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Metabolic implications of reduced heart-type fatty acid binding protein in insulin resistant cardiac muscle

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Abstract

Insulin resistance is characterized by elevated rates of cardiac fatty acid utilization resulting in reduced efficiency and cardiomyopathy. One potential therapeutic approach is to limit the uptake and oxidation of fatty acids. The aims of this study were to determine whether a quantitative reduction in heart-type fatty acid binding protein (FABP3) normalizes cardiac substrate utilization without altering cardiac function. Transgenic (FABP3$^{+/-}$) and wild-type (WT) littermates were studied following low fat (LF) or high fat (HF) diets, with HF resulting in obese, insulin-resistant mice. Cardiovascular function (systolic blood pressure, % fractional shortening) and heart dimension were measured at weaning and every month afterward for 3 mo. During this period cardiovascular function was the same independent of genotype and diet. Catheters were surgically implanted in the carotid artery and jugular vein for sampling and infusions in mice at 4 mo of age. Following 5 d recovery, mice underwent either a saline infusion or a hyperinsulinemic-euglycemic clamp (4 mU kg^{-1} min^{-1}). Indices of long chain fatty acid and glucose utilization (R_f, R_g; .μmol g wet weight^{-1} min^{-1}) were obtained using 2-deoxy[^{3}H]glucose and [^{125}I]-15-ρ-iodophenyl)-3-*R,S*-methylpentadecanoic acid. FABP3$^{+/-}$ had enhanced cardiac R_g compared with WT during saline infusion in both LF and HF. FABP3$^{+/-}$ abrogated the HF-induced decrement in insulin-stimulated cardiac R_g. On a HF diet, FABP$^{+/-}$ but not WT had an increased reliance on fatty acids (R_f) during insulin stimulation. In conclusion, cardiac insulin resistance and glucose uptake is largely corrected by a reduction in FABP3 *in vivo* without contemporaneous deleterious effects on cardiac function.

Keywords

Obesity; Glucose; Fatty acid; Hyperinsulinemic-euglycemic clamp

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1. Introduction

Chronic high fat feeding results in obesity, insulin resistance and elevated rates of fatty acid oxidation. These changes are facilitated by an increase in the transcription and translation of numerous fatty acid related genes [1,2]. Heart-type fatty acid binding protein-3 (FABP3) is one such protein that facilitates the movement of fatty acids in cardiac muscle [3–5]. Acting as a cytosolic fatty acid shuttle, it reversibly binds and moves fatty acids from the plasma membrane into storage or to the mitochondria for oxidation [6]. The protein is abundantly expressed, and modulated in a variety of physiologic and pathologic states. It is thought to play a key role in fatty acid utilization and numerous pharmaceutical inhibitors for the protein are presently at various stages of development [7,8].

We have previously reported that partial deletion of FABP3 attenuated obesity-associated insulin resistance in a murine model and analyzed the effect of the deletion on systemic glucose disposal and skeletal muscle glucose uptake [9]. Absolute genetic deficiency of FABP3 has not been reported in humans, and total abrogation of FABP3 function is an unrealistic therapeutic goal. A mouse model with a partial reduction of FABP3 is a more relevant model to test the role of FABP3 in the pathogenesis of insulin resistance and as a therapeutic target. Moreover, it is clear that abnormalities in myocardial energy metabolism play an important role in the genesis of cardiac dysfunction in insulin resistance [10]. Accordingly, it is essential to determine the effects of a partial decrease in FABP3 level on cardiac substrate utilization and cardiac function.

The high-fat fed C57BL/6J mouse is obese and insulin resistant compared to low fat fed mice of the same strain [11]. Since these mice are resistant to the development of atherosclerosis, any abnormality arising in the high-fat fed condition can likely be attributed to metabolic abnormalities rather than obstructive coronary artery disease [12]. It was hypothesized that reduction of FABP3 expression would inhibit heart fatty acid utilization and increase the dependence of the heart on glucose as a substrate.

2. Methods

2.1. FABP3$^{+/-}$ mice

All procedures performed were approved by the Vanderbilt University Animal Care and Use Committee. Generation of FABP3$^{+/-}$ mice has been previously described [9]. Mice lacking FABP3 were originally produced on a 129/Balb/c background and backcrossed for at least 10 generations to C57BL/6J. Mice carrying the FABP3 null transgene were subsequently bred and following a 3-week weaning period, littermates were separated by gender, maintained in micro-isolator cages and fed either a low fat (LF) or high fat (HF) diet *ad libitum.* Energy density of the LF diet (5001 Laboratory Rodent Diet, Purina, Richmond, IN) was 28.5% (234.0 g/kg) of energy as protein, 13.5% (45.0 g/kg) as fat and 58.0% (499.0 g/kg) as carbohydrate. In comparison, the HF diet (Custom Diet F3282; Bio-Serv; Frenchtown, NJ) contained 15.2% (197.4 g/kg) of energy as protein, 59.4% (358.0 g/kg) as fat and 24.5% (358.2 g/kg) as carbohydrate. The primary source of fat in this diet was lard. Mice were studied at ~ 4 mo of age. Genotyping for the FABP3 null transgene was performed on genomic DNA obtained from a tail biopsy as described previously [9]. Four

groups of mice were studied: WT mice fed low fat (LF-WT) or HF (HF-WT) diet and mice absent one FABP3 allele fed low fat (LF-FABP3$^{+/-}$) or HF (HF-FABP3$^{+/-}$) diet.

