Relation between blood and atrial fatty acids in patients undergoing cardiac bypass surgery¹–³

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ABSTRACT

Background: Studies relating cardiovascular outcomes to dietary or blood measures of various fatty acids rely on the implicit assumptions that dietary change results in changes in blood fatty acids that, in turn, alter cardiac fatty acids. Although dietary intakes of n–3 (omega-3), n–6 (omega-6), and trans fatty acids are reflected in their concentrations in blood, there are few human data on the relation between blood and cardiac concentrations of fatty acids.

Objective: The objective was to explore relations between blood and myocardial n–3, n–6, trans, monosaturated, and saturated fatty acids over a range of community intakes to evaluate whether blood fatty acids are useful surrogate markers of their cardiac counterparts.

Design: Patients undergoing on-pump coronary bypass surgery were recruited. Right atrial appendages and blood were collected at surgery for fatty acid analysis.

Results: Atrial appendages and matching blood samples were collected from 61 patients. Highly significant correlations were identified between atrial and erythrocyte or plasma n–3 [eg, eicosapentaenoic acid (erythrocytes: \( r = 0.93, P < 0.0001 \); plasma: \( r = 0.87, P < 0.0001 \)], some n–6 [eg, arachidonic acid (erythrocytes: \( r = 0.45, P = 0.0003 \); plasma: \( r = 0.39, P = 0.002 \)], trans [eg, total trans 18:1 (erythrocytes: \( r = 0.89, P < 0.0001 \); plasma: \( r = 0.74, P < 0.0001 \)], and monounsaturated [eg, oleic acid (erythrocytes: \( r = 0.37, P = 0.003 \)] fatty acids. There were no statistical associations between blood and cardiac saturated fatty acids.

Conclusion: Erythrocyte- and plasma phospholipid–derived fatty acids can be used to estimate cardiac fatty acid status in humans. Am J Clin Nutr 2010;91:528–34.

INTRODUCTION

Relations between dietary intakes of various fatty acids and cardiovascular outcomes have been widely studied. However, the calculation of fatty acid intake relies on estimates and assumptions usually involving recall, estimated intake of various foods, and then calculations based on estimates of the fatty acids in individual foods and meals. A biomarker for fatty acid intake may be more useful, and blood fatty acid concentrations can reflect the intake of some, but not all, fatty acids. Red blood cell (RBC) or plasma fatty acids have been used as biomarkers of the intake of the n–6 polyunsaturated fatty acid (PUFA) linoleic acid (LA, 18:2n–6) (1); the n–3 PUFAs \( \alpha \)-linolenic acid (ALA, 18:3n–3), eicosapentaenoic acid (EPA, 20:5n–3), and docosahexaenoic acid (DHA, 22:5n–3) (2); and the trans fatty acids (3). These biomarkers have been used in cohort and case-control studies to examine relations between blood fatty acids and various cardiac outcomes. For example, case-control studies have shown that the odds of sudden cardiac death were inversely related to whole blood n–3 PUFAs (4), and the odds of primary cardiac arrest were inversely related to RBC total trans fatty acids (3). However, for fatty acids whose mechanism of action is thought to directly involve the heart, myocardial fatty acid concentrations would be the ultimate measure for the independent variable in cardiovascular outcome studies.

The present study examined the relations between RBC, plasma phospholipid, and human myocardial n–6, n–3, n–9, saturated, and trans fatty acids in 61 patients undergoing cardiac bypass surgery.

SUBJECTS AND METHODS

Subjects

The subjects were patients accepted for elective on-pump cardiac surgery (coronary artery bypass graft and/or valve repair or replacement) between March 2004 and February 2005 and who had not previously had cardiac surgery, thereby having an intact right atrial appendage (RAA). Atrial appendage and matching blood samples were obtained from 61 subjects, consisting of 1) subjects enrolled in a randomized controlled trial

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(RCT) (5) and who were allocated to olive oil (n = 10), flaxseed oil (n = 10), or no oil supplement (n = 7); 2) subjects originally enrolled in the RCT but were withdrawn because of surgery being rescheduled (n = 3); 3) subjects originally enrolled in the RCT but electing to cease treatment after 1 to 2 d of oil consumption; 4) patients who were excluded from the RCT because of the self reported consumption of >2 fish meals/wk or regular consumption of fish-oil supplements (n = 23); and 5) those who declined participation in the RCT but consented to blood and atrial specimen collection (n = 1).

The RCT groups consuming high-dose fish oil were excluded from this analysis because the focus was on blood and cardiac n−3 LCPUFA concentrations applicable to the general population. Therefore, all intakes of n−3 LCPUFAs in subjects included in this analysis were achieved through dietary intake or self administered fish-oil supplementation, and no direction was given concerning required n−3 LCPUFA intakes, and no attempt was made to estimate n−3 LCPUFA intakes. Informed consent was obtained before participation in the study, which was approved by the Research Ethics Committee of the Royal Adelaide Hospital.

