Heart Fatty Acid Uptake Is Decreased in Heart Fatty Acid-binding Protein Gene-ablated Mice*

Eric J. Murphy‡§, Gwendolyn Barcelo-Coblijn‡, Bert Binas‡, and Jan F. C. Glatz**

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From the §Department of Pharmacology, Physiology, and Therapeutics, University of North Dakota, Grand Forks, North Dakota 58202-9037, the ¶Department of Pathobiology, Texas A & M University, College Station, Texas 77843, and the **Department of Molecular Genetics, Cardiovascular Research Institute Maastricht, Maastricht 6200 MD, The Netherlands

The physiological function of heart type fatty acid binding protein (H-FABP)† is not well understood, although a recently produced H-FABP gene-ablated mouse (1, 2) provides a useful tool to determine the role of H-FABP in fatty acid uptake, trafficking, and metabolism. H-FABP is a 15-kDa cytosolic protein found in heart, muscle, and lactating mammary gland (3, 4). A unique promoter region appears to account for the specific tissue localization of H-FABP expression (5). In both insulin-dependent and insulin-independent diabetes, H-FABP expression is increased (6, 7), suggesting a potential role in mediating heart fatty acid uptake and utilization in diabetes.

H-FABP binds fatty acids with a high affinity and can transport fatty acids between vesicles via a collisional mechanism involving the direct interaction between H-FABP and membrane for fatty acid binding to occur (8). However, the role of H-FABP in heart lipid metabolism in intact animals is poorly understood.

H-FABP gene-ablated mice demonstrate a normal phenotype with regard to fertility, sex ratio, and weight gain (1). However, these mice develop cardiac hypertrophy with increasing age as demonstrated by a 38-fold increase in atrial natriuretic peptide, an enlargement of myocyte nuclei, and an increase in mean muscle fiber diameter (1). A qualitative study in vivo demonstrated that H-FABP gene-ablated mice had a dramatic decrease in the uptake of 125I-labeled 15-(p-iodophenyl)-3(R,S)-methylenedecanoic acid, a nonmetabolizable fatty acid analog (1). In isolated myocytes from H-FABP gene-ablated mice, there is a dramatic 45% decrease in palmitic acid (16:0) uptake and utilization (2) and a concomitant increase in glucose utilization (1, 2). Despite the increase in glucose uptake and decrease in fatty acid uptake, there is no apparent increase in glucose transporter (GLUT4) expression or levels (2). Further, there are no compensatory increases in the expression of or levels of other FABP types or of putative membrane-associated fatty acid transport proteins (2). The apparent number of mitochondria is the same between groups, and the capacity for β-oxidation is maintained in mitochondria isolated from gene-ablated mice (2). Hence, these results indicate that the heart mitochondria are functionally normal, yet there is a severe reduction in the uptake of saturated fatty acids; however, the effect on polyunsaturated fatty acids involved in lipid-mediated signal transduction is unknown.

FABP expression in cellular systems is associated with an increase in phospholipid mass and alterations in phospholipid acyl chain composition. Liver (L)-FABP and intestinal (I)-FABP expression increased basal total phospholipid mass 1.7- and 1.3-fold in L-cell fibroblasts, respectively (9, 10). Both of these FABP increased the mass of individual phospholipid subclasses of the ethanolamine and choline glycerophospholipids (9). Overall, expression of these two FABP resulted in an increase in the degree of unsaturation in phospholipid acyl chain composition (9). Hence, FABP expression can alter steady-state phospholipid mass and acyl chain composition.

The cell culture systems have demonstrated a role for cytoplasmic fatty acid-binding proteins (FABP) in lipid metabolism, although a similar function in intact animals is unknown. We addressed this issue using heart fatty acid-binding protein (H-FABP) gene-ablated mice. H-FABP gene ablation reduced total heart fatty acid uptake 40 and 52% for [1-14C]16:0 and [1-14C]20:4 compared with controls, respectively. Similarly, the amount of fatty acid found in the aqueous fraction was reduced 40 and 52% for [1-14C]16:0 and [1-14C]20:4, respectively. Less [1-14C]16:0 entered the triacylglycerol pool, with significant redistribution of fatty acid between the triacylglycerol pool and the total phospholipid pool. Less [1-14C]20:4 entered each lipid pool measured, but these changes did not alter the distribution of tracer among these pools. In gene-ablated mice, significantly more [1-14C]16:0 was targeted to choline and ethanolamine glycerophospholipids, whereas more [1-14C]20:4 was targeted to the phosphatidylinositol (PtdIns) pool. H-FABP gene ablation significantly increased PtdIns mass 1.4-fold but reduced PtdIns 20:4 mass 30%. Consistent with a reported effect of FABP on plasmalogen mass, ethanolamine plasmalogen mass was reduced 30% in gene-ablated mice. Further, 20:4 mass was reduced in each of the three other major phospholipid classes, suggesting H-FABP has a role in maintaining steady-state 20:4 mass in heart. In summary, H-FABP was important for heart fatty acid uptake and targeting of fatty acids to specific heart lipid pools as well as for maintenance of phospholipid pool mass and acyl chain composition.