2.2. Cardiac function

Transthoracic echocardiography was performed after weaning (1 mo) and at monthly intervals to 4 mo as previously described [13,14]. To examine the effects of FABP$^{+/-}$ with prolonged high-fat feeding, cardiac function was also examined in a separate cohort of mice at 8 mo of age on HF.

Echo images were acquired using a Sonos 5500 (Agilent; Andover, MA) with a 15 MHz high frequency linear transducer. Prior to initiation of the study, the mice were trained on 2 separate occasions over 1–2 d. 2-D targeted M-Mode echocardiographic images were obtained at the level of the papillary muscles from the parasternal short-axis view. Measurements were made on screen using the digitally recorded M-mode tracings using the leading-edge technique. The fractional shortening FS%, a measure of LV systolic performance, was calculated from M-mode derived parameters.

Basal measurements of blood pressure were performed after mice were acclimated to the tail cuff plethysmography apparatus during 3 sessions on successive days to reduce stress-related perturbations and artifacts in measurement. Systolic and diastolic pressures were measured over 3 sequential determinations and recorded as absolute values and deviates, with re-measurement when excessive variance relative to strain-based controls was present.

2.3. Isotopic analogues

Glucose and long chain fatty acid (LCFA) tracers employed in the present study were 2-deoxy[^{3}H]glucose ([^{3}H]DG; New England Nuclear; Boston, MA) and [^{125}I]-15-(ρ-iodophenyl)-3-*R,S*-methylpentadecanoic acid ([^{125}I]BMIPP). BMIPP was a kind gift from Dr. Russ Knapp, Oak Ridge International Laboratories (Oak Ridge, TN). Radioiodination was performed according to a standard protocol. Briefly, BMIPP was heated in the presence of Na^{125}I solution (740 MBq/200 μl), propionic acid, and copper (II) sulphate. $Na_2S_2O_3$ was then added, and the organic phase was ether extracted and sequentially back extracted with saturated $NaHCO_3$ and water. After evaporation, the [^{125}I]BMIPP was solubilized for infusion using sonication into ursodeoxycholic acid.

2.4. Euglycemic-hyperinsulinemic clamps

Surgical catheterization of the left common carotid artery and right jugular vein was performed as previously described [15,16]. Animals were individually housed after surgery and lines were flushed daily with ~ 20 μl of saline containing 200 U/ml of heparin and 5 mg/ml of ampicillin. Experiments were performed following a post-operative recovery period of ~ 5 days, which was sufficient for body weight to be restored to within 10% of pre-surgery body weight. On the day of the study, conscious, unrestrained mice were placed in a ~ 1 L plastic container lined with bedding and fasted for 5 h. Micro-Renathane (0.033 in OD) tubing was connected to the catheter leads and infusion syringes. Following this, a baseline (t=−90 min) arterial blood sample (150 μL) was drawn for the measurement of glucose, hematocrit, and plasma insulin and LCFA. The remaining red blood cells were

washed with 0.9% heparinized saline and reinfused. Mice were then infused with saline alone (n=30) or with insulin (4 mU kg^{-1} min^{-1}; n=30). To maintain euglycemia during insulin experiments, arterial blood glucose (~5 μl; HemoCue; Mission Viejo, CA) was measured at ~ 10 min intervals and glucose (50%) was administered into the venous catheter. Mice also received saline-washed red blood cells from a donor mouse as needed in order to maintain hematocrit within 5% of initial hematocrit. Following a 90 min equilibration period (t=0 min), an arterial blood sample (150 .μL) was obtained and processed as the baseline blood sample. At t=5 min, a bolus of [^{3}H]DG and [^{125}I]BMIPP was administered to obtain indices of glucose and LCFA uptake and clearance. At t=7, 10, 15, and 20 min, arterial blood (~ 50 μL) was sampled in order to determine blood glucose, plasma [^{3}H]DG and [^{125}I]BMIPP. At t=30 min, a final arterial blood sample was obtained (150 μL) and processed as the baseline blood sample with additional blood for the measurement of [^{3}H]DG and [^{125}I]BMIPP. Mice were then anesthetized and the heart was quickly excised, rinsed in saline, and rapidly freeze clamped in liquid nitrogen. Samples were stored at −80 °C until subsequent analysis.

