Dietary-Induced Changes in Fatty Acid Composition of Human Plasma, Platelet, and Erythrocyte Lipids Follow a Similar Time Course

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ABSTRACT The dietary-induced changes in the fatty acid composition of plasma, platelet, and erythrocyte lipids were measured as a function of time. Healthy adults consumed a diet rich in saturated fat (18% total energy, TE) for 19 d and then crossed over, without washout, to a diet rich in (n-6) polyunsaturated fat (10% TE) for a further 19 d. The fatty acid composition of plasma and blood cell lipids was measured in blood samples collected from fasting subjects on d 0, 1, 2, 5, 8, 12, and 19 of consuming the diet rich in (n-6) polyunsaturated fats. The linoleic acid composition of all plasma, platelet, and erythrocyte lipids increased to a plateau within 19 d, reaching at least 70% of maximum within 5 d. The maximum increase in linoleic acid composition of erythrocyte phosphatidylcholine was 3.8 mol% at d 12; the increase at d 1 was 2 mol% and at d 5 was 3.2 mol%. The decrease in pentadecanoic acid composition followed a similar time course in all lipids with the exception of plasma phospholipids. Our results show that the time course of dietary-induced changes in erythrocyte fatty acid composition is similar to that in plasma and platelet lipids. These results provide convincing, albeit indirect evidence that the exchange of fatty acids from plasma to erythrocytes and platelets is a major determinant of their membrane fatty acid composition. J. Nutr. 136: 565–569, 2006.

KEY WORDS: • fatty acids • erythrocytes • plasma • biomarker

Biomarkers of fatty acid intake offer an objective alternative to dietary assessment because they reflect actual rather than reported intake, thus avoiding, for example, the particular problems in dietary assessment of under- or overreporting food consumption or quantifying “hidden fats” in the diet (1,2). The proportion of certain fatty acids in plasma, blood cells, and adipose tissue correlates with fatty acid intake, although the strength of correlation is not uniform across the different tissues or for all of the major classes, i.e., saturates, monounsaturates, and polyunsaturates, or individual fatty acids (3–8). In general, those fatty acids that increase, decrease, or remain unchanged in one tissue, in response to a change in fatty acid consumption, also tend to do so in other tissues (5,9–14).

One of the major unresolved matters concerning biomarkers is the extent to which the fatty acid composition of the different tissues reflect long-term (i.e., usual) or short-term (i.e., recent) fatty acid intake. Adipose tissue has a slow turnover rate compared with plasma and blood cells and is considered the best long-term biomarker; however, the invasive nature of adipose tissue aspiration usually leads investigators to collect and measure fatty acids in plasma or other blood cells when large numbers of participants are involved. There is a commonly held view that erythrocytes are a better long-term marker of fat intake than platelet or plasma lipids because the turnover of erythrocytes (120-d lifespan) is much slower than that of platelets (10 d) (17) or plasma lipids such as phospholipids (PL),1 cholesterol esters (CE), and triacylglycerols (TAG). In one of the few studies designed to compare the changes in fatty acid composition in different tissues as a function of time, Katan et al. (11) found that when monks were supplemented with fish oil for 12 mo, eicosapentaenoic acid increased in serum much more quickly than in erythrocytes, reaching half of the steady-state proportion in 4.8 and 28.1 d, respectively; adipose tissue eicosapentaenoate did not reach a plateau during the 12 mo. In contrast, the results of 2 other studies suggested that eicosapentaenoic acid composition of erythrocytes changed more rapidly, reaching steady state after 2 (18) and 3 wk (19) of fish oil supplementation. The discrepancy in these results led us to measure the fatty acid composition of RBC and platelet PC and plasma CE, TAG, and PL as a function of time over a 19-d period when participants changed from consuming a diet rich in saturated fat to one high in (n-6) polyunsaturated fat. The results of this work have particular relevance for determining the relative merits of erythrocytes or plasma as markers of dietary fat intake.