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‡To whom correspondence should be addressed: Dept. of Pharmacology, Physiology, and Therapeutics, School of Medicine and Health Sciences, University of North Dakota, 501 N. Columbia Rd., Rm. 3700, Grand Forks, ND 58202-9037. Tel.: 701-777-3450; Fax: 701-777-4490; E-mail: emurphy@medicine.und.edu.

§A Netherlands Heart Foundation Professor of Cardiac Metabolism.

The abbreviations used are: H-FABP, heart FABP; FABP, fatty acid-binding protein(s); L-FABP, liver FABP; I-FABP, intestinal FABP; TAG, triacylglycerol(s); ChoGpl, choline glycerophospholipids; EtnGpl, ethanolamine glycerophospholipids; PtdSer, phosphatidylserine; PtdIns, phosphatidylinositol; PtdEtn, ethanolamine plasmalogen; PtdCho, choline plasmalogen; 16:0, palmitic acid; 20:4, n-6-arachidonic acid.
H-FABP Expression Increases Fatty Acid Uptake

suggesting a role in cellular lipid metabolism beyond an increase in fatty acid uptake.

Previous studies using isolated hearts and myocytes suggested that 20:4n-6 is esterified into lipid pools in a concentration-dependent manner (11, 12). This conclusion resulted in confusion regarding 20:4n-6 targeting under physiological plasma fatty acid concentrations. This problem is avoided by using animals with a high specific activity radiotracer in which unlabeled plasma fatty acid levels are unaltered (13, 14). Using these conditions, we previously reported that [1-14C]20:4n-6 is esterified primarily into rat heart phospholipid, whereas [1-14C]16:0 is preferentially esterified into heart triacylglycerols and found in the aqueous fraction representing by-products of β-oxidation (14). Hence, under physiologically relevant fatty acid concentrations, such as those used in this study, there is a divergent processing of 20:4n-6 and 16:0, resulting in targeting to two vastly different heart lipid pools. This targeting for 20:4n-6 is consistent with its use in heart lipid-mediated signal transduction (15–21).

Although H-FABP has been shown to be important for heart deposition of fatty acid analogs in vivo (1) and 16:0 in isolated myocytes (1, 2), its quantitative significance, fatty acid selectivity, and potential ability to target fatty acids to different cellular fatty acid pools in vivo remain unknown. Further, it is well known that FABP expression in cells can increase cellular phospholipid mass (9), and FABP can increase phospholipid biosynthesis in vitro, yet the ability of FABP to have a similar effect in vivo is unknown. To better address the potential role for H-FABP in heart fatty acid uptake and trafficking, gene-ablated mice were infused with either [1-14C]16:0 or [1-14C]-20:4n-6 (170 μCi/kg), and heart fatty acid uptake and disposition were determined using standard lipid analytical techniques. Alterations in steady-state phospholipid mass and phospholipid acyl-chain composition were addressed using standard lipid analytical techniques. We found a significant reduction in 16:0 uptake but a surprisingly robust decrease in 20:4n-6 uptake, indicative of a previously unknown role for H-FABP in polyunsaturated fatty acid uptake in the heart. H-FABP also appears to be important in the trafficking of both fatty acids to cellular lipid pools in gene-ablated mice; more 20:4n-6 was targeted to phosphatidylglycerol (PtdIns), consistent with the increased mass in PtdIns in the gene-ablated mice, suggesting that H-FABP may have a role in directing 20:4n-6 to specific phospholipid classes.

MATERIALS AND METHODS

Animals—Male mice (25–30 g) were obtained from the Cardiovascular Research Institute Maastricht (Maastricht, The Netherlands) and were maintained on a standard laboratory chow diet and water ad libitum. The ages for the mice used in this study were between 9–15 months in both control and H-FABP gene-ablated mice. There were no correlations between age and any of the lipid parameters measured in either group. This study was conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (National Institutes of Health publication 80-23) and under an animal protocol approved by the IACUC at the University of North Dakota (Protocol 0110-1).

Mouse Surgery—After the mouse was anesthetized with halothane (1–3%), their femoral artery and vein were catheterized with polyethylene tubing (PE-10). The wound area was anesthetized with xylocaine (1%) and closed using standard surgical staples. The hind quarters of the mouse were immobilized by taping the hind legs and hind quarters to a wooden block, and the mice were maintained postoperatively in a temperature-controlled environment for 3–4 h.

Tracer Preparation—The [1-14C]16:0 and [1-14C]-20:4n-6 were synthesized by Moravek Biochemical (Brea, CA). Radiotracer (Moravek Biochemical) was prepared by taking an aliquot of tracer in ethanol and evaporating the ethanol under a constant stream of N2 at 50 °C. Prior to use, radiotracer purity was assessed by gas liquid chromatography and was found to be >97% pure. The fatty acid tracer was solubilized in 5 ml HEPES (pH 7.4) buffer containing “essentially fatty acid-free” bovine serum albumin (50 mg/ml; Sigma). Solubilization was facilitated by sonication in a bath sonicator for 45 min at 45 °C. The appropriate amount of radiotracer was prepared for each mouse using the mouse’s weight based upon the infusion parameters of 170 μCi/kg (13).

