Changes in red blood cell phospholipid acylation during the fed-to-fasted transition

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We investigated the effects of an overnight fast on composition and fatty acylation of rat red blood cell membrane phospholipids. Fasting produced decreases ($P < 0.01$) in total phospholipid and phosphatidylcholine contents, as well as decreases in acylation rates in phosphatidylcholine ($P < 0.01$) and phosphatidylethanolamine ($P < 0.05$). Among the seric biochemical indices known to vary with the feeding state, only triacylglycerol levels were positively correlated ($P < 0.05$) with red cell acylation rates both in phosphatidylcholine and in phosphatidylethanolamine. However, this correlation did not appear to reflect a direct and causal relationship. Thus, changes in membrane lipid acylation related to a nearly physiological feeding-fasting transition might modulate the lipid composition of membrane, and thereby its function.

Keywords: phospholipid; fatty acylation; red blood cells; fasting state; plasma lipids; rat

Introduction

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are major components of the outer and inner layers, respectively, of most biological membranes.[1] In nucleated cells, PC and PE participate in a state of dynamic equilibrium involving de novo synthesis, transport, and degradation, as well as in situ modifications of their base or acyl groups.[2] Phospholipid metabolism in circulating red blood cells (RBC) is essentially limited to deacylation-reacylation reactions, which are catalyzed by phospholipases A and acyl-CoA: lysophosphoglyceride acyltransferases, respectively.[3] These reactions are considered to be primarily involved in the establishment and maintenance of the molecular lipid species that govern the physicochemical structure of the membrane lipid matrix, the precise control of which appears important for proper cell functioning.[4] Although phospholipid acylation rates may be experimentally modified in rat RBC by estrogen treatment[5] or insulin deprivation,[6] the physiological regulation of deacylation-reacylation reactions is at present unknown. The present results reveal that short-term nutritional changes such as the fed-to-fasted transition influence glycerophospholipid content and acylation rates in intact circulating RBC.

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Materials and methods

Female Sprague-Dawley rats (~ 160 g) were housed at 22° C on a 0800-2000 hr lighting schedule. They were allowed to acclimate to their environmental conditions for at least 2 weeks before the experiments. They had free access to water and to a nonpurified standard diet containing, by wt, 5% lipid, 49.5% carbohydrate, and 23.5% protein until 1 hr (fed control) or 12 hr (fasted) before the sacrifice; fasted animals were given only water. Rats were sacrificed at 9:00 by decapitation under light anesthesia (25 mg/kg sodium pentobarbital, intraperitoneally).

Blood samples

Unless otherwise stated, blood was collected in 0.13 M sodium citrate as an anticoagulant and was processed within 2 hr. A platelet-free and leukocyte-free suspension of RBC was prepared from each sample by filtration on cellulose.[7] Purified RBC were centrifuged for 5 min at 750*g* and washed twice with buffer A (in mM: 140 NaCl, 5 KCl, 1 $MgSO_4$, 5 glucose and 10 Tris-HCl; pH 7.4). Cells were then adjusted to 25% hematocrit and counted in the final suspension using a Coulter Counter model S (coefficient of variation < 0.5%).

Cell incubation

Acylation rates in PC and in PE were measured in suspensions of intact RBC as previously described.[8] RBC were incubated for 45 min at 37° C in a medium (pH 7.4) containing 100 μl of RBC suspension (4×10^8 cells), 100 μl of medium B (medium A + 1 mM $CaCl_2$), 100 μl medium B containing defatted albumin (0.16%, wt/vol) and [9,10− ^{3}H] oleic acid (final concentration, 6 μM; ~ 3.4 μCi). Prior to incubation, the substrate mixture was sonicated (30 W) for 1 min at 20° C using a Branson sonicator model B-12 (Heat system Ultrasonics, Inc. Farmingdale, NY). After incubation, RBC were washed twice with 40 vol of medium A containing 2% (wt/vol) albumin and lysed with 20 vol of ice-cold water.

