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7 **METABOLIC IMPLICATIONS OF REDUCED HEART-TYPE FATTY**
8 **ACID BINDING PROTEIN IN INSULIN RESISTANT CARDIAC MUSCLE**
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11 **Jane Shearer¹, Patrick T. Fueger¹, ZhiZhang Wang^{2,3}, Deanna P. Bracy^{1,3}, David H.**
12 **Wasserman^{1,3} and Jeffrey N. Rottman^{2,3}**
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15
16 **Departments of ¹Molecular Physiology and Biophysics, ²Cardiology, ³Mouse Metabolic**
17 **Phenotyping Center. Vanderbilt University School of Medicine. Nashville, TN, USA.**
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24 **Running Title: *Role of FABP3 in Cardiac Metabolism***
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28 **Corresponding Author: Jane Shearer, PhD**
29 **Departments of Kinesiology, Biochemistry & Molecular Biology**
30 **2500 University Drive NW**
31 **University of Calgary**
32 **T2N 1N4. CANADA**
33 **T: 403.220.3431**
34 **F: 403.270.0737**
35 **E: jshearer@ucalgary.ca**
36

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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 4mo of age. Following 5d 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[³H]glucose and [¹²⁵I]-15-*p*-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. HF but not FABP3^{+/-} fed WT had an increased reliance on fatty acids (R_f). 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.

Word Count: 240 words

Keywords: Obesity, Glucose, Fatty Acid, Hyperinsulinemic-Euglycemic Clamp.

1 INTRODUCTION

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

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

20 The high-fat fed C57BL/6J mouse is obese and insulin resistant compared to low fat fed
21 mice of the same strain [11]. Since these mice are resistant to the development of atherosclerosis,
22 any abnormality arising in the high-fat fed condition can likely be attributed to metabolic
23 abnormalities rather than obstructive coronary artery disease [12]. It was hypothesized that

1 reduction of FABP3 expression would inhibit heart fatty acid utilization and increase the
2 dependence of the heart on glucose as a substrate.

3

4 **METHODS**

5 ***FABP3^{+/-} Mice.*** All procedures performed were approved by the Vanderbilt University
6 Animal Care and Use Committee. Generation of FABP3^{+/-} mice has been previously described
7 [9]. Mice lacking FABP3 were originally produced on a 129/Balb/c background and backcrossed
8 for at least 10 generations to C57BL/6J. Mice carrying the FABP3 null transgene were
9 subsequently bred and following a 3-week weaning period, littermates were separated by gender,
10 maintained in microisolator cages and fed either a low fat (LF) or high fat (HF) diet *ad libitum*.
11 Energy density of the LF diet (5001 Laboratory Rodent Diet, Purina, Richmond, IN) was 28.5%
12 (234.0g/kg) of energy as protein, 13.5% (45.0g/kg) as fat and 58.0% (499.0g/kg) as carbohydrate.
13 In comparison, the HF diet (Custom Diet F3282; Bio-Serv; Frenchtown, NJ) contained 15.2%
14 (197.4g/kg) of energy as protein, 59.4% (358.0g/kg) as fat and 24.5% (358.2g/kg) as
15 carbohydrate. The primary source of fat in this diet was lard. Mice were studied at ~ 4mo of age.
16 Genotyping for the FABP3 null transgene was performed on genomic DNA obtained from a tail
17 biopsy as described previously [9]. Four groups of mice were studied: WT mice fed low fat (LF-
18 WT) or HF (HF-WT) diet and mice absent one FABP3 allele fed low fat (LF-FABP3^{+/-}) or HF
19 (HF-FABP3^{+/-}) diet.

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

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

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

13 ***Isotopic Analogues.*** Glucose and long chain fatty acid (LCFA) tracers employed in the
14 present study were 2-deoxy[³H]glucose ([³H]DG; New England Nuclear; Boston, MA) and [¹²⁵I]-
15 15-(*p*-iodophenyl)-3-*R,S*-methylpentadecanoic acid ([¹²⁵I]BMIPP). BMIPP was a kind gift from
16 Dr. Russ Knapp, Oak Ridge International Laboratories (Oak Ridge, TN). Radioiodination was
17 performed according to a standard protocol. Briefly, BMIPP was heated in the presence of Na¹²⁵I
18 solution (740 MBq/200µl), propionic acid, and copper (II) sulphate. Na₂S₂O₃ was then added, and
19 the organic phase was ether extracted and sequentially back extracted with saturated NaHCO₃ and
20 water. After evaporation, the [¹²⁵I]BMIPP was solubilized for infusion using sonication into
21 ursodeoxycholic acid.

22 ***Euglycemic-Hyperinsulinemic Clamps.*** Surgical catheterization of the left common
23 carotid artery and right jugular vein was performed as previously described [15, 16]. Animals were
24 individually housed after surgery and lines were flushed daily with ~20 µl of saline containing 200

1 U/ml of heparin and 5 mg/ml of ampicillin. Experiments were performed following a post-
2 operative recovery period of ~5 days, which was sufficient for body weight to be restored to
3 within 10% of pre-surgery body weight. On the day of the study, conscious, unrestrained mice
4 were placed in a ~1L plastic container lined with bedding and fasted for 5h. Micro-Renathane
5 (0.033 in OD) tubing was connected to the catheter leads and infusion syringes. Following this, a
6 baseline ($t = -90\text{min}$) arterial blood sample ($150\mu\text{L}$) was drawn for the measurement of glucose,
7 hematocrit, and plasma insulin and LCFA. The remaining red blood cells were washed with 0.9%
8 heparinized saline and reinfused. Mice were then infused with saline alone ($n=30$) or with insulin
9 ($4\text{ mU}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $n=30$). To maintain euglycemia during insulin experiments, arterial blood
10 glucose ($\sim 5\mu\text{L}$; HemoCue; Mission Viejo, CA) was measured at ~ 10 min intervals and glucose
11 (50%) was administered into the venous catheter. Mice also received saline-washed red blood
12 cells from a donor mouse as needed in order to maintain hematocrit within 5% of initial
13 hematocrit. Following a 90min equilibration period ($t = 0\text{min}$), an arterial blood sample ($150\mu\text{L}$)
14 was obtained and processed as the baseline blood sample. At $t = 5\text{min}$, a bolus of [^3H]DG and
15 [^{125}I]BMIPP was administered to obtain indices of glucose and LCFA uptake and clearance. At t
16 $= 7, 10, 15,$ and 20 min , arterial blood ($\sim 50\mu\text{L}$) was sampled in order to determine blood glucose,
17 plasma [^3H]DG and [^{125}I]BMIPP. At $t = 30\text{min}$, a final arterial blood sample was obtained
18 ($150\mu\text{L}$) and processed as the baseline blood sample with additional blood for the measurement of
19 [^3H]DG and [^{125}I]BMIPP. Mice were then anesthetized and the heart was quickly excised, rinsed
20 in saline, and rapidly freeze clamped in liquid nitrogen. Samples were stored at -80°C until
21 subsequent analysis.