SUBJECTS AND METHODS

Subjects. Participants were recruited from students enrolled at the University of Otago. The Human Ethics Committee of the University

1 Supported by an Otago Research Grant.
2 To whom correspondence should be addressed. E-mail murray.skeaff@stonebow.otago.ac.nz.
3 Abbreviations used: CE, cholesterol ester; PC, phosphatidylcholine; PL, phospholipid; P:S ratio, polyunsaturated to saturated fat intake ratio; TAG, triacylglycerol; TE, total energy.
of Otago approved the study and all participants gave informed consent. Participants were excluded if they were affected by a metabolic disorder, smoked, or were taking medication known to alter plasma lipids. Twenty participants (19 women and 1 man) volunteered; they ranged in age from 20–31 y. Nineteen of the original 20 participants completed the study. Participants were weighed in light clothing at baseline and then on the last day of each dietary intervention period.

Diet. Participants consumed a diet rich in saturated fat for 19 d and then crossed over, without washout, to consume a diet rich in (n-6) polyunsaturated fat for a further 19 d, after which they returned to their usual diet. The results of this study were generated from the second 19-d period of the dietary trial.

The total fat content of the saturated and (n-6) polyunsaturated fat diets was designed to be between 30 and 33% of total energy (TE). The details of the diets were published elsewhere (20). Briefly, participants were instructed to select a background diet low in fat that would be consumed throughout the 5-wk study period. In addition to this background diet, participants were encouraged to consume butter and high-fat dairy foods while they were consuming the diet rich in saturated fat or safflower oil, and a sunflower-based table spread when they were consuming the diet rich in (n-6) polyunsaturated fat.

Assessment of dietary intake. For each diet followed, the participants completed a 3-d diet record. The energy and nutrient content of the reported diets was calculated with reference to the New Zealand food composition database, which contains the energy and nutrient content of 1800 foods commonly consumed in New Zealand.

Analysis of the fatty acid composition in blood lipids. Blood samples were taken from fasting subjects on the last day of consuming the SAT diet, which represented d 0 of the (n-6) polyunsaturated fat diet, and subsequently on d 1, 2, 5, 8, 12, and 19. After an overnight fast of at least 12 h, venous blood was collected into Vacutainers containing disodium EDTA for plasma lipid fatty acid analysis. For platelet and erythrocyte fatty acid analysis, venous blood was collected into Vacutainers containing ACD (85 mmol/L citric acid, 67 mmol/L trisodium citrate, 111 mmol/L dextrose) with 1 volume for 9 volumes of blood. Plasma to be used for the analysis of plasma fatty acids was separated by low-speed centrifugation (2000 × g) and stored at −80°C until analysis. Platelets were isolated according to the method of Lagarde et al. (21) and platelet and erythrocyte lipids were extracted within 2 h of blood collection.

After the removal of platelet-rich plasma, erythrocytes were isolated, washed and stored at −80°C as previously described (22). All blood samples were processed for storage within 1 h of blood collection.

Plasma, platelet, and erythrocyte lipids were separated according to the method of Bligh and Dyer (23). Plasma, platelet, and erythrocyte lipids were separated using TLC, and the fatty acid composition was determined as previously described (22,24). The precision of the fatty acid analysis was established by repeated measures on a pooled plasma or erythrocyte sample; ~1 pooled plasma or erythrocyte sample was extracted for every 10 experimental samples. The CV for the fatty acid analysis was established by repeated measures on a pooled plasma or erythrocyte sample. The CV for the fatty acid analysis was calculated from a statistical package SAS (version 8.0) which adjusts for correlations that occur within person, as a result of collecting multiple measurements from each person over time.

Statistical analyses. The mean differences (95% CI) in daily energy and nutrient intakes between the saturated and (n-6) polyunsaturated fat diets were calculated by regression analysis using the cluster option (Stata version 5.0). The cluster option adjusts for the correlations that occur within person as a result of collecting multiple diet records for each person.

