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# Phospholipid Composition of Liver Homogenates and Microsomes of the Lean and Obese Hyperglycemic (OB/OB) Mouse[1] (39889)

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*Introduction.* Research in this laboratory has been directed toward investigating the properties and physiological functions of insulin-degrading processes, focusing, in particular, on the role of glutathione-insulin transhydrogenase (GIT, thiol:protein-disulfide oxidoreductase; EC 1.8.4.2) [e.g., see (1-3)]. This enzyme catalyzes the inactivation of insulin via sulfhydryl-disulfide interchange by splitting the hormone at the disulfide bonds (4-7). The concentration of GIT in liver is under feedback control by the level of circulating insulin (8-13). In other words, when the insulin level in the blood is low (e.g., in starvation, diabetes), the liver GIT level is low; when the insulin level in the blood is high (e.g., in the starved-refed state, insulin-treated diabetes, or obesity), the liver GIT level is high. Studies with genetically obese hyperglycemic mice (ob/ob) and their lean litter mates have shown (12, 13) that, as previously reported for rats (14), the majority of mouse hepatic microsomal GIT occurs in a latent state; both the amount and proportion of the latent form, however, were much higher in the obese mice than in the lean (13). Since certain phospholipids [in particular, lysophosphatidylcholine (lysolecithin) and phosphatidic acid] are strong inhibitors of GIT activity *in vitro* (15), the possibility that the two phospholipids are present in higher amounts in the liver of the obese mouse, thereby resulting in the higher degree of latency of the GIT activity, was investigated. A literature search yielded no information on a comparison of the phospholipid content of livers from obese and lean mice, e.g., see (16) for a recent review. The present studies were therefore undertaken. To our knowledge, this is the first report on the phospholipid composition of the liver of the ob/ob mouse, which is a genetic mutant characterized by obesity, hyperinsulinism, and resistance to the hypoglycemic action of insulin (17).

*Methods.* Obese-hyperglycemic (ob/ob) C57BL/6J male mice and their lean litter mates (?/+) were obtained from Jackson Laboratories, Bar Harbor, Maine, and fed ad libitum on Purina rat chow. The mice were allowed to acclimatize for 10 days before they were sacrificed by decapitation. The mice were almost 8 weeks of age at the time of sacrifice. The excised livers were homogenized using a Teflon-fitted Potter-Elvehjem homogenizer, and microsomes were prepared (13, 14); these were kept frozen at −20° until used. The purity of the microsomal preparations was established by the assay of marker enzymes, which showed that they were free from mitochondrial proteins as judged by almost complete absence of succinate-INT reductase; the specific activity of glucose-6-phosphatase (a marker enzyme for the microsomes) was increased 4.5-fold over that of the homogenates.

A suspension (1.0 ml) of unfractionated liver homogenate or of isolated microsomes in 0.25 *M* sucrose-50 mM Tris, pH 7.5, was extracted by adding first 7 ml of methanol and then 14 ml of chloroform, as described by Dawson *et al.* (18), and filtered through a filter paper. The chloroform-methanol extract was washed three times by the procedure described by Folch *et al.* (19) to remove water-soluble substances. A sample of the lipid-containing lower phase was evaporated to dryness with a stream of pure nitrogen or argon and redissolved in 125 μl of chloroform-methanol. Aliquots (40 μl each) of the extracted lipid were applied to two activated precoated plates (20 × 20 cm)

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of silica gel (Q5, Quantum Industries), and duplicate aliquots of 10 μl were withdrawn for the determination of total lipid phosphorus. The plates were subjected to two-dimensional chromatography, using chloroform:methanol:ammonia (65:35:9) as the first solvent and chloroform:methanol:acetic acid:water (90:40:12:2) as the second solvent. The position of the phospholipids was detected by exposing the plates to $I_2$ vapors for a short time. The plates were then left in a hood until the iodine had vaporized, and the marked bands containing the phospholipids were scraped from the plate into test tubes. The phospholipids were quantitated by determination of phosphorus without elution (20) according to the method of Bartlette (21). For the lipids (e.g., phosphatidic acid, lysolecithin, etc.) which are present in small amounts, scrapings from two plates were combined for phosphorus determination. Individual phospholipids were identified by chromatography of reference standards which were obtained commercially.

*Results and discussion.* In Table I are given the body weights, liver weights, and liver phospholipid contents of the mice studied. The microsomal fractions from lean and obese liver contained 422 and 373 μg of total phospholipid/mg of protein ($P < 0.005$), respectively. Values of 270–350 μg/ml of protein have been reported (22, 23) for rat liver microsomes.

