Cellular and Molecular Biology™ **56**, N°1, 131-139
DOI 10.1170/T885

ISSN 1165-158X
2010 Cell. Mol. Biol.™

N-3 FATTY ACIDS AND CARDIAC AUTONOMIC FUNCTION IN HUMANS

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Received, February 15th 2009; Accepted January 25th, 2010; Published February 25th, 2010

Abstract – Studies suggest that marine n-3 polyunsaturated fatty acids (PUFA) offer some protection against sudden cardiac death (SCD). The autonomic nervous system is involved in the pathogenesis of SCD and due to the fact that n-3 PUFA is abundant in the brain and other nervous tissue it is likely that n-3 PUFA might modulate autonomic control of the heart. Heart rate variability (HRV) is a non-invasive marker of cardiac autonomic function and an attenuated HRV is a predictor for SCD and arrhythmic events. Studies on HRV and n-3 PUFA have been performed in several populations such as patients with ischaemic heart disease, patients with diabetes mellitus, patients with chronic renal failure, and in healthy subjects. Many studies have demonstrated a positive association between cellular content of n-3 PUFA and HRV as well as supplementation with n-3 PUFA seems to increase HRV and thereby decreasing the risk of arrhythmic events and SCD.

Key words: Marine n-3 polyunsaturated fatty acids, sudden cardiac death, autonomic function, heart rate variability.

INTRODUCTION

The autonomic nervous system is involved in the pathogenesis of sudden cardiac death (SCD). Thus, an increased sympathetic activity has been shown to favour the development of cardiac arrhythmias (26), while on the opposite, increased vagal tone is considered protective against SCD (1,4,6,48,49,50). The modulation of autonomic control by increasing vagal tone and/or decreasing sympathetic tone may therefore be of major importance for the prevention of SCD (33).

Possible mechanisms for protection against SCD from marine n-3 polyunsaturated fatty acids (PUFA) have mainly been addressed in animal and in-vitro studies where n-3 PUFA have shown antiarrhythmic effects. However, due to the fact that n-3 PUFA (in particular DHA) is abundant in the brain and other nervous tissue it is likely that n-3 PUFA might modulate autonomic control of the heart (60) . This paper will review the effect of n-3 PUFA on heart rate variability (HRV), a non-invasive marker of cardiac autonomic function.

Abbreviations: **CRF**, chronic renal failure; **DHA,** docosahexaenoic acid; **DM,** diabetes mellitus; **EPA,** eicosapentaenoic acid; **IHD,** ischaemic heart disease; **LF/HF,** low frequency band/high frequency band; **MI,** myocardial infarction; **PUFA,** polyunsaturated fatty acids; **SCD,** sudden cardiac death.

THE SIGNIFICANCE OF HEART RATE VARIABILITY

The heart rate continuously changes over time and during normal sinus rhythm heart rate and its inverse correlate, the RR interval, vary from beat to beat mainly in response to changes in autonomic function. This beat to beat variation termed HRV has been shown to be a noninvasive method to assess cardiac autonomic function (26). Furthermore, 24-hour HRV indices appear to be stable and free of placebo effects, and therefore, these indices may be ideal variables to assess the effect of intervention therapies on autonomic function of the heart (26).

HRV can be determined over a short period of time or from 24-hour Holter recordings as done in most studies. It can be analyzed in the time domain and frequency domain, or by

nonlinear methods. Time domain indices, which are primarily dealt with in this review, are based on normal-to-normal beat intervals (R-R). However, time and frequency domain HRV may be closely related (5,30,31).

HRV predominantly reflects modulation of vagal activity (26), but two types of time domain HRV indices can be evaluated: a) data derived directly from the R-R interbeat intervals and b) data derived from differences between successive R-R intervals. Interbeat interval measures are influenced by both short term (e.g. respiratory) and long-term (e.g. circadian) changes (32). Other time domain indices, based on comparisons of lenghts of adjacent cycles primarily reflects vagal modulation of the sinoatrial node (32). Abbreviations of some important HRV indices are listed in Table 1.