Differences in the fatty acid composition of plasma, platelet, and erythrocyte lipids between d 0 and 19 were calculated using a Students paired t test (SPSS version 6.1). Differences were considered significant when P < 0.05.

We compared the fatty acid composition of plasma, platelet, and erythrocyte lipids at d 0 with d 1, 2, 5, 8, 12, and 19 of consumption of the diet rich in (n-6) polyunsaturated fat. This comparison was performed using a random effects model to model blood fatty acids (linoleic acid or pentadecanoic acid) as a function of the day. Analyses were carried out using the MIXED procedure from the statistical package SAS (version 8.0) which adjusts for correlations that occur within person, as a result of collecting multiple measurements from each participant over time.

We then calculated the d 0–19 changes in linoleic acid and pentadecanoic acid content of plasma, platelet, and erythrocyte lipids and used Spearman Rank Correlation (SPSS, version 6.1) to determine the relation between the fatty acid changes in different tissues. The change in fatty acid composition between d 0 and 5 was chosen because we calculated the differences in least-squares means from the random effects model and then grouped days into homogeneous subsets in which mean fatty acid levels did not differ. No significant differences were noted in the linoleic acid content between d 5, 8, 12, and 19 for all fractions. Furthermore, in all tissues, the changes in linoleic acid between d 0 and 5 were more than two-thirds of the maximum change by 19 d. Correlations of the change in linoleic acid or pentadecanoic acid between the tissues were examined only when a significant change in linoleic acid or pentadecanoic acid occurred in both blood lipid fractions.

RESULTS

Healthy, young adults (1 man, 18 women) aged 22.2 ± 2.7 y (mean ± SD) completed the study. Body weight was unaffected by the diets (P > 0.05); it was 65.8 ± 6.7 kg and 66.4 ± 6.6 kg at the end of the saturated and (n-6) polyunsaturated fat diets, respectively.

Intake of energy, protein, carbohydrate, and total fat by participants did not differ between the saturated and polyunsaturated fat diets (Table 1). Participants consumed 7%TE (14 g/d) more polyunsaturated fat and 10%TE (21 g/d) less saturated fat during the (n-6) polyunsaturated fat diet, representing a 1.1 unit difference in the polyunsaturated to saturated fat (P:S) ratio.

The changes in fatty acid composition from the beginning to the end (d 0 compared with 19) of the (n-6) polyunsaturated diet were qualitatively similar in all plasma, platelet, and erythrocyte lipids (Table 2); there were marked decreases in the saturates, myristic acid (14:0), pentadecanoic acid, and palmitic acid (16:0) accompanied by large increases in linoleic acid. With only a few exceptions, there were small but significant decreases in the α-linolenic acid [18:3(n-3)] and eicosapentaenoic acid [20:5 (n-3)] composition and increases in arachidonic acid [20:4 (n-6)] in all pools.

The changes in linoleic acid and pentadecanoic acid composition of all tissues as a function of time are shown Figures 1 and 2, respectively. One day after switching from the saturated to the (n-6) polyunsaturated fat rich diet, the mean linoleic acid content of plasma TAG and erythrocyte PC

### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>SFA diet</th>
<th>PUFA diet</th>
<th>Difference ± TE</th>
<th>% TE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kJ/d</td>
<td>8398 ± 1843</td>
<td>7964 ± 1803</td>
<td>-433 (−1380, 512)</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>14 ± 3</td>
<td>15 ± 4</td>
<td>1 (−1, 4)</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>50 ± 6</td>
<td>54 ± 6</td>
<td>4 (0, 7)</td>
<td></td>
</tr>
<tr>
<td>Total fat</td>
<td>34 ± 7</td>
<td>30 ± 4</td>
<td>-4 (−8, 1)</td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td>18 ± 4</td>
<td>8 ± 2</td>
<td>-10 (−12, −7)</td>
<td></td>
</tr>
<tr>
<td>MUFAs</td>
<td>10 ± 3</td>
<td>9 ± 2</td>
<td>−1 (−2, 1)</td>
<td></td>
</tr>
<tr>
<td>PLs</td>
<td>3 ± 1</td>
<td>10 ± 4</td>
<td>7 (5, 9)</td>
<td></td>
</tr>
<tr>
<td>P:S ratio</td>
<td>0.2 ± 0.2</td>
<td>1.3 ± 0.7</td>
<td>0.1 (0.8, 1.5)</td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mg/d</td>
<td>254 ± 109</td>
<td>144 ± 73</td>
<td>-110 (−178, −43)</td>
<td></td>
</tr>
</tbody>
</table>