22 ***Tissue FABP3 Content.*** Total FABP3 protein content was determined on cardiac muscles
23 homogenized in M-PER lysis buffer (Pierce, Rockford, IL) supplemented with protease (Pierce)
24 and phosphatase (Sigma) inhibitor cocktails. After centrifugation (30 min at $4500g$) pellets were

1 discarded and supernatants were retained for protein determination using a BCA protein assay kit
2 (Pierce). Proteins (20 μ g) were separated on a 4-12% Bis-Tris SDS-PAGE gel (Invitrogen) and
3 then transferred to a PVDF membrane. Membranes were blocked, probed with rabbit anti-FABP3
4 (1:3000), and then incubated with anti-rabbit conjugated with horseradish peroxidase (1:20000;
5 Pierce, Rockford, IL). The membranes were then exposed to chemiluminescent substrate and
6 images were taken using the VersaDoc imaging system (Bio-Rad). In order to confirm equal
7 protein loading and transfer, membranes were stripped and reprobed with monoclonal anti-
8 glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:4000, Abcam) and then incubated with
9 anti-mouse conjugated with horseradish peroxidase (1:20000, Amersham). Densitometry was
10 performed using ImageJ software (NIH).

11 **Plasma Measures.** Immunoreactive insulin was assayed with a double antibody method
12 [17]. Non-esterified fatty acids (NEFA) were measured spectrophotometrically (Wako NEFA C
13 kit, Wako Chemicals Inc., Richmond, VA). [125 I]BMIPP and [3 H]DG were measured in plasma
14 and tissues as previously described [18]. Briefly, plasma was counted for [125 I]BMIPP using a
15 Beckman Gamma 5500 counter (Beckman Instruments, Fullerton, CA). Following this, the
16 plasma sample was deproteinized in Ba(OH) $_2$ and ZnSO $_4$ and subsequently centrifuged. 3 H
17 radioactivity was determined in supernatants using a Packard Tri-Carb 2900TR Liquid
18 Scintillation Analyzer (PerkinElmer, Boston, MA). Following determination of tissue 125 I
19 radioactivity, tissues were homogenized in 0.5% PCA and centrifuged for 20min. Supernatants
20 were then neutralized using 5M KOH and 3 H radioactivity was determined by liquid scintillation
21 counting.

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

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

$$2 \quad R_g = K_g \times [G]_p \quad (\text{Eq. 2})$$

3

4 The subscripts p and m refer to mean arterial plasma and total muscle accumulation respectively.

5 The measurement of R_g has been described earlier [20]. In an analogous manner LCFA clearance

6 (K_f) and metabolic (R_f) indices were calculated from the accumulation of [^{125}I]BMIPP in muscle

7 and the integral of the plasma [^{125}I]BMIPP concentration following the tracer bolus.

$$8 \quad K_f = [[^{125}\text{I}]\text{BMIPP}]_m(t) / \int_0^t [[^{125}\text{I}]\text{BMIPP}]_p dt \quad (\text{Eq. 3})$$

$$9 \quad R_f = K_f \times [\text{LCFA}]_p \quad (\text{Eq. 4})$$

10

11 [[^{125}I]BMIPP] $_m$ is the [^{125}I]BMIPP in the cell, [^{125}I]BMIPP] $_p$ is the [^{125}I]BMIPP present in the

12 plasma. The measurement of R_f and K_f have been described earlier [18, 21].

13

14 **Statistical Analyses.** A two-way analysis of variance (ANOVA) was performed to detect statistical

15 differences ($p < 0.05$). Differences within the ANOVA, were determined using a Tukey *post hoc*

16 test. All data are reported as means \pm standard error of the mean (SEM).

17

18 **RESULTS**

19 **Baseline Characteristics.** A total of 30 male and 30 female mice were used in metabolic

20 studies. Sex was equally distributed within a group and there were 14-16 animals per group.

21 Changes in body mass over time in each diet are reported in Table 1. For cardiac function studies a

22 minimum of 9 mice in each genotype and dietary condition were studied. For both sets of

1 experiments, no qualitative gender effects were observed, and accordingly, data from male and
2 female mice within a treatment group are considered together. The basic phenotype of FABP3^{+/-}
3 mice has been reported previously [9]. Total body mass of both WT and FABP3^{+/-} mice are greater
4 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
5 and 8.7±0.1 mM for WT and FABP3^{+/-}, respectively. High fat feeding caused hyperglycemia in
6 WT (10.6±0.3mM) but not FABP3^{+/-} (8.3±0.4mM). Fasting plasma NEFA for LF-WT, LF-
7 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.
8 Fasting levels of NEFA were significantly different only between LF-WT and HF-WT (p<0.05).
9 High fat feeding also resulted in fasting hyperinsulinemia regardless of genotype (p<0.05) with
10 values of 21±10, 19±10, 68±10 and 71±10 mU/ml for LF-WT, LF-FABP3^{+/-}, HF-WT and HF-
11 FABP3^{+/-}.

12 The effects of heterozygous deletion and diet on FABP3 protein level in cardiac muscle is
13 shown in **Figure 1**. On a standard LF diet heterozygous deletion of FABP3 resulted in a 32%
14 decrease in heart FABP3 protein (relative to GAPDH, 1.0±0.24 vs. 0.68±0.06 arbitrary units, p<
15 0.05). High fat feeding increased heart FABP3 level by ~ 2x (2.04±0.025) and heterozygous
16 deletion on a high fat diet blunted this increase (0.97±0.08, all differences p<0.05).

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

23 Cardiac function was also examined in an additional cohort of mice at 8 mo of age on HF
24 (n=8 WT, n=5 FABP3^{+/-}). At this time, body mass was 54±2.7 and 55±2.8 for WT and FABP3^{+/-}

1 respectively. The only significant difference between groups was a moderate decrement in basal
2 systolic function in FABP3^{+/-} mice compared with WT (WT FS% 52.4±3.1%; FABP3^{+/-} FS%
3 44.7±1.4%, p<0.001). Thus, at 8 months FABP3^{+/-} increased LV end-systolic dimension without
4 altering LV wall thickness or end-diastolic diameter.