2.5. Tissue FABP3 content

Total FABP3 protein content was determined on cardiac muscles homogenized in M-PER lysis buffer (Pierce, Rockford, IL) supplemented with protease (Pierce) and phosphatase (Sigma) inhibitor cocktails. After centrifugation (30 min at 4500 g) pellets were discarded and supernatants were retained for protein determination using a BCA protein assay kit (Pierce). Proteins (20 0μg) were separated on a 4–12% Bis-Tris SDS-PAGE gel (Invitrogen) and then transferred to a PVDF membrane. Membranes were blocked, probed with rabbit anti-FABP3 (1:3000), and then incubated with anti-rabbit conjugated with horseradish peroxidase (1:20,000; Pierce, Rockford, IL). The membranes were then exposed to chemiluminescent substrate and images were taken using the VersaDoc imaging system (Bio-Rad). In order to confirm equal protein loading and transfer, membranes were stripped and reprobed with monoclonal anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:4000, Abcam) and then incubated with anti-mouse conjugated with horseradish peroxidase (1:20,000, Amersham). Densitometry was performed using ImageJ software (NIH).

2.6. Plasma measures

Immunoreactive insulin was assayed with a double antibody method [17]. Non-esterified fatty acids (NEFA) were measured spectrophotometrically (Wako NEFA C kit, Wako Chemicals Inc., Richmond, VA). [^{125}I]BMIPP and [^{3}H]DG were measured in plasma and tissues as previously described [18]. Briefly, plasma was counted for [^{125}I]BMIPP using a Beckman Gamma 5500 counter (Beckman Instruments, Fullerton, CA). Following this, the plasma sample was deproteinized in $Ba(OH)_2$ and $ZnSO_4$ and subsequently centrifuged. ^{3}H radioactivity was determined in supernatants using a Packard Tri-Carb 2900TR Liquid Scintillation Analyzer (PerkinElmer, Boston, MA). Following determination of tissue ^{125}1 radioactivity, tissues were homogenized in 0.5% PCA and centrifuged for 20 min. Supernatants were then neutralized using 5 M KOH and ^{3}H radioactivity was determined by liquid scintillation counting.

2.7. Calculations

Glucose clearance (K_g) and metabolic (R_g) indices were calculated from the accumulation of [^{3}H]DG phosphate ([^{3}H]DGP) and the integral of the plasma [^{3}H]DG concentration following a [^{3}H]DG bolus [19,20]. The relationships are defined as:

$$K_g = \left[[^3\text{H}]\text{DGP}\right]_m(t) / \int_0^t \left[[^3\text{H}]\text{DG}\right]_p dt \quad (1)$$

$$R_g = K_g \times [G]_p \quad (2)$$

The subscripts p and m refer to mean arterial plasma and total muscle accumulation respectively. The measurement of R_g has been described earlier [20]. In an analogous manner LCFA clearance (K_f) and metabolic (R_f) indices were calculated from the accumulation of [^{125}l]BM1PP in muscle and the integral of the plasma [^{125}1]BM1PP concentration following the tracer bolus.

$$K_f = \left[[^{125}\text{I}]\text{BMIPP}\right]_m(t) / \left[\int_0^t [^{125}\text{I}]\text{BMIPP}\right]_p dt \quad (3)$$

$$R_f = K_g \times [\text{LCFA}]_p \quad (4)$$

[[^{125}1]BM1PP Jm is the [^{125}1]BM1PP in the cell, [^{125}1]BM1PP]p is the [^{125}1] BM1PP present in the plasma. The measurements of R_f and K_f have been described earlier [18,21].

2.8. Statistical analyses

A two-way analysis of variance (ANOVA) was performed to detect statistical differences ($p<0.05$). Differences within the ANOVA, were determined using a Tukey *post hoc* test. All data are reported as means±standard error of the mean (SEM).

3. Results

3.1 Baseline characteristics

A total of 30 male and 30 female mice were used in metabolic studies. Sex was equally distributed within a group and there were 14–16 animals per group. Changes in body mass over time in each diet are reported in Table 1. For cardiac function studies a minimum of 9 mice in each genotype and dietary condition were studied. For both sets of experiments, no qualitative gender effects were observed, and accordingly, data from male and female mice within a treatment group are considered together. The basic phenotype of FABP3$^{+/-}$ mice has been reported previously [9]. Total body mass of both WT and FABP3$^{+/-}$ mice are greater in HF (40 ±2,39 ±2 g) compared to LF (26 ±2, 26 ± 2 g). Fasting plasma glucose in LF are 8.5 ± 0.3 and 8.7 ± 0.1 mM for WT and FABP3$^{+/-}$, respectively. High fat feeding caused hyperglycemia in WT (10.6 ±0.3 mM) but not FABP3$^{+/-}$ (8.3 ±0.4 mM). Fasting

plasma NEFA for LF-WT, LF-FABP3$^{+/-}$, HF-WT and HF-FABP3$^{+/-}$ were 1.3±0.1, 1.7±0.1,1.8±0.1 and 1.6±0.1 mM respectively. Fasting levels of NEFA were significantly different only between LF-WT and HF-WT (p <0.05). High fat feeding also resulted in fasting hyperinsulinemia regardless of genotype (p<0.05) with values of 21 ±10, 19±10, 68±10 and 71 ± 10 mU/ml for LF-WT, LF-FABP3$^{+/-}$, HF-WT and HF-FABP3$^{+/-}$.