A diminished HRV may be associated with increased sympathetic and decreased vagal modulation, and these autonomic changes have been associated with an increase in malignant ventricular arrhythmias and SCD (26,31,53). In light of the association between an attenuated HRV and SCD a key question is whether it is possible to increase HRV and if such an increase would improve clinical outcome? At present, there is no direct evidence for this, but many pharmacological interventions resulting in an improved patient survival are associated with an increased HRV. This includes beta-adrenergic receptor blocking agents in post MI patients (37,46), angiotensin converting enzyme inhibitors and carvedilol in chronic heart failure (7,22,62), and sotalol in patients with ventricular arrhythmias (28). Also, thrombolytic therapy for AMI patients improves HRV and survival (45,61), and time domain HRV indices retain their independent prognostic significance in post-MI patients treated with fibrinolysis (65). In contrast, the antiarrhythmic agents flecainide and moricizine has been shown to increase mortality in post-MI patients and decrease HRV (18,54,64).

Based on the results from epidemiological and interventional studies of the beneficial effect of n-3 PUFA on SCD it is of considerable interest whether such an effect can be partly explained by modulation of cardiac autonomic control as evaluated by HRV.

Table 1. Time domain HRV variables obtained from 24-hour Holter recordings.

Variable	Units	Description
RR	Ms	Mean of all normal RR intervals in the 24-h recording
SDNN	Ms	Standard deviation of all normal RR intervals in the 24-h recording
SDNNindex	Ms	Mean of the standard deviation of all normal RR intervals for all 5-min segments in the 24-h recording
SDANNindex	Ms	Standard deviation of the mean of all normal RR intervals measured in successive 5-min periods
RMSSD	Ms	The square root of the mean of the sum of squares of differences between adjacent RR intervals in the 24-h recording
PNN50	%	Percentage of successive RR interval differences $\geq$ 50 ms during the 24-h recording

n-3 PUFA AND HEART RATE VARIABILITY IN HUMANS

n-3 PUFA and HRV in patients with ischaemic heart disease

Patients with established ischaemic heart disease (IHD), who have survived an MI and suffer from left ventricular dysfunction are at high risk of SCD (63), and often have depressed HRV. The association between fish consumption, the content of n-3 PUFA in cell membranes and HRV was evaluated in 52 patients with a previous MI and a left ventricular ejection fraction $\leq$ 0.40 (14). Fish consumption at least once a week was associated with a slightly (non-significant) higher SDNN compared to subjects never eating fish. Viewing a depressed SDNN as a surrogate for an increased risk of SCD, these data are in accordance with results from the DART trial (9) showing a 29 % reduction in mortality among post-MI patients eating fatty fish twice a week. Also, the results from Siscovick et al (52), and from the US Physicians Health Study (3) showed an approximately 50 % reduction in the risk of SCD by eating fish once a week, although these studies included patients without prior IHD.

It is fair to assume that it is the actual membrane level of n-3 PUFA rather than the intake of fish per se that determines the susceptibility to develop arrhythmias and SCD (51,52). In the study mentioned above (14), the content of n-3 PUFA was measured in platelets and a close positive association was found between DHA and SDNN (Table 2). Furthermore, it was also assessed whether dietary intervention with marine n-3 PUFA in these high-risk patients could increase HRV (13). The patients were randomly allocated to receive either 5.2 g of marine n-3 PUFA daily for 12 weeks or a comparable amount of olive oil (given in 8 identical capsules). SDNN increased significantly from 115 ms to 124 ms after supplementation with marine n-3 PUFA and a nonsignificant decrease in SDNN was observed among the controls. Thus, an increase in HRV by marine n-3 PUFA might partly explain the reduction in mortality (10) and in particular the reduction of SCD observed after dietary supplementation with n-3 PUFA in post-MI patients (35).

Patients with IHD are at a higher risk of SCD compared to the background population, and IHD is the predominant underlying disease behind SCD (63). In another study, the association between marine n-3 PUFA intake and HRV was evaluated in a study comprising 291 patients referred for coronary angiography due to suspected IHD (15). Significant positive correlations were found between HRV indices and cell membrane levels of marine n-3 PUFA, especially DHA. A positive association between SDNN and wine intake was also observed but this association could be explained by increased fish consumption among wine drinkers since this association disappeared after controlling for fish intake, while the association between cellular levels of n-3 PUFA and HRV remained significant after controlling for wine intake.