Infusion (Intravenous)—Awake, adult male mice were infused with 170 μCi/kg of either [1-14C]16:0 or [1-14C]-20:4n-6 into the femoral vein over 10 min at a constant rate of 50 μl/min to achieve steady-state plasma radioactivity. Prior to and during the experimental period, arterial blood samples (~20 μl) were taken to determine plasma radioactivity. Following infusion, each mouse was killed using pentobarbitral (100 mg/kg, intravenously). Its heart was rapidly removed, bisected, rinsed free of exogenous blood, and frozen in liquid nitrogen. The tissue was separated into ventricle and freezing was 40 ± 10 °C. All tissue was stored at −80 °C until use.

Plasma Extraction—Arterial blood samples, taken at fixed times during the infusion period, were stored for up to 10 min on ice before separating the plasma by centrifugation with a Beckman Microfuge (Fullerton, CA). Plasma lipids were then extracted by transferring a 10-μl aliquot of plasma into a tube containing 2 ml of chloroform/methanol (2:1, v/v) and then mixing it by vortexing (22). The addition of 0.4 ml of 0.9% KCl to these tubes resulted in two phases. These phases were thoroughly mixed and then separated overnight in a −20 °C freezer. The upper phase was removed, and the lipid-containing lower phase was rinsed with 0.45 ml of theoretical upper phase to remove any aqueous soluble contaminants (22). The radioactivity in the upper phase was determined using a Beckman LS5000 CE liquid scintillation counter (Beckman Instruments, Fullerton, CA). Lower phase radioactivity was assayed in a portion of the lower phase using a Beckman LS5000 CE liquid scintillation counter.

Blood Extraction—Following the procedure described above for plasma, whole blood was extracted to correct for radioactivity contributed by residual blood left in the tissue. For the heart, the residual blood left in the heart tissue was estimated at 22% (23).

Tissue Lipid Extraction—Frozen tissue was pulverized under liquid nitrogen temperatures. Lipids from the tissue powder were extracted in a Tenbroeck tissue homogenizer using a two-phase system (22). Briefly, tissue homogenate (g) was multiplied by a constant to convert it to an equivalent volume expressed in ml (24). This value represents 1 volume. The pulverized tissue was placed in the homogenizer, and 17 volumes of chloroform/methanol (2:1, v/v) was added. Tissue was homogenized until there was a fine particulate-like powder. The solvent was removed, and the homogenizer rinsed with 3 volumes of chloroform/methanol (2:1, v/v). The rinse was added to the original sample, and 4 volumes of 0.9% KCl solution was added to this combined lipid extract. After vigorous mixing, phase separation was facilitated by centrifugation as described above. The upper phase and proteinaceous interface was removed and saved in a 20-ml glass scintillation vial. The lower organic phase was washed twice with 2 ml of theoretical upper phase to remove any aqueous soluble contaminants (22). The radioactivity in the upper phase was determined using a Beckman LS5000 CE liquid scintillation counter. The addition of the aqueous material to the scintillation mixture did not adversely affect the counter efficiency.

Thin-Layer Chromatography—Phospholipids and neutral lipids were separated by TLC. For each separation, 100 μl of sample was spotted onto a TLC plate. Phospholipids were separated on heat-activated Whatman silica gel-60 plates (20 × 20 cm, 250 μm) and developed in chloroform/methanol/acetic acid/water (50:37.5:3.5:2 by volume) (25). This method separates all major phospholipids. Neutral lipids were separated on heat-activated silica gel-60 plates (40 × 20 cm, 250 μm) and developed in petroleum ether/diethyl ether/acetetic acid (75:25:1.3 by volume) (26). This solvent system resolves cholesterol esters, diacylglycerols, nonesterified fatty acids, and triacylglycerols. Lipid fractions were identified using authentic standards (Doosan-Nordby (Englewood Cliffs, NJ) and NuChek Prep (Elysian, MN)).

Tissue Lipid Class Determination—Individual lipid classes were determined by assaying for lipids phosphorous content of individual lipid classes separated by TLC as described above (27). Cholesterol and cholesteryl ester mass was assayed using an iron binding assay after separation by TLC as described above (28).
**Plasmalogen Mass**—The plasmalogen mass was determined following separation of individual phospholipid classes in the heart lipid extracts by high performance liquid chromatography (29). One-half of the choline glycerophospholipid (ChoGpl) and of the ethanolamine glycerophospholipid (EtnGpl) fractions were dried under nitrogen and subjected to mild acidic hydrolysis followed by separation using high pressure liquid chromatography (30). The proportion of plasmalogen in this separation was used to calculate plasmalogen mass using the EtnGpl, and ChoGpl masses were determined by TLC as reported above.

**Phospholipid Acyl Chain Composition**—The fatty acid composition of the PtDns, phosphatidylserine (PtdSer), ChoGpl, and EtnGpl was determined following transesterification in 0.5 m KOH in methanol to form the fatty acid methyl ester (31). Individual fatty acids were separated by gas liquid chromatography using an SP-2530 column (0.32-mm inner diameter $\times$ 30-m length) and a trace gas liquid chromatograph (ThermoElectron, Austin, TX) equipped with dual autosamplers and dual flame ionization detectors. Fatty acids were quantified using a standard curve from commercially purchased standards (NuChek Prep), and 17:0 was the internal standard.

**Liquid Scintillation Counting**—Bands corresponding to the appropriate lipid fractions were scraped into 20-ml liquid scintillation vials, and 0.5 ml of H$_2$O was added, followed by 10 ml of Scintiverse BD (Fisher). After mixing, the samples were quantified by liquid scintillation counting at least 1 h after the addition of the liquid scintillation mixture.