1 Values as means ± SD, n = 19.

2 Mean difference (95% CI) is [PUFA − SFA] calculated from a regression model.
TABLE 2
Fatty acid composition of plasma TAG, PL, CE, platelet, and erythrocyte PC in subjects at 0 and 19 of consuming the diet rich in polyunsaturated fat

<table>
<thead>
<tr>
<th></th>
<th>Plasma TAG</th>
<th>Plasma PL</th>
<th>Plasma CE</th>
<th>Platelet PC</th>
<th>Erythrocyte PC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d0</td>
<td>d19</td>
<td>d0</td>
<td>d19</td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>4.27 ± 1.74</td>
<td>2.97 ± 0.79*</td>
<td>0.79 ± 0.20</td>
<td>0.68 ± 0.31*</td>
<td></td>
</tr>
<tr>
<td>15:0</td>
<td>0.67 ± 0.25</td>
<td>0.42 ± 0.08**</td>
<td>0.35 ± 0.05</td>
<td>0.27 ± 0.04**</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>30.59 ± 3.00</td>
<td>28.91 ± 2.99*</td>
<td>33.14 ± 1.54</td>
<td>32.77 ± 1.60</td>
<td></td>
</tr>
<tr>
<td>16:1</td>
<td>8.38 ± 1.43</td>
<td>7.97 ± 1.06*</td>
<td>1.65 ± 0.35</td>
<td>1.67 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>3.15 ± 0.57</td>
<td>2.88 ± 0.64</td>
<td>12.28 ± 0.81</td>
<td>12.31 ± 1.18</td>
<td></td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>34.71 ± 3.92</td>
<td>36.17 ± 3.91</td>
<td>11.47 ± 0.86</td>
<td>10.5 ± 1.42**</td>
<td></td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>10.96 ± 2.87</td>
<td>14.12 ± 3.13*</td>
<td>17.55 ± 2.65</td>
<td>19.53 ± 2.85*</td>
<td></td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>1.13 ± 0.37</td>
<td>0.89 ± 0.19*</td>
<td>0.33 ± 0.09</td>
<td>0.23 ± 0.05*</td>
<td></td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>0.75 ± 0.22</td>
<td>0.92 ± 0.27*</td>
<td>7.11 ± 1.02</td>
<td>7.75 ± 1.11</td>
<td></td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>0.27 ± 0.24</td>
<td>0.22 ± 0.34</td>
<td>0.92 ± 0.32</td>
<td>0.61 ± 0.18*</td>
<td></td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>0.28 ± 0.17</td>
<td>0.24 ± 0.09</td>
<td>0.68 ± 0.14</td>
<td>0.59 ± 0.15*</td>
<td></td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>0.70 ± 0.32</td>
<td>0.60 ± 0.27</td>
<td>2.24 ± 1.26</td>
<td>1.95 ± 1.53</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 19. Symbols indicate different from d 0: *P < 0.05, **P < 0.01, ***P < 0.001 (Student’s paired t test).
as a function of time, the changes in arachidonic acid paralleled those of linoleic acid.

Significant correlations ($P < 0.05$) were observed in the d 0–5 changes in linoleic acid composition between each of the plasma, platelet, and erythrocyte lipids (Table 3). The strongest correlation ($r = 0.80$, $P < 0.001$) was between the change in platelet PC and erythrocyte PC. Other correlations ranged from $r = 0.41$ to $r = 0.77$, respectively (Fig. 1).