The effects of heterozygous deletion and diet on FABP3 protein level in cardiac muscle are shown in Fig. 1. On a standard LF diet heterozygous deletion of FABP3 resulted in a 32% decrease in heart FABP3 protein (relative to GAPDH, 1.0±0.24 vs. 0.68±0.06 arbitrary units, p<0.05). High fat feeding increased heart FABP3 level by ~2×(2.04 ±0.025) and heterozygous deletion on a high fat diet blunted this increase (0.97±0.08, all differences p<0.05).

3.2. Cardiovascular function

There was no significant difference among genotypes with respect to systolic blood pressure, ventricular function or ventricular dimensions at baseline, or after 3 months of either LF or HF (Table 2). Systolic blood pressure and % fractional shortening did not differ within a genotype after 3 months of the specified diet or at intermediate points (Fig. 2). Comparable dimensions and systolic blood pressure indicate comparable loading conditions for the measurement of systolic function.

Cardiac function was also examined in an additional cohort of mice at 8 mo of age on HF (n=8 WT, n=5 FABP3$^{+/-}$). At this time, body mass was 54±2.7 and 55±2.8 for WT and FABP3$^{+/-}$ respectively. The only significant difference between groups was a moderate decrement in basal systolic function in FABP3$^{+/-}$ mice compared with WT (WT FS% 52.4 ±3.1%; FABP3$^{+/-}$ FS% 44.7 ±1.4%, p <0.001). Thus, at 8 months FABP3$^{+/-}$ increased LV end-systolic dimension without altering LV wall thickness or end-diastolic diameter.

3.3. Plasma measures and glucose infusion rates

Plasma measures and glucose infusion rates during clamps have been previously reported [9] and are briefly summarized here. Mean insulin concentrations during insulin clamps were ~ 60 μU/ml in LF fed mice. High fat feeding resulted in ~ two-fold higher clamp insulin concentrations (i.e. ~ 120 μU/ml). Infusion of insulin resulted in a suppression of LCFA from baseline values in LF fed mice. In HF mice, LCFA were not suppressed significantly by insulin. HF reduced GIR by ~ 70% and ~ 50% in WT and FABP3$^{+/-}$, respectively, compared to the respective LF fed mice of the same genotype.

3.4. Metabolic indices of cardiac glucose and fatty acid metabolism

Cardiac R_g measurements are shown in Fig. 3. Basal cardiac R_g in LF-WT was not different from rates in HF-WT. LF-FABP3$^{+/-}$ mice had a >two-fold increase in basal cardiac R_g when compared to LF-WT. Basal R_g in HF-FABP3$^{+/-}$ was reduced compared to LF-FABP3$^{+/-}$. Cardiac R_g was increased during insulin clamps in all groups. In relation to LF, HF resulted in insulin resistance and a reduction in cardiac R_g (Fig. 3). FABP3$^{+/-}$ prevented this decline as there was no difference between R_g in LF-FABP3$^{+/-}$ and HF-FABP3$^{+/-}$ mice.

Results of cardiac R_f are shown in Fig. 4. Basal cardiac R_f was elevated in HF-WT compared to LF-WT. However, there was no such increase in HF-FABP3$^{+/-}$ in relation to LF-FABP3$^{+/-}$ due to the high variance in the LF-FABP3$^{+/-}$ measure of R_f. Here, differences in NEFA levels between genotypes may be a contributing factor. Cardiac R_f was increased in HF-WT during insulin clamps compared to LF-WT. As in the basal state, HF feeding induced an increase in cardiac R_f, which was alleviated by partial deletion of FABP3, as rates were not different between HF-FABP3$^{+/-}$ and LF-FABP3$^{+/-}$. K_g and K_f are indices of glucose and LCFA flux that are calculated independently of their respective concentrations. Results of K_g and K_f during insulin clamps are shown in Fig. 5. Cardiac K_g and K_f were decreased and increased, respectively in HF-WT compared to LF-WT. A partial reduction in FABP3$^{+/-}$ blunted these effects of HF feeding.