**Calculations**—Integrated areas for the plasma radioactivity curves were determined using the trapezoidal method (Sigma Plot, Jandel Scientific, San Rafael, CA). Total radioactivity for each individual heart fraction was normalized to the wet weight (g wet weight) and divided by the integrated area of plasma radioactivity. This calculation essentially normalizes the tissue radioactivity to the exposure to plasma tracer. The resulting coefficient is called the unilateral incorporation coefficient or $k^*$, with values expressed as s$^{-1}$. Hence, the $k^*$ represents the radioactivity of each fraction normalized to the amount of radioactivity in the plasma to which the tissue was exposed. The following equation was used to calculate the unilateral incorporation coefficient,

$$k^* = c^*_{\text{tissue}} \int_0^T c^*_{\text{plasma}} dt$$

where $k^*$ represents the incorporation coefficient of tracer into a heart compartment, $c^*_{\text{tissue}}$ is tracer radioactivity in the heart compartment, $c^*_{\text{plasma}}$ is tracer radioactivity in the plasma, and $T$ is time of tissue sampling.

**Statistics**—Statistical analysis was done using Instat2 from GraphPad (San Diego, CA). Statistical significance was assessed using a unpaired, two-way, Student’s $t$ test, with $p < 0.05$ considered to be statistically significant.

**RESULTS**

**Plasma Curves**—The plasma curves for [1$-$14C]16:0- and [1$-$14C]20:4n-6-infused mice reached a steady state between 2 and 4 min following the start of infusion (Fig. 1). For [1$-$14C]16:0-infused mice, the average integrated plasma curve area was significantly different between groups ($p < 0.05$), with values of 943 $\pm$ 61 and 1172 $\pm$ 127 nCi $\times$ min$^{-1}$ $\times$ ml$^{-1}$ for control and gene-ablated mice, respectively. For [1$-$14C]20:4n-6-infused mice, the average integrated plasma curve area was 1457 $\pm$ 484 and 1420 $\pm$ 286 nCi $\times$ min$^{-1}$ $\times$ ml$^{-1}$ for control and gene-ablated mice, respectively.

**Heart Fatty Acid Uptake**—Heart radiotracer uptake was normalized to the integrated area under the curve and expressed as the incorporation coefficient, $k^*$. For [1$-$14C]16:0-infused mice, there was a significant reduction in the $k^*$ for the total, aqueous, and organic fractions (Fig. 2). The total uptake was significantly reduced 40% in gene-ablated mice, with a reduction of 40% in the aqueous fraction and a 24% reduction in the organic fraction. Calculating the percentage of the radiotracer in the plasma extracted by the heart, 6.2 $\pm$ 0.4% of the [1$-$14C]16:0 was extracted from the plasma in control mice, whereas 4.2 $\pm$ 0.9% was extracted from the plasma in gene-ablated mice. Hence, two different measures of fatty acid uptake demonstrate a 35–40% reduction in 16:0 uptake in gene-ablated mice.

For [1$-$14C]20:4n-6-infused mice, there was also a significant reduction in $k^*$ for the total, aqueous, and organic fractions (Fig. 2). In all cases, the values were decreased 52%, indicating no preferential reduction in the tracer entering either the aqueous or organic fractions. For control mice, 14.2 $\pm$ 3.0% of the [1$-$14C]20:4n-6 was extracted from the plasma, whereas 6.6 $\pm$ 1.2% was extracted from the plasma in gene-ablated mice. Thus, two different measures of fatty acid uptake demonstrate a greater than 50% reduction in 20:4n-6 uptake into heart by H-FABP gene ablation.

**Distribution of Fatty Acid into Metabolic Compartments**—The effects of H-FABP gene ablation on fatty acid distribution into the aqueous and organic fractions was determined. For [1$-$14C]16:0-infused mice, there was a significant redistribution of fatty acid into the organic fraction relative to the aqueous fraction in H-FABP gene-ablated mice. Nearly 12% more 16:0 was targeted for esterification into stable lipid compartments found in the organic fraction than to the aqueous compartment in gene-ablated mice. For [1$-$14C]20:4n-6-infused mice, there was no redistribution of 20:4n-6 targeted to the organic or aqueous fraction between groups. Thus, H-FABP gene ablation only resulted in an alteration in targeting of 16:0 between the aqueous and organic fractions.

**Fatty Acid Targeting into Esterified Lipid Compartments**—The incorporation and distribution of tracer in the esterified neutral lipid and phospholipid fractions was determined (Fig. 3). For [1$-$14C]16:0-infused mice, there was a significant reduction (52%) in the incorporation of tracer into the neutral lipid compartment in gene-ablated mice, whereas there was no al-
FIG. 2. Uptake of [1-14C]16:0 or [1-14C]20:4-n-6 into heart tissue from control (■) and H-FABP gene-ablated (□) mice expressed as total uptake or uptake into the aqueous and organic fractions. All values are expressed as k (s−1) and were corrected for radioactivity associated with the residual blood left in the heart tissue. Values represent mean ± S.D. (n = 5–6). *, statistical significance from control mice, p < 0.05.

teration in the incorporation of tracer into the phospholipid compartment (Fig. 3A). This resulted in a significant redistribution of tracer between the neutral lipids and phospholipid compartments, with a net increase in the tracer targeted for esterification into the total phospholipid pool (Fig. 3B). For [1-14C]20:4-n-6-infused mice, there was a significant reduction in the incorporation of tracer into both the esterified neutral lipid compartment (40%) and phospholipid compartment (40%) (Fig. 3A). Hence, there was no significant redistribution of [1-14C]20:4-n-6 between the esterified neutral lipid and phospholipid compartments (Fig. 3B). Thus, H-FABP gene ablation resulted in a significant decrease in fatty acid entering lipid compartments; however, the effect on fatty acid targeting to specific esterified lipid pools was fatty acid-specific.