**DISCUSSION**

The results of our study indicate that dietary-induced changes in the fatty acid composition of plasma PL and CE and platelet and erythrocyte PC as a function of time are qualitatively similar and largely complete within 2 wk. Furthermore, we showed that the pentadecanoic acid composition of plasma, platelet, and erythrocyte lipids was decreased when participants consumed the diet high in polyunsaturated, and low in dairy fat.

To induce the changes in tissue fatty acids, we chose to replace saturated fat, derived predominantly from dairy fat, with (n-6) polyunsaturated fat from vegetable oils because this is a practice consistent with most dietary guidelines, and our results would be relevant and applicable to monitoring population and individual compliance with such recommendations. We limited reporting of the fatty acid changes as a function of time (Figs. 1 and 2) to linoleic and pentadecanoic acids because results from previous cross-sectional studies showed that these 2 fatty acids were good biomarkers of (n-6) PUFA and dairy fat intake, respectively (7,8,10).

The rapid increase in linoleic acid in plasma erythrocyte PC during the first 5 d of consuming the (n-6) polyunsaturated fat diet is convincing evidence of fatty acid transfer from plasma to erythrocytes; the increase cannot be explained by entry into the circulation of new erythrocytes. With $<5\%$ of erythrocytes being replaced in 5 d (120-d life span) (16) the linoleic acid composition of PC in new erythrocytes would have had to be 100 mol% to account entirely for the observed change by d 5. The exchange of fatty acids from plasma to erythrocytes or platelets can occur by several routes: transfer of albumin-bound nonesterified fatty acids through fatty acid binding proteins, transfer as spillover during lipoprotein lipase catalyzed hydrolysis of TAG in chylomicrons or VLDL, or direct transfer of PC from plasma lipoproteins to erythrocyte and platelet membranes (25–29). Our results suggest that fatty acid transfer from plasma to erythrocytes plays a predominant role in determining erythrocyte membrane fatty acid composition when dietary fat intake is altered.

We cannot exclude the possibility that with several more months of intervention, a small and steady increase in linoleic acid composition of erythrocyte PC would have occurred while the entire population of erythrocytes was replaced. However, this seems unlikely given the sharp leveling-off of linoleic acid in erythrocytes between d 5 and 19. In one of the few biomarker studies of long duration (12 mo) Katan et al. (11) found that eicosapentanoic acid in erythrocytes increased until reaching a plateau at 6 mo; only half of the increase occurred in the first 28 d. This contrasts with other fish oil supplementation trials, admittedly of shorter duration, in which no further increase in erythrocyte eicosapentanoate was found after 2–3 wk (18,19); the latter time course was more similar to our results.

In the case of linoleic acid, the platelet PC exchange from plasma to platelets must also contribute to the rapid increase between d 0 and 5 although the contribution of newly generated platelets to the change may be greater than for erythrocytes because the life span of platelets is $\sim$ 10 d (17). The strong correlation between the d 0–5 change in platelet and erythrocyte linoleate ($r = 0.80$, $P < 0.001$) suggests that very similar mechanisms of fatty acid incorporation are operating in the 2 types of blood cells.

We are confident that participant compliance to the diets was high throughout the trial and that diet records accurately reflected what was consumed. This confidence is based on the quality of the diet records, the close agreement between the predicted and actual changes in plasma total cholesterol [previously reported (20)], and the close agreement between the predicted and actual change in plasma CE linoleate between d 0 and 19, a 6.2 weight% predicted change (10) and a 6.9 weight% (6.8 mol%) actual change. Furthermore, the pattern of changes in fatty acid composition of plasma, platelets, and erythrocytes at the end of the (n-6) polyunsaturated fat diet was comparable to results from studies of longer duration in which diets high in saturated or (n-6) polyunsaturated fat were consumed for periods ranging from 2 wk to 6 mo (5,13,14, 30–33).