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

12 **Metabolic Indices of Cardiac Glucose and Fatty Acid Metabolism.** Cardiac R_g
13 measurements are shown in **Figure 3**. Basal cardiac R_g in LF-WT was not different from rates in
14 HF-WT. LF-FABP3^{+/-} mice had a >two-fold increase in basal cardiac R_g when compared to LF-
15 WT. Basal R_g in HF-FABP3^{+/-} was reduced compared to LF -FABP3^{+/-}. Cardiac R_g was increased
16 during insulin clamps in all groups. In relation to LF, HF resulted in insulin resistance and a
17 reduction in cardiac R_g (**Figure 3**). FABP3^{+/-} prevented this decline as there was no difference
18 between R_g in LF -FABP3^{+/-} and HF-FABP3^{+/-} mice.

19 Results of cardiac R_f are shown in **Figure 4**. Basal cardiac R_f was elevated in HF-WT
20 compared to LF -WT. However, there was no such increase in HF-FABP3^{+/-} in relation to LF -
21 FABP3^{+/-} due to the high variance in the LF- FABP3^{+/-} measure of R_f. Here, differences in NEFA
22 levels between genotypes may be a contributing factor. Cardiac R_f was increased in HF-WT
23 during insulin clamps compared to LF-WT. As in the basal state, HF feeding induced an increase
24 in cardiac R_f, which was alleviated by partial deletion of FABP3, as rates were not different

1 between HF-FABP3^{+/-} and LF-FABP3^{+/-}. K_g and K_f are indices of glucose and LCFA flux that
2 are calculated independently of their respective concentrations. Results of K_g and K_f during
3 insulin clamps are shown in **Figure 5**. Cardiac K_g and K_f were decreased and increased,
4 respectively in HF-WT compared to LF-WT. A partial reduction in FABP3^{+/-} blunted these effects
5 of HF feeding.

6

7 **DISCUSSION**

8 Insulin resistance results in cardiac dysfunction due to an oversupply of FFA that drives
9 changes in metabolism, gene expression and function [22-24]. One therapeutic approach to correct
10 perturbed metabolism in insulin resistant tissue is to limit the uptake and oxidation of fatty acids
11 [25, 26]. In the present study, HF feeding was successfully employed to induce obesity and
12 insulin resistance in WT and FABP3^{+/-} mice. Although only HF-WT developed hyperglycemia
13 and hyperlipidemia, both genotypes were insulin resistant as evidenced by a reduction in whole
14 body glucose infusion rates during a hyperinsulinemic-euglycemic clamp. HF-WT resulted in a
15 ~70% impairment in glucose infusion rates compared to a 50% reduction in HF-FABP3^{+/-} [9].
16 Together these results suggest that a small reduction in FABP3^{+/-} may mitigate the development of
17 insulin resistance caused by HF feeding.

18 A partial reduction in FABP3 increased basal cardiac glucose utilization in LF fed
19 animals. High fat feeding blunted this response indicating the genetic reduction was not sufficient
20 to restore total glucose utilization in an obese, insulin resistant state. Insulin-stimulated glucose
21 utilization was reduced in HF-WT animals but not HF-FABP3^{+/-} compared to the respective LF
22 controls. Together, these results show that altering fatty acid utilization by targeting or inhibiting
23 FABP3^{+/-} levels may be a viable means to counter perturbed substrate flux seen in obesity and
24 insulin resistance. These results are in line with previous studies showing both ablation and a

1 heterozygous reduction in FABP3 are protective towards dietary-induced insulin resistance [6, 9].
2 However, a potential caveat is that the direct tissue specific effects of FABP3 cannot be readily
3 distinguished from secondary consequences of changes in systemic metabolism, although from a
4 therapeutic standpoint this distinction may not be critical.

5 To examine whether a reciprocal relationship between glucose and fatty acid utilization
6 existed in FABP3^{+/-} mice, I¹²⁵-BMIPP, a partially metabolizable fatty acid tracer was employed.
7 Basal fatty acid utilization was greater in WT-HF compared to WT-LF. Increases in fatty acid
8 utilization with obesity are well known and result from an increased efficiency of fatty acid uptake
9 [27]. This increased efficiency also contributes to the accumulation of intracellular
10 triacylglycerides which are implicated in the etiology of metabolic dysfunction [28-30]. Upon
11 insulin stimulation, WT-HF showed the hypothesized, reciprocal change in substrate utilization
12 with a decrease in glucose uptake and a corresponding increase in fatty acid utilization. This
13 finding is important to the insulin resistant heart as inhibiting fatty acid uptake and oxidation are
14 established anti-anginal approaches [31]. Ranolazine, trimetazidine and perhexiline are agents that
15 inhibit fatty acid utilization and are known to improve both the symptoms from and decrease the
16 frequency of angina pectoris [25].

17 When the combined results of cardiac glucose and fatty acids utilization are analyzed in
18 terms of substrate clearance rather than uptake, results show that FABP3^{+/-} mice do not have a
19 significantly altered basal rates. This is in agreement with the findings of Luiken et al [32, 33], in
20 which heterozygous deletion of FABP3 was described as “permissive” on fatty acid uptake using
21 giant vesicle techniques. However, when exposed to high fat feeding, this balance is disrupted and
22 only partially restored by lowering FABP3 levels. Beyond its role in directly regulating substrate
23 utilization, FABP3 has been shown to play important roles in intracellular binding, targeting,
24 signal transduction and gene expression [34]. Of interest, Murphy and colleagues [6] have shown

1 FABP3 to target specific fatty acids towards β -oxidation or esterification. A lack of FABP3 in
2 cardiac muscle differentially affects the fate and handling of both saturated and polyunsaturated
3 fatty acids with 20:4n-6 directed towards phospholipid fractions and 16:0 directed to β -oxidation.
4 By mediating the availability of fatty acids available for oxidation, FABP3 is concluded to play an
5 important role in fatty acid mediated signal transduction in the heart, consistent with, or
6 potentially extending, the effects expected from the “Randle cycle.”

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

20 As a strong interdependence exists between heart metabolism and function, we determined
21 the effects of diet-induced insulin resistance and FABP3^{+/-} on cardiac function at numerous time
22 points throughout the experiment. Echocardiographic assessment was conducted at baseline and
23 following 4,8, and 12 wk on each respective diet, spanning the metabolic evaluation reported.
24 Results demonstrated that this level of quantitative decrease in FABP3, with either a LF or HF

1 diet, did *not* alter basal heart function or dimension in conditions of comparable afterload (systolic
2 blood pressure) at 12 wk. This extends previous studies that have shown either no change or only
3 slight cardiac dysfunction with high fat feeding [43, 44]. The lack of change in cardiac function
4 with insulin resistance in the present study at 4 mo may be due to the circumscribed duration of
5 the dietary manipulation. Indeed, prolonged high-fat feeding (8 mo) in FABP3^{+/-} was associated
6 with a mild decrement LV end-systolic dimension without altering LV wall thickness or end-
7 diastolic size.

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

1 Lastly, plasma samples obtained from the animals were of limited volume. As such, other
2 measures of interest including leptin and adiponectin could not be assessed.