Villa *et al* performed a cross-over trial in 10 patients with IHD and found a positive correlation between HRV and DHA after dietary supplementation with n-3 PUFA (59). They found a decrease in the low frequency band/high frequency band (LF/HF) after giving these patients 6 g of n-3 PUFA for 4 weeks. The LF/HF is a HRV measurement in the frequency domain and a low LF/HF is considered to reflect a favourable vagal predominance meaning a protection against SCD.

Table 2. Heart rate variability indices stratified by quartiles according to the platelet levels of DHA in patients with a previous MI and left ventricular dysfunction. Mean values ($\pm$SD) are given. Modified from reference # 14.

	Content of DHA in platelets(%)		
	1st quartile	2nd - 3rd quartile	4th quartile
DHA (%)	< 2.26	2.26 - 3.14	> 3.14
Number	13	26	13
RR (ms)	802 ($\pm$105)	791 ($\pm$147)	866 ($\pm$165)
SDNN (ms)	98 ($\pm$32)	116 ($\pm$48)	140 ($\pm$33)*
SDNN index (ms)	36 ($\pm$17)	47 ($\pm$35)	54 ($\pm$26)*

*: $p < 0.01$

In a study by O´Keefe et al (43) 18 men with a history of MI and ejection fractions <40% were randomized to placebo or n-3 PUFA (585 mg of DHA and 225 mg of EPA) for two 4-month periods in a cross-over design. At the end of each period, heart rate, HRV, and the rate of heart rate recovery after exercise were determined. n-3 PUFA significantly decreased heart rate at rest from 73 to 68 beats/min and increased HRV in the high-frequency (HF) band. Thus, these changes are also consistent with an increase in vagal activity and may in part explain the observed decrease in risk for SCD seen after treatment with n-3 PUFA.

A smaller single blinded uncontrolled study in 38 post-MI patients using 20-min ECG recordings did not show any changes in HRV indices in the time domain nor in the frequency domain after 3 months of supplementation with n-3 PUFA or usual care (24). Many of these patients were on beta-blocking and/or ACE-inhibitor therapy, agents known to improve HRV. Furthermore, high risk patients with ventricular ejection fractions < 40 % and probably a low HRV, were excluded from this study. In fact, it might be these patients who would benefit most from n-3 PUFA supplementation.

n-3 PUFA and heart rate variability in patients with diabetes mellitus and in overweight persons

Patients with diabetes mellitus (DM) are at increased risk of developing IHD and they have an excess post-MI mortality (34). The background for the increased risk is multifactorial but autonomic neuropathy involving the heart is probably important. Thus, cardiac autonomic neuropathy carries an excess risk of mortality in patients with DM, including a high risk of SCD (36). Therefore, detection of autonomic dysfunction may be of importance for risk stratification and subsequent management of DM patients, and HRV analysis is a well-established tool in the early detection of autonomic neuropathy in patients with DM (53).

The association between the platelet content of marine n-3 PUFA and HRV has been examined in 43 type 1 and 38 type 2 diabetes patients (16). A close positive relation was found between fish intake and platelet levels of n-3 PUFA, and furthermore, HRV increased with increasing levels of DHA in patients with type 1 DM. In patients with type 1 DM solely receiving insulin therapy the positive correlation between HRV and platelet DHA was more pronounced. However, this study could not demonstrate a significant association between n-3 PUFA and HRV in the patients with type 2 DM. However, a small Italian study found that 6 months of n-3 PUFA treatment in a group of 13 type 2 diabetic patients partially improved HRV in the frequency domain (47).