Laboratory methods

Tissue collection procedures were as described previously (5). Briefly, blood was collected into lithium heparin-containing tubes for plasma and red blood cell (RBC) fatty acid analysis at enrollment and again on admission to the hospital before surgery. The right atrial appendage was placed immediately into cold saline and placed on ice, transferred to the laboratory, removed from the saline, and stored at −70°C until analyzed. RBC lipids were extracted as described (6). Atrial samples were cleaned of adipose tissue and clotted blood. Approximately 0.2 g tissue was homogenized in 2 mL saline before mixing with 3 mL methanol. Chloroform (6 mL) was added and centrifuged and the lower chloroform phase was transferred to a 20-mL vial and evaporated to dryness. Phospholipids were separated by thin-layer chromatography and, after methanolysis of the phospholipids, fatty acid methyl esters were analyzed by gas-liquid chromatography as described (2). The CVs for the assay ranged from 0.02% to 11.1% for the fatty acids reported.

Fatty acids

Total n−3 long-chain PUFAs (LCPUFAs) refers to the sum of EPA, docosapentaenoic acid (DPA, 22:5n−3), and DHA. Total n−6 LCPUFAs refers to the sum of n−6 PUFAs containing ≥20 carbons and ≥3 double bonds, namely, the sum of dihomoy-linolenic acid (DGLA, 20:3n−6), arachidonic acid (AA, 20:4n−6), 22:4n−6, and 22:5n−6. Total trans fatty acids (TFAs) refers to the sum of trans 16:1, trans 18:1n−7, trans 18:1n−9, and trans 18:2. Total trans 18:1 is the sum of trans 18:1n−7 and trans 18:1n−9.

Statistical analysis

Univariate regression and multivariate analyses were performed to assess the associations between atrial fatty acids and characteristics of the participants. In the case of multivariate analysis, the following items were included into the calculation as independent predictors: age, sex, BMI, smoking status, diabetes status, and the proportions of the corresponding fatty acid/combination of fatty acids in RBCs and plasma phospholipids. The atrial fatty acids/combination examined were as follows: n−3 PUFAs (EPA, DPA, DHA, EPA+DHA, and total n−3 LCPUFAs), n−6 PUFAs (LA, AA, total n−6 PUFAs, and total n−6 LCPUFAs), saturated fatty acids (palmitic acid, stearic acid, and total saturates), TFAs (trans 18:1n−9, trans 18:1n−7, trans 18:1, and total TFAs), and oleic acid (OA, 18:1n−9). We did not collect detailed dietary information in this study; therefore, estimates of fish consumption or n−3 PUFA intakes are not available. Data were analyzed by using Statistica version 7.1 (StatSoft Inc, Tulsa, OK).

RESULTS

Subjects

The fat intakes of subjects in this study were achieved through normal dietary intake or self-administered fish-oil supplementation, and no direction was given about required n−3 LCPUFA intakes. Patient age, BMI, type of surgery, medication use, and medical history are presented in Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>65.2 ± 10.8²</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.7 ± 3.9</td>
</tr>
<tr>
<td>Male sex [n (%)]</td>
<td>49 (80)</td>
</tr>
<tr>
<td>Type of surgery [n (%)]</td>
<td>37 (61)</td>
</tr>
<tr>
<td>CABG only</td>
<td>37 (61)</td>
</tr>
<tr>
<td>Valve only</td>
<td>11 (18)</td>
</tr>
<tr>
<td>CABG + valve</td>
<td>10 (16)</td>
</tr>
<tr>
<td>Bentall’s procedure</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Medications [n (%)]</td>
<td>8 (13)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>45 (74)</td>
</tr>
<tr>
<td>ACE inhibitor/ARB</td>
<td>36 (59)</td>
</tr>
<tr>
<td>Antianginal</td>
<td>22 (36)</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>28 (46)</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>22 (36)</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>5 (8)</td>
</tr>
<tr>
<td>Digoxin</td>
<td>6 (10)</td>
</tr>
<tr>
<td>Statin</td>
<td>40 (66)</td>
</tr>
<tr>
<td>Warfarin</td>
<td>7 (11)</td>
</tr>
<tr>
<td>LV dysfunction [n (%)]</td>
<td>7 (11)</td>
</tr>
<tr>
<td>LV of those with LV dysfunction (%)</td>
<td>47.0 ± 5.2</td>
</tr>
<tr>
<td>History [n (%)]</td>
<td>55 (91)</td>
</tr>
<tr>
<td>Angina</td>
<td>35 (57)</td>
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<tr>
<td>Diabetes</td>
<td>14 (23)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>38 (62)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>22 (36)</td>
</tr>
<tr>
<td>Smoker (current/former)</td>
<td>39 (64)</td>
</tr>
</tbody>
</table>

¹ CABG, coronary artery bypass graft; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; LV, left ventricular; LVEF, left ventricular ejection fraction.
² Mean ± SD (all such values).
³ LVF < 55%.