Isolation of RBC phospholipids

Aliquots [~ 40 nmol phospholipid phosphorus (PL-P)] from total lipids extracted from the lysates[3] were concentrated and submitted to high-performance thin-layer chromatography on silicagel 60 plates (Merck, S.A., Darmstadt, Germany) developed in chloroform/methanol/water (75/25/3, by vol.) to isolate PC (Rf: 0.25) and PE (Rf: 0.42). The gel areas containing PC and PE, identified with reference standards, were located by iodine vapor, scraped, and analyzed for Pi and radioactivity contents. Acylation rates were calculated from the specific activities of the exogenously added [^{3}H]oleic acid, which was assumed to be constant during incubation. Results of assays carried out in triplicate agreed within 4% and 5% of acid incorporated for PC and for PE, respectively.

Other procedures

The RBC content in cholesterol was measured in lipid extracts using an enzymatic assay (Merck, S.A.); PL-P was measured according to the procedure of Rouser et al.[9] Concentrations of glucose, cholesterol, triacylglycerol (TAG), phospholipid, and inorganic phosphorus (Pi) in serum were determined by enzymatic methods; fatty acids (FA) and insulin were quantified by the method of Barash and Akow[10] and by radioimmunoassay (Cis-Bioindustries, Gif-sur-Yvette, France), respectively. Whole blood content in ATP was determined on trichloracetic extracts immediately after blood withdrawal by enzymatic assay using a commercial kit (Sigma Diagnostics, St. Louis, MO); ATP values were expressed on a per-cell basis (nmol/10^{10} RBC) since almost all the blood ATP is found in RBC.[11]

Expression of results

Values were expressed as mean ± SD. Differences between means in fasted and fed animals were compared by Student's t test. A value of $P < 0.05$ was considered as significant.

Materials

[9,10− ^{3}H] Oleic acid (4.8 Ci/mmol) was from Amersham International (UK). Unlabeled oleic acid was from Sigma Chemical Co. (St. Louis, MO) and defatted albumin from Calbiochem-Behring Co. (La Jolla, CA). Radioactivity was determined in a LKB liquid scintillation counter using PCS II (Amersham International) as counting solution (coefficient of efficacy for ^{3}H: 60%).

Results

Acylation reactions were performed with [^{3}H]oleic acid, the most abundant fatty acid found in association with albumin[12] at a value of the albumin-to-fatty acid molar ratio of 2.0, which is well within the physiological range of 1.25–5.0 found in rat plasma. Intact RBC submitted to 45-min incubation at 37° C incorporated [^{3} H]oleic acid into PC and PE, the specific activities of which increased according to zero-order kinetics. Acylation rates were proportional to the number of RBC within the range $0.5–5 \times 10^8$ cells.

As shown in *Figure 1*, mean molar acylation rates in PC and PE were found to be 36% and 25% lower, respectively, in RBC from rats fasted overnight than in fed rats. This was associated, in fasted animals, with 21% and 18% decreases in total phospholipids and in PC, respectively; other membrane lipids were unchanged (*Table 1*). As expressed on a per-cell basis (pmol acyl incorporated/10^{10} RBC) and taking into account the cumulative effect of decreases both in content and in molar acylation rate of PC in the fasted state, the net amount of oleic acid entering PC was 47% lower in fasted than in fed rats (79 ± 8 versus 148 ± 28, respectively); the corresponding values were 58 ± 4 versus 77 ± 9 for PE.

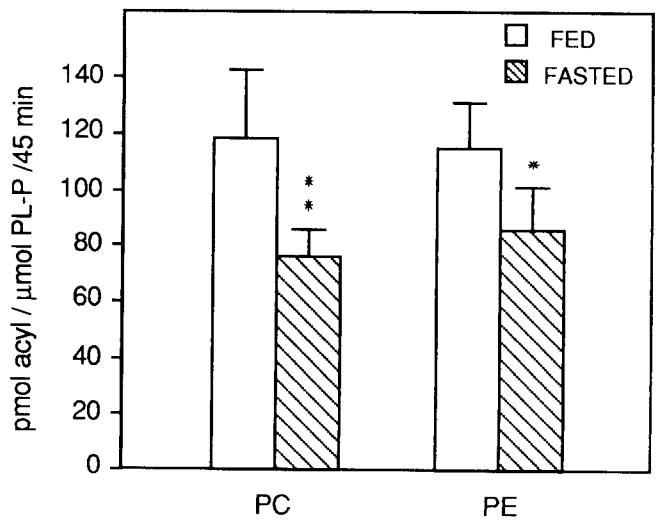

Figure 1 Acylation rates in PC and PE of red blood cells from fed (open columns, $n = 5$) and fasted (hatched columns, $n = 5$) rats. Phospholipid acylation was measured with [^{3}H]oleic acid as described in Materials and methods. Significantly different from the fed group $^*P < 0.05$; $^{**}P < 0.01$.