4. Discussion

Insulin resistance results in cardiac dysfunction due to an oversupply of FFA that drives changes in metabolism, gene expression and function [22–24]. One therapeutic approach to correct perturbed metabolism in insulin-resistant tissue is to limit the uptake and oxidation of fatty acids [25,26]. In the present study, HF feeding was successfully employed to induce obesity and insulin resistance in WT and FABP3$^{+/-}$ mice. Although only HF-WT developed hyperglycemia and hyperlipidemia, both genotypes were insulin resistant as evidenced by a reduction in whole body glucose infusion rates during a hyperinsulinemic-euglycemic clamp. HF-WT resulted in a ~ 70% impairment in glucose infusion rates compared to a 50% reduction in HF-FABP3$^{+/-}$ [9]. Together these results suggest that a small reduction in FABP3$^{+/-}$ may mitigate the development of insulin resistance caused by HF feeding.

A partial reduction in FABP3 increased basal cardiac glucose utilization in LF fed animals. High fat feeding blunted this response indicating that the genetic reduction was not sufficient to restore total glucose utilization in an obese, insulin-resistant state. Insulin-stimulated glucose utilization was reduced in HF-WT animals but not HF-FABP3$^{+/-}$ compared to the respective LF controls. Together, these results show that altering fatty acid utilization by targeting or inhibiting FABP3$^{+/-}$ levels may be a viable means to counter perturbed substrate flux seen in obesity and insulin resistance. These results are in line with previous studies showing both ablation and a heterozygous reduction in FABP3 are protective towards dietary-induced insulin resistance [6,9]. However, a potential caveat is that the direct tissue specific effects of FABP3 cannot be readily distinguished from secondary consequences of changes in systemic metabolism, although from a therapeutic standpoint this distinction may not be critical.

To examine whether a reciprocal relationship between glucose and fatty acid utilization existed in FABP3$^{+/-}$ mice, I^{125}-BM1PP, a partially metabolizable fatty acid tracer was employed. Basal fatty acid utilization was greater in WT-HF compared to WT-LF. Increases in fatty acid utilization with obesity are well known and result from an increased efficiency of fatty acid uptake [27]. This increased efficiency also contributes to the accumulation of intracellular triacyglycerides which are implicated in the etiology of metabolic dysfunction [28–30]. Upon insulin stimulation, WT-HF showed the hypothesized, reciprocal change in substrate utilization with a decrease in glucose uptake and a corresponding increase in fatty

acid utilization. This finding is important to the insulin-resistant heart as inhibiting fatty acid uptake and oxidation are established anti-anginal approaches [31]. Ranolazine, trimetazidine and perhexiline are agents that inhibit fatty acid utilization and are known to improve both the symptoms from and decrease the frequency of angina pectoris [25].

When the combined results of cardiac glucose and fatty acids utilization are analyzed in terms of substrate clearance rather than uptake, results show that FABP3$^{+/-}$ mice do not have significantly altered basal rates. This is in agreement with the findings of Luiken et al [32,33], in which heterozygous deletion of FABP3 was described as “permissive” on fatty acid uptake using giant vesicle techniques. However, when exposed to high fat feeding, this balance is disrupted and only partially restored by lowering FABP3 levels. Beyond its role in directly regulating substrate utilization, FABP3 has been shown to play important roles in intracellular binding, targeting, signal transduction and gene expression [34]. Of interest, Murphy and colleagues [6] have shown FABP3 to target specific fatty acids towards β-oxidation or esterification. A lack of FABP3 in cardiac muscle differentially affects the fate and handling of both saturated and polyunsaturated fatty acids with 20:4n-6 directed towards phospholipid fractions and 16:0 directed to β-oxidation. By mediating the availability of fatty acids available for oxidation, FABP3 is concluded to play an important role in fatty acid mediated signal transduction in the heart, consistent with, or potentially extending, the effects expected from the “Randle cycle.”

Additional results show cardiac FABP3 protein levels to change in response to HF feeding. High fat feeding resulted in more than a doubling of cardiac FABP3 levels regardless of genotype suggesting this protein plays a pivotal role in shifting metabolism to favor fatty acids. Between genotypes studied here, germline deletion of a single allele decreased FABP3 protein level compared to WT in both LF and HF. However, the magnitude of the difference was less than 50%. FABP3 is a prominent element in a cohort of highly regulated metabolic genes linked to specific transcription factors in the heart [35,36], and this attenuated decrease was potentially due to induced counter-regulatory transcriptional effects. Indeed, previous studies confirm FABP3 levels are highly variable, with protein levels changing in concert with metabolic demands. This is evident in the fetal heart, where the expression of FABP3 increases rapidly following birth, allowing the heart to transition from glucose to fatty acids as a predominant energy source [37]. In addition, weight loss, chronic exercise training and omega-3 supplemented diets are all known to increase levels of FABP3 in skeletal muscle [38–42].