Fatty Acid Targeting into Individual Lipid Classes—The effect of H-FABP gene ablation on fatty acid incorporation into specific individual lipid classes was determined as well as the targeting (percentage distribution) of each fatty acid to these individual lipid classes.

For [1-14C]16:0-infused mice, 53% less tracer entered the triacylglycerol (TAG) pool and 54% less tracer entered the sphingomyelin pool, whereas there was a 2.6-fold increase in the incorporation of tracer into the EtnGpl pool (Table I, top). This caused a redistribution of tracer between lipid compartments with significantly less tracer entering the TAG pool and much more tracer entering the EtnGpl and ChoGpl pool (Table I, bottom). Thus, for 16:0, H-FABP appears to be crucial for directing fatty acid to the TAG pool, and in the absence of H-FABP more 16:0 was directed to specific phospholipid classes.

For [1-14C]20:4-n-6-infused mice, there was a significant reduction (40–45%) in the incorporation of tracer into nearly every individual lipid class (Table I, top), although there was no reduction in the incorporation of tracer into either the PtdIns pool or to the PtdSer pool. This resulted in a net increase in the proportion of tracer targeted to the PtdIns pool (Table I, bottom). Thus, H-FABP appears to be crucial for directing 20:4-n-6 into a number of specific individual lipid classes.

The incorporation and targeting of 20:4-n-6 to the plasmalogen subclasses of the ChoGpl and EtnGpl was determined, because plasmalogens are known reservoirs of 20:4-n-6 in heart (32, 33). The incorporation of [1-14C]20:4-n-6 into the plasmalogen subclasses was independent of H-FABP expression. For the nonplasmalogen portion of the EtnGpl, the k* × 10−5 (s−1) was significantly less (p < 0.05) in gene-ablated compared with control mice, with values of 20.9 ± 5.2 and 11.2 ± 4.0, respectively. For the nonplasmalogen portion of the ChoGpl, the k* × 10−5 (s−1) was significantly less (p < 0.05) in gene-ablated compared with control mice, with values of 245.2 ± 64.7 and 131.3 ± 36.6, respectively. For the ethanolamine plasmalogen (PtdEtn), the k* × 10−5 (s−1) was unchanged between groups and was 1.8 ± 1.4 and 1.0 ± 0.8 for control and gene-ablated mice, respectively. For the choline plasmalogen (PtdCho), the k* × 10−5 (s−1) was unchanged between groups and was 12.2 ± 3.7 and 8.2 ± 1.7 for control and gene-ablated mice, respectively. Hence, the reduction in incorporation of 20:4-n-6 in H-FABP gene-ablated mice was limited to the acid-stable compartments. There were no significant differences in targeting to the individual EtnGpl and ChoGpl subclasses. Thus, H-FABP does not appear to be crucial for targeting 20:4-n-6 into the plasmalogen subclasses; rather, it is more important for targeting fatty acid to the acid-stable fraction of the ethanolamine and choline glycerophospholipids.

Phospholipid Mass—Heart phospholipid mass and composition was assessed in H-FABP gene-ablated and control mice. There was no net change in total phospholipid mass, although there were significant changes in individual phospholipid mass (Table II). PtdIns and PtdSer mass was increased 1.4-fold in gene-ablated mice, whereas phosphatidylglycerol and phosphatidic acid masses were increased 2.7- and 4.2-fold, respectively. The increase in these masses caused significant changes in phospholipid composition. Interestingly, there was a 15% reduction in EtnGpl and with increases in the proportions of PtdIns, PtdSer, phosphatidylglycerol, and phosphatidic acid. These changes suggest that the increase in PtdIns, PtdSer, phosphatidylglycerol, and phosphatidic acid masses are at the expense of reduced EtnGpl.

Because FABP expression can alter plasmalogen subclasses (9), the plasmalogen mass and composition of the EtnGpl and ChoGpl were determined. There was a marked reduction (30%) in PtdEtn mass in gene-ablated mice (Table III), causing a shift in the proportion of acid-stable and acid-labile (plasmalogen) subclasses in the EtnGpl. However, there were no significant changes observed in PtdCho mass or composition. Hence, H-FABP gene ablation resulted in a significant reduction in PtdEtn in the heart.

Cholesterol and Cholesteryl Ester Mass—The cholesterol, cholesteryl ester, and cholesterol/phospholipid ratio were also determined. Heart cholesterol mass (nmol/g wet weight) was 2647 ± 400 and 2716 ± 419, and cholesteryl ester mass (nmol/g wet weight) was 1025 ± 327 and 1066 ± 324 for control and gene-ablated mice, respectively. The cholesterol/phospholipid ratio was 0.094 ± 0.026 and 0.086 ± 0.014 in control and gene-ablated mice, respectively. There were no significant alterations in any of these parameters in the gene-ablated compared with control mice.