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

REFERENCES

- 1
2
3 [1] H. Taegtmeyer, P. McNulty and M.E. Young, Adaptation and maladaptation of the heart in
4 diabetes: Part I: general concepts *Circulation* 105 (2002) 1727-33.
- 5 [2] M.E. Young, P. McNulty and H. Taegtmeyer, Adaptation and maladaptation of the heart in
6 diabetes: Part II: potential mechanisms *Circulation* 105 (2002) 1861-70.
- 7 [3] B. Binas, H. Danneberg, J. McWhir, L. Mullins and A.J. Clark, Requirement for the heart-
8 type fatty acid binding protein in cardiac fatty acid utilization *Faseb J* 13 (1999) 805-12.
- 9 [4] A. Bonen, C.R. Benton, S.E. Campbell, A. Chabowski, D.C. Clarke, X.X. Han, J.F. Glatz
10 and J.J. Luiken, Plasmalemmal fatty acid transport is regulated in heart and skeletal muscle
11 by contraction, insulin and leptin, and in obesity and diabetes *Acta Physiol Scand* 178
12 (2003) 347-56.
- 13 [5] A. Bonen, J.J. Luiken and J.F. Glatz, Regulation of fatty acid transport and membrane
14 transporters in health and disease *Mol Cell Biochem* 239 (2002) 181-92.
- 15 [6] E.J. Murphy, G. Barcelo-Coblijn, B. Binas and J.F. Glatz, Heart fatty acid uptake is
16 decreased in heart fatty acid-binding protein gene-ablated mice *J Biol Chem* 279 (2004)
17 34481-8.
- 18 [7] F. Lehmann, S. Haile, E. Axen, C. Medina, J. Uppenberg, S. Svensson, T. Lundback, L.
19 Rondahl and T. Barf, Discovery of inhibitors of human adipocyte fatty acid-binding
20 protein, a potential type 2 diabetes target *Bioorg Med Chem Lett* 14 (2004) 4445-8.
- 21 [8] M. Furuhashi, G. Tuncman, C.Z. Gorgun, L. Makowski, G. Atsumi, E. Vaillancourt, K.
22 Kono, V.R. Babaev, S. Fazio, M.F. Linton, R. Sulsky, J.A. Robl, R.A. Parker and G.S.
23 Hotamisligil, Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding
24 protein aP2 *Nature* 447 (2007) 959.

- 1 [9] J. Shearer, P.T. Fueger, D.P. Bracy, D.H. Wasserman and J.N. Rottman, Partial gene
2 deletion of heart-type fatty acid-binding protein limits the severity of dietary-induced
3 insulin resistance *Diabetes* 54 (2005) 3133-9.
- 4 [10] B.N. Finck, The role of the peroxisome proliferator-activated receptor alpha pathway in
5 pathological remodeling of the diabetic heart *Curr Opin Clin Nutr Metab Care* 7 (2004)
6 391-6.
- 7 [11] P.T. Fueger, D.P. Bracy, C.M. Malabanan, R.R. Pencek, D.K. Granner and D.H.
8 Wasserman, Hexokinase II overexpression improves exercise-stimulated but not insulin-
9 stimulated muscle glucose uptake in high-fat-fed C57BL/6J mice *Diabetes* 53 (2004) 306-
10 14.
- 11 [12] D.L. Severson, Diabetic cardiomyopathy: recent evidence from mouse models of type 1
12 and type 2 diabetes *Can J Physiol Pharmacol* 82 (2004) 813-23.
- 13 [13] V.J. Exil, R.L. Roberts, H. Sims, J.E. McLaughlin, R.A. Malkin, C.D. Gardner, G. Ni, J.N.
14 Rottman and A.W. Strauss, Very-long-chain acyl-coenzyme a dehydrogenase deficiency in
15 mice *Circ Res* 93 (2003) 448-55.
- 16 [14] J.N. Rottman, G. Ni, M. Khoo, Z. Wang, W. Zhang, M.E. Anderson and E.C. Madu,
17 Temporal changes in ventricular function assessed echocardiographically in conscious and
18 anesthetized mice *J Am Soc Echocardiogr* 16 (2003) 1150-7.
- 19 [15] A.E. Halseth, D.P. Bracy and D.H. Wasserman, Limitations to basal and insulin-stimulated
20 skeletal muscle glucose uptake in the high-fat-fed rat *Am J Physiol Endocrinol Metab* 279
21 (2000) E1064-71.
- 22 [16] P.T. Fueger, D.P. Bracy, C.M. Malabanan, R.R. Pencek and D.H. Wasserman, Regulation
23 of Glucose Uptake by the Working Muscle of Conscious Mice: Distribution of Control

- 1 between Transport and Phosphorylation. *Am J Physiol Endocrinol Metab* (2003)
2 309.2003.
- 3 [17] C.R. Morgan and A. Lazarow, Immunoassay of pancreatic and plasma insulin following
4 alloxan injection of rats *Diabetes* 14 (1965) 669-71.
- 5 [18] J.N. Rottman, D. Bracy, C. Malabanan, Z. Yue, J. Clanton and D.H. Wasserman,
6 Contrasting effects of exercise and NOS inhibition on tissue-specific fatty acid and glucose
7 uptake in mice *Am J Physiol Endocrinol Metab* 283 (2002) E116-123.
- 8 [19] S.M. Furler, A.B. Jenkins and E.W. Kraegen, Effect of insulin on [3H]deoxy-D-glucose
9 pharmacokinetics in the rat. *Am. J. Physiol.* 255 (1988) E806-E811.
- 10 [20] E.W. Kraegen, D.E. James, A.B. Jenkins and D.J. Chisholm, Dose-response curves for in
11 vivo insulin sensitivity in individual tissues in rats *Am J Physiol Endocrinol Metab* 248
12 (1985) E353-362.
- 13 [21] C.T. Coburn, F.F. Knapp, Jr., M. Febbraio, A.L. Beets, R.L. Silverstein and N.A.
14 Abumrad, Defective Uptake and Utilization of Long Chain Fatty Acids in Muscle and
15 Adipose Tissues of CD36 Knockout Mice *J. Biol. Chem.* 275 (2000) 32523-32529.
- 16 [22] A.J. Murray, M. Panagia, D. Hauton, G.F. Gibbons and K. Clarke, Plasma free fatty acids
17 and peroxisome proliferator-activated receptor alpha in the control of myocardial
18 uncoupling protein levels *Diabetes* 54 (2005) 3496-502.
- 19 [23] A.N. Carley and D.L. Severson, Fatty acid metabolism is enhanced in type 2 diabetic
20 hearts *Biochim Biophys Acta* 1734 (2005) 112-26.