As a strong interdependence exists between heart metabolism and function, we determined the effects of diet-induced insulin resistance and FABP3$^{+/-}$ on cardiac function at numerous time points throughout the experiment. Echocardiographic assessment was conducted at baseline and following 4, 8, and 12 wk on each respective diet, spanning the metabolic evaluation reported. Results demonstrated that this level of quantitative decrease in FABP3, with either a LF or HF diet, did *not* alter basal heart function or dimension in conditions of comparable afterload (systolic blood pressure) at 12 wk. This extends previous studies that have shown either no change or only slight cardiac dysfunction with high fat feeding [43,44]. The lack of change in cardiac function with insulin resistance in the present study at 4 mo may be due to the circumscribed duration of the dietary manipulation. Indeed,

prolonged high-fat feeding (8 mo) in FABP3$^{+/-}$ was associated with a mild decrement LV end-systolic dimension without altering LV wall thickness or end-diastolic size.

This contrasts with data published in the null mutation of FABP3, which resulted in morphologic cardiac hypertrophy, with increased thickness of both the septum and ventricle [3]. While null mutations of FABP3 appear to be deleterious to heart function, these results show no effect of a heterozygous mutation for several months, suggesting that a full complement of the protein is not continuously necessary under normal conditions. This is essential if FABP3 is to be a credible therapeutic target for metabolic manipulation, or if interventions targeted at other FABP's have a lesser but detectable effect on FABP3. This study examined mice absent a single FABP3 allele. This creates a model with more physiological and pathophysiological relevance than mice with a full knockout of the FABP3 gene, as the latter has not been reported in humans. Of course, insulin resistance is also often accompanied by associated cardiovascular alterations such as hypertension, myocardial infarction and congestive failure. The applicability of present observations to these states requires further investigation. Limitations of the present study include our inability to distinguish between the effects of high fat feeding from obesity and insulin resistance. Diet alone is known to influence cardiac metabolism, independent of obesity. In addition, cardiac metabolism was only assessed at a single time point (4 mo) as the tracer technique employed requires animal euthanasia, and the catheter technique carries substantially increases mortality in old mice. In contrast, cardiac function was serially measured over time. Lastly, plasma samples obtained from the animals were of limited volume. As such, other measures of interest including leptin and adiponectin could not be assessed.

In conclusion, results of the present study show FABP3 to have a central role in cardiac fuel selection and utilization in both health and obesity. Acting in a permissive capacity, FABP3 levels are highly variable and dependent on metabolic demands. Here we show insulin resistance to cause perturbed cardiac substrate utilization *in vivo*, but that the severity of impairment is limited in the context of reduced FABP3 content. Importantly, this metabolic change is not associated with changes in basal cardiac dimension or systolic function.

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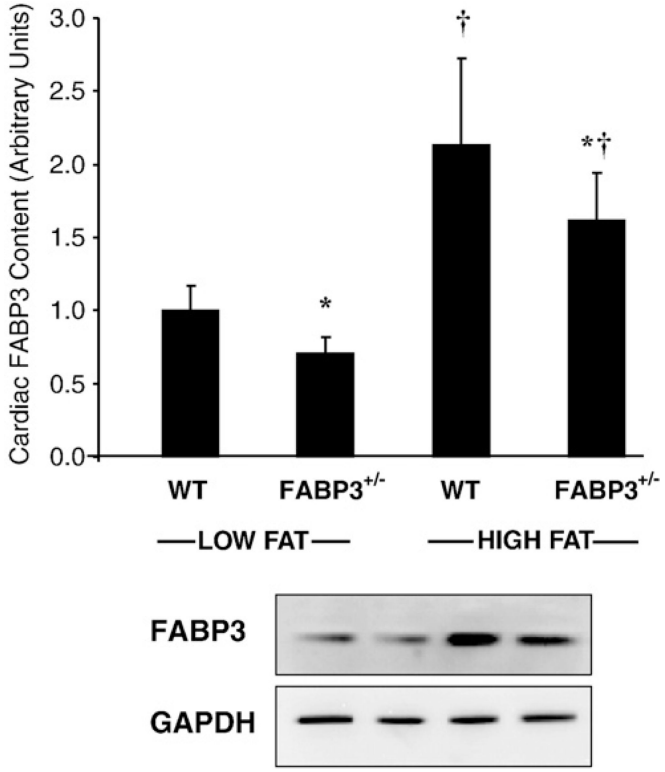

Fig. 1.

Total FABP3 content in the heart muscle in WT and FABP3$^{+/-}$ fed either a LF or an HF (60% of kcal from lipid) for 12 wk. Immunoblotting was performed to measure total FABP3 protein content. FABP3 levels were normalized to GADPH content. Order of loading on the immunoblot follows the graphical data. Densitometry data are means±SE, n=7 per group. *Indicates p<0.05 between genotype within diet, †Indicates p<0.05 between diets.