Table 1 Red blood cell membrane lipids in the fed and fasted states

Red blood cell component[a]	Fed rats	Fasted rats
Cholesterol	1875 ± 179	1660 ± 183
Phospholipids	2680 ± 152	2125 ± 233[b]
Phosphatidylcholine	1245 ± 106	1025 ± 86[b]
Phosphatidylethanolamine	660 ± 65	680 ± 66

[a] Each value (in nmol/10^{10} cells) is the mean ± SD for 5 rats.
[b] Fasted values are statistically different ($P < 0.01$) from fed values.

Table 2 Concentrations of serum components in the fed and fasted states

Plasma component[a]	Fed rats	Fasted rats
Glucose (mmol/l)	6.50 ± 0.71	4.50 ± 0.47[c]
Fatty acids (mmol/l)	0.54 ± 0.08	0.84 ± 0.06[c]
Triacylglycerol (mmol/l)	0.93 ± 0.11	0.41 ± 0.10[c]
Cholesterol (mmol/l)	0.87 ± 0.06	0.78 ± 0.21
Phospholipids (mmol/l)	1.18 ± 0.30	1.21 ± 0.20
Phosphorus (mmol/l)	1.05 ± 0.14	1.90 ± 0.18[c]
Insulin (pmol/l)	160 ± 80	63 ± 11[b]

[a] Values are mean ± SD for five rats.
[b] Fasted values are statistically different from fed values at $P < 0.01$.
[c] Fasted values are statistically different from fed values at $P < 0.001$.

As predicted, the serum levels of glucose, TAG, and insulin were respectively 31%, 56%, and 61% lower in the fasted state, whereas FA and phosphorus were 56% and 81% higher, respectively; cholesterol and phospholipids were unchanged (*Table 2*). TAG levels in serum were positively correlated with acylation rates in RBC both for PC ($r = 0.70$; $P < 0.05$; $n = 10$) and for PE ($r = 0.72$; $P < 0.05$; $n = 10$) (*Figure 2*).

The mean ATP content of RBC was significantly ($P < 0.01$) higher in fasted than in fed rats (493 ± 40 versus 344 ± 53 nmol/10^{10} RBC, respectively). Confirming a previous report,[13] ATP values were positively correlated ($r = 0.81$; $P < 0.01$; $n = 10$) with Pi levels in serum.

Discussion

The present results indicate that lipid composition and phospholipid acylation rates in circulating RBC are influenced by a short-term and nearly physiological change such as an overnight fast. The decrement in membrane PC content is likely to account, at least in part, for the parallel decrease in total phospholipids;

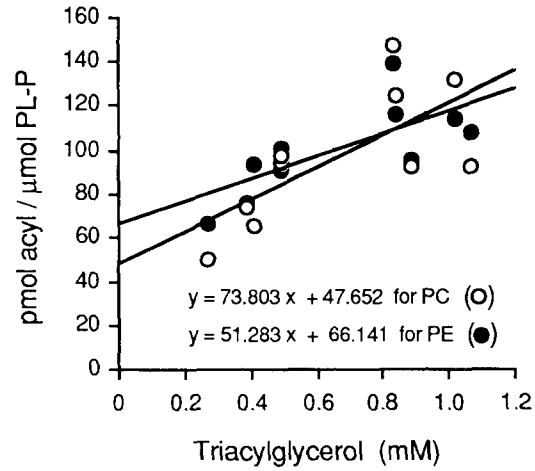

Figure 2 Relationship between acylation rates measured in vitro with [^{3}H]oleic acid in PC (○) and PE (●) of intact red blood cells and triacylglycerol concentrations in serum. Regression lines were calculated by method of least squares. PL-P, phospholipid phosphorus.