The significant decrease in linoleic acid composition of plasma TAG between d 12 and 19 suggests a change in dietary compliance during this period; however, the downward trends were much smaller in plasma PL and CE. This latter observation along with the fact that plasma TAG linoleate turnover is high, i.e., the composition reached a maximum after little more than 1 d with consumption of the polyunsaturated fat diet, argues that if compliance did change, it was probably within 1 or 2 rather than 5 d of the final blood sample. Durrington et al. (13) reported a similar rebound, although, as in our study, the participants were not provided with controlled diets.

The decrease in the pentadecanoic acid composition of lipids as a function of time followed a similar time course to that of linoleic acid, with the exception of plasma PL in which there was a continuous decline during the 19 d with no evidence of a plateau being reached. These time course results are novel and show that pentadecanoic acid in plasma, platelet, or erythrocyte lipids is a good biomarker of changing intake of dairy fat. Previous evidence for pentadecanoic acid as a biomarker was derived from cross-sectional studies in which intakes of saturated and dairy fat were correlated with the pentadecanoic acid composition of adipose tissue, as well plasma CE and PL (7,8).

**TABLE 3**

Correlations between d 0–5 changes in the fatty acid composition of the different lipids when participants consumed the diet rich in polyunsaturated fat

<table>
<thead>
<tr>
<th></th>
<th>Plasma PL</th>
<th>Plasma CE</th>
<th>Platelet PC</th>
<th>RBC PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2 (n-6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma TAG</td>
<td>0.53*</td>
<td>0.52*</td>
<td>0.66**</td>
<td>0.62**</td>
</tr>
<tr>
<td>Plasma PL</td>
<td>0.41</td>
<td>0.77***</td>
<td>0.53*</td>
<td></td>
</tr>
<tr>
<td>Plasma CE</td>
<td>0.51*</td>
<td>0.55**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet PC</td>
<td>0.80***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma TAG</td>
<td>NT²</td>
<td>0.03</td>
<td>0.41</td>
<td>0.64**</td>
</tr>
<tr>
<td>Plasma PL</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>Plasma CE</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>Platelet PC</td>
<td>NT</td>
<td>-0.55*</td>
<td>0.40</td>
<td></td>
</tr>
</tbody>
</table>

1 $n = 19$.
2 NT, not tested because there was no significant difference in 15:0 composition of plasma PL between d 0 and 5.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. 

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The fatty acid composition as a function of time was measured when switching from the diet high in saturated fat to one high in polyunsaturated fat; therefore, it cannot be assumed that administering the diets in reverse order would produce the opposite changes in fatty acid composition, although in a study in which participants crossed over 3 times (4 wk duration each) between diets high in saturated or (n-6) polyunsaturated fats, the changes in linoleic acid composition of plasma TAG were the same (30).

The d-0–5 changes in linoleic acid composition of plasma, platelet, and erythrocyte lipids were correlated, in general explaining ~40–60% of the variation in change between the biomarkers. In simple terms, these results indicate that for a given fat intake, individuals who have a large change in fatty acid composition of one tissue also tend to have large changes in the other fractions. Previously, the only evidence showing the relation between the fatty acid composition of plasma, platelet, and erythrocyte lipids has come from cross-sectional studies (34–38).

One of the more common questions about fatty acid biomarkers is whether erythrocyte or plasma fatty acids should be measured. Our results suggest that in the context of monitoring saturated, particularly dairy fat, and (n-6) polyunsaturated fat intake, the fatty acid composition of erythrocyte PC provides no clear advantage over plasma CE and PL; all lipids showed a similar rate of change in linoleic acid as a function of time and the changes in each lipid were well correlated. Thus, neither appears to reflect fat intake longer than the previous 1–2 wk. This has implications for dietary intervention trials and observational studies because, at best, erythrocytes and plasma lipids can reflect only recent rather than long-term compliance.

**LITERATURE CITED**