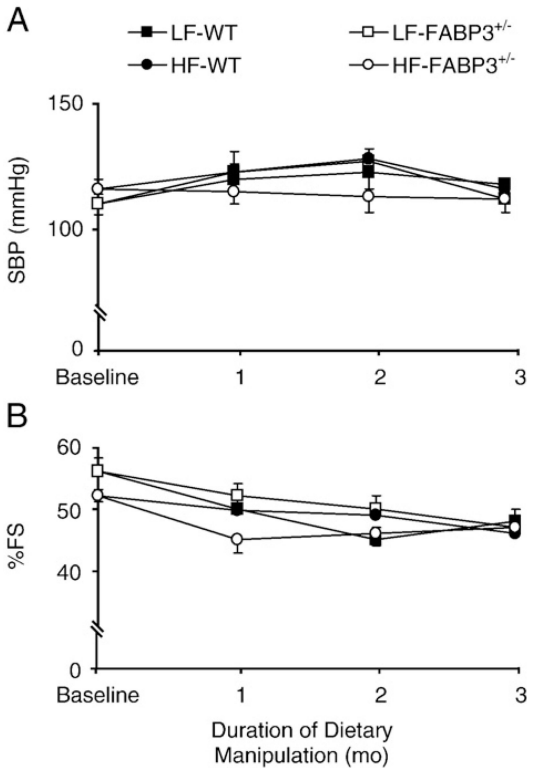

Fig. 2.

A: Systolic blood pressure (SBP) (mmHg) and B: % fractional shortening (%FS) at baseline (1 mo of age) and following 1, 2, 3 mo of either a LF or HF diet. No significant differences were noted between diet, age or genotype. Data represent means ±SE. n=8–10 mice/treatment.

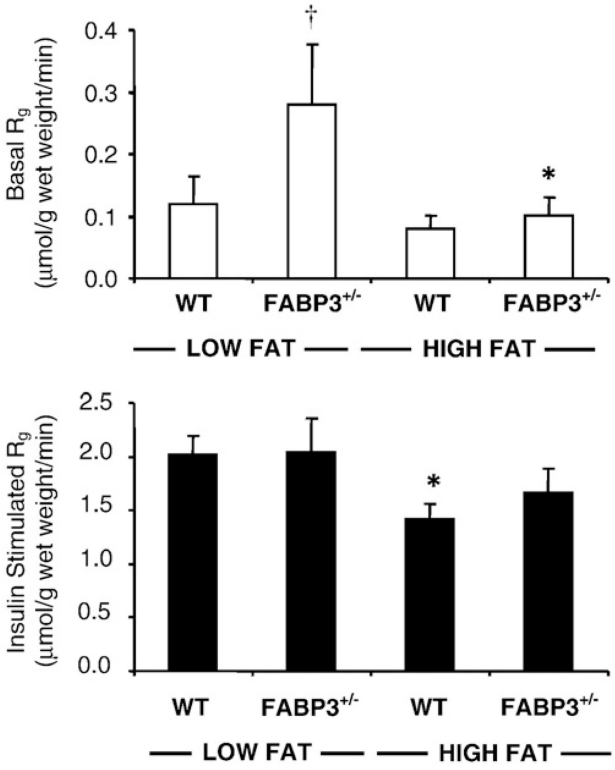

Fig. 3.

Glucose utilization (R_g) in cardiac muscle during saline infusion (top panel) and a euglycemic-hyperinsulinemic clamp (4 mU/kg/min) (bottom panel). Wild type (WT) and heterozygous mice ($FABP^{+/-}$) are shown. *Indicates $p<0.05$ HF vs. LF fed within a genotype, $^{\dagger}p<0.05$ WT vs. $FABP^{+/-}$ within a diet. All data are reported as means ±SEM, $n=7–8$ mice/group.

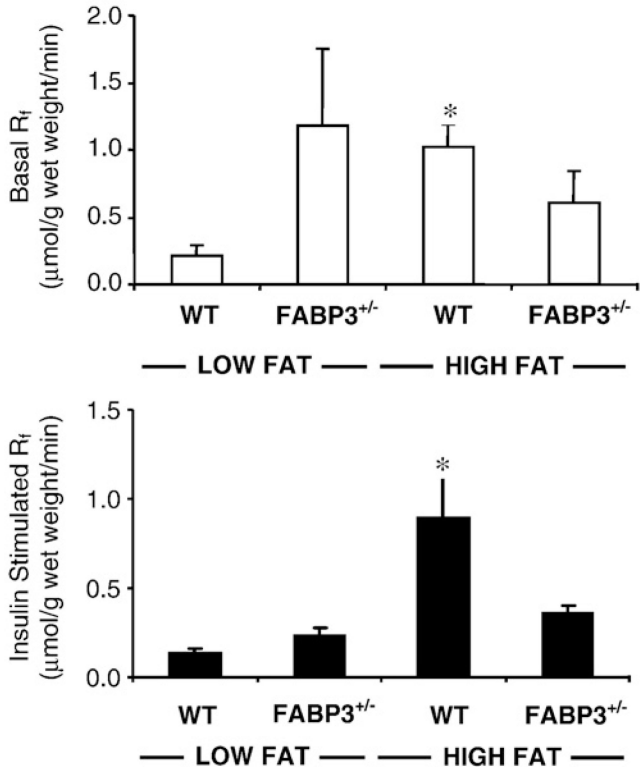

Fig. 4.