- 1 [24] A.N. Carley, L.L. Atkinson, A. Bonen, M.E. Harper, S. Kunnathu, G.D. Lopaschuk and
2 D.L. Severson, Mechanisms responsible for enhanced fatty acid utilization by perfused
3 hearts from type 2 diabetic db/db mice Arch Physiol Biochem 113 (2007) 65-75.
- 4 [25] A. Lam and G.D. Lopaschuk, Anti-anginal effects of partial fatty acid oxidation inhibitors
5 Curr Opin Pharmacol 7 (2007) 179-85.
- 6 [26] M. Roden, Blocking Fatty acids' mystery tour: a therapy for metabolic syndrome? Cell
7 Metab 6 (2007) 89-91.
- 8 [27] B.D. Hegarty, G.J. Cooney, E.W. Kraegen and S.M. Furler, Increased efficiency of fatty
9 acid uptake contributes to lipid accumulation in skeletal muscle of high fat-fed insulin-
10 resistant rats Diabetes 51 (2002) 1477-84.
- 11 [28] J. He, B.H. Goodpaster and D.E. Kelley, Effects of Weight Loss and Physical Activity on
12 Muscle Lipid Content and Droplet Size Obes Res 12 (2004) 761-769.
- 13 [29] P. Malenfant, D.R. Joannisse, R. Theriault, B.H. Goodpaster, D.E. Kelley and J.A.
14 Simoneau, Fat content in individual muscle fibers of lean and obese subjects Int J Obes
15 Relat Metab Disord 25 (2001) 1316-21.
- 16 [30] P. Malenfant, A. Tremblay, E. Doucet, P. Imbeault, J.A. Simoneau and D.R. Joannisse,
17 Elevated intramyocellular lipid concentration in obese subjects is not reduced after diet and
18 exercise training Am J Physiol Endocrinol Metab 280 (2001) E632-9.
- 19 [31] S.L. Coort, W.A. Coumans, A. Bonen, G.J. van der Vusse, J.F. Glatz and J.J. Luiken,
20 Divergent effects of rosiglitazone on protein-mediated fatty acid uptake in adipose and in
21 muscle tissues of Zucker rats J Lipid Res 46 (2005) 1295-302.

- 1 [32] J.J. Luiken, D.P. Koonen, W.A. Coumans, M.M. Pelters, B. Binas, A. Bonen and J.F.
2 Glatz, Long-chain fatty acid uptake by skeletal muscle is impaired in homozygous, but not
3 heterozygous, heart-type-FABP null mice *Lipids* 38 (2003) 491-6.
- 4 [33] J.F. Glatz, F.G. Schaap, B. Binas, A. Bonen, G.J. van der Vusse and J.J. Luiken,
5 Cytoplasmic fatty acid-binding protein facilitates fatty acid utilization by skeletal muscle
6 *Acta Physiol Scand* 178 (2003) 367-71.
- 7 [34] A.W. Zimmerman and J.H. Veerkamp, New insights into the structure and function of fatty
8 acid-binding proteins *Cell Mol Life Sci* 59 (2002) 1096-116.
- 9 [35] W.A. Alaynick, R.P. Kondo, W. Xie, W. He, C.R. Dufour, M. Downes, J.W. Jonker, W.
10 Giles, R.K. Naviaux, V. Giguere and R.M. Evans, ERRgamma Directs and Maintains the
11 Transition to Oxidative Metabolism in the Postnatal Heart *Cell Metab* 6 (2007) 13-24.
- 12 [36] C.R. Dufour, B.J. Wilson, J.M. Huss, D.P. Kelly, W.A. Alaynick, M. Downes, R.M.
13 Evans, M. Blanchette and V. Giguere, Genome-wide orchestration of cardiac functions by
14 the orphan nuclear receptors ERRalpha and gamma *Cell Metab* 5 (2007) 345-56.
- 15 [37] R.O. Heuckeroth, E.H. Birkenmeier, M.S. Levin and J.I. Gordon, Analysis of the tissue-
16 specific expression, developmental regulation, and linkage relationships of a rodent gene
17 encoding heart fatty acid binding protein *J Biol Chem* 262 (1987) 9709-17.
- 18 [38] K.P. Kempen, W.H. Saris, H. Kuipers, J.F. Glatz and G.J. Van Der Vusse, Skeletal muscle
19 metabolic characteristics before and after energy restriction in human obesity: fibre type,
20 enzymatic beta-oxidative capacity and fatty acid-binding protein content *Eur J Clin Invest*
21 28 (1998) 1030-7.

- 1 [39] B. Schmitt, M. Fluck, J. Decombaz, R. Kreis, C. Boesch, M. Wittwer, F. Graber, M. Vogt,
2 H. Howald and H. Hoppeler, Transcriptional adaptations of lipid metabolism in tibialis
3 anterior muscle of endurance-trained athletes *Physiol. Genomics* 15 (2003) 148-157.
- 4 [40] Y. Yuan, A.W. Kwong, W.A. Kaptein, C. Fong, M. Tse, J.F. Glatz, C. Chan and R.
5 Renneberg, The responses of fatty acid-binding protein and creatine kinase to acute and
6 chronic exercise in junior rowers *Res Q Exerc Sport* 74 (2003) 277-83.
- 7 [41] S. Clavel, L. Farout, M. Briand, Y. Briand and P. Jouanel, Effect of endurance training
8 and/or fish oil supplemented diet on cytoplasmic fatty acid binding protein in rat skeletal
9 muscles and heart *Eur J Appl Physiol* 87 (2002) 193-201.
- 10 [42] D. Hittel and K.B. Storey, Differential expression of adipose- and heart-type fatty acid
11 binding proteins in hibernating ground squirrels *Biochim Biophys Acta* 1522 (2001) 238-
12 43.
- 13 [43] P.T. Fueger, R.S. Lee-Young, J. Shearer, D.P. Bracy, S. Heikkinen, M. Laakso, J.N.
14 Rottman and D.H. Wasserman, Phosphorylation barriers to skeletal and cardiac muscle
15 glucose uptakes in high-fat fed mice: studies in mice with a 50% reduction of hexokinase
16 II *Diabetes* 56 (2007) 2476-84.
- 17 [44] S.Y. Park, Y.R. Cho, H.J. Kim, T. Higashimori, C. Danton, M.K. Lee, A. Dey, B.
18 Rothermel, Y.B. Kim, A. Kalinowski, K.S. Russell and J.K. Kim, Unraveling the temporal
19 pattern of diet-induced insulin resistance in individual organs and cardiac dysfunction in
20 C57BL/6 mice *Diabetes* 54 (2005) 3530-40.
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Diet	Genotype	<i>n</i>	Baseline	6wk	9wk	12wk
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*

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. Animal sex was equally distributed between groups. *Indicates significant difference (p<0.05) between diets at a given time point. Data represent means ± SE.