Table II shows the percentages of various phospholipid classes determined on unfractionated homogenates and microsomal fractions of livers obtained from lean and obese mice. Values obtained for mouse liver homogenates are similar to those reported for rat liver (24). Except for sphingomyelin, which was 38% lower ($P \leq 0.01$) in the obese liver than in the lean liver, the phospholipid content was similar in lean and obese liver homogenates. Examination of the microsomal fractions, however, revealed that the obese mouse contains a 46% higher level of phosphatidic acid ($P \leq 0.005$) and a 20% lower level of phosphatidylethanolamine ($P \leq 0.001$) than the lean mouse.

When these data are calculated as concentrations (micrograms per milligram of protein) of the various phospholipid classes, the

TABLE I. BODY WEIGHTS, LIVER WEIGHTS, AND PHOSPHOLIPID CONTENTS.[a]

| | Lean | Obese | *P* value |
|---|---|---|---|
| Body weight (g) | 21.01 ± 2.05 | 34.57 ± 1.65 | <0.001 |
| Liver wet weight (g) | 1.34 ± 0.19 | 2.74 ± 0.21 | <0.001 |
| Phospholipids (μg/mg of protein) | | | |
| Homogenates | 127.11 ± 23.15 | 124.45 ± 10.26 | n.s.[b] |
| Microsomes | 421.59 ± 62.72 | 373.35 ± 65.69 | <0.005 |

[a] Data shown are means ± SD for seven mice in lean group and nine mice in obese group. *P* values were determined by the two-tailed Student's *t* test.

[b] Statistically not significant (i.e., $P > 0.05$).

TABLE II. PHOSPHOLIPID CLASS PERCENTAGES OF THE UNFRACTIONATED HOMOGENATES AND MICROSOMES OF LIVERS FROM LEAN AND OBESE (OB/OB) MICE.[a]

| Lipid | Unfractionated homogenates | | | Microsomes | | |
|---|---|---|---|---|---|---|
| | Lean | Obese | *P* Value | Lean | Obese | *P* Value |
| Lysolecithin | 3.1 ± 1.53 (7) | 2.19 ± 0.82 (8) | n.s.[b] | 1.60 ± 0.50 (7) | 1.99 ± 0.69 (9) | n.s. |
| Sphingomyelin | 3.8 ± 0.94 (7) | 2.35 ± 0.58 (8) | 0.01 | 2.07 ± 0.35 (7) | 1.65 ± 0.42 (9) | n.s. |
| Phosphatidic acid | 1.69 ± 0.48 (7) | 1.45 ± 0.35 (8) | n.s. | 1.39 ± 0.19 (7) | 2.03 ± 0.28 (9) | 0.005 |
| Phosphatidylinositol (plus serine) | 10.75 ± 1.18 (14) | 10.42 ± 1.16 (16) | n.s. | 13.61 ± 1.20 (14) | 13.81 ± 1.47 (18) | n.s. |
| Lecithin | 41.55 ± 5.44 (14) | 45.53 ± 5.89 (16) | n.s. | 56.23 ± 4.19 (13) | 60.84 ± 4.81 (17) | n.s. |
| Phosphatidylethanolamine | 18.09 ± 4.33 (14) | 20.34 ± 3.09 (16) | n.s. | 18.38 ± 1.72 (13) | 14.75 ± 2.68 (18) | 0.001 |
| Cardiolipin | 4.50 ± 1.21 (6) | 5.14 ± 0.89 (8) | n.s. | ~1 | ~1 ± | |
| PE/PA[c] | 11.86 ± 5.89 (7) | 14.43 ± 4.08 (9) | n.s. | 13.47 ± 2.24 (7) | 7.38 ± 1.74 (9) | ≪0.001 |

[a] The values shown are the means ± SD of the number of determinations shown in parentheses; lean group contained seven mice and obese group nine mice. Phospholipid contents are expressed as percentages of total lipid phosphorus in each preparation. About 7.5% P was associated with the area at which two solvent fronts meet in the case of homogenates and about 2% P was associated with this area in the case of microsomes.

[b] Statistically not significant (i.e., $P > 0.05$).