TABLE 1

Characteristics of the subjects (n = 61)

The mean values for atrial, RBC, and plasma phospholipid fatty acids are presented in Table 2. There were differences and
Correlations with atrial \textit{trans} fatty acids

RBC or plasma total 18:1 was significantly correlated with the corresponding atrial fatty acid (Figure 3). On multivariate analysis, age was positively associated with atrial total 18:1 ($\beta = 0.14$; 95% CI: 0.01, 0.26; $P = 0.03$). RBC or plasma 18:2 was significantly correlated with the corresponding atrial fatty acid, although atrial \textit{trans} 18:2 was below the level of detection in 11 (18%) samples, and RBC \textit{trans} 18:2 was below the level of detection in one sample (Figure 3).

Atrial saturated fatty acids

Neither RBC nor plasma stearic acid [18:0: RBC ($r = 0.21$, $P = 0.10$); plasma ($r = 0.13$, $P = 0.31$)], palmitic acid [16:0: RBC ($r = 0.04$, $P = 0.79$); plasma ($r = -0.04$, $P = 0.78$)], or total saturated fatty acids (RBC: $r = -0.07$, $P = 0.61$; plasma: $r = 0.003$, $P = 0.98$) was significantly correlated with their atrial counterparts. On multivariate analysis, presence of diabetes was positively associated with atrial total saturated fatty acids ($\beta = 0.33$; 95% CI: 0.07, 0.55; $P = 0.01$).

Atrial monounsaturated fatty acids

RBC but not plasma oleic acid was significantly correlated with atrial oleic acid (Figure 4). On multivariate analysis, atrial oleic acid was positively associated with female sex ($\beta = 0.35$; 95% CI: 0.12, 0.58; $P = 0.004$) and age ($\beta = 0.29$; 95% CI: 0.06, 0.52; $P = 0.02$).
DISCUSSION

The main outcome of this study was the demonstration of relations between blood n-3, n-6, and trans fatty acids and their cardiac counterparts. This has been lacking in the literature, but is important for providing a linkage between recommendations on intakes of various fatty acids, such as n-3 and trans fatty acids, and the ability to estimate cardiac concentrations from plasma and RBC markers with high accuracy has important clinical significance, particularly for high-risk patients.

One mechanism by which n-3 fatty acids achieve their cardiovascular benefits may be through incorporation into cardiac membrane phospholipids and subsequent release by phospholipase A2 under provocation (8). Because many studies have related cardiovascular outcomes to blood concentrations of n-3 fatty acids, it is important to establish that these measures are acceptable surrogates for cardiac n-3 fatty acids (4, 9, 10). In addition, the proportion of EPA+DHA in RBC phospholipids, also known as the Omega-3 Index, has been proposed as an independent risk factor for coronary heart disease mortality (7). Although these studies, especially those that examined sudden cardiac death (4) and primary cardiac arrest (10), assume that there is a relation between cardiac and blood n-3 fatty acid concentrations, there has been only one report on this topic (11). That study reported the correlation between RBC and cardiac EPA+DHA in biopsy samples obtained from only 20 heart transplant patients (11). However, the content of EPA+DHA in these transplanted hearts (mean: 2.2% of total fatty acids) was considerably lower than the cardiac EPA+DHA concentrations reported elsewhere, with mean values of 5.3% and 7.5% in atrial tissue obtained from cardiac bypass patients (5, 12), 5.7% in the left ventricle obtained during mitral valve replacement (13), and ~3.4% in unspecified normal myocardium obtained at autopsy (14).

We previously showed that supplementation with flaxseed oil results in significant increases in atrial, RBC, and plasma phospholipid ALA (5), and we showed here that there are strong linear relations between atrial ALA and the 2 blood markers. The evidence for a beneficial cardiovascular effect of ALA is mixed. Increased dietary ALA has been associated with a reduced risk of fatal ischemic heart disease (15), sudden cardiac death (16), and nonfatal myocardial infarction (17), although a recent systematic review failed to identify any significant association between dietary ALA and coronary heart disease (18).