Phospholipid Acyl Chain Composition—The individual phospholipid acyl chain composition was determined for the
ChoGpl, EtnGpl, PtdIns, and PtdSer classes. For the ChoGpl class, there was a significant reduction (50%) in 20:4 \(\text{n-6}\) proportion accompanied by a marked increase in docosahexaenoic acid (22:6 \(\text{n-3}\)) proportion in the gene-ablated mice (Table IV). There was also a significant increase in 16:0 and a reduction in monoenoic fatty acids. These changes caused a shift in the total \(\text{n-3}\) and \(\text{n-6}\) fatty acids, with a dramatic increase in the \(\text{n-3}/\text{n-6}\) ratio. For EtnGpl, the proportion of 20:4 \(\text{n-6}\) was reduced 27%, with no observable change in 22:6 \(\text{n-3}\) proportions (Table IV). Similar to ChoGpl, there was an increase in the proportion of 16:0 and an increase in the \(\text{n-3}/\text{n-6}\) ratio. For PtdIns, there was a marked 30% reduction in 20:4 \(\text{n-6}\) proportion, accompanied by

Fig. 3. Uptake of tracer (A) and distribution of tracer (B) into the esterified neutral lipids and phospholipids of control (■) and H-FABP gene-ablated (□) mice. All values are expressed as \(k^* (s^{-1})\) and were corrected for radioactivity associated with the residual blood left in the heart tissue. Distribution was determined based upon \(k^*\) values found in A. Values represent mean ± S.D. (\(n = 5–6\)). *, statistical significance from control mice, \(p < 0.05\).

**Table I**

| Class             | \([1-14C]16:0\) | \([1-14C]20:4\text{n-6}\) |
|-------------------|-----------------|---------------------------|
|                   | Control (\(n = 5\)) | Gene-ablated (\(n = 6\)) | Control (\(n = 6\)) | Gene-ablated (\(n = 5\)) |
| TAG               | Mean            | S.D.                      | Mean            | S.D.                      |
|                   | 119.4           | 13.1                      | 55.6            | 19.8\(^a\)               |
| DAG               | 12.7            | 5.6                       | 13.6            | 4.2                       |
| EtnGpl            | 2.6             | 1.0                       | 6.7             | 2.5\(^b\)                |
| PtdIns            | 0.7             | 2.3                       | 1.0             | 0.9                       |
| PtdSer            | 0.4             | 0.8                       | 1.0             | 0.5                       |
| ChoGpl            | 21.7            | 0.3                       | 24.4            | 5.3                       |
| Sphingomyelin     | 19.9            | 2.6                       | 9.1             | 2.3\(^b\)                |
| Total             | 182.1           | 17.2                      | 112.9           | 27.4\(^a\)               |

**Distribution (%)**

| Class             | \([1-14C]16:0\) | \([1-14C]20:4\text{n-6}\) |
|-------------------|-----------------|---------------------------|
|                   | Control         | Gene-ablated              | Control         | Gene-ablated              |
| TAG               | Mean            | S.D.                      | Mean            | S.D.                      |
|                   | 65.6            | 4.8                       | 48.4            | 6.5\(^a\)                |
| DAG               | 8.4             | 1.6                       | 12.3            | 3.6                       |
| EtnGpl            | 1.7             | 0.9                       | 5.9             | 1.7\(^a\)                |
| PtdIns            | 0.4             | 0.4                       | 0.9             | 0.8                       |
| PtdSer            | 0.2             | 0.1                       | 1.0             | 0.5                       |
| ChoGpl            | 12.0            | 1.6                       | 22.2            | 4.5\(^a\)                |
| Sphingomyelin     | 10.9            | 2.7                       | 8.2             | 2.0                       |

\(^a\) Indicates statistical significance from control, \(p < 0.05\).
a 2.9-fold increase in the proportion of 22:6n-3 in the gene-ablated mice (Table IV). Similar to the ChoGpl, there was an increase in 16:0, but there was also an increase in monoenoic fatty acids between different vesicles (8, 43–46). In L-cell fibroblasts, L-FABP expression enhances fatty acid uptake and targets fatty acid for esterification into phospholipid and neutral lipid compartments (35, 36, 38). I-FABP expression in L-cells does not enhance fatty acid uptake but rather increases the targeting to neutral lipids (37, 38). Others have demonstrated a similar effect for I-FABP when expressed in an intestinal cell line (39). Clearly both 1- and L-FABP enhance fatty acid trafficking, presumably by enhancing intracellular fatty acid diffusion (34, 35). Collectively, these studies demonstrate that FABP expression in cells may enhance fatty acid uptake depending upon the FABP but certainly influences fatty acid targeting into cellular lipid pools.