1

	Baseline	Low Fat, 3 mo	High Fat, 3 mo
WT	SBP (mmHg)	110 ± 4	
	HR (bpm)	590 ± 34	118 ± 4
	FS%	56.5 ± 1.9	642 ± 14
	IVSd (cm)	0.072 ± 0.003	48.3 ± 1.9
	LVIDd (cm)	0.265 ± 0.009	0.096 ± 0.001
	LVPWd (cm)	0.079 ± 0.003	0.095 ± 0.003
	IVSs (cm)	0.146 ± 0.005	0.167 ± 0.004
	LVIDs (cm)	0.116 ± 0.008	0.149 ± 0.011
	LVPWs (cm)	0.116 ± 0.007	0.167 ± 0.003
	LVIDs (cm)	0.116 ± 0.008	0.127 ± 0.007
FABP ^{+/-}	SBP (mmHg)	116 ± 4	112 ± 5
	HR (bpm)	652 ± 16	116 ± 3
	FS%	51.9 ± 1.0	643 ± 13
	IVSd (cm)	0.077 ± 0.002	46.5 ± 1.1
	LVIDd (cm)	0.262 ± 0.005	0.099 ± 0.001
	LVPWd (cm)	0.076 ± 0.002	0.096 ± 0.003
	IVSs (cm)	0.144 ± 0.004	0.170 ± 0.003
	LVIDs (cm)	0.126 ± 0.003	0.178 ± 0.005
	LVPWs (cm)	0.106 ± 0.003	0.161 ± 0.005
	LVIDs (cm)	0.126 ± 0.003	0.161 ± 0.005
LVPWs (cm)	0.106 ± 0.003	0.129 ± 0.004	
		0.139 ± 0.007	

2 **Table 2.** Effects of genotype and diet on cardiovascular parameters at baseline, 12 wk or 35 wk
3 on either a low or high fat diet. SBP, systolic blood pressure; HR, heart rate; FS%, fractional
4 shortening; IVSd, interventricular septal thickness in diastole; LVIDd, LV end-diastolic
5 dimension; LVPWd, LV posterior wall thickness in diastole; IVSs, interventricular septal

1 thickness in systole; LVIDs, LV end-systolic dimension; LVPWs, LV posterior wall
2 thickness in systole. Data represent means \pm SE. $n=8-10$ mice/treatment.

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5 **FIGURE LEGENDS**

6 **Figure 1.** Total FABP3 content in the heart muscle in WT and FABP3^{+/-} fed either a LF or an HF
7 (60% of kcal from lipid) for 12 weeks. Immunoblotting was performed to measure total FABP3
8 protein content. FABP3 levels were normalized to GADPH content. Order of loading on the
9 immunoblot follows the graphical data. Densitometry data are means \pm SE, $n=7$ per group.
10 *Indicates $p<0.05$ between genotype within diet, †Indicates $p<0.05$ between diets.

11 **Figure 2.** **A:** Systolic blood pressure (SBP)(mmHg) and **B:** % fractional shortening (%FS) at
12 baseline (1mo of age) and following 1,2,3 mo of either a LF or HF diet. No significant
13 differences were noted between diet, age or genotype. Data represent means \pm SE. $n=$
14 8-10 mice/treatment.

15 **Figure 3.** Glucose utilization (R_g) in cardiac muscle during saline infusion (top panel) and a
16 euglycemic-hyperinsulinemic clamp (4mU/kg/min)(bottom panel). Wild type (WT) and
17 heterozygous mice (FABP^{+/-}) are shown. *Indicates $p<0.05$ HF vs. LF fed within a genotype, †
18 $p<0.05$ WT vs. FABP^{+/-} within a diet. All data are reported as means \pm SEM, $n=7-8$ mice/group.

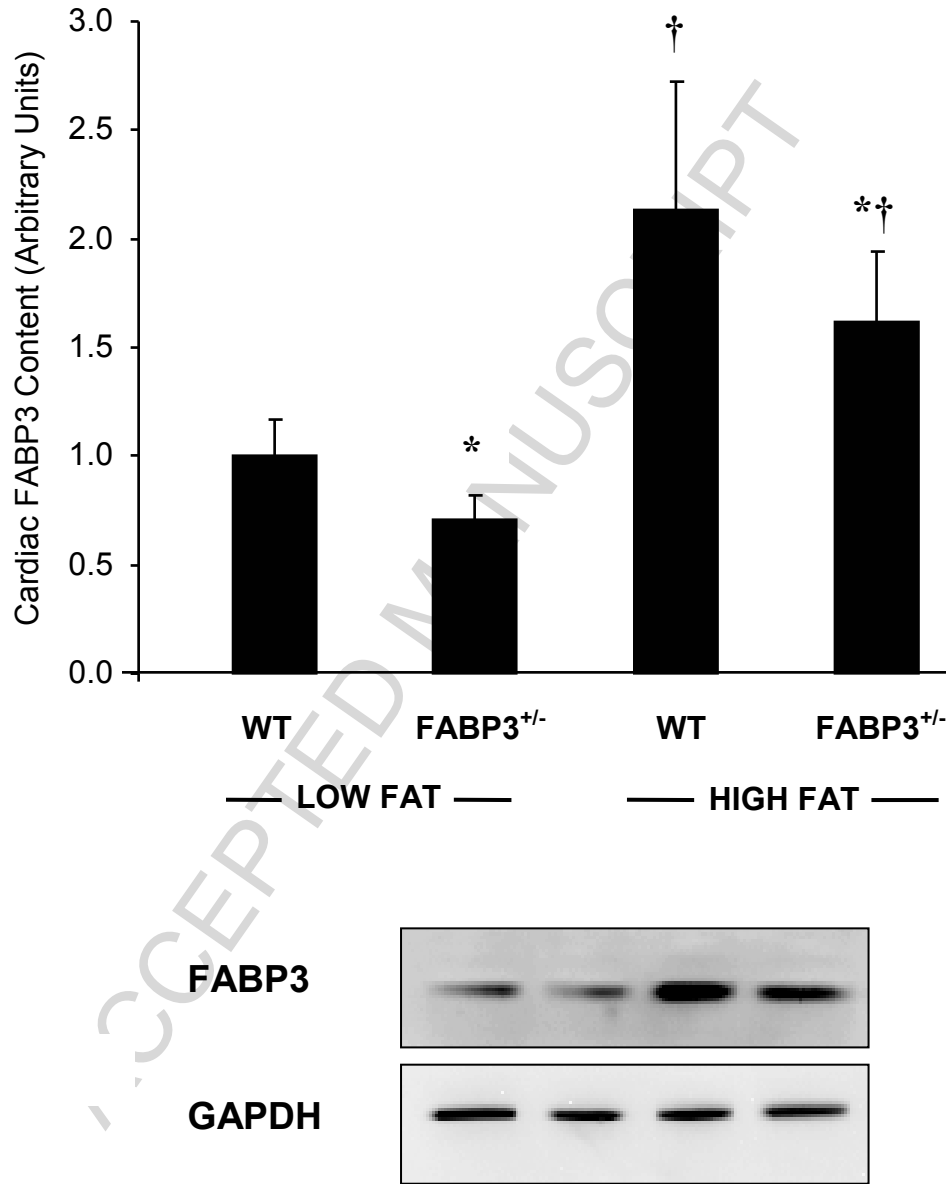
19 **Figure 4.** Fatty acid utilization (R_f) in cardiac muscle during saline infusion (top panel) and a
20 euglycemic-hyperinsulinemic clamp (4mU/kg/min, bottom panel). Wild type (WT) and
21 heterozygous mice (FABP3^{+/-}) are shown. *Indicates $p<0.05$ high fat vs. low fat fed within a
22 genotype.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