Overweight persons have a high risk of developing type 2 DM and they also have an impaired HRV (41). In a randomised, double-blind, parallel comparison, 65 overweight volunteers consumed DHA 1.56 g/d and EPA 0.36 g/d or sunflower-seed oil (placebo) for 12 weeks (41). Resting heart rate and the heart rate response to submaximal exercise were measured 3 times, and in 46 subjects HRV was assessed in the frequency domain using 20 min ECG recordings (at baseline and after 12 weeks). n-3 PUFA supplementation compared with placebo improved HRV by increasing high-frequency power, representing para-sympathetic activity, and it also reduced heart rate at rest and during submaximal exercise. Thus, the conclusion of this study was that dietary supplementation with DHA-rich fish oil reduced HR and modulated HRV in a favourable way (an improved parasympathetic-sympathetic balance) in overweight subjects with a high risk of developing IHD.

n-3 PUFA and heart rate variability in patients with chronic renal failure

IHD accounts for approximately 50 % of the mortality in patients with chronic renal failure (CRF) receiving dialysis and SCD is a major problem in this population (27). Cardiac autonomic dysfunction is very frequent in these patients (4) with a high prevalence of ventricular arrhythmias. A depressed HRV confers significant prognostic value in CRF patients (42) , and it may identify patients at increased risk of SCD (25).

While supplementation with n-3 PUFA to dialysis patients have shown several beneficial effects (20), the effect of n-3 PUFA on HRV in these patients have only been addressed in three studies. In a study comprising 29 CRF patients treated with chronic haemodialysis or continuous ambulatory peritoneal dialysis the association between the content of n-3 PUFA in granulocyte membranes and HRV was examined (11). The patients were randomly allocated to dietary supplementation with either 5.2 g of n-3 PUFA or olive oil for 12 weeks. Only 17 patients completed the study (11 from the n-3 PUFA group and 6 controls). This hampered comparisons

between the two groups, but in the n-3 PUFA group no increase in SDNN was observed after supplementation whereas the mean RR-interval increased indicating a beneficial decrease in heart rate.

These CRF patients had significantly lower HRV indices compared to the post-MI patients with left ventricular dysfunction (14). Interestingly, levels of marine n-3 PUFA at baseline were significantly lower in CRF patients compared to post-MI patients. These limited ranges of both HRV indices and marine n-3 PUFA values may partly explain lack of association between HRV and n-3 PUFA at baseline since an increase in the range of marine n-3 PUFA levels after dietary supplementation led to a significant positive correlation between n-3 PUFA in granulocytes and SDNN ($r = 0.71$, $p < 0.01$). Also, dichotomizing these 17 patients according to their median SDNN revealed significantly higher n-3 PUFA levels among those with the highest SDNN (Table 3).

Table 3. Level of marine n-3 PUFA in granulocyte membranes (%) in patients with chronic renal failure after dichotomizing according to their mean SDNN. Values are mean (± SD). Modified from reference # 11.

	SDNN	
	< 76 ms	≥ 76 ms
EPA	0.96 (1.2)	2.70 (1.6)*
DHA	1.45 (0.5)	1.93 (0.5)*
Total n-3 PUFA	4.2 (0.6)	7.8 (1.9)*

* : $p < 0.05$

In another published trial, Fiedler *et al* gave 1.2 g of n-3 PUFA daily for 12 weeks to 11 haemodialysis patients in an uncontrolled design (19). The authors measured a series of cardiovascular risk factors among them HRV, but found no effect of n-3 PUFA on HRV.

In a subgroup of a larger population with end-stage kidney disease and documented cardiovascular disease, Svensson et al, randomized 30 patients to 1.7 g of n-3 PUFA daily or control oil (olive oil) for 3 months (55). The authors could not show any effect on HRV whereas there was a trend towards a reduced heart rate in the n-3 PUFA arm. Thus, further studies are warranted regarding the effects of n-3 PUFA on HRV and autonomic modulation in these high-risk patients.

n-3 PUFA and heart rate variability in healthy subjects

A low HRV may predict a poor outcome in healthy populations. In the "Men Born in 1913 Study" randomly selected men aged 50 years, had an increased risk of death from IHD during a 10 year follow-up, if their HRV was decreased when analyzed from 10 seconds ECG strips at entry (56). In the Zutphen study, the 5-year age-adjusted relative risk of mortality for subjects with a low HRV measured from 25-30 seconds ECG strips was 2.1 in middle-aged and 1.4 in elderly men (17), and there was an inverse association between HRV and the risk of SCD. Reports from the Framingham study based on 2-hour recordings confirm the predictive value of a decreased HRV in the general population (57,58). Also, Mølgaard et al found an attenuated HRV in apparently healthy subjects subsequently suffering SCD (38).

