex $p > 0.05$, n = 6-11 cells/diet group). N3D groups also exhibited significantly reduced systolic Ca^{2+} (Ca^{2+} ratio; male: N3D: 2.03 ± 0.04, N6D: 2.33 ± 0.12; female: N3D: 1.92 ± 0.09, N6D: 2.21 ± 0.08; diet $p < 0.05$; sex $p > 0.05$, n = 3 hearts/diet group). Myocyte shortening was not significantly influenced by diet or sex, although there was a trend for female N3D myocytes to exhibit reduced shortening.

In summary, although minimal functional effect of N3D was observed under basal conditions in *ex vivo* perfused hearts, significant differences in Ca^{2+} handling were observed for myocytes of N3D and N6D groups. These results are the first to demonstrate altered Ca^{2+} handling in female cardiomyocytes as a result of dietary ω3 intervention. These results also provide evidence for membrane-incorporated ω3 PUFA to modulate cardiac function and cardiomyocyte calcium flux in both sexes. Further characterisation of the role of PUFA dietary intervention in modulation of myocardial function in females is warranted.

Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, Elwood PC, Deadman NM. (1989) *Lancet*, **2:** 757-61.

Den Ruijter HM, Berecki G, Verkerk AO, Bakker D, Baartscheer A, Schumacher CA, Belterman CNW, de Jonge N, Fiolet JWT, Brouwer IA, Coronel R. (2008) *Circulation*, **117:** 536-44.