1
2 **Figure 5.** Relative rates of cardiac glucose (K_g) and fatty acid (K_f) clearance during a
3 euglycemic-hyperinsulinemic clamp across genotype and treatment. K_g is depicted by the closed
4 symbols (\bullet) while the open symbols (o) represent K_f . All data are reported as means \pm SEM, $n=7-$
5 8 mice/group. *Indicates $p<0.05$ HF vs. LF fed within a genotype, † $p<0.05$ WT vs. FABP3^{+/-}
6 within a diet.

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ACCEPTED MANUSCRIPT

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Figure 1

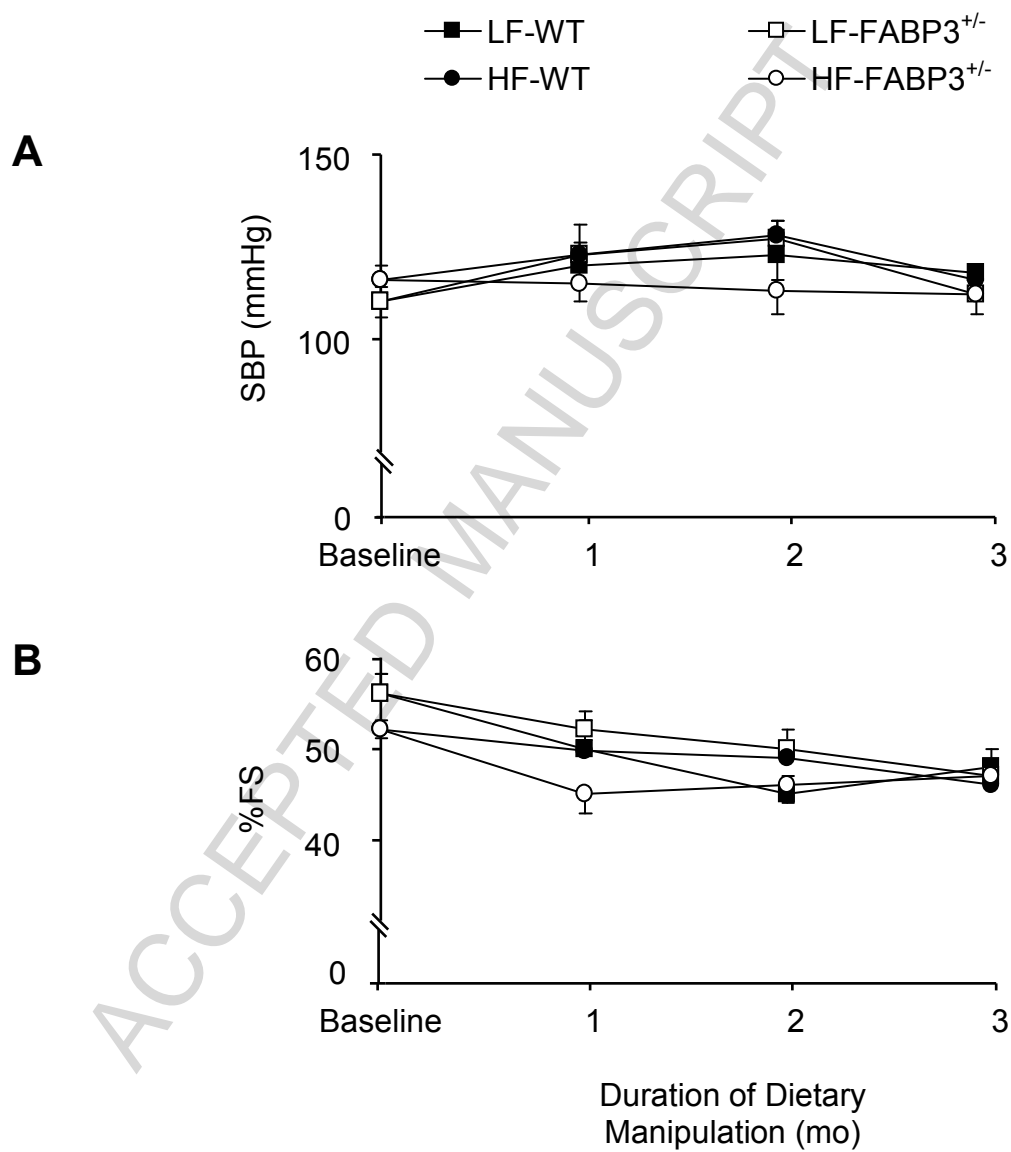


Figure 2

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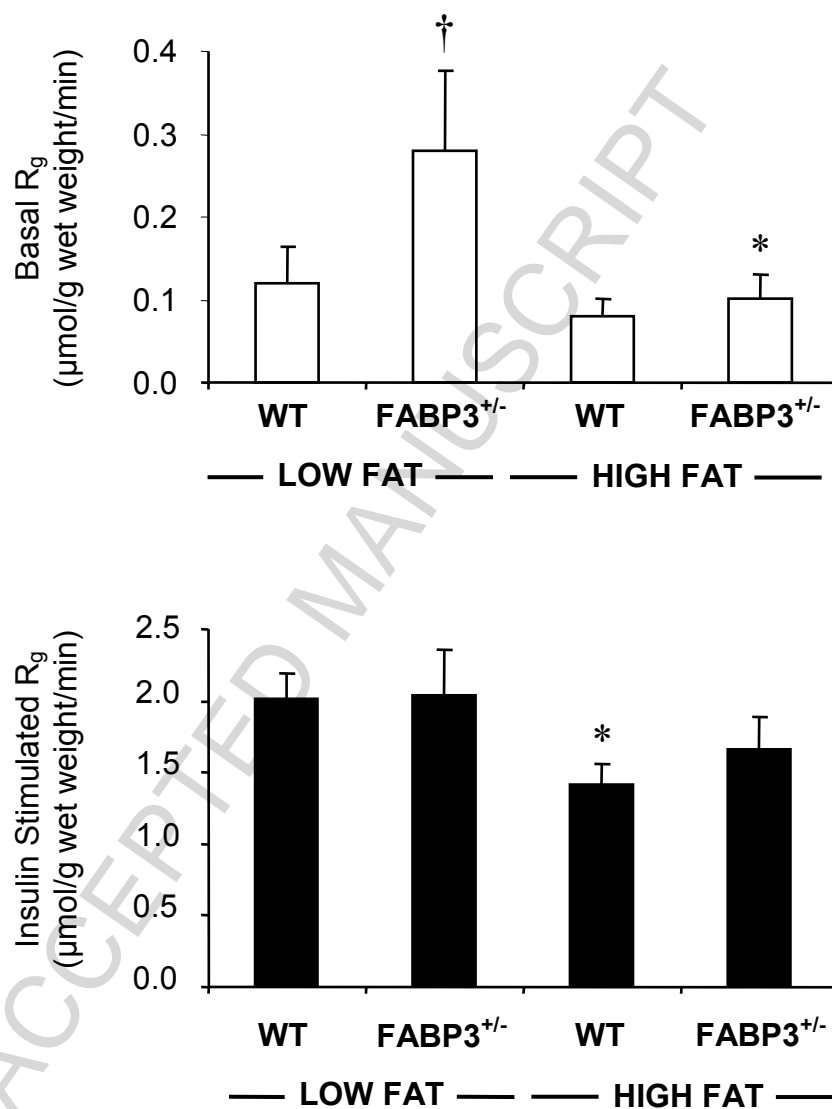


Figure 3

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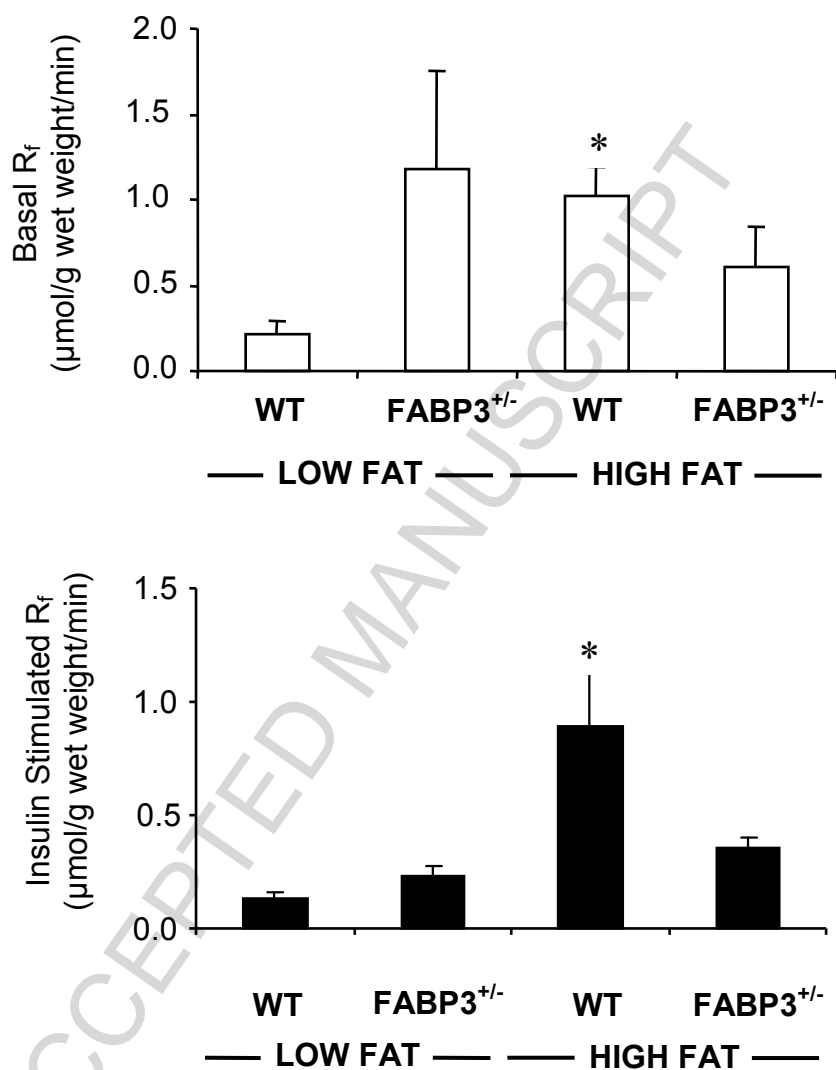


Figure 4

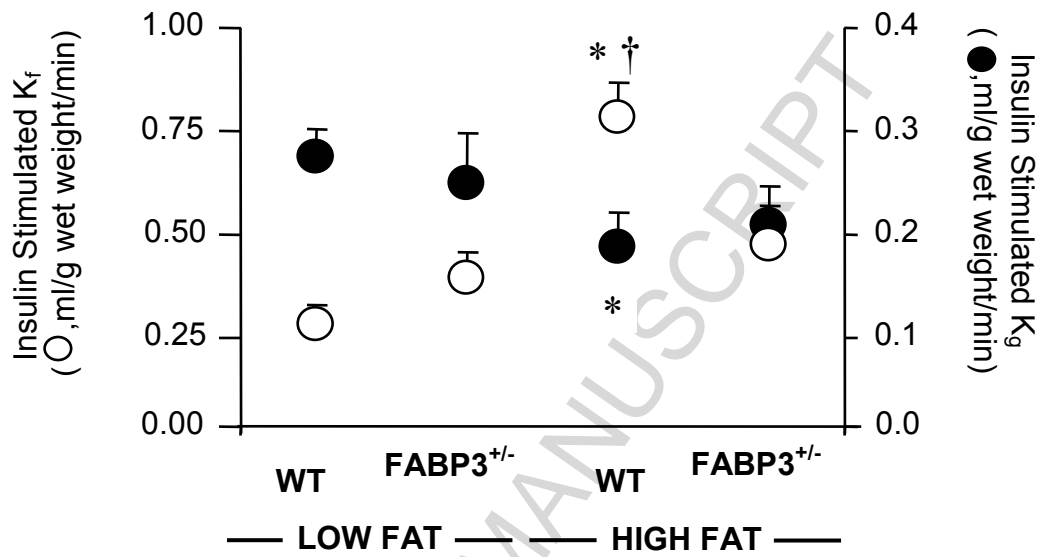
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Figure 5