similar decreases have been described in rat RBC membranes after prolonged fasting.[14] The net entry of oleic acid into phospholipids is markedly decreased in the fasted state, particularly in PC, consonant with the fact that PC is the major fatty acid acceptor in circulating RBC.[15] Although the mechanism of this diet-related decrease in acyl incorporation is at present uncertain, it is in all likelihood related to diet-induced changes in plasma. At first sight, our finding of a positive correlation between acylation rates in RBC and TAG concentrations in plasma supports this hypothesis. However, a direct and causal relationship between the two metabolic events is not certain. We have observed that animals fasted for 12 hr and provided in their drinking water with glucose (20 g/L, equivalent to approximately 3% of the total caloric intake supplied by their usual standard mixed diet) elicited values of acylation rates comparable to those measured in RBC from fed animals, although no change in plasma TAG could be detected (data not shown). Note that acylation processes in RBC phospholipids occur at levels lower by several orders of magnitude than the mean concentrations at which major lipids (including TAG) circulate in plasma; thus, quantitative or qualitative changes undetectable in plasma lipids might be able to influence deacylation-reacylation reactions in RBC. Perhaps relevant to this hypothesis is the suggestion by Branchey and Buyden-Branchey[16] that chronic ethanol intake, a status also associated with increased plasma lipids, might modify the lipid composition of RBC membrane via plasma lipid changes. Equilibrium exchanges between phospholipids[17] and cholesterol[18] in RBC and those in plasma have been demonstrated.

It has been shown[19] that in rat, Pi concentration in plasma follows a circadian variation, with a rapid fall at the onset of feeding period followed by a rise thereafter. To our knowledge, a similar circadian variation

has not been directly characterized for ATP in RBC. A significant positive correlation appears to have been measured between Pi and ATP in RBC.[20] Taken together, these results are consistent with our finding of a relatively high ATP concentration in RBC after an overnight fast period. To be esterified, fatty acids need to be activated as acyl-CoA in an ATP-dependent process.[3] The coexistence in the fed state of high acylation rates with low ATP levels, confirms other reports[21,22] suggesting that ATP is not a limiting factor of acylation processes in RBC membranes.

Although deacylation-reacylation cycles occur continually in vivo at the expense of plasma fatty acids, the phospholipid composition of the membrane lipid matrix appears to be tightly controlled.[23] Modifications of lipid molecular species in RBC have been shown to induce morphological changes and altered osmotic sensitivity.[24] Since partially lethal lysophospholipids do not accumulate, it can be assumed that: (1) activities of the enzymes catalyzing acylation equilibrate with those catalyzing hydrolysis; (2) determination of acylation rates provide a reasonable estimation of the turnover of the phospholipid fatty acid moieties. The results presented indicate that significant modifications of acyl turnover occur in relation to short-term nutritional changes comparable, if not identical, to those induced by the physiological feeding-fasting cycle. The possibility that these modifications may modulate the lipid composition of cell membrane, and thereby its function, deserves further consideration.

References

1 Op Den Kamp, J.A.F. (1979). Lipid asymmetry in membranes. *Ann. Rev. Biochem.* **48,** 47–71

2 Yeagle, P.L. (1989). Lipid regulation of cell membrane structure and function. *FASEB J.* **3,** 1833–1842

3 Dise, C.A., Goodman, D.B.P., and Rasmussen, H. (1980). Definition of the pathway for membrane phospholipid fatty acid turnover in human erythrocytes. *J. Lipid Res.* **21,** 292–300

4 Stubbs, C.D., and Smith, A.D. (1984). The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim. Biophys. Acta* **779,** 89–137

5 Le Petit-Thévenin, J., Rahmani-Jourdheuil, D., Nobili, O., and Boyer, J. (1986). Ethinyl estradiol alters lipid composition and phosphatidylethanolamine metabolism in red blood cells. *J. Steroid Biochem.* **25,** 601–603

6 Le Petit-Thévenin, J., Nobili, O., and Boyer, J. (1988). Decreased acylation of phosphatidylcholine in diabetic rat erythrocytes. *Diabetes* **37,** 142–146

