Palmitoleic Acid in Erythrocytes from Carriers of Duchenne Muscular Dystrophy

Howland and Iyer (*1*) reported that erythrocyte membranes from carriers and patients of Duchenne muscular dystrophy had a decreased level of palmitoleic acid (1.4 percent and 1.2 percent, respectively, in total fatty acids) compared to membranes from normal subjects (4.9 percent).

We prepared fatty acid methyl esters from normal human erythrocytes by several published methods (*2*) and analyzed their composition by gas-liquid chromatography. In red cells, or in ghosts or lipids extracted from them, the amount of palmitoleic acid in the total fatty acids varied from only 0.8 to 1.2 percent. Several other investigators also found palmitoleic acid levels in human erythrocytes of 0.2 to 1.6 percent (*3*). Thus, normal human red cells contain palmitoleic acid at levels equal to or lower than those observed by Howland and Iyer in the red cells of Duchenne muscular dystrophy carriers and patients.

Palmitoleic acid is not a normal component of dietary fat, but is produced by the action of tissue Δ9-desaturase on palmitic acid. In animals on diets that are free of fat or linoleate, both of which stimulate hepatic Δ9-desaturase activities, palmitoleic acid in erythrocyte lipids increases to 2.5 to 3.5 percent of the total fatty acids (*4*), and there are only small amounts of linoleic acid (1 to 2 percent of total fatty acids). The large amount of palmitoleic acid observed by Howland and Iyer in normal subjects can not be related to diet because linoleic acid was not decreased to such low levels in erythrocytes.

The major lipids of human red cell membrane are phospholipids and cholesterol. Erythrocytes do not contain or have only trace amounts of glycerides and sterol esters (*5*). Hence the triglyceride content of normal human erythrocyte membranes reported by Howland and Iyer (*1*) must be 1.54 nanomoles rather than moles per milligram protein.

Howland and Iyer considered that in erythrocytes, palmitoleic acid is mostly in the triglycerides and concluded that a decrease in the composition of palmitoleic acid is, in part, related to a low membrane triglyceride content (*1*). We found that when the palmitoleic acid of erythrocytes is increased by dietary manipulation, the increase is reflected in the phosphatidyl choline and phosphatidyl ethanolamine fractions of erythrocyte membrane lipids. These results, and the fact that erythrocytes contain negligible amounts of triglycerides, suggest that if membranes from carriers of Duchenne muscular dystrophy had decreased amounts of palmitoleic acid, decreased amount of this acid in the phospholipids of erythrocyte lipids would more probably be the cause.

Human red cells contain appreciable amounts of plasmalogens (*6*) which produce dimethyl acetal derivatives during the preparation of fatty acid methyl esters. Elution of the heptadecanaldehyde dimethyl acetal derivative along with methyl palmitoleate during the gas-liquid chromatographic analysis could have given an abnormally high value for the amount of palmitoleic acid. Such a conclusion is supported by our observations that when the fatty acid methyl esters prepared from phosphatidyl ethanolamine fractions of erythrocytes were subjected to gas-liquid chromatography, the palmitoleic acid content was 3 to 4 percent. However, if the fatty acid methyl esters were purified by thin-layer chromatography and then analyzed, the palmitoleic acid content was less than 0.5 percent (*7*). Thus, the difference in the erythrocyte lipids of normal subjects and carriers of Duchenne muscular dystophy could be due to the content of plasmalogens rather than palmitoleic acid.

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References and Notes

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We had earlier considered the possibility that dimethyl acetals might account for the apparent differences in palmitoleic acid when comparing normal and dystrophic cells (*1*). This explanation seems unattractive for the following reasons. (i) The total plasmalogen content is the same in erythrocytes obtained from normal and Duchenne dystrophic individuals. Virtually all erythrocyte plasmalogen is in the phosphatidyl ethanolamine (PE) fraction. In normal cells, PE accounted for 21.2 ± 0.4 percent of phospholipids; the PE plasmalogen accounted for 7.5 ± 0.3 percent of total phospholipids. In hemizygous Duchenne-dystrophic cells the PE content was 20.3 ± 0.7 percent and the PE plasmalogen content 7.8 ± 1.0 percent. Thus, no significant differences were noted. (ii) Examination of the phospholipid dimethyl acetal composition by gas-liquid chromatography revealed no differences in the distribution of chain lengths between normal and Duchenne cells. (iii) Our routine preparation of membrane lipid for fatty acid analysis includes a saponification step. The deacylated plasmalogen residues occur in the nonsaponifiable fraction and, thus, do not interfere with fatty acid analysis (*2*).

It is true, as Rao and co-workers point out, that we report values for palmitoleic acid that are higher than theirs [but close to other literature values—for example, see (*3*)]. In this connection, we can only comment that variation in washing of cells, preparation of membranes, and estimation of chromatographic baselines can give rise to differences in composition data, especially for relatively minor fatty acids.

Finally, it should be said that the enzymic cause of differences in fatty acid content between normal and dystrophic cells remains undiscovered.

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