A similar influence of FABP on fatty acid uptake and trafficking at the tissue level in an intact animal has not been determined using physiologically metabolizable fatty acids. A qualitative study using the fatty acid analog 15-(p-iodophenyl)-3(R,S)-methylpentadecanoic acid demonstrated a marked reduction in fatty acid uptake in H-FABP gene-ablated mice in vivo (1). Herein, we report for the first time that H-FABP gene ablation significantly reduced fatty acid uptake and altered targeting as compared with control mice and that the magnitude of these effects was fatty acid-specific. Previous work using isolated myocytes from the H-FABP gene-ablated mice demonstrated a 45% reduction in 16:0 uptake (2). Other studies using giant vesicles made from isolated muscle tissue demonstrate a partial reduction in vesicle fatty acid uptake in H-FABP gene-ablated mice (48, 49). However, this reduction was much less than what was observed in isolated myocytes or in our results presented here. Because 16:0 is used for energy production via β-oxidation in the myocyte, the decrease in uptake could be the result of mitochondrial dysfunction secondary to the gene ablation. This is not the case, since isolated mitochondria from gene-ablated mice have a normal capacity for β-oxidation, indicating that the mitochondria function normally (2).

Similar to the studies using isolated myocytes demonstrating a 45% reduction in 16:0 uptake (2), we observed a 35–40% reduction in 16:0 uptake in gene-ablated mice. However, the reduction of polyunsaturated fatty acid uptake has not been demonstrated in isolated myocytes from these gene-ablated mice. We report herein a striking 52% reduction in 20:4n-6 uptake in gene-ablated mice (Fig. 2). Hence, this large decrease in 20:4n-6 uptake by the heart in H-FABP gene-ablated mice may dramatically impact the availability of this crucial fatty acid for heart lipid-mediated signal transduction (16, 17, 50, 51).

Because β-oxidation is an important source of energy in the heart, we examined the incorporation and distribution of tracer entering either the aqueous or organic metabolic compartments. H-FABP gene ablation decreased the amount of 16:0 entering into the aqueous compartment 40%, whereas the amount entering the organic fraction was reduced only 24% (Fig. 2). These results clearly demonstrate that H-FABP has a greater role for targeting 16:0 for β-oxidation over a reduction in targeting to the organic fraction containing stable lipid metabolic compartments. For 20:4n-6, there was a 52% reduction in fatty acid entering either the aqueous or organic compartments (Fig. 2), resulting in no change in targeting between these two metabolic compartments. Thus, H-FABP appears to be more important in the targeting of 16:0 to specific metabolic

### Table II

| Class       | Mass (nmol/g wet weight) | Control (n = 7) | Gene-ablated (n = 8) | Mol % |
|-------------|-------------------------|----------------|---------------------|-------|
|             | Mean                    | S.D.           | Mean                | S.D.  |
| PtdGro      | 2127                    | 294            | 1992                | 216   |
| PtdGro      | 427                     | 203            | 1145                | 502   |
| PtdOH       | 141                     | 68             | 665                 | 261   |
| EtnGpl      | 6435                    | 1006           | 5874                | 592   |
| PtdIns      | 1084                    | 93             | 1488                | 386   |
| PtdSer      | 922                     | 148            | 1296                | 307   |
| ChoGpl      | 12.296                  | 1788           | 12.698              | 686   |
| Sphingomyelin | 5678                   | 1538           | 6456                | 644   |
| Total       | 29,110                  | 4454           | 31,554              | 2846  |

### Table III

| Class       | Mol % of glycerophospholipid class | Control (n = 5–7) | Gene-ablated (n = 7) |
|-------------|-----------------------------------|-------------------|---------------------|
|             | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| EtnGpl      | 68.1 | 3.6  | 75.2 | 2.1  |
| PlsEtn      | 31.9 | 3.6  | 24.4 | 2.1  |
| ChoGpl      | 88.0 | 1.4  | 90.2 | 1.9  |
| PlsCho      | 12.0 | 1.4  | 9.8  | 1.9  |

### Statistical significance from control, p < 0.05.
MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

The first report of a single FABP, in a single tissue, different from control. Despite the greater dependence of 20:4\(^{-}n-6\) on H-FABP for uptake into the heart, the organic fraction is composed of esterified neutral lipids and phospholipids that have different roles in heart metabolism. To better understand the role of H-FABP in fatty acid targeting to different esterified lipid pools, we determined the incorporation of tracer and distribution of tracer between the two pools (Fig. 3). It appears that H-FABP is more important for directing 16:0 to specific cellular metabolic compartments than it is for directing 20:4\(^{-}n-6\) to these compartments. In the absence of H-FABP, 16:0 was used less for \(\beta\)-oxidation and redirected for esterification into phospholipid pools rather than esterification into neutral lipid pools. Whereas it is well established that different FABPs can direct fatty acids to different lipid compartments (35–38), this is the first report of a single FABP, in a single tissue, differentially affecting fatty acid uptake and targeting.