7 Beutler, E., and Gelbart, T. (1986). The mechanism of removal of leukocytes by cellulose columns. *Blood Cells* **12,** 57–64

8 Gastaldi, M., Lerique, B., Feugère, T., Le Petit-Thévenin, J., Nobili, O., and Boyer, J. (1988). Altered acylation of erythrocyte phospholipids in alcoholism. *Alcohol. Clin. Exp. Res.* **12,** 356–359

9 Rouser, G., Fleischer, S. and Yamamoto, A. (1970). Two dimensional thin-layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids* **5,** 494–496

10 Barash, H., and Akov, S. (1987). Improved ^{63}Ni radiochemical assay of free fatty acids in plasma. *Clin. Chem.* **33,** 176–179

11 Beutler, E. (1984). In *Red Cell Metabolism*, p. 122. Grune and Stratton, Inc., Orlando

12 Noel, J.K.F., and Hunter, M.J. (1972). Bovine mercaptalbumin and non-mercaptalbumin monomers interconversions and structural differences. *J. Biol. Chem.* **247,** 7391–7406

13 Travis, S.F., Surgerman, H.J., Ruberg, R.L., Dudrick, S.J., Delivoria-Papadopoulos, M., Miller, L.D., and Oski, F.A. (1971). Alterations of red-cell glycolytic intermediates and oxygen transport as a consequence of hypophosphatemia in patients receiving intraveinous hyperalimentation. *N. Engl. J. Med.* **285,** 763–768

14 Lima, V.L.M., Gillett, M.P.T., Silva, M.N., Maia, M.D., and Filho, M.C. (1986). Changes in the lipid composition of erythrocytes during prolonged fasting in lizard (*Tropidurus torquatos*) and rats (*Rattus norvegicus*). *Comp. Biochem. Physiol.* **83,** 691–695

15 Shohet, S.B. (1976). Mechanisms of red cell membrane lipid renewal. In *Membranes and Diseases* (L. Bolis, J.F. Hoffman, and A. Leaf, eds.), p. 61–74. Raven Press, New York

16 Branchey, M.H., and Buydens-Branchey, L. (1990). Are the effects of chronic ethanol administration on erythrocyte membrane mediated by changes in plasma lipids? *Drug Alcohol Depend.* **25,** 67–71

17 Van den Bosch, H. (1974). Phosphoglyceride metabolism. *Annu. Rev. Biochem.* **43,** 243–277

18 Hagerman, J.S., and Gould, R.G. (1951). The in vitro interchange of cholesterol between plasma and red cells. *Proc. Soc. Exp. Biol. Med.* **78,** 329–332

19 Talmage, R.V., Doppelt, S.H. and Cooper, C.W. (1975). Relationship of blood concentrations of calcium, phosphate, gastrin and calcitonin to the onset of feeding in the rat. *Proc. Soc. Exp. Biol. Med.* **149,** 855–859

20 Johnson, M.A., Tekkanat, K., Schmaltz, S.P., and Fox, I.H. (1989). Adenosine triphosphate turnover in humans. Decreased degradation during relative hyperphosphatemia. *J. Clin. Invest.* **84,** 990–995

21 Shastri, K., and Rubinstein, D. (1969). The metabolism of the erythrocyte. XIX. Factors affecting esterification of linoleate by human erythrocytes. *Can J. Biochem.* **47,** 967–975

22 Bröring, K., Haest, C.W.M., and Deuticke, B. (1989). Translocation of oleic acid across the erythrocyte membrane. Evidence for a fast process. *Biochim. Biophys. Acta* **986,** 321–331

23 Kuypers, FA., Roelofsen, B., Ob den Kamp, J.A.F., and Van Deenen, L.L.M. (1984). The membrane of intact human erythrocytes tolerates only limited changes in the fatty acid composition of its phosphatidylcholine. *Biochim. Biophys. Acta* **769,** 337–347

24 Van Deenen, L.L.M., Kuypers, F.A., Ob Den Kamp, J.A.F., and Roelofsen, B. (1987). Effects of altered phospholipid molecular species on erythrocyte membrane. *Ann. N.Y. Acad. Sci.* **492,** 145–155