Previous studies have suggested a substantial reduction in the risk of SCD among healthy subjects eating fish once a week or more (3,51,52). In a dose-response study, the effect of dietary supplementation with n-3 PUFA on HRV was examined in healthy subjects (12) . Sixty healthy subjects were randomly divided into three groups receiving either 1) 2.0 g of marine n-3 PUFA, 2) 6.6 g of marine n-3 PUFA or 3) olive oil (placebo) daily for 12 weeks. Baseline examination revealed positive correlations between HRV indices and DHA in men, an observation also found by other authors (8). Overall, intervention with n-3 PUFA had no effect on HRV, but in healthy subjects with a baseline SDNN below the median (< 150 ms) dietary supplementation with n-3 PUFA (both 2.0 and 6.6 g) increased RR. By stratifying the subjects according to gender, a dose-dependent increase in several HRV indices among men with a baseline SDNN < 150 ms was seen whereas no effect was observed in women. The result from the male participants may emphasize the importance of the actual cellular membrane level of n-3 PUFA as a major determinant of the risk of SCD (2,51).

The effect found on RR is similar to results by Grimsgaard *et al* showing a reduction in heart rate (inversely related to RR) after supplementation with DHA to healthy men (23). Indeed, several studies have shown that n-3 PUFA reduce heart rate (39) , which is of importance because an increased heart rate is strongly associated with a poor cardiovascular outcome (44).

Geelen *et al* included 84 middle-aged subjects in a 12 week intervention trial where the participants were given 3.5 g of n-3 PUFA or placebo daily for 12 weeks (21). They measured HRV from short ECG recordings (10 minutes) and found no effect of n-3 PUFA on HRV. A possible explanation for this could be the short recording time because it might be essential to include night time measurements as the vagal predominance during night time may be a factor which is modifiable by n-3 PUFA. However, recently Holguin *et al* managed to demonstrate that even in short term ECG recordings (6 minutes) supplementation with 2 g of n-3 PUFA daily to elderly people was associated with a significant increase in HRV (29).

Population-based investigation, n-3 PUFA, and heart rate variability

A large study based on dietary n-3 PUFA intake and HRV in a well-defined population was recently published (40). More than 4.000 subjects, aged $\geq$ 65 years were included from the Cardiovascular Heart Study. Approximately 25 % had DM and 20 % had a history of IHD. SDNN and RMSSD were measured from 10-second ECG recordings in 4263 subjects and in a subset of 1361 participants, HRV in the time domain and in the frequency domain was derived from 24-hour Holter recordings. In a subset of the subjects, plasma phospholipids levels of EPA and DHA was measured and these levels correlated significantly with the fish consumed ($r = 0.55$, $p < 0.001$). According to the fish intake, the participants were divided into 5 groups and in general, HRV was highest among the participants with the highest fish intake. From the subanalyses of HRV it was suggested that a high fish consumption was associated with an enhanced vagal activity and parasympathetic predominance. Indeed, this large population based study seems to confirm that n-3 PUFA supplementation can modulate cardiac autonomic function in a favourable way, and this may protect against SCD.

CONCLUSIONS

Both brain tissue and heart tissue have a high content of n-3 PUFA (especially DHA) and this is consistent with the finding that this marine n-3 fatty acid may be associated with HRV. Thus, n-3 PUFA may modulate HRV both at the level of the brain and the heart. The results presented in this review support an antiarrhythmic effect of n-3 PUFA acids in humans and this effect seems to be due to a modulation of cardiac autonomic control with an increase in vagal tone thereby reducing the risk of arrhythmias. However, some studies are not consistent regarding the effect of n-3 PUFA on HRV and this may be due to small size in some studies, short duration of intake, limited periods of HRV assessment, or variable doses of n-3 PUFA. Thus, further research is required to confirm the obtained results in this field.

Other articles in this theme issue include references (66-77).

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