[c] PE, phosphatidylethanolamine; PA, phosphatidic acid.

qualitative pattern of phospholipid distribution is the same as that found on the basis of percentages with some differences in the magnitude and degree of significance. Thus, in homogenates, the concentration of sphingomyelin was 41% lower ($P \leq 0.005$) in the obese liver than in the lean liver. In the microsomal fractions, the concentrations in the obese liver were 25% higher for phosphatidic acid ($P < 0.05$) and 21% lower for phosphatidylethanolamine ($P < 0.05$) than those in the lean liver. The inference that the qualitative pattern is the same is further supported by the fact that the ratios (i.e., relative proportions) of various phospholipids are strikingly similar whether the data are calculated as the percentage of total lipid phosphorus or as the concentration per unit of protein. Comparison of the ratio values indicated that the phosphatidylethanolamine to phosphatidic acid ratio was greatly different ($P <<< 0.001$) for the microsomal fraction of obese and lean livers (Table II, last line). This observation is of particular interest since in any given type of membrane, the ratios among different phospholipids are constant (and thus appear to be genetically determined) and are not greatly altered by dietary or other environmental (developmental) factors (25, 26). Future investigations on the mechanism(s) resulting in the alteration of the turnover (i.e., biosynthesis and degradation) rates of individual lipids might provide important information in understanding the syndrome of obesity in the ob/ob mouse.

A possible effect of the alterations in phospholipid composition (i.e., relative ratios among various lipids) upon the insulin degradation catalyzed by GSH-insulin transhydrogenase is discussed below. Previously, we have demonstrated (15) that, of the several lipids tested, only one (phosphatidylethanolamine) causes significant increase in the GIT activity and two (lysolecithin and phosphatidic acid) cause marked inhibition of the GIT activity. Since the microsomal fraction contains most of the liver GIT (13, 14), these results, i.e., a higher level of phosphatidic acid (an inhibitor of GIT) and a lower level of phosphatidylethanolamine (an activator of GIT) in the microsomes of the obese mouse than in the microsomes of the lean mouse, are consistent with the possibility that the phospholipid composition might be one of the factors responsible for the higher degree of latency [i.e., the proportion of the enzymatic activity available after a detergent treatment; see (13)] of the microsomal GIT activity in the obese mouse. The "concentration" of phosphatidic acid in rat liver may be calculated to be about 2–3 m$M$ (15, 27) and that of phosphatidylethanolamine about 20 m$M$. However, the concentration of phosphatidic acid causing 50% inhibition of the GIT activity is only 25 $\mu M$ (15) and the concentration of phosphatidylethanolamine resulting in 50% activation is only 8.2 $\mu M$ (15). Thus, alterations in the two lipids are of sufficient magnitude to account for the observed differences in the insulin-inactivating activity between lean and obese mice (13). The content of lysolecithin, the second lipid that is highly inhibitory to the GIT activity, was the same in the two groups. However, the possibility of the modulation of the GIT activity because of the amount of individual lipid species per se is probably less likely, since GIT and lipids occur membrane bound and organized. The latency of GIT activity appears to be related to phospholipid-oriented structural constraints in the membrane (14, 15). It is therefore more likely that an alteration in the membrane phospholipid composition alters the microenvironment and thereby modulates the GIT activity. That the microenvironment provided by membrane lipids does in fact modulate the functional properties of several enzymes in biological membranes has been previously reported [see (28) for a review]. Consistent with the modulation of the GIT activity due to phospholipid microenvironment is the finding that the microsomal GIT contains spatially separated multiple sites (allosteric and catalytic) of reaction (15). It should be emphasized however, that the above discussion concerning the possible effect upon the GIT activity is based upon small changes in relative content of phospholipids; although of significant magnitude, these results should be taken with caution until confirmed by an independent method such as with the use of [$^{32}$P]phosphorus.

*Summary.* The phospholipid composition of livers obtained from obese-hyperglycemic (ob/ob) male mice and from their lean mates

was compared. In unfractionated liver homogenates, the content (percentage of the total lipid phosphorus) of phosphatidylcholine, phosphatidylinositol, lysophosphatidylcholine, phosphatidic acid, phosphatidylethanolamine, and cardiolipin was similar in obese and lean mice; the content of sphingomyelin was 38% lower ($P \leq 0.01$) in the obese liver than in the lean liver. In the microsomal fractions, the obese mouse liver contained a 20% lower content of phosphatidylethanolamine ($P \leq 0.001$) and a 46% higher content of phosphatidic acid ($P \leq 0.005$) than the lean mouse liver; the content of the remaining four phospholipids was similar in the two groups. The total phospholipid (micrograms per milligram of protein) concentrations in lean and obese livers were 127 and 124 for unfractionated homogenates (without statistical significance) and 422 and 373 for the microsomal fraction ($P < 0.005$), respectively. The possibility is discussed that the alterations in the phospholipid composition of obese liver microsomes might be partly responsible for the greater amount of a latent form of the insulin-degrading enzyme, glutathione-insulin transhydrogenase, in the obese liver microsomes.

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