Targeting to individual lipid classes was also determined in the H-FABP gene-ablated and control mice. For 16:0, there were large decreases in the incorporation of fatty acid into the TAG (54%) accompanied by a 2.6-fold increase in the incorporation into the EtnGpl (Table I, top). The net result was a significant reduction in the distribution of 16:0 into the TAG and a significant increase in the proportion of 16:0 entering into the ChoGpl and EtnGpl (Table I, bottom). This is consistent with our findings demonstrating a greater reduction in the amount used for \(\beta\)-oxidation if one considers the TAG pool as a temporary storage location for fatty acids targeted for \(\beta\)-oxidation. For 20:4\(^{-}n-6\), the incorporation into individual lipid classes was reduced 40–45%, except for PtdIns and PtdSer, where there was no change (Table I, top). This is consistent with the observed increase in PtdIns and PtdSer mass in the gene-ablated mice (Table II). In the presence of reduced amounts of 20:4\(^{-}n-6\) entering into the individual lipid classes, there were no changes in targeting except for PtdIns, where there was a net increase in the proportion of 20:4\(^{-}n-6\) targeted to PtdIns (Table I, bottom). The increase in targeting may be compensatory for the reduction observed in steady-state 20:4\(^{-}n-6\) levels observed in PtdIns in the gene-ablated mice (Table IV). This suggests that H-FABP expression may control the targeting of 20:4\(^{-}n-6\) in the heart, thereby regulating the amount of 20:4\(^{-}n-6\) made available for esterification into the PtdIns fraction. Because there are receptors in the heart operative through phospholipase C-mediated signaling mechanisms (52, 53), the implication of our results is that H-FABP may maintain specific lipid pools involved in heart lipid-mediated signal transduction.

Because plasmalogens are reservoirs for 20:4\(^{-}n-6\) (32, 33) and are active signaling molecules in the heart (16, 17, 50, 51), we examined the potential role for H-FABP in targeting 20:4\(^{-}n-6\) for esterification into heart plasmalogen pools as well as a role in maintaining steady-state mass of these pools. This is important, because I- and L-FABP expression increases plasmalogen mass in L-cell fibroblasts (9). In H-FABP gene-ablated mice, targeting of 20:4\(^{-}n-6\) to the plasmalogens was not altered. Instead, all of the changes were limited to the acid stable fraction containing primarily the diacyl subclass. However, H-FABP gene deletion did reduce PlsEtn mass, although a change in PlsCho did not reach statistical significance. A similar effect was observed when I- and L-FABP were separately expressed in L-cell fibroblasts (9). Thus, although H-FABP did not target 20:4\(^{-}n-6\) to the plasmalogen subclass, H-FABP was associated with a reduction in the PlsEtn mass (Table III). The lack of a reduction in PlsCho mass may be indicative of 1) increased use of PlsEtn to maintain PlsCho mass at the basal steady-state level or 2) decreased use of PlsCho in lipid-mediated signal transduction, thereby maintaining PlsCho mass at control lev-

### Table IV

Effect of H-FABP expression on heart phospholipid class fatty acid composition

| Fatty acid | ChoGpl | EtnGpl | PtdSer | PtdIns |
|-----------|--------|--------|--------|--------|
| Control   | Gene-ablated |
| Mean      | Mean    |
| MUFA      | Unsaturated |
| 16:0      | 21.2    | 1.1    |
| 18:0      | 23.0    | 1.0    |
| 18:1n-9   | 5.9     | 0.8    |
| 18:1n-12  | 2.3     | 0.3    |
| 18:2n-6   | 6.9     | 1.1    |
| 20:3n-6   | 0.5     | 0.1    |
| 20:4n-6   | 8.9     | 1.2    |
| 22:5n-3   | 2.3     | 0.2    |
| 22:6n-3   | 28.7    | 3.1    |
| 24:1      | 0.4     | 0.3    |

Saturated 44.2 1.3 44.8 1.0 30.7 1.6 33.1 0.8 41.3 3.2 42.9 3.6 50.4 2.4 47.8 1.5

Unsaturated 55.9 1.3 55.2 1.0 69.3 1.6 67.0 0.8 58.7 3.2 57.1 3.6 49.6 2.4 52.2 1.5

MUFA 8.7 1.4 6.4 0.9 6.5 1.0 6.8 0.6 7.6 3.5 5.4 0.7 3.4 0.3 4.7 0.3

PUFA 47.2 1.4 48.8 0.7 62.8 1.7 60.2 0.6 51.2 4.5 51.7 3.3 46.2 2.3 47.5 1.7

n-3 31.0 3.0 38.4 1.1 52.6 2.1 52.2 0.9 34.2 9.5 40.4 4.1 7.1 1.5 18.7 2.6

n-6 16.2 1.9 19.0 0.9 10.1 1.8 8.0 0.7 14.9 2.3 11.5 0.9 38.7 2.0 28.8 1.2

n-3/n-6 1.96 0.42 3.71 0.38 5.33 0.94 6.57 0.64 2.51 0.74 3.82 0.65 0.19 0.04 0.65 0.12

Unsaturated/saturated 1.27 0.07 1.23 0.05 2.52 0.67 2.02 0.07 1.44 0.33 1.34 0.19 0.99 0.10 1.09 0.07

PUFA/MUFA 5.59 1.17 7.76 1.08 9.85 1.70 8.99 0.70 7.92 3.50 9.62 1.24 13.60 1.31 10.03 0.82

MUFA/saturated 0.20 0.03 0.14 0.03 0.22 0.04 0.18 0.07 0.19 0.09 0.13 0.02 0.07 0.01 0.10 0.01

MUFA/saturated 1.07 0.06 1.09 0.03 2.05 0.16 1.82 0.06 1.25 0.20 1.21 0.18 0.92 0.09 1.00 0.07

\(\%\) Significantly different from control.
H-FABP Expression Increases Fatty Acid Uptake

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