The range of n-3 LCPUFAs observed in plasma and RBCs in this study was large, which indicated that the dietary intake of the subjects reflected a range of n-3 intakes ranging from low fish consumption to self administration of fish-oil supplements. Thus, the results indicate that our study population was representative of the extremes of n-3 fat intakes in Australian society. Importantly, the blood concentrations of most fatty acid classes are excellent surrogate markers of cardiac membrane phospholipid fatty acids in subjects consuming stable diets providing a wide range of n-3 intakes. Specifically, both RBC and plasma n-3 fatty acids are highly correlated with their corresponding atrial fatty acids, as are the RBC trans 18:1 fatty acids. In general, relations between blood and atrial n-6 fatty acids were less robust than those for the n-3 LCPUFAs.
whereas no relation between blood and atrial saturated fatty acids was observed.

Age has been reported to be positively associated with RBC EPA+DHA in healthy volunteers, independent of the number of monthly fish servings (19) or dietary EPA+DHA (20), and also in hemodialysis patients (21). Although we found a significant independent inverse relation between age and atrial DPA content, there was no association between age and atrial EPA, DHA, EPA+DHA, or total n-3 LCPUFAs. The lack of association between atrial n-3 LCPUFAs and age may have been due in part to the much narrower age range of our study population, 85% of whom were aged between 50 and 80 y.

Although the number of females in this study was small, we found that women had significantly lower proportions of DHA in the atrium than did men and that these differences were independent of the corresponding RBC fatty acid concentrations. Because DHA is by far the predominant n-3 LCPUFA in atrial phospholipids, the observed sex effect also carried over to the sum of EPA+DHA and total n-3 LCPUFAs. In a healthy Japanese population, women were found to have significantly lower RBC EPA+DHA concentrations than men on both univariate and multivariate analysis, independent of dietary intake and age (20), whereas there was no effect of sex on RBC EPA+DHA among healthy volunteers in a study from the United States, where the range of n-3 fat intakes was presumably less than in Japan (19). In schizophrenic patients, women were found to have significantly higher RBC EPA and DHA concentrations than men, but only in nonsmokers (22).

In this study, we found no effect of smoking on atrial n-3 fatty acid concentrations on either univariate or multivariate analysis.

Wilhelm et al (23) found an inverse relation between the Omega-3 index (RBC EPA+DHA) and left ventricular ejection fraction in patients with New York Heart Association class II/III heart failure. We found no association between atrial n-3 PUFAs and left ventricular dysfunction, although only 11% of subjects had left ventricular dysfunction, and those that did had only mild dysfunction. The lowest left ventricular ejection fraction recorded in our study population was 40%.

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There are reports of associations between RBC trans 18:1n-9 and total trans fatty acids and acute coronary syndrome (24) and coronary heart disease between RBC total trans fatty acids and trans 18:2, but not trans 18:1n-9, and risk of primary cardiac arrest (3). We found highly significant positive associations between RBC and atrial trans fatty acids. Whether or not cardiac trans fatty acids are important in the mechanism of risk is not known. Potential mechanisms for the increased cardiovascular disease risk of trans fatty acid intakes include plasma lipids,
systemic inflammation, altered endothelial function, and effects on intracellular signaling (25). Despite there being no attribution to a direct cardiac effect, this is a possibility because of the relation between RBC trans fatty acids and risk of primary cardiac arrest (3). The proportions of trans fatty acids observed in RBC and plasma phospholipids in our study are considerably lower than those reported in the United States (24, 26, 27), which may reflect differences in trans fatty acid intakes between the 2 countries, and the proportion of cardiac trans 18:1 fatty acids that we observed in this study is ≈50% of that reported in papillary muscle obtained from mitral valve replacement (13). Intakes in Australia are reported to be 0.15% (28) and 0.6% (29) of energy compared with >1.5% of energy as reported in North America (30, 31).

FIGURE 3. A, B: Univariate associations (linear regression and Pearson’s product-moment correlation coefficients) between atrial trans fatty acids and corresponding red blood cell (RBC) and plasma phospholipid trans fatty acids and total trans 18:1 and trans 18:2. n = 61.

FIGURE 4. Univariate associations (linear regression and Pearson’s product-moment correlation coefficients) between atrial oleic acid (OA) and corresponding red blood cell (RBC) and plasma phospholipid OA. n = 61.
In conclusion, the results show the reliability of estimating human cardiac n−3, n−6, and trans fatty acid status from blood-derived biomarkers over a range of fatty acid intakes that represent community dietary values.

We thank all of the administrative, theater, and ward staff of the Cardiothoracic Surgical Unit of the Royal Adelaide Hospital for their invaluable assistance in conducting this trial.

The authors’ responsibilities were as follows—RGM, MJJ, LGC, and GDY: designed the study and obtained funding; RGM and KCR-T: conducted the study; RAG: analyzed the fatty acids; RGM: analyzed the data and wrote the first draft of the manuscript; and JRME, RS, and PS: provided significant advice and consultation. All authors contributed to the final draft of the manuscript. None of the authors had any conflicts of interest.

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