Fatty acid utilization (R_f) in cardiac muscle during saline infusion (top panel) and a euglycemic-hyperinsulinemic clamp (4 mU/kg/min, bottom panel). Wild type (WT) and heterozygous mice (FABP3$^{+/-}$) are shown. *Indicates $p<0.05$ high fat vs. low fat fed within a genotype. All data are reported as means ±SEM, n=7–8 mice/group.

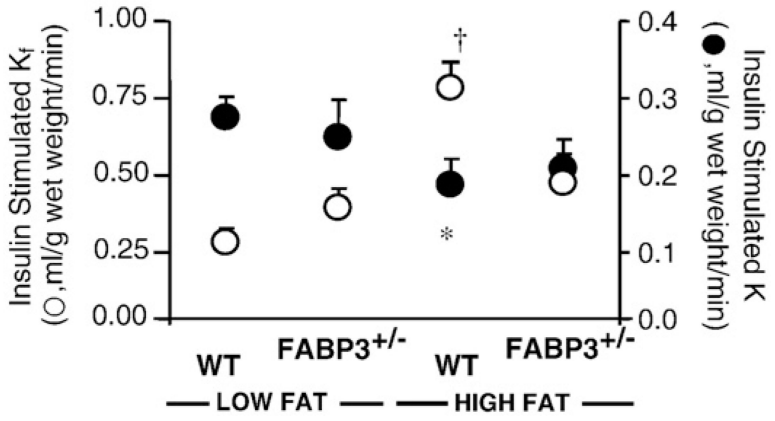

Fig. 5.

Relative rates of cardiac glucose (K_g) and fatty acid (K_f) clearance during a euglycemic-hyperinsulinemic clamp across genotype and treatment. K_g is depicted by the closed symbols (●) while the open symbols (○) represent K_f. All data are reported as means ±SEM, n=7–8 mice/group. *Indicates $p<0.05$ HF vs. LF fed within a genotype, $^{\dagger}p<0.05$ WT vs. FABP3$^{+/-}$ within a diet.

Table 1

Body mass of wild-type (WT) and fatty acid binding protein heterozygotes (FABP3$^{+/-}$) animals used in metabolic studies at baseline (post-weaning, no dietary intervention) and following 6, 9, or 12 wk of a low or high fat diet

Diet	Genotype	*n*	Baseline	6 wk	9 wk	12 wk
Low fat	WT	14	19±1	22±1	24±1	26±2
	FABP3$^{+/-}$	16	21±1	24±1	25±1	26±2
High fat	WT	16	21±1	26±1	32±1*	40±2*
	FABP3$^{+/-}$	14	21±1	27±2	31±1*	39±2*

Animal sex was equally distributed between groups.

Data represent means ±SE.

*Indicates significant difference (p<0.05) between diets at a given time point.

Table 2

Effects of genotype and diet on cardiovascular parameters at baseline, 12 wk or 35 wk on either a low or high fat diet

		Baseline	Low fat, 3 mo	High fat, 3 mo
WT	SBP (mmHg)	110±4	118±4	112±5
	HR (bpm)	590±34	642±14	723±9
	FS%	56.5±1.9	48.3±1.9	46.9±0.9
	IVSd (cm)	0.072±0.003	0.096±0.001	0.097±0.002
	LVIDd (cm)	0.265±0.009	0.285±0.012	0.316±0.011
	LVPWd (cm)	0.079±0.003	0.095±0.003	0.097±0.004
	IVSs (cm)	0.146±0.005	0.167±0.004	0.167±0.003
	LVIDs (cm)	0.116±0.008	0.149±0.011	0.171±0.009
	LVPWs (cm)	0.116±0.007	0.127±0.007	0.129±0.005
FABP$^{+/-}$	SBP (mmHg)	116±4	116±3	112±5
	HR (bpm)	652±16	643±13	742±7
	FS%	51.9±1.0	46.5±1.1	47.1±1.2
	IVSd (cm)	0.077±0.002	0.099±0.001	0.103±0.003
	LVIDd (cm)	0.262±0.005	0.300±0.007	0.311±0.005
	LVPWd (cm)	0.076±0.002	0.096±0.003	0.104±0.003
	IVSs (cm)	0.144±0.004	0.170±0.003	0.178±0.005
	LVIDs (cm)	0.126±0.003	0.161 ±0.005	0.164±0.005
	LVPWs (cm)	0.106±0.003	0.129±0.004	0.139±0.007

SBP, systolic blood pressure; HR, heart rate; FS%, fractional shortening; IVSd, interventricular septal thickness in diastole; LVIDd, LV end-diastolic dimension; LVPWd, LV posterior wall thickness in diastole; IVSs, interventricular septal thickness in systole; LVIDs, LV end-systolic dimension; LVPWs, LV posterior wall thickness in systole. Data represent means±SE. n=8–10 mice/treatment.